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Theme

**Study of the Pharmacological Effect of *Ocimum basilicum* L.
and Manganese Nanosizes Against Physiological
and Biochemical Alterations in Wistar Rats**

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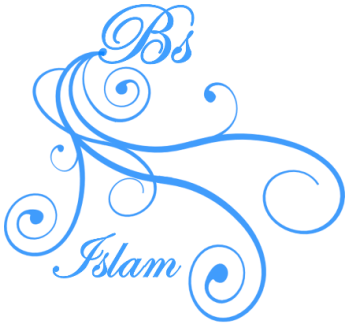
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إهداء

تأمل قوله تعالى (فنادى في الظلمات) .. حتى في بطن الحوت كان هناك أمل ..
الأمل هو تلك النقطة البيضاء التي لا ترى بالعين المجردة .. النقطة البيضاء التي يجب على وهجها
ألا يحتفي من حياتنا
الأمل هو أول خطوات النجاح .. فالنجاح ليس قفزة إنما خطوة تليها خطوة .. نحو قمة لا يرتقي سلمها إلا
أصحاب الهمم العالية لأن همته تقودهم إلى المواصله وإن تعثرت خطاهم
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Abstract

The primary aim of this study was to develop liposomes as a novel drug delivery system (NDDS) encapsulating basil aqueous extract, along with the green synthesis of MnO NPs. The research sought to assess their curative capability against changes caused by sub-chronic exposure to metribuzin in male Wistar albino rats. For *in vitro* study, bioactive molecules were extracted and phytochemical compounds were identified and quantified utilizing multiple analytical methods. Moreover, the green synthesis of MnO NPs was confirmed by using UV-Vis, FTIR, Zeta potential, XRD, XPS, SEM, TEM and EDS techniques. *In vitro* biological activities of basil including antioxidant, anti-inflammatory, and thrombolytic activities were studied. *In vitro* biological applications of MnO NPs including antioxidant, anti-inflammatory, thrombolytic, anticancer and catalytic properties were examined. Liposomes encapsulating basil extract were formulated. Furthermore, an *in vivo* acute toxicity test of basil and MnO NPs were done on Wistar rats. For *in vivo* rats study, thirty-six albino Wistar rats, weighing between 202.81 ± 5.44 g, were divided into 6 groups (n=6); G 1: rats used as control group (Control), G 2: metribuzin treated rats' group (MTZ), G 3: rats were exposed to metribuzin, given direct basil extract (BE), G 4: rats were exposed to metribuzin and received intraperitoneal injections of BE-loaded liposomes (BE-LPs), G 5: rats were exposed to metribuzin and given intraperitoneal administrations of MnO NPs (MnO NPs), G 6: rats were exposed to metribuzin and received intraperitoneal injections of BE- and MnO NPs-loaded liposomes (BE-MnO NPs-LPs). G 1, G 2 and G 3 were injected with physiological saline solution. Metribuzin intoxication was induced through oral administration in drinking water (mg/kg) over an 8-week period. For last 4 weeks, the rats were treated with BE, BE-LPs, MnO NPs, and BE-MnO NPs-LPs. The weight gain of each organ was measured. SOD, GPx, GSTs, CAT, GSH and MDA levels in each were measured in order to evaluate oxidative stress status. The tissue histology of the organs was examined. Various biochemical parameters and hematological markers were estimated. *In vitro* results of phytochemical analysis revealed that *Ocimum basilicum* L. contains multitude of bioactive compounds, especially phenolic compounds. The results indicated that the MnO NPs exhibited a spherical shape with an average size of 6.52 ± 0.88 nm. Basil extract and MnO NPs have significant biological activities. No mortality or any symptoms of toxicity effects were noted on the acute toxicity test at all basil extract and MnO NPs' doses tested. Microscopic images revealed that liposomes containing *Ocimum basilicum* L. extract were tiny, round vesicles. *In vivo* results indicate that exposure to metribuzin leads to various adverse effects, including hyperglycemia, microcytic anemia, inflammation, learning and memory deficits. Additionally, metribuzin exhibits toxic effects on multiple organ systems, such as the liver,

brain, lungs, heart, kidneys, testis, and muscles. The treatment of BE, BE-LPs, MnO NPs, and BE-MnO NPs-LPs significantly improved all the above-mentioned parameters. Basil-containing liposomes possess a variety of bioactive compounds that act as potent antioxidants and anti-inflammatory agents. MnO NPs offer therapeutic and preventive benefits to various organs due to their unique properties. These liposomes and MnO NPs are likely to reduce the harmful effects of metribuzin by modulating oxidative stress and inflammation, which are the main causes of cell damage. In conclusion, our research revealed that rats exposed to metribuzin herbicide exhibited several adverse effects, including increased organ weight, oxidative stress, hematological and biochemical alteration, inflammation, and structural changes in their organ tissues. We successfully synthesized MnO NPs and formulated liposomes as a novel drug delivery system loaded basil extract, which is rich in bioactive compounds, both to both treat the toxic effects caused by metribuzin exposure.

Keywords: *Ocimum basilicum L.*; Novel Drug Delivery System (NDDS); Liposomes; Nanotechnology; MnO NPs; Metribuzin.

كان الهدف الأساسي من هذه الدراسة هو تطوير الجسيمات الدهنية كنظام جديد لتوصيل الدواء (NDDS) مغلفًا المستخلص المائي للريحان، إلى جانب التخليق الأخضر لجزيئات أكسيد المنغنيز النانوية. وقد سعيينا في هذا البحث إلى تقييم قدراتها العلاجية ضد التغيرات الناجمة عن التعرض شبه المزمن للميتريبيزين في ذكور جرذان ويستار ألبينو. بالنسبة للدراسة المختبرية، تم استخلاص الجزيئات النشطة بيولوجيًا وتم تحديد المركبات الكيميائية للنبات وتحديد كميتها باستخدام طرق تحليلية متعددة. وعلاوة على ذلك، تم التأكد من التركيب الأخضر لجزيئات أكسيد المنغنيز النانوية باستخدام تقنيات UV-Vis و FTIR و Zeta potential و XRD و XPS و SEM و TEM و EDS. تمت دراسة الأنشطة البيولوجية المختبرية للريحان بما في ذلك الأنشطة المضادة للأكسدة والمضادة للالتهابات، والمضادة للتخثر. تم فحص التطبيقات البيولوجية لجزيئات أكسيد المنغنيز النانوية بما في ذلك مضادات الأكسدة ومضادات الالتهابات، ومضادات التخثر، ومضادات السرطان، والخصائص التحفيزية. تمت صياغة الجسيمات الدهنية المغلفة بمستخلص الريحان. وعلاوة على ذلك، تم إجراء اختبار السمية الحادة للريحان وجزيئات أكسيد المنغنيز النانوية على جرذان ويستار. للدراسة داخل الجسم الحي، استعملنا ستة وثلاثين جرذًا من جرذان ويستار ألبينو يتراوح وزنها بين 202.81 ± 44.5 غ، تم تقسيمها إلى 6 مجموعات (العدد=6)؛ المجموعة 1: الجرذان المستخدمة كمجموعة ضابطة (Control)، المجموعة 2: مجموعة الجرذان المعالجة بالميتريبيزين (MTZ)، المجموعة 3: تم تعريض الجرذان للميتريبيزين وإعطائها مستخلص الريحان بطريقة مباشرة (BE)، المجموعة 4: تم تعريض الجرذان للميتريبيزين وحقنها داخل الصفاق بالجسيمات الدهنية المحملة بمستخلص الريحان (BE-LPs)، المجموعة 5: تم تعريض الجرذان للميتريبيزين وحقنها داخل الصفاق بجزيئات أكسيد المنغنيز النانوية (MnO NPs)، المجموعة 6: تم تعريض الجرذان للميتريبيزين وحقنها داخل الصفاق بالجسيمات الدهنية المحملة بمستخلص الريحان وجزيئات أكسيد المنغنيز النانوية (BE-MnO NPs-LPs). تم حقن المجموعة 1 و 2 و 3 بمحلول ملحي فسيولوجي. تم إحداث التسمم الناجم عن الميتريبيزين من خلال تناوله عن طريق الفم في مياه الشرب (مغ/كغ) على مدى 8 أسابيع. وفي آخر 4 أسابيع، عولجت الجرذان بمستخلص الريحان وبالجسيمات الدهنية المحملة بمستخلص الريحان وجزيئات أكسيد المنغنيز النانوية وبالجسيمات الدهنية المحملة بمستخلص الريحان وجزيئات أكسيد المنغنيز النانوية. تم قياس زيادة وزن كل عضو. تم قياس مستويات SOD و GPx و GSTs و CAT و GHS و MDA في كل عضو من أجل تقييم حالة الإجهاد التأكسدي. تم فحص أنسجة الأعضاء. تم تقدير مختلف العلامات الكيميائية الحيوية والدموية. كشفت النتائج المختبرية للتحليل الكيميائي النباتي أن *Ocimum basilicum* L. يحتوي على العديد من المركبات النشطة بيولوجيًا، وخاصة المركبات الفينولية. وأشارت النتائج إلى أن MnO NPs أظهرت شكلًا كرويًا بمتوسط حجم 6.52 ± 0.88 نانومتر. يتميز مستخلص الريحان و MnO NPs بأنشطة بيولوجية هامة. ولم يلاحظ أي وفيات أو أي أعراض لتأثيرات

سمية في اختبار السمية الحادة في جميع جرعات مستخلص الريحان و MnO NPs التي تم اختبارها. وكشفت الصور الميكروسكوبية أن الجسيمات الدهنية التي تحتوي على مستخلص الريحان *Ocimum basilicum* L. كانت عبارة عن حويصلات صغيرة مستديرة. تشير النتائج في الجسم الحي إلى أن التعرض للميتريبيزين يؤدي إلى تأثيرات ضارة مختلفة، بما في ذلك ارتفاع سكر الدم وفقر الدم (microcytic anemia)، والالتهابات، والعجز في التعلم والذاكرة. بالإضافة إلى ذلك، يُظهر الميتريبيزين تأثيرات سامة على أجهزة أعضاء متعددة، مثل الكبد والدماغ والرئتين والقلب والكليتين والخصيتين والعضلات. العلاج ب BE و BE-LPs و MnO NPs و BE-MnO NPs-LPs حسن بشكل ملحوظ جميع المعايير المذكورة أعلاه. تحتوي الجسيمات الدهنية المحتوية على الريحان على مجموعة متنوعة من المركبات النشطة بيولوجيًا التي تعمل كعوامل مضادة للأكسدة ومضادة للالتهابات قوية. وتوفر MnO NPs فوائد علاجية ووقائية لمختلف الأعضاء بسبب خصائصها الفريدة. ومن المحتمل أن تقلل هذه الجسيمات الدهنية و MnO NPs من الآثار الضارة للميتريبيزين عن طريق تعديل الإجهاد التأكسدي والالتهابات، وهما السببان الرئيسيان لتلف الخلايا. في الختام، كشفت أبحاثنا أن الجرذان التي تعرضت لمبيد أعشاب الميتريبيزين أظهرت العديد من الآثار الضارة، بما في ذلك زيادة وزن الأعضاء، والإجهاد التأكسدي، والتغيرات الدموية والكيميائية الحيوية، والالتهابات، والتغيرات الهيكلية في أنسجة أعضائها. وقد نجحنا في تصنيع MnO NPs وصياغة الجسيمات الدهنية كنظام جديد لتوصيل الدواء محمل بمستخلص الريحان الغني بالمركبات النشطة بيولوجيًا لعلاج ومنع الآثار السامة الناجمة عن التعرض للميتريبيزين.

الكلمات الرئيسية: نبات الريحان؛ نظام جديد لتوصيل الأدوية (NDDS)؛ الجسيمات الدهنية؛

تكنولوجيا النانو؛ جزيئات أكسيد المنغنيز النانوية؛ الميتريبيزين.

Figures list

Figure 01: Global pesticide application and distribution for 1990-2018, based on data from FAO in 2019.....	7
Figure 02: Main classes of pesticides.....	8
Figure 03: The negative consequences of pesticides on human health across various living systems	9
Figure 04: Chemical structure of metribuzin	10
Figure 05: Human pesticide exposure pathways	13
Figure 06: Possible mechanisms of pesticide-induced toxicity in both animals and humans	15
Figure 07: Pesticide use and its impact on human reproductive function.....	16
Figure 08: Influence of environmental pollutants on neurodegenerative diseases.....	17
Figure 09: Significant molecular pathways implicated in the carcinogenic effects induced by pesticides	18
Figure 10: Pesticide transport pathways after usage	22
Figure 11: Leaves of <i>Ocimum basilicum</i> L.	28
Figure 12: Liposome as noval drug delivery system.....	31
Figure 13: Various methods of metal oxide NPs synthesis.....	34
Figure 14: Green synthesis mechanism of MnO NPs	35
Figure 15: Green synthesis of MnO NPs using extract of various plant parts	36
Figure 16: Experimental design of <i>in vivo</i> study.....	46
Figure 17: Experimental design of <i>Ocimum basilicum</i> L. <i>in vitro</i> essays.....	47
Figure 18: Experimental design of MnO NPs <i>in vitro</i> essays	51
Figure 19: Liposomes as a novel drug delivery system (NDDS) photo in the eight groups...59	
Figure 20: Liposomes as a novel drug delivery system (NDDS) photo in the eight groups after pre-processing	خطأ! الإشارة المرجعية غير معروفة.
Figure 21: MobileNet model training and architecture for Nano Drug Delivery Systems (NDDS) classification in a graphical format.....	خطأ! الإشارة المرجعية غير معروفة.
Figure 22: Total phenols, total flavonoids and total hydrolysable tannins concentrations in basil extract.....	73
Figure 23: HPLC-Q-TOF-MS chromatogram of (A) positive and (B) negative phenolic compounds from <i>Ocimum basilicum</i> L.	73
Figure 24: Chromatogram of phenolic acid found in <i>Ocimum basilicum</i> L. extract.....	75
Figure 25: GC-MS spectrum of volatile compounds present in basil leaves extract	76
Figure 26: Biosynthesis of MnO NPs using basil extract at different time.....	82

Figure 27: UV-Vis spectrum of MnO NPs.....	83
Figure 28: FTIR spectrum of MnO NPs.....	83
Figure 29: Zeta potential analysis of MnO NPs	84
Figure 30: XRD patterns of MnO NPs	84
Figure 31: EDS spectrum of MnO NPs.....	85
Figure 32: SEM and TEM image of MnO NPs and their average particle size	86
Figure 33: (A) XPS survey spectrum (B) High resolution core level O 1s peak (C) High resolution core level Mn2p peak of Mn ₃ O ₄ material surface	87
Figure 34: IC ₅₀ values for antioxidant activity of basil extract, MnO NPs and different standards.....	87
Figure 35: IC ₅₀ values for anti-inflammatory activity (protein anti-denaturation and RBC membrane stabilization assays) of basil extract, MnO NPs and diclofenac [®]	88
Figure 36: Blood clot lysis Before (A) and after (B) addition of <i>Ocimum basilicum</i> L. extract, MnO NPs and Aspegic [®]	89
Figure 37: Optical density and cytotoxicity of different concentration of MnO NPs measured by MTT assay on MCF-7 cell line	90
Figure 38: Medium aspect (A) and microscopic observation (B) of MCF-7 cells control (1) and MCF-7 cells treated by MnO NPs (2)	90
Figure 39: Photocatalytic and sonocatalytic activity of MnO NPs	91
Figure 40: Congo red degradation using photocatalytic (A) and sonocatalytic (B) activities of MnO NPs.....	91
Figure 41: Basil extract-loaded liposomes as a novel drug delivery system (NDDS)	92
Figure 42: Training and validation accuracy and loss curves	93
Figure 43: Sample predictive performance on test samples	93
Figure 44: Body weight gain (g/d/rat) in control and experimental rats' groups	98
Figure 45: Passive avoidance learning across of control and experimental groups	99
Figure 46: AChE activity in brain in control and experimental rats' groups.....	99
Figure 47: Blood glucose levels in control and experimental rats' groups.....	101
Figure 48: Albumin levels in control and experimental rats' groups	102
Figure 49: Renal markers levels in control and experimental rats' groups	102
Figure 50: Lipid profile levels in control and experimental rats' groups	103
Figure 51: Enzymatic activity in control and experimental rats' groups.....	104
Figure 52: GSH levels in control and experimental rats' groups in various organs	106
Figure 53: MDA levels in control and experimental rats' groups in various organs.....	107
Figure 54: SOD activity in control and experimental rats' groups in various organs	108

Figure 55: GPx activity in control and experimental rats' groups in various organs	109
Figure 56: GSTs activity in control and experimental rats' groups in various organs	110
Figure 57: CAT activity in control and experimental rats' groups in various organs	112
Figure 58: Histological photomicrographs of liver section of all experimental groups.....	114
Figure 59: Histological photomicrographs of brain section of all experimental groups	116
Figure 60: Histological photomicrographs of lungs section of all experimental groups	118
Figure 61: Histological photomicrographs of heart section of all experimental groups	120
Figure 62: Histological photomicrographs of kidneys section of all experimental groups...	122
Figure 63: Histological photomicrographs of testes section of all experimental groups	124

Tables list

Table 01: Physicochemical properties of metribuzin	10
Table 02: Human routes of pesticide exposure	12
Table 03: Toxic effect of metribuzin across a multitude of species	14
Table 04: Oxidative stress effects of metribuzin across various species.....	21
Table 05: Deleterious impacts of pesticides on the environment	22
Table 06: Strategies for removing pesticides with their advantages and disadvantages	23
Table 07: Some plants used in phytotherapy	27
Table 08: Properties of the nanomaterial and bulk material.....	33
Table 09: Green synthesis of manganese oxide nanoparticles using various plants	37
Table 10: Standard diet composition	44
Table 11: Dataset split summary	خطأ! الإشارة المرجعية غير معروفة.
Table 12: TensorFlow dataset conversion parameters.....	62
Table 13: MobileNetV2 configuration details.....	خطأ! الإشارة المرجعية غير معروفة.
Table 14: Training parameters and evaluation metrics.....	خطأ! الإشارة المرجعية غير معروفة.
Table 15: Percentage yield of crude extract	72
Table 16: Phytochemical compounds of <i>Ocimum basilicum</i> L. aqueous extract.....	72
Table 17: Characterization of phytochemicals found in extract of <i>Ocimum basilicum</i> L. by HPLC-Q-TOF-MS	74
Table 18: Phenolic compounds found by LC-ESI-MS analysis in basil extract	75
Table 19: Volatile compounds found by GC-MS analysis in basil extract	76
Table 20: Trace metal distribution in <i>Ocimum basilicum</i> L.	82
Table 21: Thrombolysis activity (clot lysis) of basil extract, MnO NPs and Aspegic®	88
Table 22: Model training and validation performance	خطأ! الإشارة المرجعية غير معروفة.
Table 23: Effect of basil's extract on mortality and physiological parameters of Wistar rats..	94
Table 24: Mortality, physiological parameters and behavior observations after acute toxicity using MnO NPs.....	96
Table 25: Relative organs weight in control and experimental groups	98
Table 26: Leukocyte and erythrocyte line markers level in control and experimental groups	100
Table 27: Photomicrographs of liver section of control and experimental groups.....	114
Table 28: Photomicrographs of brain section of control and experimental groups.....	116

Table 29: Photomicrographs of lungs section of control and experimental groups.....	118
Table 30: Photomicrographs of heart section of control and experimental groups.....	120
Table 31: Photomicrographs of kidneys section of control and experimental groups.....	122

Abbreviation list

ACh:	Acetylcholine
AChE:	Acetylcholinesterase
AI:	Artificial Intelligence
ALP:	Alkaline phosphatase
ALT:	Alanine transaminase
AML:	Acute myeloid leukemia
AOPs:	Advanced oxidation processes
AST:	Aspartate transferase
ATCC:	American type culture collection
Aβ:	Amyloid-beta
BC:	Before christ
BHA:	β -hydroxy acid
BHT:	Butylated hydroxytoluene
BSA:	Bovine serum albumin
CAMA-1:	Cancer of the mammary gland, adenocarcinoma, 1
CAT:	Catalase
CDNB:	1 - chloro 2,4 - dinitrobenzene
CLAHE:	Contrast limited adaptive histogram equalization
CNNs:	Convolutional neural network
CPK:	Creatine phosphokinase
CRC:	Colorectal cancer
Cu Kα:	Copper K-alpha
CuO NPs:	Copper oxide nanoparticles
DNA:	Deoxyribonucleic acid
DPPH:	1,1-diphenyl-2-picrylhydrazyl
DTNB:	5,5'-dithiobis-(2-nitrobenzoic acid)
EDCs:	Endocrine disrupting chemicals
EDTA:	Ethylenediaminetetraacetate
EDS:	Energy dispersive X-ray spectroscopy
EPA:	Environmental protection agency
Erk1/2:	Extracellular signal-regulated kinases 1 and 2
ESI:	Electron spray ionization modes
FAK:	Focal adhesion kinase

FAO:	Food and agriculture organization
FBS:	Fetal bovine serum
FeCl₃:	Ferric chloride
FNS:	Numeration of the blood formula
FRAP:	Ferric reducing antioxidant power
FTIR:	Fourier transform infrared spectroscopy
FWHM:	Full-width half maximum
GABA:	Gamma-aminobutyric acid
GC-MS:	Gas chromatography/mass spectrometry
GGT:	Gamma-glutamyl transferase
GOT:	Glutamyl oxaloacetic transaminase
GPT:	Glutamyl pyruvic transaminase
GPx:	Glutathione peroxidase
GR:	Glutathione reductase
Gran:	Granulocyte
GSH:	Reduced glutathione
GSK-3β:	Glycogen synthase kinase 3 beta
GSSG:	Glutathione disulfide
GSTs:	Glutathione S-transferase
H₂O₂:	Hydrogen peroxide
HCl:	Hydrochloric acid
HCT:	Hematocrit
HEK293:	Human embryonic kidney 293 cells
HepG2:	Human liver carcinoma cells
HGB:	Hemoglobin
HPLC-Q-TOF-MS:	High performance liquid chromatography- quadrupole time-of flight mass spectrometry
IC₅₀:	Half-maximal inhibitory concentration
IP:	Inhibition percentage
ISE:	Iterative stochastic elimination
JCPDS:	Joint committee on powder diffraction standards
JPG:	Joint photographic experts group
K₃Fe (CN)₆:	Potassium ferricyanide
KMnO₄:	Potassium permanganate

KNN:	K-nearest neighbors
LC-ESI-MS:	Liquid chromatography-electrospray ionization-mass spectrometry
LDH:	Lactate dehydrogenase
LDL:	Low-density lipoprotein
Lymph:	Lymphocytes
Mag:	Magnification
MCF-7:	Human breast cancer cells
MCH:	Mean corpuscular hemoglobin
MCHC:	Mean corpuscular hemoglobin concentration
MCV:	Mean corpuscular volume
MDA-MB-231:	Triple-negative breast cancer cells
MgO NPs:	Magnesium oxide nanoparticles
Mid:	
ML:	Machine learning
MnO NPs:	Manganese oxide nanoparticles
mRNA:	Messenger ribonucleic acid
MS:	Mass spectrometric
MTT:	3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide
NBT:	Nitro blue tetrazolium chloride
NDDS:	Novel drug delivery system
NHL:	Non-hodgkin lymphoma
NIST:	National institute standard and technology
NPs:	Nanoparticles
O₂^{•-}:	Superoxide anion
OECD:	Organisation for economic co-operation and development
OD:	Optical density
OH[•]:	Hydroxyl radicals
PAL:	Passive avoidance learning
PBS:	Phosphate-buffered saline
PCB:	Polychlorinated biphenyl
PEG:	Polyethylene glycol
PLT:	Platelet
PP-2A:	Phosphoprotein phosphatase 2A

ppm:	Parts per million
QSPR:	Quantitative structure-property relationship
RBC:	Red blood cell
RGB:	Red, green and blue
RNA:	Ribonucleic acid
RNS:	Reactive nitrogen species
ROS:	Reactive oxygen species
rpm:	Rotation per minute
RPMI:	Roswell park memorial institute
RT:	Retention time
SCh:	Thiocholine
SEM:	Scanning electron microscopy
SeO NPs:	Selenium oxide nanoparticles
SOD:	Superoxide dismutase
STL:	Step-through latency
TBA:	Thiobarbituric acid
TBARS:	Thiobarbituric acid reactive substances
TBS:	Tert-butyldimethyl silyl ethers
TCA:	Trichloroacetic acid
TDC:	Time spent in the dark chamber
TEM:	Transmission electron microscopy
TNB:	2-nitro-5-thiobenzoic acid
UHPLC:	Ultra-performance liquid chromatography
UV-Vis:	Ultraviolet-visible spectroscopy
WBC:	White blood cell
WHO:	World Health Organization
XRD:	X-ray powder diffraction
XPS:	X-ray photoelectron spectroscopy
ZnO NPs:	Zinc oxide nanoparticles

Content table

Acknowledgment	
Abstract	
Figures list	
Tables list.....	
Abbreviation list	
Content table.....	
Introduction	1

Chapter I : Pesticides

I. Pesticides.....	6
I.1. Definition of Pesticides	6
I.2. Utilization of Pesticides Around the World	6
I.3. Classification of Pesticides	7
I.4. Adverse Effects of Pesticides	8
II. Metribuzin.....	9
II.1. Difinition of Metribuzin.....	9
II.2. Physical and Chemical Characteristics of The Herbicides Metribuzin.....	10
II.3. Metribuzin Exposure Routes in Humans	11
II.4. Metribuzin Toxicity on Living Organisms	13
II.5. Effects of Metribuzin on Human Health.....	15
II.5.1. Metribuzin-Induced Infertility and Reproduction Problems	15
II.5.2. Metribuzin-Induced Neurotoxicity	17
II.5.3. Metribuzin-Induced Cancer	18
II.5.4. Metribuzin-Induced Endocrine System Alteration	19
II.5.5. Metribuzin-Induced Oxidative stress.....	19
II.5.5.1. Definition of Oxidative Stress	19
II.5.5.2. Oxidants.....	19
II.5.5.3. Antioxidants	20
II.5.5.4. Oxidative Stress and Metribuzin	20
II.6. Effects of Metribuzin on the Environment.....	21
II.7. Removal of Metribuzin from Environment.....	23

Chapter II: *Ocimum basilicum* L.

I. Phytotherapy	27
I.1. Definition and Distribution of <i>Ocimum basilicum</i> L.....	28
I.2. Botanical and Taxonomical Description	29

I.3. Chemical Composition and Nutritional Properties.....	29
I.4. Therapeutic Effect and Economic Value.....	30
I.5. Herbal Extract-Loaded Liposomes as A Novel Drug Delivery System (NDDS)	30

Chapter III : Nanotechnology

I. Nanotechnology	33
II. Nanomaterials.....	33
III. General Method of Synthesis for Metal Oxide Nanoparticles.....	34
IV. Green Synthesis of MnO NPs.....	35
V. Green Synthesis of MnO NPs Using Plant Extract	36
VI. Analytical Characterization Techniques of MnO NPs	39
VII. Biomedical Applications of MnO NPs	39
VII.1.1. Antioxidant Activity of MnO NPs	39
VII.1.2. Antiinflammatory Activity of MnO NPs	39
VII.1.3. Anti-Cancer Activity of MnO NPs.....	40
VII.1.4. Anti-Diabetic Activity of MnO NPs	40
VII.1.5. Anti-Bacterial Activity of MnO NPs	40
VII.1.6. Anti-Viral Activity of MnO NPs.....	41
VII.1.7. Anti-Fungal Activity of MnO NPs.....	41

Experimental Part

Chapter I: Material and Methods

I. Material.....	44
I.1. Plant Materials	44
I.2. Animal Materials.....	44
I.2.1. Animal and Husbandry Condition	44
I.2.2. Animals Treatment	45
I.2.3. Sacrifice, Blood Sampling and Tissues Collection.....	45
II. Methods.....	47
II.1. In Vitro Study	47
II.1.1. In Vitro Essays of <i>Ocimum basilicum</i> L.	47
II.1.1.1. Method of Aqueous Extract Preparation	47
II.1.1.2. Calculation of Percentage Yield of Aqueous Plant Extract.....	48
II.1.1.3. Phytochemical Screening.....	48
II.1.1.4. Quantification of Phytochemical Compounds.....	48
II.1.1.5. HPLC-Q-TOF-MS Analysis for Phenolic Compounds.....	49
II.1.1.6. LC-ESI-MS Analysis.....	49

II.1.1.7. GC-MS Analysis for Volatile Compounds.....	50
II.1.1.8. Analysis of Trace Metal Distribution in <i>Ocimum basilicum</i> L.....	50
II.1.2. In Vitro Essays of MnO NPs	51
II.1.2.1. Biosynthesis of MnO NPs	51
II.1.2.2. Characterization of MnO NPs	52
II.1.2.2.5. X-Ray Photoelectron Spectroscopy (XPS).....	52
II.1.2.2.7. Energy X-Ray Dispersive Spectroscopy (EDS)	53
II.1.3. Applications of <i>Ocimum basilicum</i> L. and MnO NPs.....	53
II.1.3.1. Antioxidant Activity	53
II.1.3.2. Anti-Inflammatory Activity	54
II.1.3.3. Thrombolytic Activity (Clot Lysis).....	55
II.1.3.4. Anticancer Activity Against MCF-7 Breast Cancer Cell Line	55
II.1.3.5. Catalytic Activity (Dye Degradation).....	56
II.1.4. In Vitro Essays of Liposomes and Artificial Intelligence	57
II.1.4.1. Biosynthesis of Liposomes	57
II.1.4.2. Liposomes Bioconjugation by Orcinol and Glutamine	57
II.1.4.3. Characterization of Liposomes Using Optical Microscope	57
II.1.4.4. MobileNet Models as a Tool for Liposomes Classification	57
II.2. In Vivo Study.....	64
II.2.1. In Vivo Acute Toxicity Evaluation of Basil Extract	64
II.2.2. In Vivo Acute Toxicity Evaluation of MnO NPs.....	65
II.2.3. Step-through Passive Avoidance Test (Passive Avoidance Learning Test).....	65
II.2.3.1. Passive Avoidance Training	65
II.2.3.2. Retention Test.....	66
II.2.5. Relative Organ Weight	66
II.2.6. Biochemical and Hematological Markers	66
II.2.7. Oxidative Stress Parameters	67
II.2.7.1. Preparation of Homogenates.....	67
II.2.7.2. Reduced Glutathione (GSH) Levels	67
II.2.7.3. Malondialdehyde (MDA) Levels.....	67
II.2.7.4. Superoxide Dismutase (SOD) Activity	68
II.2.7.5. Glutathione Peroxidase (GPx) Activity	68
II.2.7.6. Glutathione-S-Transferase (GSTs) Activity	68
II.2.7.7. Catalase (CAT) Activity	69
II.2.8. Acetylcholinesterase (AChE) Activity	69

II.2.9. Histological Study	70
II.2.10. Statistical Analysis	70

Chapter II: Results

I. Results	72
I.1. In Vitro Study	72
I.1.1. Phytochemical Study of <i>Ocimum basilicum</i> L.....	72
I.1.1.1. Percentage Yield of Aqueous Plant Extract.....	72
I.1.1.2. Qualitative Phytochemical Compounds.....	72
I.1.1.3. Quantitative Phytochemical Compounds.....	72
I.1.1.4. HPLC-Q-TOF-MS Analysis for Phenolic Compounds	73
I.1.1.5. LC-ESI-MS Analysis for Phenolic Acids and Flavonoids	75
I.1.1.6. GC-MS Analysis for Volatile Compounds.....	76
I.1.1.7. Trace Metal Distribution in <i>Ocimum basilicum</i> L.	82
I.1.2. Biosynthesis and Characterization of MnO NPs	82
I.1.2.1. Biosynthesis of MnO NPs (Visual observation).....	82
I.1.2.2. Characterization of MnO NPs.....	83
I.1.2.2.1. UV-Visible (UV-Vis) Spectroscopy	83
I.1.2.2.2. Fourier Infrared (FTIR) Spectroscopy	83
I.1.2.2.3. Zeta Potential	84
I.1.2.2.4. X-Ray Diffractometry (XRD).....	84
I.1.2.2.5. Energy X-Ray Dispersive Spectroscopy (EDS)	85
I.1.2.2.6. Scanning and Transmission Electron Microscopy (SEM and TEM).....	85
I.1.2.2.7. X-Ray Photoelectron Spectroscopy (XPS)	86
I.1.3. Biological Applications of <i>Ocimum basilicum</i> L. and MnO NPs.....	87
I.1.3.1. Anti-Oxidant Activity	87
I.1.3.2. Anti-Inflammatory Activity	87
I.1.3.3. Thrombolytic Activity (Clot Lysis)	88
I.1.3.4. Anticancer Activity Against MCF-7 Breast Cancer Cell Line	89
I.1.3.5. Catalytic Activity (Dye Degradation).....	90
I.1.4. In Vitro Essays of Liposomes and Artificial Intelligence	91
I.1.4.1. Characterization of Liposomes Using Optical Microscope	91
I.1.4.2. Liposomes Classification by MobileNet Models	92
I.2. In Vivo Study	94
I.2.1. In Vivo Acute Toxicity Evaluation of Basil Extract.....	94
I.2.2. In Vivo Acute Toxicity Evaluation of MnO NPs	95

I.2.3. Body Weight Gain	98
I.2.4. Relative Organs Weight	98
I.2.5. Step-through Passive Avoidance Test (passive avoidance learning).....	99
I.2.6. Acetylcholinesterase (AChE) Activity.....	99
I.2.7. Hematological Markers.....	100
I.2.8. Biochemical Parameters.....	101
I.2.8.1. Blood Glucose	101
I.2.8.2. Albumin	101
I.2.8.3. Renal Markers.....	102
I.2.8.4. Lipid Profile.....	103
I.2.8.5. Enzymatic Activity	104
I.2.9. Oxidative Stress Parameters.....	105
I.2.9.1. Reduced Glutathione (GSH) Levels	105
I.2.9.2. Malondialdehyde (MDA) Levels.....	106
I.2.9.3. Superoxide Dismutase (SOD) Activity	107
I.2.9.4. Glutathione Peroxydase (GPx) Activity	108
I.2.9.5. Glutathione-S-Transferase (GSTs) Activity	110
I.2.9.6. Catalase (CAT) Activity	111
I.2.10. Histological Study.....	112
I.2.10.1. Photomicrographs of Liver Section	112
I.2.10.2. Photomicrographs of Brain Section.....	114
I.2.10.3. Photomicrographs of Lungs Section.....	116
I.2.10.4. Photomicrographs of Heart Section.....	118
I.2.10.5. Photomicrographs of Kidneys Section	120
I.2.10.6. Photomicrographs of Testes Section	122

Chapter III: Discussion

I. Discussion.....	126
I.1. Qualitative and Quantitative Phytochemical Compounds.....	126
I.2. Chromatographic Methods Analysis Phytochemical Compounds	127
I.3. Trace Metal Distribution in <i>Ocimum basilicum</i> L.....	128
I.4. Biosynthesis and Characterization of MnO NPs	128
I.5. Anti-Oxidant Activity of <i>Ocimum basilicum</i> L. and MnO NPs.....	132
I.6. Anti-Inflammatory Activity of <i>Ocimum basilicum</i> L. and MnO NPs.....	133
I.7. Thrombolytic Activity (Clot Lysis) of <i>Ocimum basilicum</i> L. and MnO NPs.....	134
I.8. Anticancer Activity of MnO NPs	135

I.9. Catalytic Activity (Dye Degradation) of MnO NPs	136
I.10. Biosynthesis and Characterization of Liposomes.....	137
I.11. Liposomes Classification by MobileNet Models	137
I.12. In Vivo Acute Toxicity Evaluation of <i>Ocimum basilicum</i> L. and MnO NPs.....	138
I.13. Body Weight Gain and Relative Organs Weight	139
I.14. Brain toxicity markers "Step-through Passive Avoidance Test (passive avoidance learning) and Acetylcholinesterase (AChE) Activity"	140
I.15. Hematological Markers	141
I.16. Biochemical Parameters	143
I.16.1. Blood Glucose.....	143
I.16.2. Renal Toxicity Markers.....	145
I.16.3. Lipid Profile	146
I.16.4. Enzymatic Activity	147
I.17. Oxidative Stress Parameters	149
I.18. Histological Study	151
Conclusion & Perspectives.....	153
References	157
Annex.....	209

Introduction

Introduction



esticides are defined as chemical substances employed in agricultural practices for the purpose of eradicating or managing pest populations, including those that serve as vectors for diseases affecting humans or animals (Derouiche, Rezzag Mohcem, & Serouti, 2020). Pesticides are widely acknowledged as major contributors to environmental contamination in today's world. These compounds, designed to be lethal to pests, can have harmful effects on unintended targets, including humans, when consumed (Kadeche et al., 2017). Agricultural pesticide use has increased in recent years (Derouiche, Serouti, & Rezzag Mohcen, 2019). According to the Algerian Association for the Protection of the Environment, Algeria is among the countries with the highest pesticide consumption, using approximately 30,000 tons annually (Derouiche, Rezzag Mohcen, & Serouti, 2018). The presence of pesticide residues in food has raised concerns about their potential negative impact on human health. Some pesticides have been linked to various health issues, including developmental problems, hormonal disruptions, neurological damage, and certain cancers. As a result, substances intended to enhance and protect food production may unintentionally endanger human well-being (Sajad et al., 2024).

Metribuzin, a compound classified as an asymmetric triazine with the chemical formula 4-amino-6-(1,1-dimethyl)-3-(methylthio)-1,2,4-triazin 5(4H)-one, received approval for herbicidal use in the United States in 1973 (Rubio-Armendáriz, Paz-Montelongo, Gutiérrez-Fernández, Luis-González, & Hardisson, 2024). It functions by inhibiting electron transport essential for photosynthesis, effectively managing broadleaf weeds and grasses in various crops, turfgrass, and non-agricultural areas (Jamil et al., 2018). Common applications of metribuzin include the cultivation of potatoes, soybeans, peas, tomatoes, and lentils (Chiali, Merzouk, Merzouk, Medjdoub, & Narce, 2013). This herbicide can be applied both before and after plant emergence to combat annual grasses and broadleaf weeds (Lahlaf, Belkacem, Laabed, & Khenifi, 2024), with the dosage varying based on soil composition and geographic location (Tuti & Das, 2011). However, the use of metribuzin has been associated with negative effects on humans, fish, and domestic animals (Kadeche et al., 2016).

Metribuzin, a foreign chemical compound, exemplifies the toxicity mechanisms common to most xenobiotics, including herbicides and environmental contaminants. These mechanisms involve disrupting the body's overall antioxidant defense and promoting lipid peroxidation through increased free radical production (Derouiche et al., 2020). Reactive oxygen species (ROS) primarily mediate metribuzin's harmful effects by interacting with various biological molecules (Zeng, Du, Ding, & Jiang, 2021). Studies have shown that metribuzin triggers

intracellular oxidative stress (Derouiche et al., 2018). Investigations into metribuzin's toxic impact have revealed heightened oxidative stress and alterations in antioxidant levels in both laboratory settings and living organisms (Kadeche et al., 2016). Oxidative stress occurs when there is an imbalance between the generation and accumulation of ROS in cells and tissues, and the biological system's capacity to neutralize these reactive substances (Pizzino et al., 2017). This phenomenon is a key factor contributing to physiological and metabolic changes, as well as various diseases in the body (Chetehouna, Atoussi, Boulaares, Guemari, & Derouiche, 2020).

The natural world consistently provides insights into the fundamental principles of coexistence. Humans have long utilized natural substances from plants, animals, and minerals to treat various ailments (Boulaares & Derouiche, 2021). The use of plant metabolites dates back to 2600 BC, with secondary metabolites from plants serving as primary sources of food, medicine, and poison for over four millennia (Twaij & Hasan, 2022). Many herbal products demonstrate therapeutic properties, including antioxidant, anticancer, anti-inflammatory, antimicrobial, and antiviral effects (Parham et al., 2020). Additionally, medicinal plants find applications in diverse industries such as food, beverage, and pharmaceuticals (Tang, Dunshea, & Suleria, 2019). Plant-derived bioactive compounds, especially polyphenols, have garnered significant attention due to their potential health benefits (Ali, Bashmil, Cottrell, Suleria, & Dunshea, 2021). Among aromatic plants, common basil holds a prominent position (Taha, Alharby, Bamagoos, Medani, & Rady, 2020). It belongs to the Lamiaceae family and is cultivated annually in numerous regions worldwide (Derouiche, Guemari, & Boulaares, 2020). Common basil is a key species within the *Ocimum* genus (Zahedifar, Moosavi, Zarei, Shafigh, & Karimian, 2019), which encompasses over 60 species, including *basilicum* (Murali & Prabakaran, 2018). *Ocimum basilicum* L. has been cultivated and utilized since ancient times (Georgiadou et al., 2018). It is commonly referred to as sweet basil (Ahmed et al., 2019), it has gained recognition as both a medicinal plant and culinary herb due to its phytochemical composition (Rumengan, Mandey, Citraningtiyas, & Luntungan, 2019).

A Novel Drug Delivery System (NDDS) represents an innovative approach that employs cutting-edge technologies and creative concepts to administer active compounds at safe and effective levels, producing the desired pharmacological outcomes (Kumar, Mandal, & Mahmood, 2023). The drug delivery system enables the release of active pharmaceutical ingredients to generate the necessary therapeutic effects (Adepu & Ramakrishna, 2021). Liposome-based drug delivery systems have been instrumental in formulating potent drugs to enhance therapeutic efficacy (Thenge, Chandak, & Adhao, 2020). Regarded as promising and versatile drug carriers, liposomes offer advantages over conventional delivery methods, including targeted delivery, sustained or controlled release, protection against degradation and

clearance, improved therapeutic effects, and reduced toxicity (Liu, Chen, & Zhang, 2022). These spherical vesicles, composed of one or more phospholipid bilayers, are extensively researched as carriers for various substances and bioactive agents in medical, pharmacological, nutritional, and biological studies (Salimi, 2018). The components encapsulated within liposomal formulations are designed to enhance solubility, minimize adverse effects, prolong drug release, target specific regions, protect drugs, and improve therapeutic efficacy (Rahmi, Rosidah, Widyawati, & Sumaiyah, 2022). As a result, liposomes have proven effective in delivering plant-derived compounds to combat systemic toxins (Kulabhusan, Agrawal, Jeevanandam, & Danquah, 2020).

Nanotechnology and nanoscience are the most commonly utilized scientific disciplines in areas such as human healthcare, tissue engineering, food production, and agriculture (Rajput, Sevalkar, Pardeshi, & Pingale, 2023). As an advanced research domain, nanotechnology employs environmentally friendly methods to create nanoparticles that are chemically stable, non-toxic, biocompatible, and appropriate for biomedical uses (Kavaz, Abubakar, Rizaner, & Umar, 2021). In recent years, there has been growing interest in manganese oxide nanoparticles for various applications, including water purification, catalytic processes, solar energy conversion, and biomedical uses such as biosensing, bioimaging, cancer diagnosis and treatment, and drug delivery systems (Alsaleh et al., 2024). The widespread use of manganese oxides in electrochemistry, medicine, and catalysis can be attributed to their cost-effectiveness, environmental friendliness, diverse manganese forms, and natural abundance (Shaik et al., 2021).

In the current investigation, the objective was the formulation of liposomes as a novel drug delivery system (NDDS) encapsulating basil extract and the environmentally friendly synthesis of MnO NPs, with the intent to evaluate their therapeutic efficacy against alterations induced by sub-chronic exposure to metribuzin in male Wistar albino rats.

Literature Review

Chapter I
Pesticides
Metribuzin
and
Oxidative Stress

I. Pesticides

I.1. Definition of Pesticides

Pesticides refer to agents designed to eradicate pest organisms. The term is etymologically rooted in the suffix "cide," which is derived from the Latin term "cida," signifying an entity that kills (Oguh, Okunowo, Musa, & Osuji, 2020). Pesticides are chemical substances that are used in agriculture to eliminate or combat pests, including vectors of human or animal diseases (Derouiche et al., 2020). Pesticides play a beneficial role in enhancing agricultural productivity. They contribute to higher crop yields, increased accessibility and affordability of food items, and reduced infestations of pests, weeds, and plant diseases (AbuQamar et al., 2024). The most commonly used pesticides are fungicides, insecticides, and herbicides, which are used to control weeds and pests on agricultural sites (Sood, 2024). Pesticides are a type of chemical that are widely recognized to be significant contributors to environmental pollution in the current era (Kadeche et al., 2017).

I.2. Utilization of Pesticides Around the World

The use of pesticides in agriculture has increased over the past decade (Derouiche et al., 2019). A significant proportion exceeding fifty percent of global pesticide application is documented in Asian nations, with subsequent utilization observed in the Americas and Europe (Ponnuchamy et al., 2021). A multitude exceeding one thousand distinct pesticide compounds are utilized globally (Fusar Poli & Fontefrancesco, 2024). Annually, around four million tons of pesticides are used worldwide, as per the Food and Agriculture Organization (FAO) (Sood, 2024). Algeria ranks among the countries with the highest pesticide usage, as reported by the Algerian Association for the Protection of the Environment. Approximately 30,000 tons of pesticides are applied in Algeria each year (Derouiche et al., 2018).

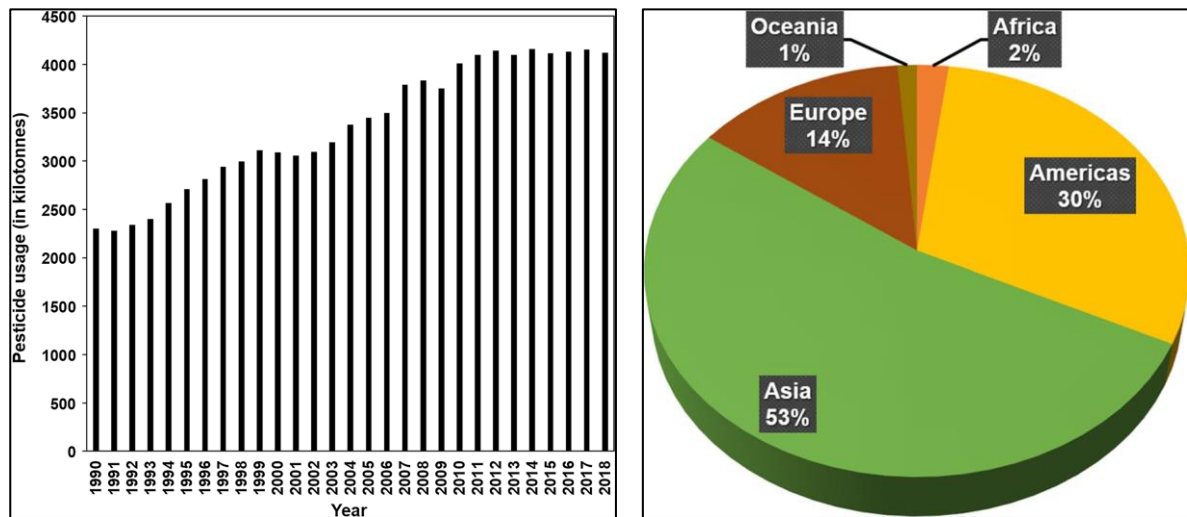


Figure 01: Global pesticide application and distribution for 1990-2018, based on data from FAO in 2019 (Ponnuchamy et al., 2021)

I.3. Classification of Pesticides

Pesticides are typically classified based on several criteria, including their chemical nature (Hassaan & El Nemr, 2020), target pests (Tudi et al., 2021), and mode of action (Sood, 2024). According to their chemical composition, pesticides are classified into various categories, such as organiochlorine, organic phosphorous, carbamate, pyrethrin, and pyrethroid (Hassaan & El Nemr, 2020). Additionally, pesticides are classified based on their targets, including fungicides, insecticides, herbicides, and rodenticides. For instance, fungicides are designed to eliminate fungi, while insecticides are used to eliminate insects, and herbicides are used to eliminate weeds (Tudi et al., 2021). Pesticides are also categorized based on their mode of action, which includes acetylcholine esterases, chloride channel antagonists and GABA gated channels, sodium channel modulators, juvenile hormone mimics, and mitochondrial complex I electron transport inhibitors (Sood, 2024).

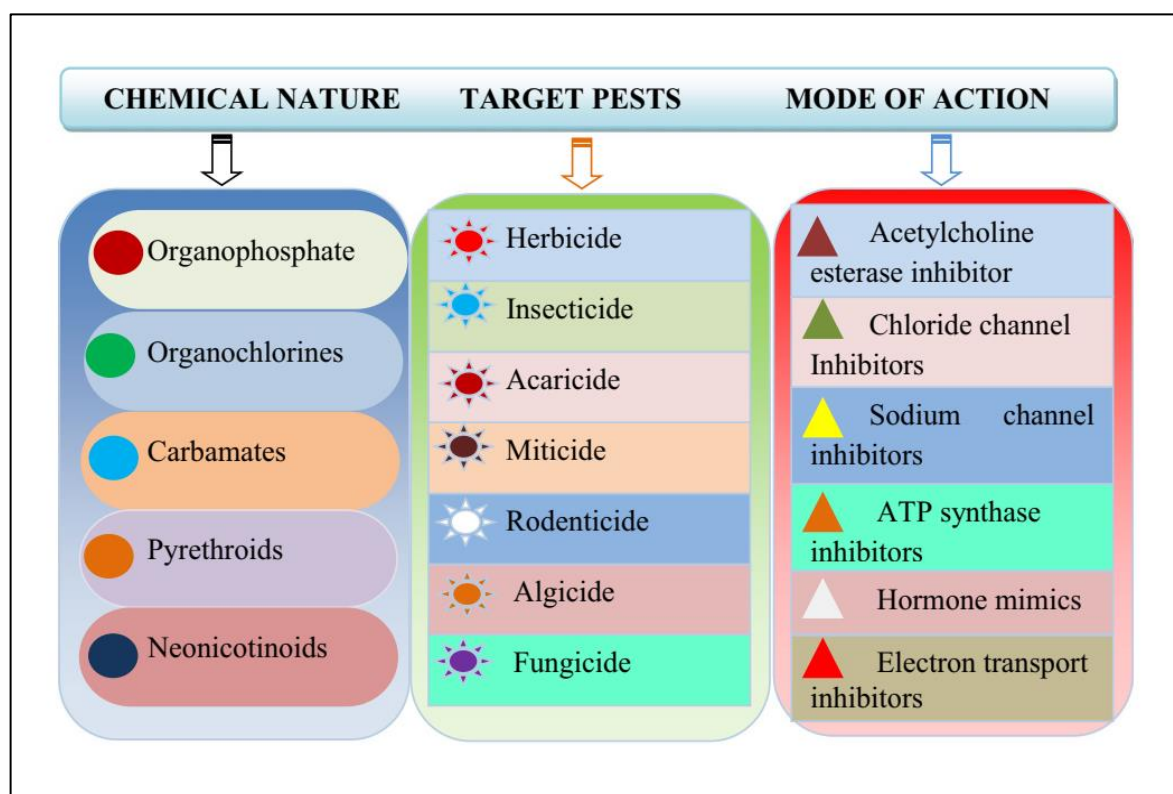


Figure 02: Main classes of pesticides (Dhuldhaj, Singh, & Singh, 2023)

I.4. Adverse Effects of Pesticides

Despite their effectiveness in eliminating or preventing the growth of target organisms, pesticides are widely believed to cause significant unintended harm to both humans and non-target organisms (Donley et al., 2024). The concern of pesticide residues in food has generated anxieties about their possible detrimental impacts on human well-being (Sajad et al., 2024). The diverse adverse health consequences associated with the exposure to chemical pesticides include toxicity, carcinogenicity, dermatological, gastrointestinal, neurological, respiratory, reproductive, and endocrine effects (Mohd Ghazi et al., 2023). As a result, substances that are intended to improve and protect food production may inadvertently pose a threat to human health (Sajad et al., 2024). Toxicity of pesticides is the basis for the World Health Organization's (WHO) classification of them into four categories: extremely hazardous, highly hazardous, moderately hazardous, and slightly hazardous (Yadav & Devi, 2017).

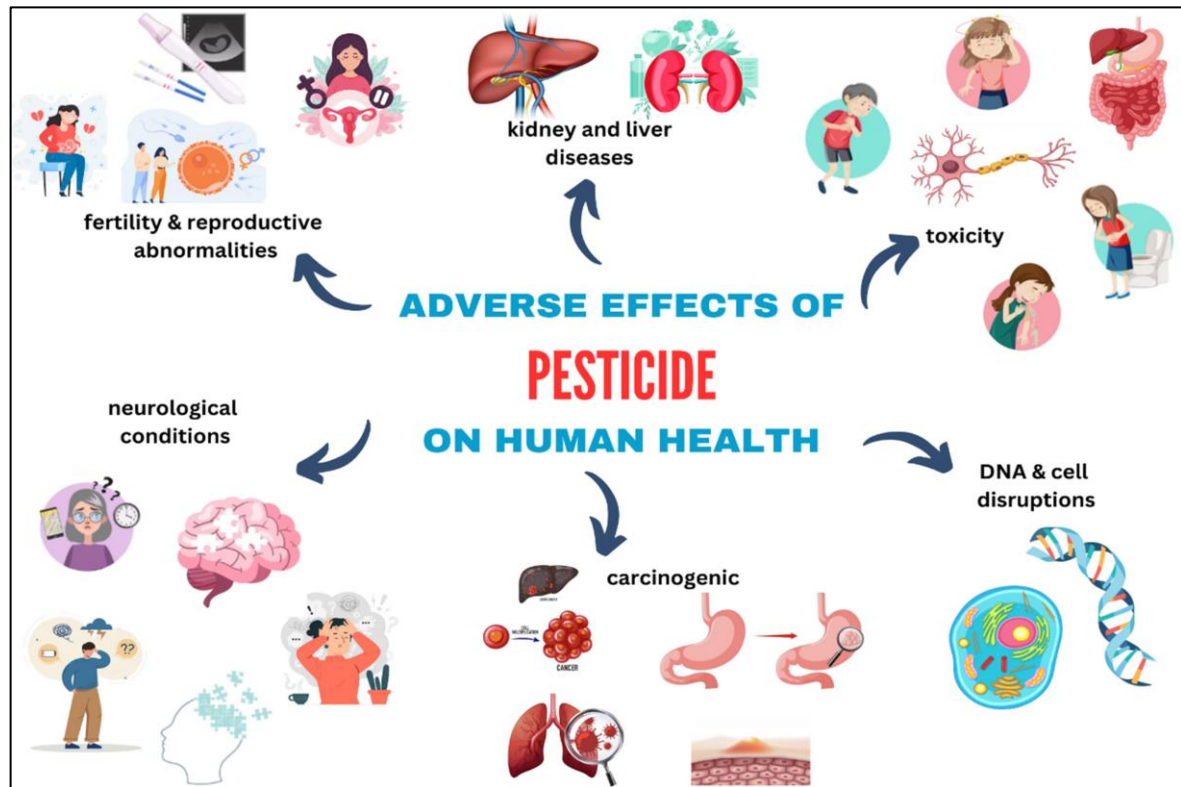


Figure 03: The negative consequences of pesticides on human health across various living systems (Mohd Ghazi et al., 2023)

II. Metribuzin

II.1. Difinition of Metribuzin

Metribuzin (4-amino-6-tert-butyl-4,5-dihydro-3-methylthio-1,2,4-triazin-5-one), a systemic herbicide (Mirzavand, Aeini, Najafgholi, & Rezamahalleh, 2024), is categorized within the triazinone chemical class (Lahlaf et al., 2024). The efficacy of metribuzin is contingent upon its interference with the photosynthetic mechanism, specifically by obstructing the electron transport chain during this process (Ay, Dikmen, & Koyuncu, 2024). This herbicide is employed in both pre- and post-emergence to control annual grasses and broadleaf weeds (Lahlaf et al., 2024). Its application is notably prevalent in agricultural practices aimed at regulating the growth and dissemination of broad-leaved weeds and grasses, particularly in the cultivation of potatoes, carrots, soybeans, and various cereals (Ay et al., 2024). Metribuzin received formal approval for application within the United States in 1973, explicitly for herbicidal purposes (Rubio-Armendáriz et al., 2024). It is toxic at even low doses, leading the European Commission to classify it as a candidate for substitution under directive 540/2011 (Ay et al., 2024).

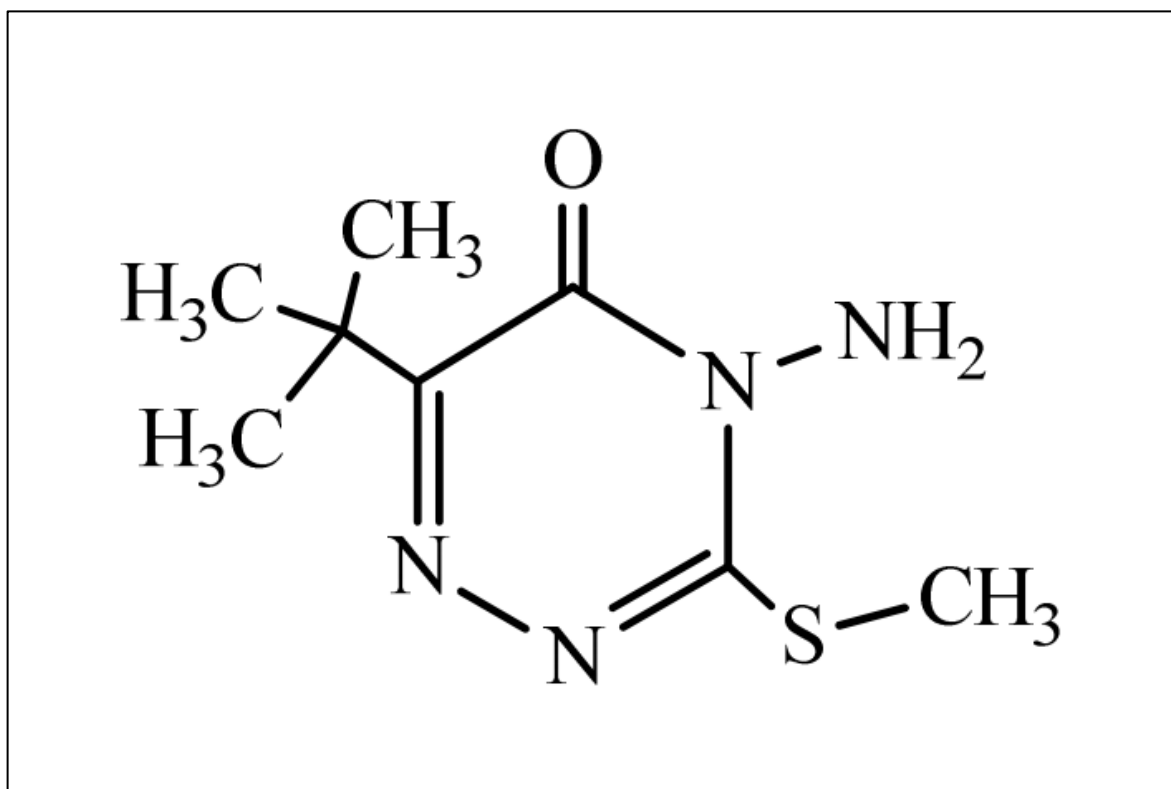


Figure 04: Chemical structure of metribuzin (Fouad, Aly, El-Aswad, & Badawy, 2024)

II.2. Physical and Chemical Characteristics of The Herbicides Metribuzin

The comprehensive physical and chemical properties that pertain to the herbicide known as metribuzin have been meticulously outlined and are presented in an organized manner within the confines of Table 01.

Table 01: Physicochemical properties of metribuzin

Characteristics	Metribuzin	Reference
Chemical name	4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one	(Kumar et al., 2023)
Type of pesticide	Herbicide	(Saygılı, Kadioğlu, Belgüzar, & Yanar, 2024)
Chemical family	Triazinone	(Gitsopoulos, Georgoulas, Vazanelli, & Botsoglou, 2024)
Chemical group	Triazine	(Abou-Khater et al., 2021)
Chemical formula	C ₈ H ₁₄ N ₄ OS	(Kadam & Pejaver, 2023)

Molecular weight	214.3	(Volova et al., 2016)
Mode of action	PSII inhibition	(Li & McIntyre, 2024)
Appearance	White crystalline solid	(Kumar et al., 2020)
Odour	Slight sulphur odour	(Yahiaoui et al., 2011)
Half-life	Between 5-50 days	(Kadam, Jadhav, Pandit, & Pejaver, 2021)
Melting point	125-126 °C	(Boyandin & Kazantseva, 2021)
Solubility in water	1.05 g/L	(Abdel Rahman, Ismail, Seloma, & Shalaby, 2023)
Absorbance wavelength λ (nm)	294 $\pm$ 1.0 nm	(Honório, de Liz Junior, de FPM Moreira, de Sena, & José, 2013)
Vapor pressure	4 $\times$ 10 ⁷ mmHg	(Pohanish, 2015)

II.3. Metribuzin Exposure Routes in Humans

Pesticide exposure can manifest through both direct and indirect pathways. Direct exposure transpires through agricultural practices, domestic applications, and professional usage, while pesticides are indirectly transferred via the food supply (Upadhayay, Rana, Juyal, Bisht, & Joshi, 2020). Significant dietary exposure commences early in postnatal development through breastfeeding, as evidenced by the detection of elevated levels of organochlorine pesticides in human breast milk (Clementi et al., 2008). The primary avenues for human pesticide exposure include the food chain, atmospheric conditions, water sources, soil, and both plant and animal life (Kim, Kabir, & Jahan, 2017). Individuals subjected to direct and/or indirect pesticide exposure may experience acute toxicological effects as well as chronic health conditions (Tudi et al., 2022). Simões et al. indicated that extensive research is necessary to investigate the implications of (non-occupational) exposure to these pesticides on human health, particularly regarding vulnerable populations such as pregnant women and their infants (Simões, Vermeulen, Portengen, Janssen, & Huss, 2023).

There exist four predominant routes through which pesticides may penetrate the human organism: dermal, oral, ocular, and respiratory pathways (Kim et al., 2017). Dermal exposure represents a significant concern for individuals involved in pesticide application (MacFarlane, Carey, Keegel, El-Zaemay, & Fritschi, 2013). Oral exposure may similarly transpire when individuals neglect adequate hand hygiene prior to consuming food or engaging in smoking

activities (Damalas & Koutroubas, 2016). Respiratory exposure, which occurs via inhalation or breathing, arises due to the volatile constituents of pesticides and poses considerable health risks to workers, particularly affecting the nasal, pharyngeal, and pulmonary tissues if substantial quantities of pesticides are inhaled from environmental sources such as air, water, and soil (Tudi et al., 2022). The exposure of unprotected ocular surfaces to pesticides can result from inadvertent splashes of these substances, leading to their infiltration into ocular tissues and potentially culminating in significant ocular toxicity (García-García et al., 2016). Table 02 delineates the pathways through which humans are subjected to pesticide exposure (Upadhayay et al., 2020).

Table 02: Human routes of pesticide exposure (Upadhayay et al., 2020)

Routes	Mechanism of exposure
Dermal exposure	Absorption transpires as a consequence of a splash, spill, or spray drift, particularly during the processes of mixing, loading, disposal, and cleaning of pesticides.
Oral exposure	When transferring from a labeled container to an unlabeled one, pesticides in soft drink or drinking water bottles that have been contaminated with pesticides may be at risk of transfer.
Respiratory exposure	Application of pesticides through spraying results in the generation of smaller droplets, particularly when utilizing conventional application equipment.
Eye exposure	Certain pesticides have been documented to be absorbed through the ocular region, leading to serious and potentially fatal health complications.

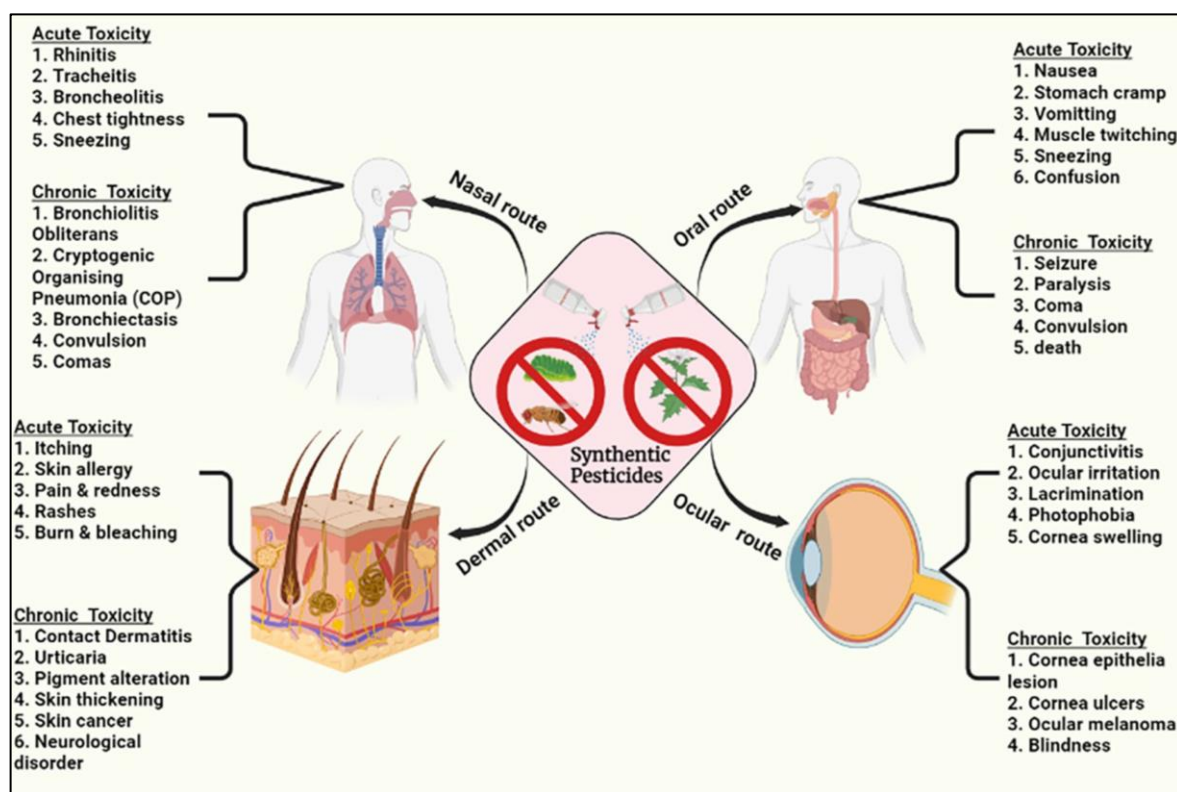


Figure 05: Human pesticide exposure pathways (Okagu, Okeke, Ezeorba, Ndefo, & Ezeorba, 2023)

II.4. Metribuzin Toxicity on Living Organisms

Metribuzin, a widely-used pesticide on vegetable crops to control broadleaf and grassy weed species, has also demonstrated significant toxicity to humans and animals (Kadeche et al., 2017). It is toxic even at low doses (Ay et al., 2024). The toxic effects of metribuzin are primarily mediated by ROS, which can react with various biological molecules (Zeng et al., 2021). Herbicides such as metribuzin exhibit variable toxicity, leading to acute intoxication characterized by respiratory difficulties and drowsiness, while high exposures may result in gastrointestinal discomfort, fatigue, and central nervous system depression; furthermore, chronic exposure can induce hepatic enzyme alterations, renal disturbances, reproductive effects, endocrine dysfunction, and an imbalance in the oxidant/antioxidant system (Derouiche et al., 2020). Medjdoub et al. also reported that animal studies have shown evidence of adverse health effects caused by metribuzin, including liver enzyme activity changes, histopathological alterations, kidney damage, endocrine modifications, and fetal toxicity (Medjdoub, Merzouk, Merzouk, Chiali, & Narce, 2011). The deleterious effects and adverse impacts associated with the chemical compound metribuzin, have been documented and reported across a multitude of species, and these findings are comprehensively summarized in the detailed presentation of data found in Table 03.

Table 03: Toxic effect of metribuzin across a multitude of species

Species	Sex	Dose	Route	Experimental period	Reference
Earthworm	Hermaphrodite	1000, 500, 200, 100, 10 and 1 µg/g soil	In the soil	10 days	(Fouad, Shamsan, & Abdel-Raheem, 2023)
Rabbits	Female	3.22 and 6.44 mg/kg	Oral	60 days	(Derouiche et al., 2019)
Rabbits	Male	0.1, 1 and 2 µg/l	Oral	/	(Sarsar et al., 2017)
Rats	Male	110, 220 and 440 mg/kg	Oral	90 days	(Elalfy, Mona, Aboumosalam, & Ali, 2017)
Rats	Male	133.33 mg/kg	Oral	21 days	(Kadeche et al., 2017)
Rats	Male/ Female	1.3 and 13 mg/kg	Oral	90 days	(Chiali et al., 2013)
Fish	/	1.5, 5, 16, 33, and 53 mg/L	In the aquaria	28 days	(Plhalova et al., 2012)
Fish	/	175.1 and 250.2 mg/L	In the aquaria	04 days	(Velisek, Svobodova, Piackova, & Sudova, 2009)
Fish	/	0.175 and 1.75 mg/L	In the aquaria	28 days	(Modra et al., 2008)

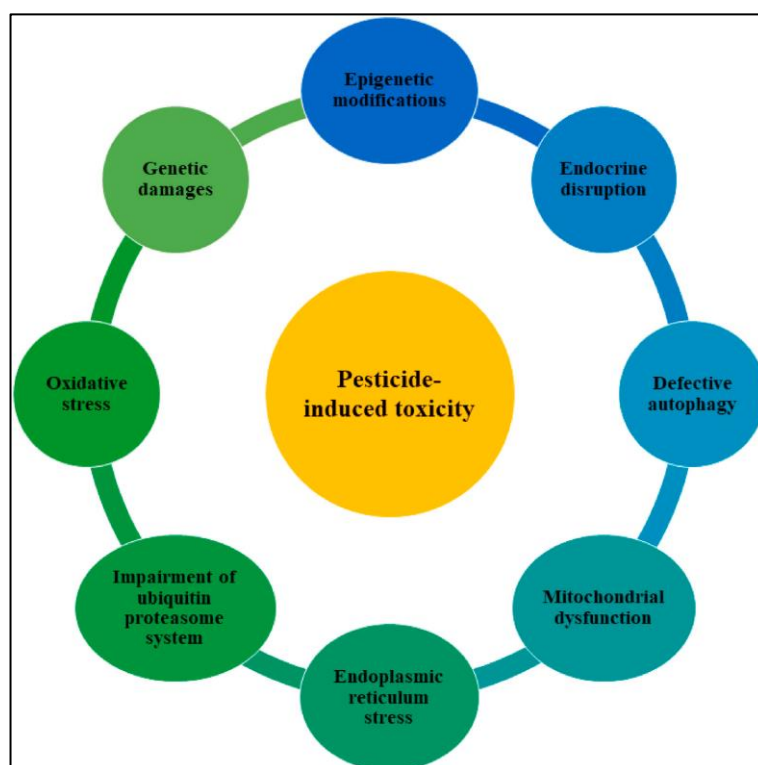


Figure 06: Possible mechanisms of pesticide-induced toxicity in both animals and humans (Zeng et al., 2021)

II.5. Effects of Metribuzin on Human Health

II.5.1. Metribuzin-Induced Infertility and Reproduction Problems

A reduction in fertility attributed to pesticide exposure has been documented across numerous studies (Vessa, Perlman, McGovern, & Morelli, 2022). Pesticides possess the capability to function as endocrine disrupting chemicals (EDCs), thus interfering with the normal operations of endogenous hormones (which play a critical role in the regulation of the reproductive system) (Moreira et al., 2021), consequently resulting in subfertility in both male and female populations (Sengupta & Banerjee, 2014).

When focusing specifically on pesticides, it is evident that they can adversely affect the male reproductive system through various mechanisms: (i) reproductive toxicity that leads to direct damage to cellular structures; (ii) alterations in DNA architecture that may induce gene mutations, potentially resulting in congenital anomalies or infertility; (iii) epigenetic modifications that affect gene expression patterns (Moreira et al., 2021). Conversely, Clementi et al. elucidated that pesticides may exert detrimental effects on male fertility through multiple pathways, which encompass the induction of developmental anomalies within the male reproductive system, as well as the direct or indirect impairment of the functionality of properly developed gonads (Clementi et al., 2008). Additionally, research has indicated that pesticides

possess the capability to obstruct the morphological alterations that are essential for sperm capacitation, leading to suboptimal sperm capacitation and, ultimately, male infertility (Uwamahoro et al., 2024). In summary, the available literature indicates that exposure to pesticides correlates with a decline in semen quality, revealing diverse associations across different semen parameters (Rodprasert, Toppari, & Virtanen, 2023).

Pesticides and various environmental agents possess the capacity to disrupt female reproductive function through several mechanisms, which encompass the alteration of hormonal equilibrium, direct impairment of the female gamete, interference with the processes of fertilization and implantation, as well as abnormal development and function of the reproductive tract (Clementi et al., 2008). Furthermore, there exist indications that exposure to specific pesticides may precipitate ovarian dysfunction (Bretveld, Thomas, Scheepers, Zielhuis, & Roeleveld, 2006). Bhardwaj et al. reported a positive correlation between oxidative stress induced by pesticides and female infertility, which culminates in a multitude of reproductive disorders that modify both the structural and physiological processes integral to reproduction (Bhardwaj, Mittal, Saraf, & Kumari, 2020).

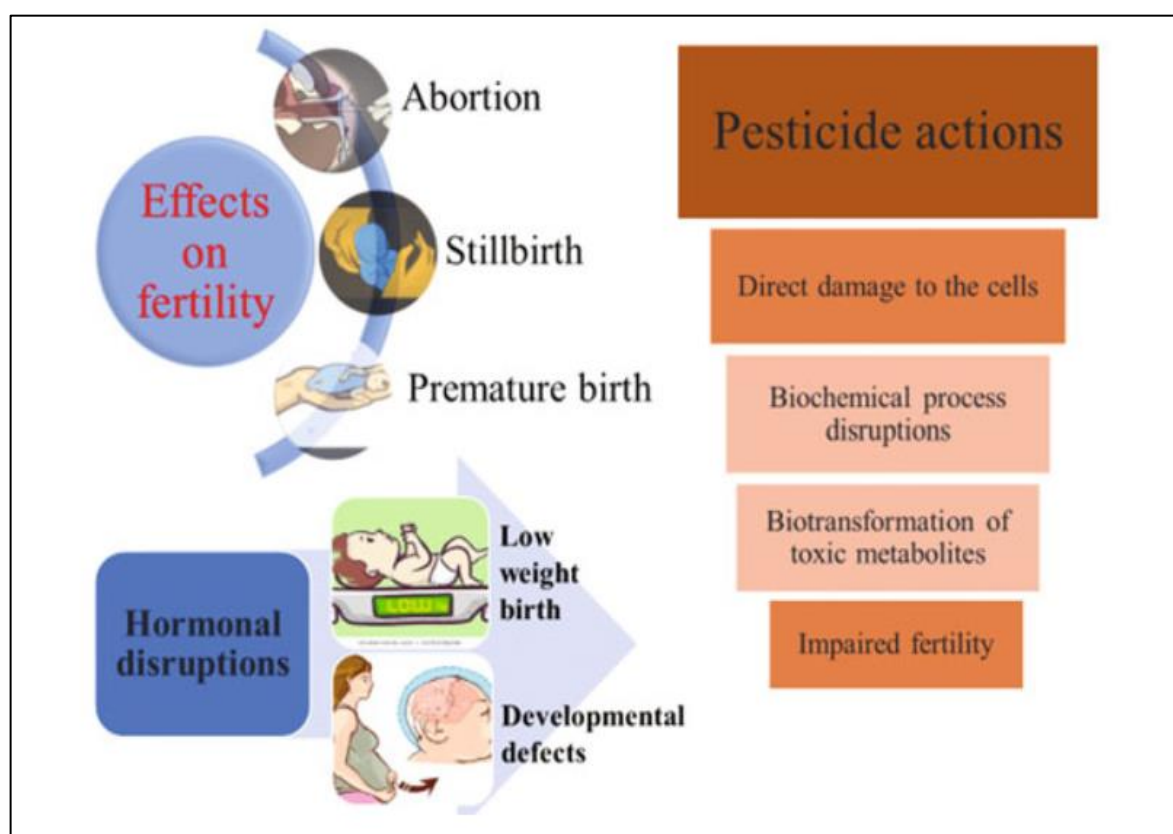


Figure 07: Pesticide use and its impact on human reproductive function (Ali, Ullah, Sajjad, Shakeel, & Hussain, 2021)

II.5.2. Metribuzin-Induced Neurotoxicity

Compounds that can harm the central nervous system and brain are classified as neurotoxins (Sajad et al., 2024). Research has demonstrated that pesticides can influence acetylcholinesterase (AChE) activity (Pathak et al., 2022). The neurotoxic damage caused by these pesticides is a result of their inhibitory effects on AChE (Farkhondeh, Mehrpour, Forouzanfar, Roshanravan, & Samarghandian, 2020). Pesticide-induced neurotoxicity manifests in various neurological disorders, including neurodegenerative conditions (such as Alzheimer's, Parkinson's, amyotrophic lateral sclerosis, and multiple sclerosis), neurodevelopmental issues (like attention deficit hyperactivity disorder, autism spectrum disorders, developmental delay, and intellectual disability), and neurobehavioral and neuropsychiatric problems (including depression/suicide attempt, anxiety/insomnia, and cognitive impairment). Many of these disorders are among the most severe human health challenges (Arab & Mostafalou, 2022). For instance, metribuzin has been shown to have a facilitating effect on Parkinson's disease (Wan & Lin, 2016). Furthermore, long-term exposure to metribuzin is linked to a higher incidence of neurodegenerative diseases (Jyoti, Chhaya, Kumar, Kumar, & Kumar, 2021).

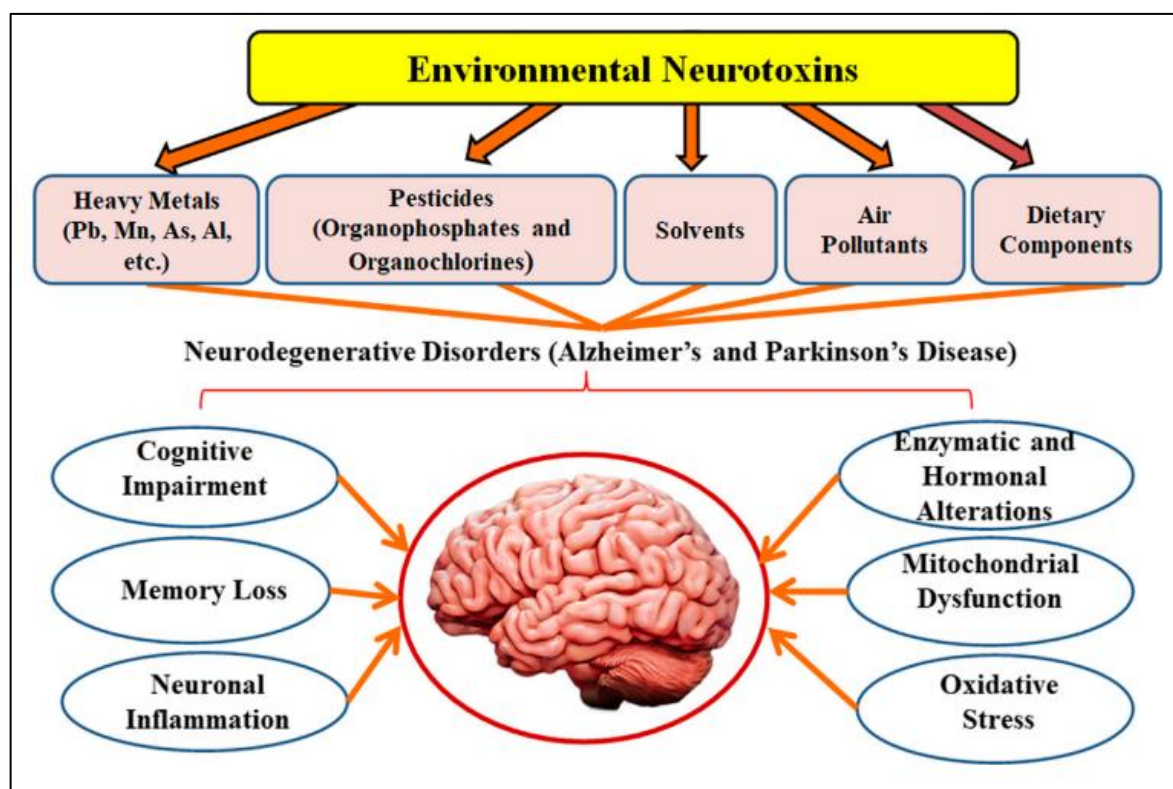


Figure 08: Influence of environmental pollutants on neurodegenerative diseases (Nabi & Tabassum, 2022)

II.5.3. Metribuzin-Induced Cancer

Certain pesticides have been associated with an elevated risk of cancer (Sajad et al., 2024). Primarily focusing on farmers, epidemiological investigations have identified connections between pesticide exposure and increased cancer incidence in specific areas, including prostate, lung, ovarian, rectal, testicular, skin, and breast cancers, as well as Non-Hodgkin Lymphoma (NHL), multiple myelomas, and leukemia (de Graaf et al., 2022). Cavalier et al. noted that recent evidence points to causal relationships between pesticide exposure and cancer, with the most compelling data supporting links to acute myeloid leukemia (AML) and colorectal cancer (CRC), as these associations were consistently observed across multiple studies (Cavalier, Trasande, & Porta, 2023). In 1998, the Environmental Protection Agency (EPA) categorized metribuzin as group D, indicating that it is not classifiable regarding human carcinogenicity due to insufficient evidence supporting its carcinogenic potential (Armendáriz, de la Torre, Fernández, & González, 2014). However, findings from the investigation conducted by Delancey et al. indicated a possible correlation between the application of metribuzin and specific lymphohematopoietic malignancies among males (Delancey et al., 2009).

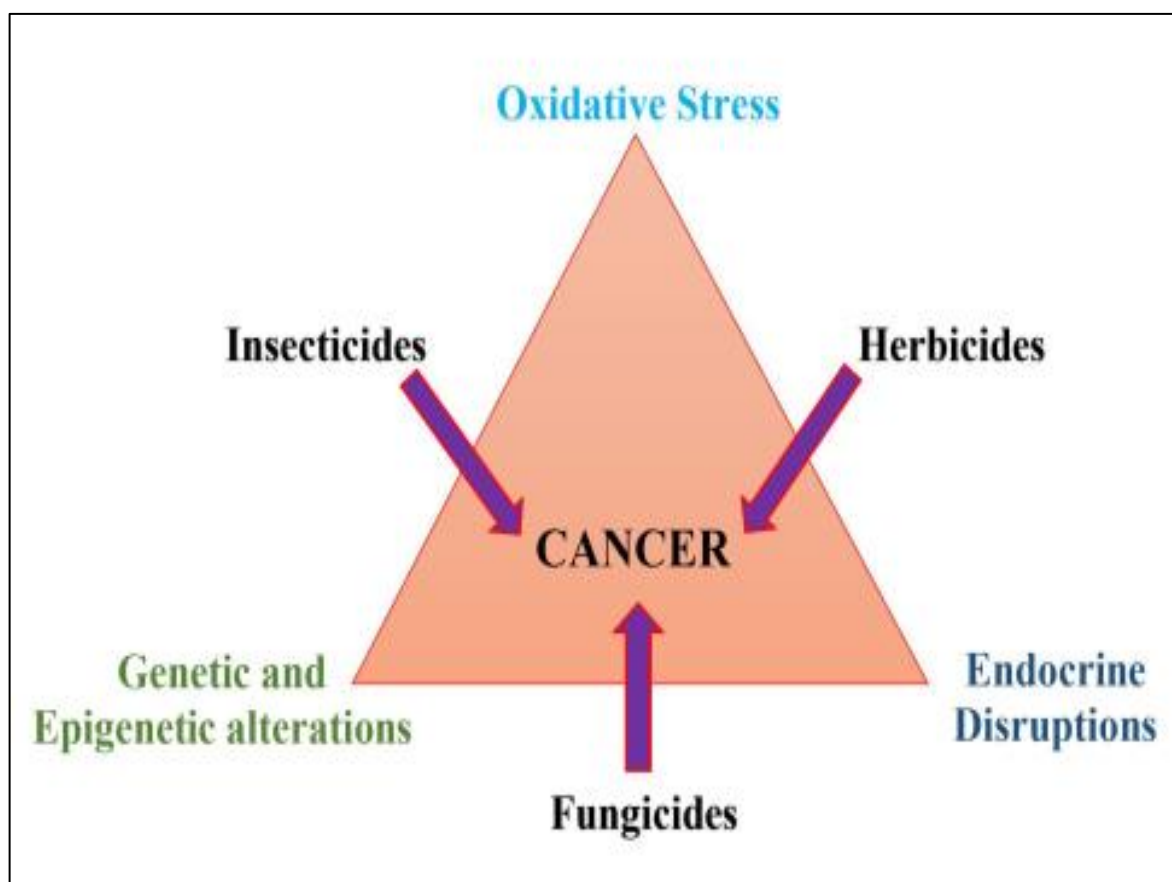


Figure 09: Significant molecular pathways implicated in the carcinogenic effects induced by pesticides (Pathak et al., 2022)

II.5.4. Metribuzin-Induced Endocrine System Alteration

Pesticides are recognized for their potential to act as endocrine disrupting chemicals (EDCs), thereby disrupting the normal functionality of endogenous hormones (Moreira et al., 2021). The etiology of endocrine disorders may be attributed to the low-dose hormone-like effects of pesticides, resulting in alterations in the synthesis and metabolism of the body's endogenous hormones (Gerunova, Bardina, Gerunov, & Sechkina, 2019). Primarily, pesticides affect hormonal signaling pathways and the neuroendocrine system, also inducing oxidative stress that contributes to various metabolic diseases (Kamal et al., 2021). In addition to eliciting cytotoxic effects in the pancreas, pesticides modulate hormone secretion, thereby precipitating metabolic disorders (He, Ni, Jin, & Fu, 2020). Acting as antagonists to sex hormone functions, pesticides may induce abnormal sexual development and disrupt other critical physiological processes (Lushchak, Matviishyn, Husak, Storey, & Storey, 2018). Numerous studies have identified triazine herbicides as prospective endocrine disruptors (Hostovsky, Blahova, Plhalova, Kopriva, & Svobodova, 2014). Furthermore, evidence derived from animal studies suggests that metribuzin may engender detrimental health effects, including endocrine alterations (Derouiche et al., 2018). Additionally, Jyoti et al. indicated that chronic exposure to metribuzin is correlated with an increased incidence of endocrine anomalies (Jyoti et al., 2021).

II.5.5. Metribuzin-Induced Oxidative stress

II.5.5.1. Definition of Oxidative Stress

Oxidative stress is characterized as a perturbation in the equilibrium between antioxidants and pro-oxidants, predominantly favoring pro-oxidants (Guemari, Boulaares, & Derouiche, 2024), which results in the alteration of redox signaling and regulation and/or molecular injury (Jakubek, Parchem, Wieckowski, & Bartoszek, 2024). Oxidative stress is intricately linked to a diverse array of diseases and health complications, encompassing neoplasms, diabetes mellitus, cardiovascular pathologies, neurodegenerative ailments, inflammatory conditions, and the aging process (Hu et al., 2024).

II.5.5.2. Oxidants

Atoms or molecules with unpaired electrons that can exist independently are known as oxidants or free radicals (Derouiche, Abid, & Tahraoui, 2024). In human physiology, two main categories of free radicals are typically involved: reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Chandra, Sharma, & Arora, 2020). These free radicals can cause harm

to cellular components, including the cell lipids (especially the phospholipids of cell membranes), proteins, and nucleic acids (Miller & Zachary, 2017). The free radicals, encompassing both ROS and RNS, originate from a myriad of endogenous sources, including mitochondria, peroxisomes, the endoplasmic reticulum, and phagocytic cells, as well as from various exogenous sources such as environmental pollution, alcoholic beverages, tobacco combustion products, heavy metals, transition metals, industrial solvents, pesticides, specific pharmaceuticals like halothane and paracetamol, and radiation (Phaniendra, Jestadi, & Periyasamy, 2015).

II.5.5.3. Antioxidants

Antioxidants serve a crucial function in safeguarding against oxidative damage at the molecular level (Guemari et al., 2024). These protective compounds (antioxidants) are categorized into two types: enzymatic and non-enzymatic (Uniyal, Kumar, & Joshi, 2024). The most effective initial defense mechanism involves enzymatic antioxidants, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Jomova et al., 2024). Non-enzymatic antioxidants are further classified into metabolic and nutrient antioxidants. Metabolic antioxidants, which are endogenous, are generated through bodily metabolic processes. In contrast, nutrient antioxidants are exogenous compounds that the body cannot produce and must be obtained from dietary sources or supplements (Gupta et al., 2014).

II.5.5.4. Oxidative Stress and Metribuzin

Pesticides trigger oxidative stress, resulting in free radical production and modifications to the body's antioxidant or oxygen-free radical scavenging enzyme systems (Medithi, Jonnalagadda, & Jee, 2021). Research has shown that various pesticide types share a common effect: inducing oxidative stress in diverse cell types and animal models (Sule, Condon, & Gomes, 2022). Certain pesticides can directly generate reactive oxygen species (ROS), including superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^{\cdot}$) (Sajad et al., 2024). Excessive ROS can harm lipids, proteins, and DNA, leading to mitochondrial stress, followed by apoptosis and cellular death (Gómez et al., 2021). The scavenging systems comprise superoxide dismutase (SOD), catalase (CAT), peroxidases, other antioxidant enzymes, and low-molecular weight antioxidants in living cells (Zhang, He, Sen, & Wang, 2020). Exposure to pesticides, either individually or in combination, has been shown to inhibit antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) (Anupama Ojha, Yaduvanshi, & Srivastava, 2011). Pesticide

exposure may impact the fatty acid composition of organisms by enhancing lipid peroxidation (Gonçalves, Rocha, Marques, & Gonçalves, 2021). Lipid peroxidation (LPO), a major contributor to cell function loss, along with DNA damage, enzyme inactivation, and hormone oxidation, are indicators of oxidative cellular damage (M. Ali et al., 2014). Metribuzin has been found to induce intracellular oxidative (Derouiche et al., 2018). Research on metribuzin's toxic effects has demonstrated increased oxidative stress and changes in antioxidant levels in both laboratory and living organisms (Kadeche et al., 2016). Table 04 illustrated the oxidative stress effects of metribuzin across various species.

Table 04: Oxidative stress effects of metribuzin across various species

Species	Dose	Experimental period	Organs	Parameters	Reference
Rabbits	3.22 and 6.44 mg/kg	60 days	Kidneys	MDA, GSH and GST	(Derouiche et al., 2019)
Rabbits	3.22 and 6.44 mg/kg	60 days	Liver	MDA, GSH and GST	(Derouiche et al., 2018)
Rats	133.33 mg/kg	21 days	Liver, kidney, brain and testis	MDA, GSH and GPx	(Kadeche et al., 2017)
Rats	1.3 and 13 mg/kg	90 days	Liver, adipose tissue, muscle and intestine	MDA, carbonyl proteins and GSH	(Chiali et al., 2013)

II.6. Effects of Metribuzin on the Environment

While pesticides are considered crucial for protecting and enhancing crop growth, they pose significant risks to the environment. Excessive use of pesticides can result in the loss of biodiversity and environmental degradation (Khan et al., 2023). The primary environmental impacts of pesticides stem from their toxicity to non-target organisms and their persistence, leading to long-term consequences (Kalyabina, Esimbekova, Kopylova, & Kratasyuk, 2021). Currently, pesticide residues can be found in various environmental components, including air, water, soil, and sediment (Zeng et al., 2021). Soil contaminated by pesticides can cause fluctuations and deterioration in soil quality (Cara et al., 2021). Additionally, the misuse of herbicides pollutes surface water bodies such as ponds, streams, rivers, and lakes, as well as groundwater (Basu & Rao, 2020). Metribuzin, a specific pesticide, has weak soil adsorption properties, allowing it to easily leach into deeper soil layers (Armendáriz et al., 2014). This compound is highly toxic to freshwater macrophytes and algae (Phillips & Boopathy, 2022).

Due to its high solubility in water, metribuzin is more likely to contaminate surface and subsurface water through leaching (Chaparadza, Rambwawasvika, Chinorumba, & Mutatu, 2022). The deleterious impacts of pesticides on the ecosystem are illustrated in Table 05.

Table 05: Deleterious impacts of pesticides on the environment (Derouiche et al., 2020)

Agricultural procedures	Pesticides
Soils	Pesticides' accumulation and degradation products
Groundwater	Leaching phenomenon of mobile pesticide residues alongside degradation products
Surface water	
Flora and fauna	Impact on soil microbiota; resilience of specific weed species Toxicological resistance
Additional factors: atmospheric conditions, acoustic pollution, landscape integrity	Evaporation phenomena, detrimental effects, aerial dispersion, residual substances

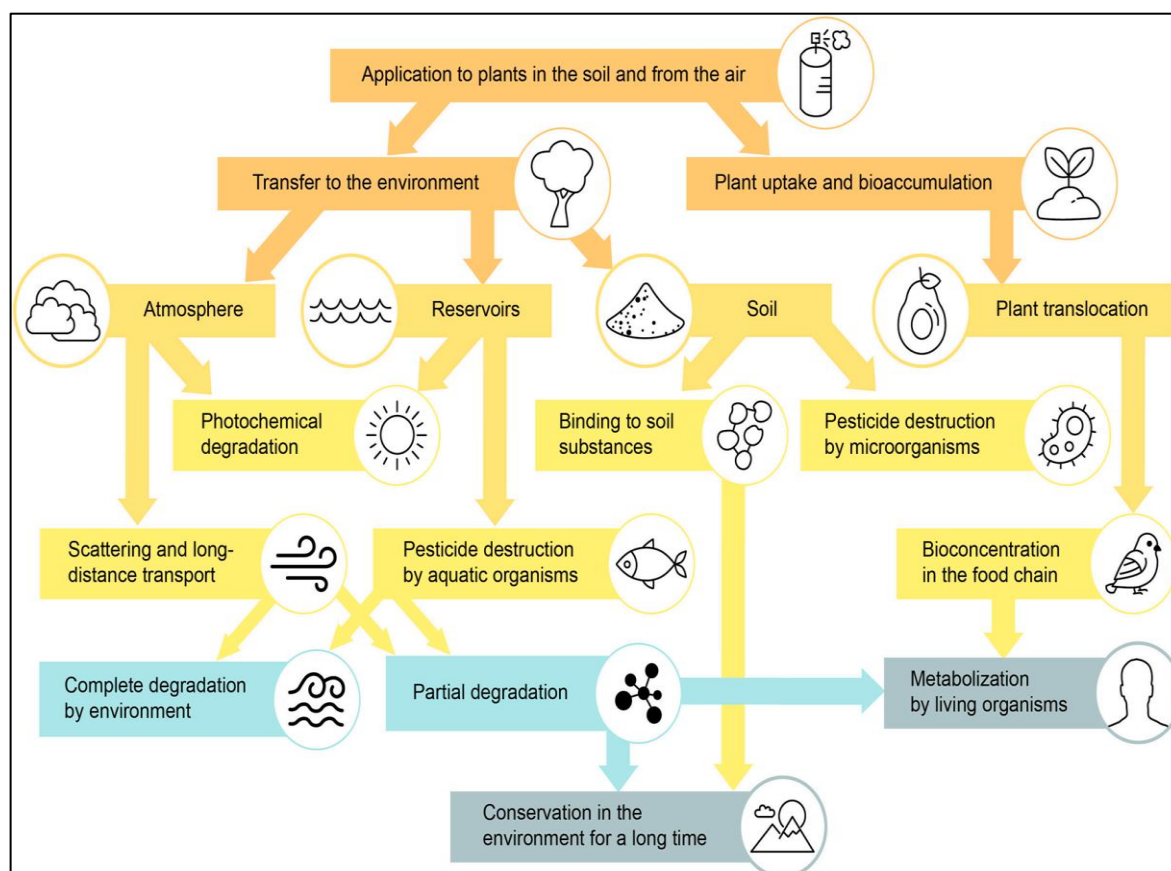


Figure 10: Pesticide transport pathways after usage (Kalyabina et al., 2021)

II.7. Removal of Metribuzin from Environment

To protect human health and the environment, various remediation methods can be employed when pesticide levels surpass established standards (Bhat, Qadri, Cui, & Li, 2020). Numerous techniques have been developed to eliminate pesticide contaminants from water, including hydrogen peroxides, ultrasonic waves, hybrid processes, bioremediation, photocatalytic degradation, adsorption, membrane separation, bio-purification systems, composite materials, ion exchange resins, carbon nanotubes, graphene, and nanocrystalline metal oxides (Nazir et al., 2021). It is crucial to recognize that many of these remediation approaches are currently in the pilot stage, necessitating further research to advance them to full commercial (Ajiboye, Kuvarega, & Onwudiwe, 2020). Soil remediation methods involving toxic removal and debris treatments are often time-intensive and costly (Rajmohan, Chandrasekaran, & Varjani, 2020). Among the various remediation options available, biological approaches have gained significant attention for treating pesticides in soil and water systems due to their environmentally friendly nature (Giri et al., 2021). Compared to physical or chemical methods, biological treatment is considered a cost-effective and eco-friendly approach for contaminant removal (Azelee et al., 2022). The biological method of remediating polluted sites utilizes living organisms, such as plants or microorganisms, to mitigate or clean up pollutants. Bioremediation mechanisms work by degrading, mineralizing, transforming, or detoxifying pollutants, thereby reducing their concentration to harmless levels (Alori, Gabasawa, Elenwo, & Agbeyegbe, 2022). Cara *et al.* used mesoporous carbon rich from wheat straw to remediate the environmental effects of the herbicide metribuzin (Cara et al., 2021). The pros and cons of various pesticide removal techniques are presented in Table 06

Table 06: Strategies for removing pesticides with their advantages and disadvantages (Ajiboye et al., 2020)

Strategies	Advantages	Disadvantages
Phytoremediation: Using of plants to extract organochlorine from soil and water	Economically efficient; On-site treatment is feasible; No need for excavation equipment, although excavator plants are utilized	Difficulties in identifying excavator plants capable of effectively absorbing pesticides
Bioremediation: Employing microbes to break down	Fully decomposing pollutants into CO ₂ and H ₂ O without accumulating intermediates;	It is ineffective when environmental conditions do not support microbial growth;

organochlorine pesticides	No need for initial contamination site evaluation	may disrupt the ecological equilibrium
Incineration: Thermal decomposition of organochlorine pesticides through heat and oxygen exposure	Thorough breakdown of pesticides into ash	Challenges with ash management; generation and environmental release of cyanide when pesticides contain nitrogen
Thermal desorption at low temperatures	Pesticides can be extracted from sludges, soils, and sediments	The process requires specialized equipment, making it expensive; pesticides are not fully eliminated
Adsorption: Utilization of adsorbents	Rapid in nature and applicable in situ	Pesticides are not eradicated; rather, they are relocated from one medium to another
Supercritical oxidation	Toxic gases and particulate matter are not emitted during the construction of supercritical systems	Challenges are associated with the oxidation unit
Ozonation: Utilization of ozone	The operation of the system is straightforward, and it exhibits rapid functionality	It requires substantial energy consumption, rendering it costly
Dechlorination: Removal of chlorine	High boiling point organic compounds, such as PCB, can be subjected to degradation	The efficiency of degradation is relatively low when compared to the majority of existing techniques
Electrochemical oxidation	It can break down pesticide organic feed in any phase, including soluble and insoluble	Synthesis of various intermediate compounds
Gas-phase chemical reduction	The likelihood of dioxin and furan formation is eliminated due to the occurrence of these	The extraction of chlorine donors from both the combustion air and resultant effluent gases is a requisite

	processes in an anoxic atmospheric environment	
Ultrasonic technology: Utilization of sound	No need for additional chemicals, no secondary pollutants, ease of usage, and a faster pace of remediation	The maintenance of optimal temperature levels presents significant challenges; issues related to attenuation are prevalent
Plasma arc techniques	It is suitable when metal has come into contact with pesticides and can be used to break down resistant organochlorine pesticides	Obtaining the right scrubber to eliminate metal vaporization and incomplete combustion products is a challenge.
Catalytic hydrogenation	A broad spectrum of organochlorine pesticides is amenable to degradation through this process	Toxicity from noble metals
Molten salt oxidation	Complete conversion of organic compounds to CO ₂ and H ₂ O; it can be implemented on a small scale	Organochlorine compounds combined with a high proportion of inert materials are not amenable to treatment
Fenton oxidation	Demonstrates efficacy in the remediation of pesticide-laden wastewater on an industrial scale	Exhibits sensitivity to pH variations
Zero-valence iron	Iron is relatively low-cost	As an oxide layer forms and blocks the active site, its effectiveness gradually wanes

Chapter II
Phytotherapy
Ocimum basilicum L.

I. Phytotherapy

Phytotherapy constitutes a scientific discipline that focuses on the treatment and prevention of ailments through the application of medicinal flora and herbal formulations (Miraldi & Bains, 2018). These phytotherapeutic agents represent crude extracts derived from desiccated plants or any constituent thereof, encompassing leaf, stem, root, flower, or seed (Rocha-Miranda & Venâncio, 2019). Phytotherapy ranks among the most ancient therapeutic modalities employed by humankind. Its historical roots extend back to 8,500 BC, being founded upon traditional knowledge and empirical scientific inquiry (ethnopharmacology/ethnobotany) (Leite, Camargos, & Castilho, 2021). Basil is principally cultivated for its aromatic foliage, which is utilized in both fresh and desiccated forms for essential oil extraction and as flavoring agents or spices in a diverse array of culinary applications, in addition to its extensive utilization as a spice, in aromatherapy, cosmetics, and phytotherapy globally (Egata, 2021). Certain botanical species employed in phytotherapeutic practices is presented in Table 07.

Table 07: Some plants used in phytotherapy

No	Plant	Part used	Medicinal properties	References
1	<i>Phragmites australis</i>	Leaves	Antioxidant and anti-inflammatory properties	(Frahtia, Derouiche, & Niemann, 2024)
2	<i>Sonchus maritimus</i> L.	Leaves	Antioxidant and antidiabetic properties	(Chetehouna, Derouiche, & Régami, 2024)
3	<i>Ephedra alata</i>	Aerial part	Antioxidant and anti-inflammatory Properties	(Zouari Ahmed, Chadou, Ben Ghalia, & Derouiche, 2024)
4	<i>Aristolochia albida</i>	Root	Antibacterial and antioxidant activities	(Gambo et al., 2024)
5	<i>Aguilaria microcarpa</i>	Leaves	Antibacterial activity	(Indrawati, Baharuddin, & Isnaeni, 2024)

I.1. Definition and Distribution of *Ocimum basilicum* L.

Ocimum basilicum L., derived from the Greek term 'basileus' meaning 'king,' is a widely appreciated aromatic herb (Malakar & Mandal, 2024) and stands as one of the most ancient spices among annual herbaceous plants (Alhasan, Abbas, & Al-Ameri, 2021). *Ocimum basilicum* L., which is widely recognized as sweet basil (Rashid et al., 2023), is one of the most public aromatic and medicinal plants which belong to Lamiaceae family and the *Ocimum* genus is distinguished with its extremely aromatic leaves (Jimayu, 2021). It is renowned for its applications in both medicine and cuisine (Qamar, Sana, Naveed, & Faizi, 2023). Basil cultivation primarily occurs in regions with warmer climates (Juškevičienė, Radzevičius, Viškelis, Maročkienė, & Karklelienė, 2022) (Figure 11).

Ocimum basilicum L. is a renowned herb with a long history of cultivation and use dating back to ancient times (Georgiadou et al., 2018). This plant thrives in various parts of the world world (Murali & Prabakaran, 2018), including certain regions of Asia such as Iran, India, and Turkey, as well as in warm and subtropical areas of Africa and central South America (Choi, Heo, Bae, Kim, & Moon, 2020).



Figure 11: Leaves of *Ocimum basilicum* L. (Nadeem, Hanif, Bhatti, Jilani, & Al-Yahyai, 2020)

I.2. Botanical and Taxonomical Description

Ocimum basilicum L. is an herbaceous plant with multiple branches. It typically grows to a height between 0.6 and 0.9 m. The plant features stems and branches that are square and smooth, usually displaying a green to light purple coloration. Basil's leaves are uncomplicated and positioned opposite each other on the stem, measuring between 2.5 and 5 cm or more in length. These elongated leaves exhibit an egg-like shape with a pointed tip and edges that are either lobed or serrated (Nadeem et al., 2020).

Basil's taxonomic classification is recognized as extensive and intricate (Chowdhury, Mandal, Roy, & De Sarker, 2017). This intricate taxonomy is primarily due to the plant's genetic variability, which results from cross-pollination and various environmental factors (Devika & Krishnan, 2024).

Kingdom :	Plantae
Phylum :	Magnoliophyta
Class :	Magnoliopsida
Order :	Lamiales
Family :	Lamiaceae
Genus :	<i>Ocimum</i>
Species :	<i>basilicum</i>
Common name:	Basil (Ghosh et al., 2024)

I.3. Chemical Composition and Nutritional Properties

Studies on the chemical composition of *Ocimum basilicum* L. have revealed a diverse array of compounds, including terpenoids, alkaloids, phenolics, flavonoids, tannins, saponin, reducing sugars, cardiac glycosides, steroids, and glycosides (Al-Snafi, 2021). The extract of basil is rich in phenolic and flavonoid compounds, such as cinnamic acid, caffeic acid, ferulic acid, rosmarinic acid, and sinapic acid (Sathasivam et al., 2022). Additionally, it contains essential nutritional components like carbohydrates, oils, proteins, as well as various vitamins and minerals (Al-Snafi, 2021). Basil is a source of several vitamins, including A, C, B6, thiamine, and carotene, and also provides significant amounts of micro and macronutrients (Eser & Adiloglu, 2020). The nutritional benefits and medicinal properties of basil are closely related to its tissue nutrient composition, which includes elements such as nitrogen (N), phosphorus (P), potassium (K), and carotenoids (Song, Yang, & Jeong, 2024).

I.4. Therapeutic Effect and Economic Value

In this context, sweet basil (*Ocimum basilicum* L.) is recognized as a significant medicinal and aromatic plant, cultivated in many parts of the world for its culinary and economic value (Bahcesular, Yildirim, Karaçocuk, Kulak, & Karaman, 2020), thanks to its diverse chemical composition and sensory attributes (Ciriello, Kyriacou, De Pascale, & Rouphael, 2022), as well as its extensive biological and pharmacological effects (Sathasivam et al., 2022).

Ocimum basilicum L. has been the subject of numerous studies investigating its diverse and powerful effects. These include antifungal, antimicrobial, antiparasitic, insecticidal, antioxidant, anti-inflammatory, immunomodulatory, anti-osteoporotic, hepatoprotective, neuroprotective, cardioprotective, and anti-cancer properties, along with other health benefits (Dhama et al., 2023). In traditional medicine, basil has been employed to address various health issues, such as headaches, coughing, diarrhea, constipation, warts, worms, and kidney dysfunction. For external use, basil can be applied as a salve for insect bites, while its oil is utilized as a topical treatment for acne (Brandão et al., 2022). The leaves of the basil plant possess pharmaceutical qualities due to their content of polyphenols, phenolic acids, and flavonoids (Romano et al., 2022).

Research has demonstrated that phenolic compounds offer various cardioprotective benefits, helping to mitigate several risk factors associated with cardiovascular diseases, including inflammation, high blood pressure, hyperlipidemia, and endothelial dysfunction (Torres-Fuentes et al., 2022). Studies have also revealed that numerous terpenoids possess significant disease-preventing and therapeutic effects, exhibiting antitumor, anti-inflammatory, antibacterial, antiviral, and antimalarial properties, as well as potential benefits in immune regulation, neuroprotection, and antiallergy (Yang et al., 2020). Additionally, multiple investigations have shown that alkaloids, which are naturally occurring substances, exhibit neuroprotective qualities and therapeutic potential in neurodegenerative disorders due to their advantageous properties (Dolanbay, Kocanci, & Aslim, 2021). Furthermore, flavonoids found in the diet have been found to possess strong antioxidant and free-radical scavenging capabilities, playing crucial roles in disease prevention (Alsawaf et al., 2022).

I.5. Herbal Extract-Loaded Liposomes as A Novel Drug Delivery System (NDDS)

Herbal extracts present a promising alternative to synthetic drugs and supplements due to their potential health benefits, including antioxidant, antimicrobial, anticancer, anti-inflammatory, hepatoprotective, and neuroprotective effects (Sogut, Sezer, & Sezer, 2021). Nevertheless, these plant-based actives face several challenges, such as poor water or lipid

solubility, limited permeation, lack of targeting specificity, instability in highly acidic environments, and liver metabolism, etc (Pandey, Bhairam, Shukla, & Gidwani, 2021). To address these issues, liposome technology has been applied to natural extracts, helping to overcome their highly hygroscopic structure, low solubility, poor bioavailability, instability, and high dose-induced toxicity (Choi et al., 2023). Also, the components encapsulated within liposomal formulations are designed to enhance solubility, minimize adverse effects, prolong drug release, target specific regions, protect drugs, and improve therapeutic efficacy (Rahmi et al., 2022). Numerous characteristics of liposomes render them a more advantageous delivery mechanism for these herbal bioactives, including their biocompatibility and their efficacy in drug loading for hydrophilic, lipophilic, and amphiphilic substances (Alexander, Ajazuddin, Patel, Saraf, & Saraf, 2016). The use of nanotechnology to deliver phytochemicals or plant extracts is gaining traction among researchers, as it enhances their solubility, pharmacokinetics, pharmacodynamics, bioavailability, efficacy, and organoleptic properties such as odor and taste (Karpuz, Gunay, & Ozer, 2020).

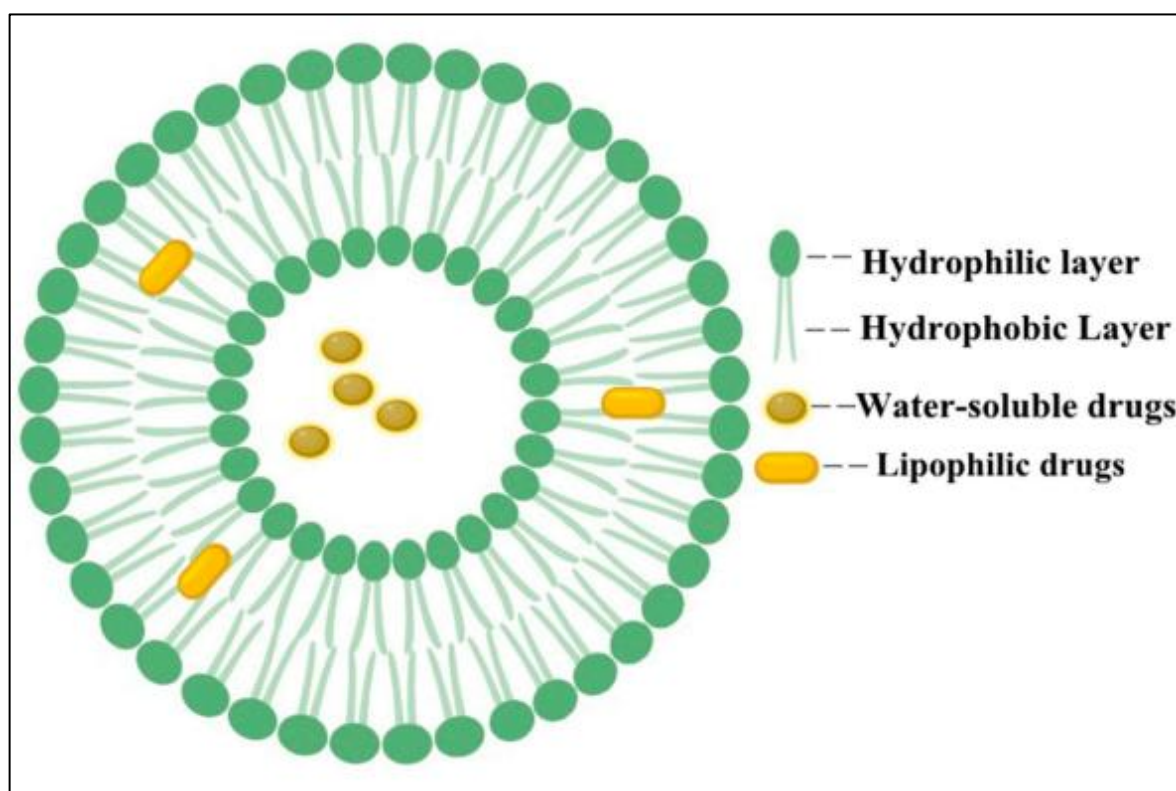


Figure 12: Liposome as noval drug delivery system (Chen et al., 2024)

Chapter III
Nanotechnology
MnO NPs

I. Nanotechnology

Nanotechnology has become a prominent scientific discipline with diverse applications across multiple fields (Gatoo et al., 2014). It combines knowledge from various areas, such as chemistry, physics, materials science, engineering, biology, and health sciences (Godwin, Shri, & Balaji, 2015). Nanotechnology field focuses on examining methods for working with materials at the nanoscale, specifically within the range of 1 to 100 nanometers (Kavaz, Umar, & Zimuto, 2019). As an advanced research field, nanotechnology utilizes eco-friendly techniques to produce nanoparticles that are chemically stable, non-toxic, biocompatible, and suitable for biomedical applications (Kavaz et al., 2021). Consequently, nanotechnology has widespread use in the medical sector as nanomedicine (Haleem, Javaid, Singh, Rab, & Suman, 2023). This field offers numerous benefits in addressing persistent human ailments by enabling precise drug delivery and targeting specific locations (Patra et al., 2018).

II. Nanomaterials

Bulk materials are characterized as particles whose dimensions surpass 100 nm across all axes (Saleh, 2022). In contrast, nanomaterials are categorized as substances possessing at least one external dimension within the size range of approximately 1-100 nm (Bhardwaj & Arora, 2018). In summary, the primary distinction between nanomaterials and bulk materials lies in the fact that the dominant thermal, mechanical, optical, electrical, and magnetic characteristics of the material exhibit significantly greater variation at the nanoscale compared to the bulk scale (Table 08) (Saleh, 2022).

Table 08: Properties of the nanomaterial and bulk material (Ahire et al., 2022)

Nanomaterial	Bulk material
Shows different properties at a small scale	Properties remain same
Chemically more reactive	Chemically less reactive
Large surface area	Small surface area
Lighter material	Heavier material
Faster in performance	Slower in performance
More energy efficient	Not energy efficient
Cheaper than bulk	Expensive

III. General Method of Synthesis for Metal Oxide Nanoparticles

The synthesis of NPs can be accomplished through three primary methodologies: physical, chemical, and biological techniques (Altammar, 2023) (Figure 13). The physical approach has numerous limitations, including high expenses, energy demands, radiation exposure, substantial waste production, temperature and pressure requirements, reduced stability, high dilution, challenges in size and shape control, and modifications to the surface chemistry and physicochemical properties of NPs (Bloch, Pardesi, Satriano, & Ghosh, 2021). Additionally, most metal nanoparticles are produced through chemical processes, which can lead to unforeseen issues such as excessive energy consumption, environmental pollution, and potential health hazards (Ying et al., 2022). Green synthesis is beneficial due to its rapid assembly for large-scale applications, cost-effectiveness, eco-friendliness, and avoidance of hazardous substances, high temperatures, and excessive energy consumption (kazemi et al., 2023). In physical methods, the production of nanomaterials follows a top-down approach, whereas biological and chemical techniques employ a bottom-up strategy (Al-Harbi & Abd-Elrahman, 2024). This green synthesis approach falls under the category of bottom-up methods, which involve biological processes. In a bottom-up system, atoms aggregate to form clusters, eventually leading to the creation of nanostructures (Arya, Bhar, Richa, Kataria, & Mehta, 2022).

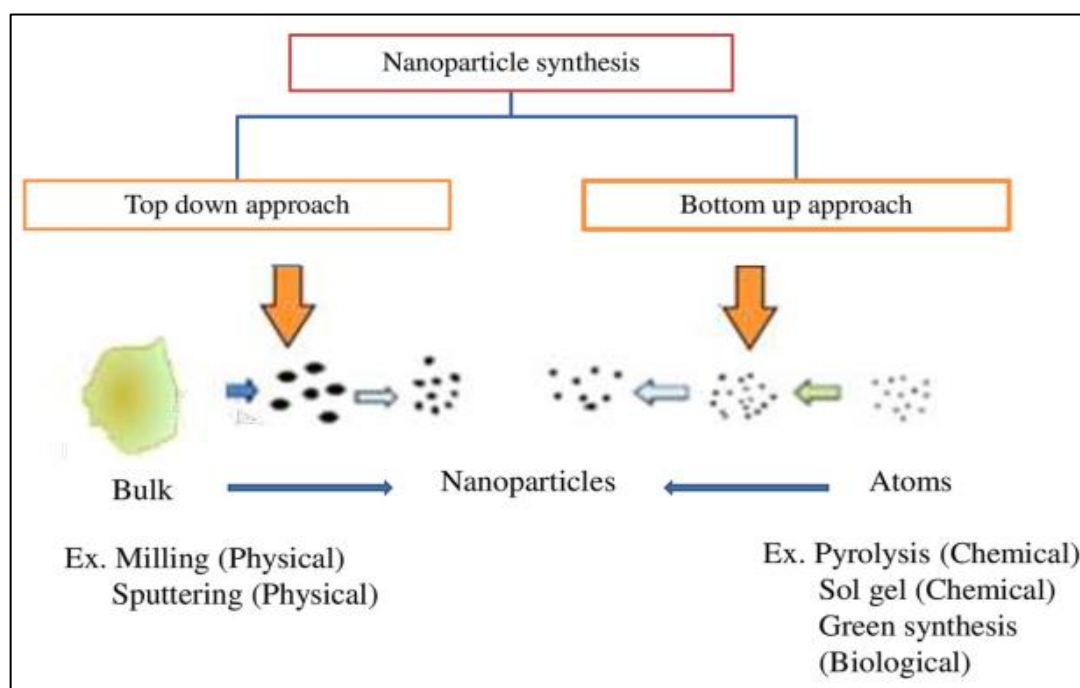


Figure 13: Various methods of metal oxide NPs synthesis (Patil & Chandrasekaran, 2020)

IV. Green Synthesis of MnO NPs

Nano-biotechnology, a newly emerging branch of nanotechnology, utilizes bioactive compounds extracted from organisms such as plants, algae, bacteria, and fungi to create nanoparticles (El-Moslami, Yahia, Zahran, & Kamoun, 2023). This biological synthesis process employs oxidation and reduction mechanisms driven by plant phytochemicals or microbial enzymes (Chakraborty et al., 2022). Plants are preferred over fungi and bacteria for metal ion reduction due to their faster processing time, as the latter require extended incubation periods because of water-soluble phytochemicals (Mohamed El Shafey, 2020). Currently, plant-based nanoparticle green synthesis is considered the most effective method, owing to its adaptability and user-friendly nature (Hano & Abbasi, 2022). However, MnO NPs have gained prominence in synthesis and production processes due to their reduced toxicity (Khan, Shahid, Shahid, Fatima, & Abbasi, 2020). The green synthesis of Mn NPs offers several advantages, including non-toxicity, environmental friendliness, cleanliness, and cost-effectiveness. It can be performed at room temperature and normal pressure using various raw materials such as fruits, vegetables, plant extracts, microbes, and fungi (Nie, Zhu, Guo, Yue, & Lin, 2022). The mechanism for green synthesis of MnO NPs is illustrated in (Figure 14).

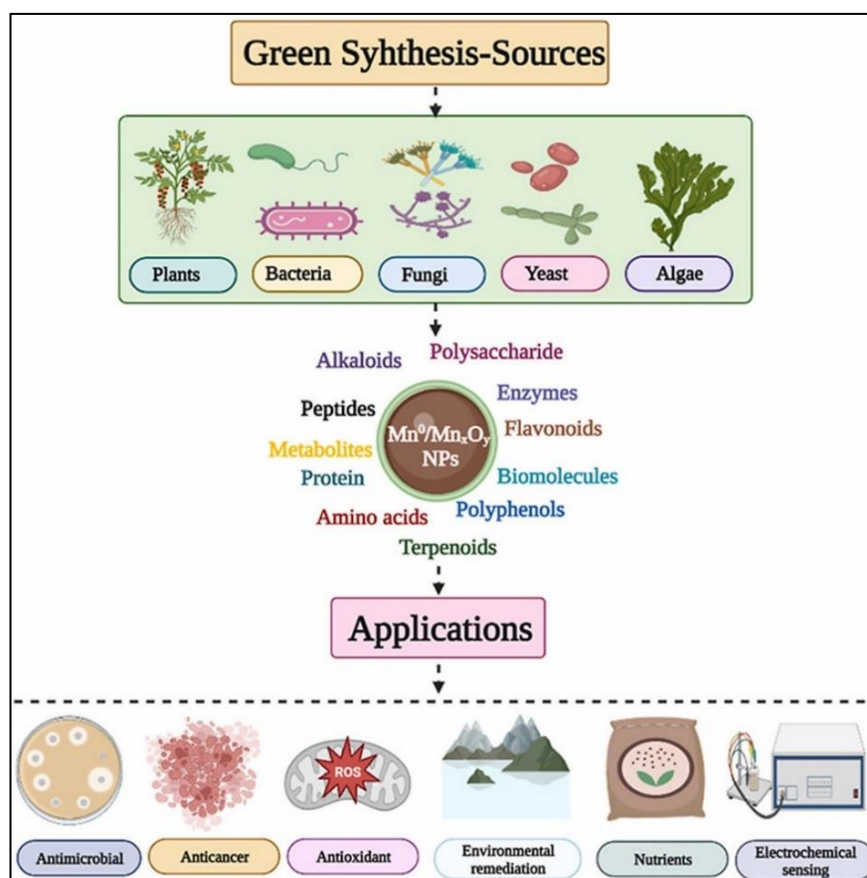


Figure 14: Green synthesis mechanism of MnO NPs (Zhang, Sathiyaseelan, Naveen, Lu, & Wang, 2023)

V. Green Synthesis of MnO NPs Using Plant Extract

Among the green biological methods, the green synthesis of nanoparticles using plants is now regarded as the premier approach due to its adaptability and simplicity (Hano & Abbasi, 2022). Not only leaves but also other plant components such as fruits and stems, along with their extracts, have been employed to produce metal nanoparticles (Chopra et al., 2022). Research has shown that plant-derived phytochemicals, including terpenoids, alkaloids, polyphenols, and flavonoids, facilitate the reduction of metal ions, ultimately forming metal nanoparticles (Lu, Zhang, Khan, Li, & Wan, 2022). The synthesis of MnO₂ NPs was visually monitored by observing color changes that occurred when a precursor was added to the leaf extract (Faisal et al., 2022). The green synthesis of nanoparticles is influenced by various parameters, such as temperature, pH, reaction duration, and the volumes of metal salt and plant extract used. The interplay of these factors plays a crucial role in determining the morphology and dimensions of the synthesized NPs (Ansari et al., 2023).

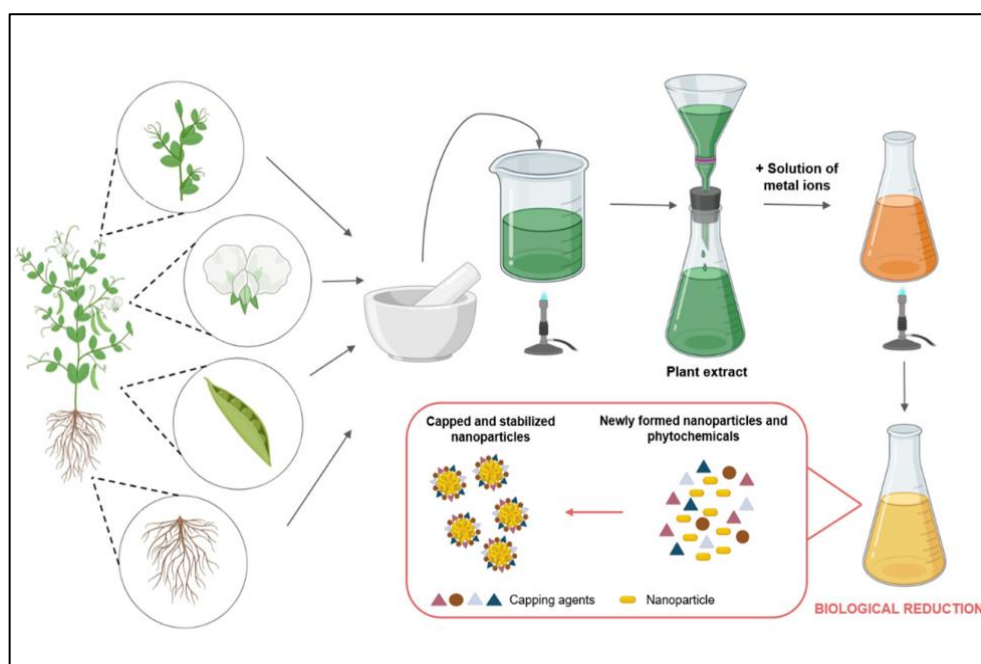


Figure 15: Green synthesis of MnO NPs using extract of various plant parts (Rónavári et al., 2021)

Various categories of nanoparticles have been persistently synthesized utilizing an array of plant species, including: MgO NPs from *Ocimum basilicum* L. leaves (Derouiche et al., 2020), SeO NPs from *Sonchus maritimus* leaves (Chetehouna, Derouiche, & Réggami, 2023), CuO NPs from *Portulaca oleracea* L. leaves (Atoussi, Chetehouna, & Derouiche, 2020), ZnO NPs from *Spinacia oleracea* leaves (Djouadi & Derouiche, 2021). The methodology for generating MnO NPs from plant extracts is illustrated in (Figure 15), while Table 09 enumerates the diverse plant species capable of yielding MnO NPs.

Table 09: Green synthesis of manganese oxide nanoparticles using various plants

Sl. No.	Plants/ plant extract	Precursor	Part used	Size of NPs (nm)	Shape / structure / morphology	Characterization techniques used	Reference
01	Green tea (<i>Camellia sinensis</i>)	Manganese sulfate (MnSO₄)	Leaves	Around 18	-	UV-Vis, XRD, FTIR and SEM	(Saod, Hamid, Alaallah, & Ramizy, 2022)
02	<i>Fagonia cretica</i>	Manganese acetate	Leaves	15.5 ± 0.85	Spherical with homogenous dispersity	UV-Vis, XRD, SEM, EDS, and FTIR	(Faisal et al., 2022)
03	<i>Gmelina arborea</i>	Manganese acetate	Fruit	Between 50 and 60	-	XRD, SEM, TEM and HR-TEM	(Srinivasa et al., 2022)
04	<i>Phyllanthus acidus</i>	Potassium permanganate (KMnO₄)	Leaves	10-20	Spherical in shape	FTIR, SEM, and UV-Vis	(Sakthi & Saravanakumari, 2021)
05	Banana Peel (<i>Musa paradiasca</i>)	Potassium permanganate (KMnO₄)	Peel	~ 1	Crystalline	UV-vis, EDS, XRD and FTIR	(Amatya, Shrestha, & Aryal, 2021)
06	Cabbage (<i>Brassica oleraceae</i>)	Potassium permanganate (KMnO₄)	Leaves	10.70	Spherical and ellipsoidal	Visual observation, UV-vis, XRD, FTIR, SEM and EDS	(Amatya & Shrestha, 2021)
07	<i>Viola betonicifolia</i>	Manganese acetate	Leaves	10.5 ± 0.85	Spherical with homogeneous dispersity	XRD, EDS, TEM and Zetasizer Dynamic Light Scattering	(Lu et al., 2022)
08	<i>Cassia tora</i>	Potassium permanganate (KMnO₄)	Leaves	75	Spherically agglomerated	UV-vis, FTIR, SEM and XRD	(Usman Ahmed, Noseh Dahiru, Yada Sudi, Gabriel, & Kulini John, 2020)
09	<i>Aloe vera</i>	Potassium permanganate (KMnO₄)	Aerial parts	-	Agglomerated sphere-shaped	FTIR, XRD and FESEM	(Joshi, Siddiqui, Salman, & Singh, 2020)
10	<i>Trigonella foenum-graecum</i>	Manganese (II) Acetate tetrahydrate [(CH₃COO) 2Mn.4H₂O]	Seed	50 – 75 and 15 - 20	Spherical in shape that are dense and agglomerate	SEM, XRD, TEM, FTIR, EDS and UV-Vis	(Rinita, Jose, & Jothi, 2021)
11	<i>Abutilon indicum</i>	Manganese sulfate monohydrate (MnSO₄.H₂O)	Leaves	80 ± 0.5	Spherical morphology and crystalline nature	SEM, XRD, EDS, UV-Vis and FTIR	(Khan et al., 2020)

12	<i>Euphorbia heterophylla</i> L.	Manganese (II) chloride tetrahydrate (MnCl₂·4H₂O)	Leaves	56.68	Irregular shape	FTIR, Particle Size Analyzer, XRD, UV-Vis SEM, EDS and TEM	(Dewi & Yulizar, 2020)
13	<i>Matricaria chamomilla</i> L.	-	Flower	16.5	Irregularly spherical shaped	UV-Vis, FTIR, XRD, TEM and SEM	(Ogunyemi et al., 2019)
14	<i>Gardenia resinifera</i>	Manganese acetate	Leaves	20 - 30	Spherical in shape	UV-Vis, PSA, FTIR, XRD, SEM-EDAX, and HR-TEM	(Manjula, Thenmozhi, Thilagavathi, Srinivasan, & Kathirvel, 2020)
15	<i>Yucca gloriosa</i>	Manganese acetate [(CH ₃ COO) ₂ Mn·6H ₂ O]	Leaves	Around size of 32	Spherical shape	XRD, FESEM and TEM	(Souri, Hoseinpour, Ghaemi, & Shakeri, 2019)
16	<i>Phyllanthus amarus</i>	Manganese acetate	-	40 - 50	Rod-like	UV-vis, FTIR, XRD, SEM and TEM	(Prasad & Patra, 2017)
17	<i>Ctenolepis garcini</i>	Potassium permanganate (KMnO₄)	-	57 - 69	Spherical and granular nature	UV-vis, FTIR, XRD, SEM and EDS	(Paul, Sakunthala, & Udhaya, 2017)
18	<i>Aegle marmelos</i>	Potassium permanganate (KMnO₄)	Fruit	23.7	With smooth edged and tetragonal in shape	UV-vis, SEM, FTIR and XRD	(Sivanesan et al., 2017)
19	Lemon <i>Citrus limon</i>	Manganese acetate	Fruit	50	Nearly spherical and eclipse morphology	UV-vis, FTIR, SEM and HRTEM	(Jayandran, Haneefa, & Balasubramanian, 2015)
20	<i>Kalopanax pictus</i>	Potassium permanganate (KMnO₄)	Leaves	19.2	Uniformly dispersed spherical shaped	EDS, XPS, TEM and FTIR	(Moon, Salunke, Alkotaini, Sathiyamoorthi, & Kim, 2015)
21	<i>Datura stramonium</i>	Potassium permanganate (KMnO₄)	Leaves	-	Polymorphic	UV-vis, FTIR, XRD, FESEM and EDS	(Joshi, Joshi, & Singh, 2020)
22	<i>Dalbergiella welwitschia</i>	Manganese (II) chloride tetrahydrate (MnCl₂·4H₂O)	Leaves	35.58	Spherically shaped	DRX, (SEM/TEM) and EDS	(Adeyemi, Onwudiwe, & Oyedeji, 2022)
23	<i>Tinospora Cardifolia</i>	Manganese sulfate (MnSO₄)	Stems	62.59±2.1	Spherical shapes	FTIR, TEM, EDS, DRX and SEM	(Dixit, Bala, Pareek, Chaudhary, & Jaswal, 2021)
24	<i>Punica granatum</i>	Manganese sulfate (MnSO₄)	Leaves	40-80	-	FTIR, UV-vis, SEM and TEM	(Shah et al., 2022)
25	<i>Hyphaene thebaica</i>	Manganese sulfate pentahydrate (Mn₂SO₄·5H₂O)	Fruit	18.33	Polymorphic	SEM, TEM, XRD, FTIR, and UV-vis	(Munir, Mohamed, Hkiri, Ghotekar, & Maaza, 2024)

VI. Analytical Characterization Techniques of MnO NPs

Various analytical methods are commonly employed to examine metal nanoparticles following their synthesis and to assess their specific physicochemical attributes (including surface area, morphology, structure, size, dispersity, composition, and crystallinity). These techniques include ultraviolet-visible spectroscopy (UV-Vis), fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray powder diffraction (XRD), and energy dispersive X-ray spectroscopy (EDS), Dynamic Light Scattering (DLS) (Chetehouna, Derouiche, & Reggami, 2024).

VII. Biomedical Applications of MnO NPs

VII.1.1. Antioxidant Activity of MnO NPs

Oxidative stress is a major contributor to metabolic and physiological changes, as well as numerous diseases (Chetehouna et al., 2020). The primary cause of oxidative stress is hydrogen peroxide (H_2O_2), which is produced by inflammatory and vascular cells (Coyle & Kader, 2007). While abnormal ROS generation mechanisms exist, manganese dioxide (MnO_2) nanoparticles can potentially consume excess H_2O_2 and convert it into O_2 in situ (Li et al., 2021). Zhang et al. confirmed the concept proposed by Ding et al. that MnO_2 catalyzes H_2O_2 decomposition into oxygen and water, thereby reducing oxidative stress reactions and providing cells with an oxygen equivalent (Zhang et al., 2022). The synthesized MONPs demonstrated strong antioxidant properties against DPPH free radical scavenging and exhibited biocompatibility (Kuthati, Busa, Goutham Davuluri, & Wong, 2019).

VII.1.2. Antiinflammatory Activity of MnO NPs

Inflammation, a widespread health concern, is initiated by microbes and irritants (Derouiche, Chetehouna, Djouadi, Boulaares, & Guemari, 2022). Various types of nanoparticles (NPs) have shown potential anti-inflammatory properties (Wang et al., 2021). Concurrently, MnO_2 particles can influence cytokine production by regulating gene expression, thus reducing inflammation severity. During this process, MnO_2 slowly breaks down into Mn^{2+} ions, which are then expelled through bodily fluids, helping to restore the body's internal environment to an optimal state (Zhang et al., 2022). Additionally, immune cells may enhance the delivery of nanoparticles to inflamed areas (Mahdavi Gorabi et al., 2019). A study conducted by Kumar et al. demonstrated that manganese dioxide nanoparticles shield cartilage from inflammation-induced oxidative stress (Kumar, Adjei, Brown, Liseth, & Sharma, 2019).

VII.1.3. Anti-Cancer Activity of MnO NPs

Globally, cancer stands as a significant public health issue (Boulaares, Derouiche, & Guemari, 2024) and the leading cause of death (Boulaares, Guemari, & Derouiche, 2020). Various traditional methods exist for treating and controlling cancer (Abbas & Rehman, 2018). Nanoparticles primarily trigger apoptosis in cancer cells through multiple pathways, with the most extensively studied being reactive oxygen species-mediated apoptosis. Additionally, other mechanisms contribute to nanoparticle-induced apoptosis in cancer cells, including immune system interventions, inhibition of transcription, alterations in protein expression levels, and targeted cytotoxicity (Mundekkad & Cho, 2022). The small size of nanoparticles allows them to more readily breach the tumor cell membrane (Das, Kumar, Dhas, Karthick, & Kumar, 2023). According to Sobańska et al., Mn nanoparticles have also shown promise in the field of cancer treatment (Sobańska, Roszak, Kowalczyk, & Stępnik, 2021).

VII.1.4. Anti-Diabetic Activity of MnO NPs

Diabetes mellitus, a widespread chronic condition, poses a significant challenge to public health (Liu et al., 2020). Studies have shown that manganese oxide compounds demonstrate efficacy in combating diabetes mellitus (Shaukat, Hussain, Irfan, Ijaz, & Anwar, 2022). Investigations have revealed that nanoparticles of manganese oxide significantly influence glucose level control in diabetic animal models (Ali, Hussein, & Abbas, 2024). According to Adeyemi et al., the observed inhibitory effects of synthesized Mn_3O_4 NPs suggest their potential as a viable alternative in diabetes treatment (Adeyemi et al., 2022). Research by Ahmad et al. indicated that manganese nanoparticles produced through eco-friendly methods show promise as an anti-diabetic agent, with α -amylase and α -glucosidase assays yielding IC_{50} values of 26.65 $\mu\text{g/ml}$ and 27.37 $\mu\text{g/ml}$, respectively (Ahmad, Noor, Muhammad, & Hussain, 2024).

VII.1.5. Anti-Bacterial Activity of MnO NPs

MnO_2 NPs exhibit antibacterial properties due to their minuscule size, which allows them to swiftly enter bacterial cells and disrupt their cell membranes (Joshi, Siddiqui, et al., 2020). The secondary metabolites in Mn NPs greenly synthesized that possess various therapeutic properties, including anti-diabetic, anti-cancer, anti-inflammatory, anti-plasmodium, anti-fungal, and anti-bacterial effects, enabling them to combat multiple bacterial strains (Ramesh, Rajendran, & Manogar, 2021). Both gram-negative and gram-positive bacteria are highly susceptible to the antibacterial potential of MnO_2 NPs (Manjula et al., 2020). Research has demonstrated the efficacy of MnO_2 NPs as an antibacterial agent against several bacterial

species, including *S. aureus*, *E. coli*, *K. pneumonia*, *B. subtilis*, and *P. aeruginosa*, with varying inhibition zone diameters observed (Ogunyemi et al., 2019).

VII.1.6. Anti-Viral Activity of MnO NPs

Globally, viral infections remain a significant cause of illness and death (Harper et al., 2021). MnO₂ NPs, known for their remarkable stability and immunogenic properties, are gaining attention as potential candidates for antiviral therapies (Baghban et al., 2024). These nanoparticles exhibit antiviral effects through multiple mechanisms, such as attaching to viral membranes to interfere with DNA/RNA functions, penetrating host cells to disrupt their structure, and generating reactive oxygen species (I. Gupta, Azizighannad, Farinas, & Mitra, 2022). Additionally, research has shown that the Mn²⁺ released by MnO₂ NPs plays a key role in regulating immune responses during viral infections (Baghban et al., 2024).

VII.1.7. Anti-Fungal Activity of MnO NPs

Infections caused by fungi are particularly concerning due to their chronic nature and resistance to treatment. Some fungal infections can result in severe disfigurement and even death after a prolonged and agonizing illness (Hyde et al., 2018). Nanoparticles play a significant role as antifungal agents (Dheeb et al., 2019). Research has shown that nanoparticles' fungicidal activity disrupts the fungal membrane (Shah et al., 2022). According to Faisal et al., MnO₂ nanoparticles, which were produced using an environmentally friendly method by *Fagonia cretica*, demonstrated varying levels of antifungal activity against different fungal species, including *Penicillium expansum*, *Monilinia fructigena*, *Penicillium chrysogenum*, and *Aspergillus oryzae* (Faisal et al., 2022).

Experimental Part

Chapter I

Materials & Methods

I. Material

I.1. Plant Materials

The basil was harvested in the El-Oued (Guemar) area of Algeria during August 2022. Professor Youcef Hellis from the Arid Region Scientific and Technical Research Center, Station of Touggourt, conducted the identification of the plant material. After cleaning, the basil leaves were left to dry at ambient temperature, away from direct sunlight. A mechanical grinder was employed to pulverize the dried leaves into a fine powder. The resulting *Ocimum basilicum* L. powder was stored in sealed containers at room temperature until the commencement of the experiment (Derouiche et al., 2020).

I.2. Animal Materials

I.2.1. Animal and Husbandry Condition

Our study employed 36 male Wistar rats, weighing an average of 202.81 ± 5.44 g. These 8-week-old rats were obtained from the Pasteur Institute in Algiers. The animals were reared in the animal husbandry laboratory at the Faculty of Natural and Life Sciences, Echahid University Hamma Lakhdar-El-Oued. The rats were kept in plastic cages covered with metal mesh and given a standard diet as outlined by (Southon, Gee, & Johnson, 1984). They had unrestricted access to food and water and were maintained under uniform conditions, including a 12-hour light-dark cycle and consistent room temperature. The experiment lasted for a duration of 8 weeks.

Table 10: Standard diet composition (Southon et al., 1984)

Raw materials	Quantity (g / kg)	Percentage (%)
Maize	326	32.6
Sucrose	326	32.6
Protein	168	16.8
Oil	80	8
Cellulose	40	4
Minerals	40	4
Vitamin	20	2

I.2.2. Animals Treatment

Following a two-week adaptation period, the subjects were allocated into six experimental groups, each containing six animals:

Group 1 (Control):

Group 2 (MTZ):

Group 3 (BE):

Group 4 (BE-LPs):

Group 5 (MnO NPs):

Group 6 (BE-MnO NPs-LPs):

I.2.3. Sacrifice, Blood Sampling and Tissues Collection

Following an 8-week treatment period and 16 hours of fasting, the rats were euthanized using mild chloroform (94%) anesthesia via inhalation. During the animal sacrifice, blood samples were collected in EDTA tubes for FNS and in dry tubes. Serum was extracted by centrifugation at 3000 rpm for 10 minutes and utilized for biochemical analysis. Blood glucose levels were measured at the time of sacrifice using a glucometer. The liver, brain, lungs, heart, kidneys, testes, and muscles were carefully extracted, rinsed with normal saline (NaCl), weighed, and stored at -20°C for oxidative stress parameter analysis. Additionally, the organs were preserved in 10% formaldehyde for histological examination.

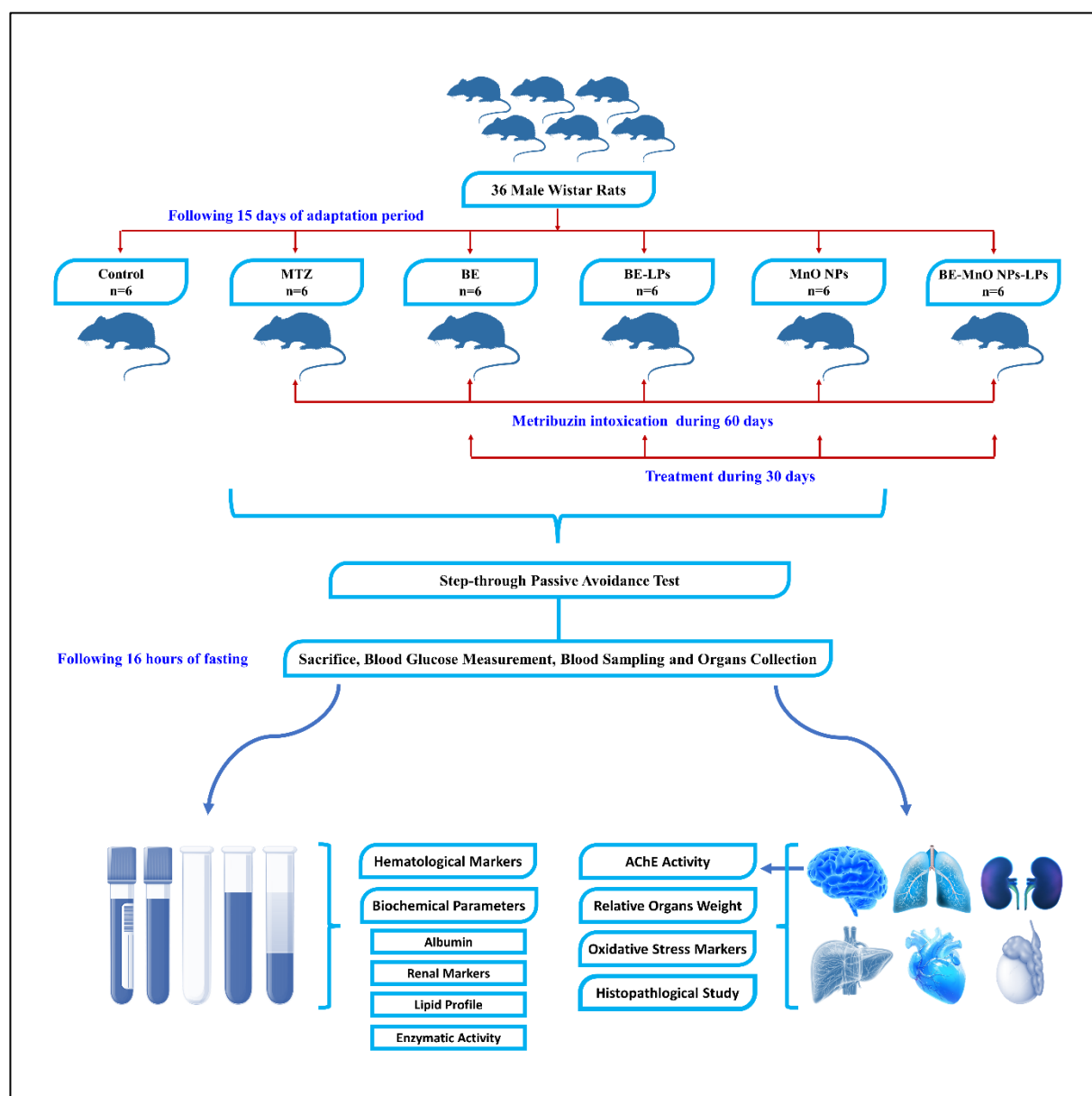


Figure 16: Experimental design of *in vivo* study

II. Methods

II.1. *In Vitro* Study

II.1.1. *In Vitro* Essays of *Ocimum basilicum* L.

II.1.1.1. Method of Aqueous Extract Preparation

To create the basil aqueous extract, 10 g of dried leaves powder was combined with 100 mL of distilled water and heated at 50 °C for 02 hours. The mixture was then left to macerate at ambient temperature for 24 hours before being filtered using Whatman filter paper. Subsequently, the filtered extract underwent evaporation in a rotary evaporator and was finally dried in an oven (Derouiche et al., 2020).

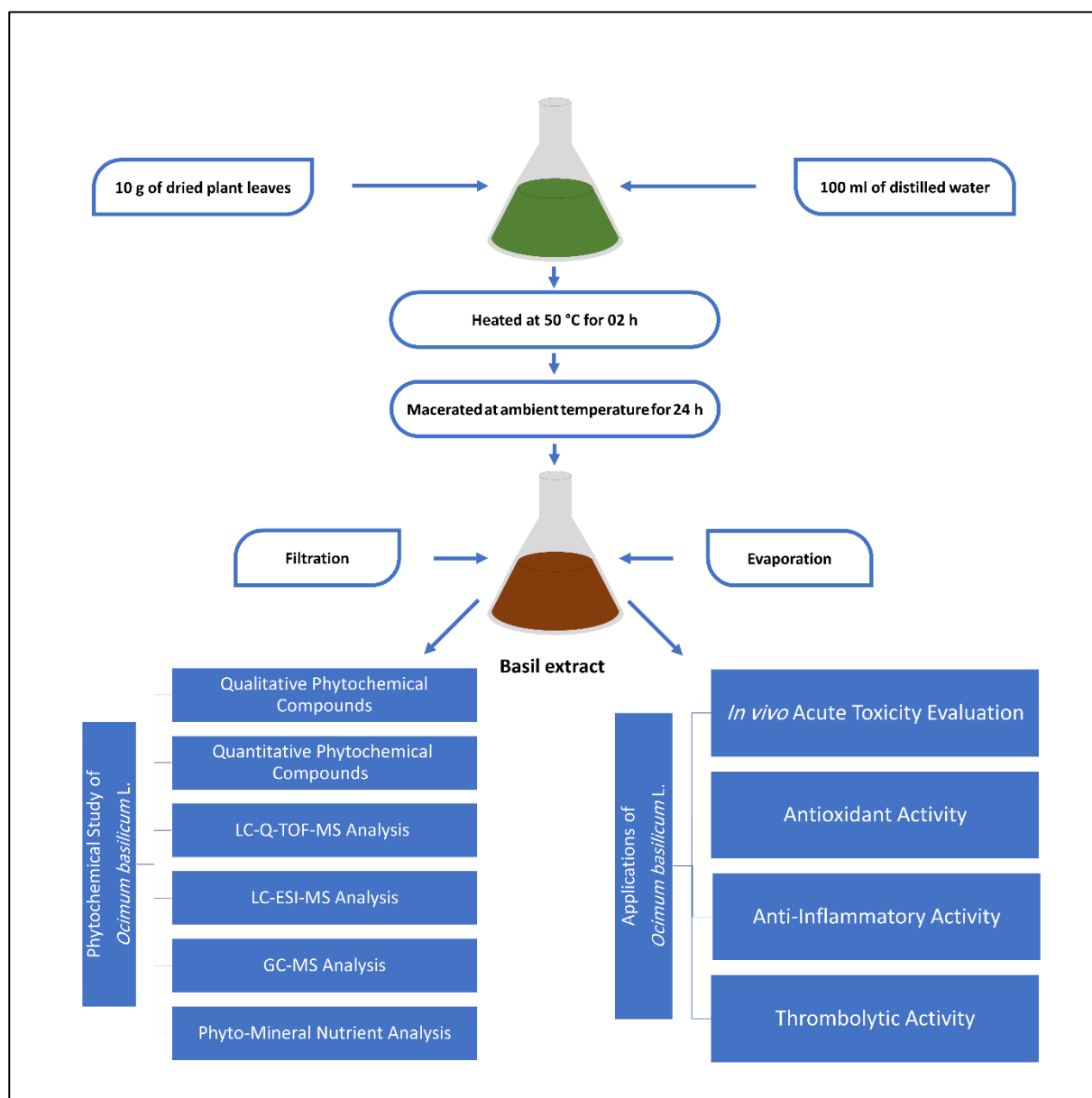


Figure 17: Experimental design of *Ocimum basilicum* L. *in vitro* essays

II.1.1.2. Calculation of Percentage Yield of Aqueous Plant Extract

The calculation of the plant extract product's yield percentage was performed using an equation employed by (Okoduwa, Umar, James, Inuwa, & Habila, 2016):

$$\text{Percentage yield \% } \left(\frac{w}{w} \right) = \left(\frac{\text{Weight of extract (g)}}{\text{Weight of plant sample (g)}} \right) \times 100 \quad (1)$$

II.1.1.3. Phytochemical Screening

The aqueous plant extract underwent a comprehensive analysis utilizing a standardized protocol to detect various phytochemicals, following the protocols followed by Boulaares et al. in their 2024 research (Boulaares et al., 2024).

II.1.1.4. Quantification of Phytochemical Compounds

II.1.1.4.1. Total Phenols

To determine the total phenolic content, researchers employed the Folin-Ciocalteu technique (Li et al., 2007). The process involved combining 1000 μL of 10% Folin-Ciocalteu reagent with 200 μL of basil aqueous extract. After a 4-minute interval, 800 μL of 7.5% saturated sodium carbonate solution was introduced. Following a 2-hour incubation at ambient temperature, the absorbance was measured at 765 nm. Gallic acid served as the standard for calibration curve preparation. The findings were reported as milligrams of gallic acid equivalents per gram of dry extract (mg of GAE / g). Each experiment was conducted three times to ensure reliability.

II.1.1.4.2. Total Flavonoids

To assess the flavonoid content of the plant extract, the technique described by (Ahn et al., 2007) was employed. In summary, equal volumes (500 μL) of the basil extract and AlCl_3 (2%) reagent were combined. After allowing the mixture to stand at room temperature for 1 hour, its absorbance was evaluated at 420 nm. Quercetin was utilized to establish the calibration curve. The findings were reported as milligrams of quercetin equivalents per gram of dry extract (mg of QE / g). Each experiment was conducted three times to ensure reliability.

II.1.1.4.3. Total Hydrolysable Tannins

The total hydrolysable tannin content was determined using the Folin-Ciocalteu colorimetric method. Tannic acid was utilized as the standard to establish a calibration curve.

The procedure involved combining 1 ml of plant extract with 0.7 ml of Na₂CO₃ (7%) solution, 0.5 mL of Folin-Ciocalteu (10%) reagent, and 8.4 mL of distilled water in a 10 mL test tube. Following a 30-minute incubation period, absorbance was measured at 700 nm against a blank (Poudel & Rajbhandari, 2020). Results were reported as milligrams of tannic acid equivalents per gram of dry extract (mg of TAE / g). Each experiment was conducted three times to ensure reliability.

II.1.1.5. HPLC-Q-TOF-MS Analysis for Phenolic Compounds

The HPLC analysis was performed using an Agilent 1290 UHPLC system (Waldbronn, Germany) coupled with a G1316A temperature-controlled column compartment, a G4226A auto-sampler, a G1330B thermostat, and a G1312B binary pump. Separation was achieved on a Venusil XBP phenyl column (150mm× 2.1mm, 5 µm; Agela Technologies, Tianjin, China). The mobile phase comprised a 0.1% formic acid aqueous solution (A) and an acetonitrile–methanol mixture (1:1, v/v; B), delivered at 0.4 mL/min with the following gradient: 75–25% B for 0–7.50 min, 50% B for 7.50–15 min, and 25–75% B for 15–22.50 min. A Triple TOF 5600+ system (Applied Biosystems Sciex, Framingham, MA, USA) with an ESI interface in positive ionization mode was used to obtain mass spectra. The "TOFMS" scan parameters were: source temperature, 550°C; ion source gases 1 and 2, 50 psi; curtain gas, 35 psi; ion spray voltage, 5500 V; collision energy, 10V; declustering potential, 80 V. The "product ion" scan used identical parameters except for collision energy, set at 35 V with a 15 V spread. Peakview® software was employed for data acquisition and processing.

II.1.1.6. LC-ESI-MS Analysis

The analysis of phenolic compounds in *Ocimum basilicum* L. extracts was conducted using Liquid Chromatography-Electrospray Ionization-Mass Spectrometry (LC-ESI-MS). A 5 µL sample was examined with a quadrupole mass spectrometer (LC-MS-2020, Shimadzu, Kyoto, Japan) equipped with a negative mode electro-nebulization ionization source (ESI). The mass spectrometer was linked to an ultra-fast liquid chromatography system, which included an LC-20AD XR binary pump system, a SIL-20AC XR autosampler, a CTO-20AC column oven, and a DGU-20A 3R degasser (Shimadzu, Kyoto, Japan). The analysis employed an Inertsustain C18 column (GL Sciences Japan) measuring 150 mm × 3 mm with 3 µm particles. The mobile phase consisted of a 1:1 mixture of 0.02% acetic acid in water and acetonitrile, using linear isocratic elution over a 10-minute acquisition period. The mobile phase flow rate was set at 0.4 mL/min, with the column temperature maintained at 40 °C and an injection

volume of 20 μ L. Spectra were monitored using SIM (Selected Ion Monitoring) mode and processed with Shimadzu Lab Solutions LC-MS software. High-purity nitrogen served as both the auxiliary gas and nebulizer. Phenolic compounds were identified by comparing retention times and mass spectra with chemical standards of >98% purity obtained from Sigma Chemical Co. (St. Louis, MO, USA) (Chetehouna, Derouiche, Boulaares, & Réggami, 2024).

II.1.1.7. GC-MS Analysis for Volatile Compounds

The volatile compounds in plant leaf extract were quantified using GC-MS analysis. A water-based extract was prepared, and 1 μ L was used for analysis. Headspace solid-phase micro-extraction (SPME) with DVB/CAR/PDMS fiber was employed to extract volatiles. The fiber underwent conditioning at 270°C for 4 h in the GC injection port. It was then exposed to the sample in a vial for 15 min at room temperature, followed by desorption in the gas chromatograph injection port for 10 min at 260°C in splitless mode. The analysis utilized a 7890A GC system linked to a 5975C VL Triple-Axis mass detector (both from Agilent Technologies, Santa Clara, United States). Compound separation occurred on a DB-5MS capillary column (25 m $\times$ 0.2 mm; 0.33 μ m film thickness; J&W, Folsom, California) with helium as the carrier gas flowing at 0.6 mL/min. The injector and transfer line temperatures were set at 260°C and 280°C, respectively. The oven temperature program began at 40°C for 3 min, increased to 160°C at 4°C/min, then to 280°C at 10°C/min, and held at the final temperature for 3 min. Mass scanning ranged from 33 to 333 Da, with ionization energy at 70eV.

The interpretation of GC-MS data was facilitated by the National Institute Standard and Technology (NIST) database. This comparative assessment helped identify unknown volatile compounds in the basil extract by matching them against the NIST library's stored data.

II.1.1.8. Analysis of Trace Metal Distribution in *Ocimum basilicum* L.

An atomic absorption spectrophotometer (Shimadzu AA-6800) equipped with an auto sampler was utilized to measure the phyto-mineral nutrient content in *Ocimum basilicum* L. The analysis included potassium (K), sodium (Na), calcium (Ca), iron (Fe), copper (Cu), zinc (Zn), magnesium (Mg), and manganese (Mn). The preparation process involved incinerating *Ocimum basilicum* L. leaf powder in a muffle furnace (Nabertherm, Germany) at 600°C for 4 hours. Subsequently, the resulting ashes were dissolved in 3 mL of hot, concentrated nitric acid. The solution was then filtered using Whatman's filter paper, and the filtrate was diluted to 10 mL with distilled water (Chetehouna, Derouiche, Reggami, Boulaares, & Frahtia, 2024).

II.1.2. *In Vitro* Essays of MnO NPs

II.1.2.1. Biosynthesis of MnO NPs

The synthesis of manganese oxide nanoparticles using *Ocimum basilicum* L. leaf extract MnO NPs was conducted following a modified version of the method described by Saod et al. (Saod et al., 2022). The process involved combining basil's aqueous extract with a manganese (II) chloride ($\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$) solution. To achieve a pH of 8, sodium hydroxide (NaOH) solution was gradually introduced to the mixture under constant stirring, leading to the formation of small particles. The solution was then subjected to magnetic stirring at 65 °C for 6 hours, during which its color transitioned from golden yellow to dark brown, signaling the successful biosynthesis of MnO NPs. Subsequently, the mixture underwent centrifugation at 5000 rpm for 20 minutes, after which the supernatant was removed. The resulting precipitate was washed three times using distilled water and ethanol before being dried to obtain the final product.

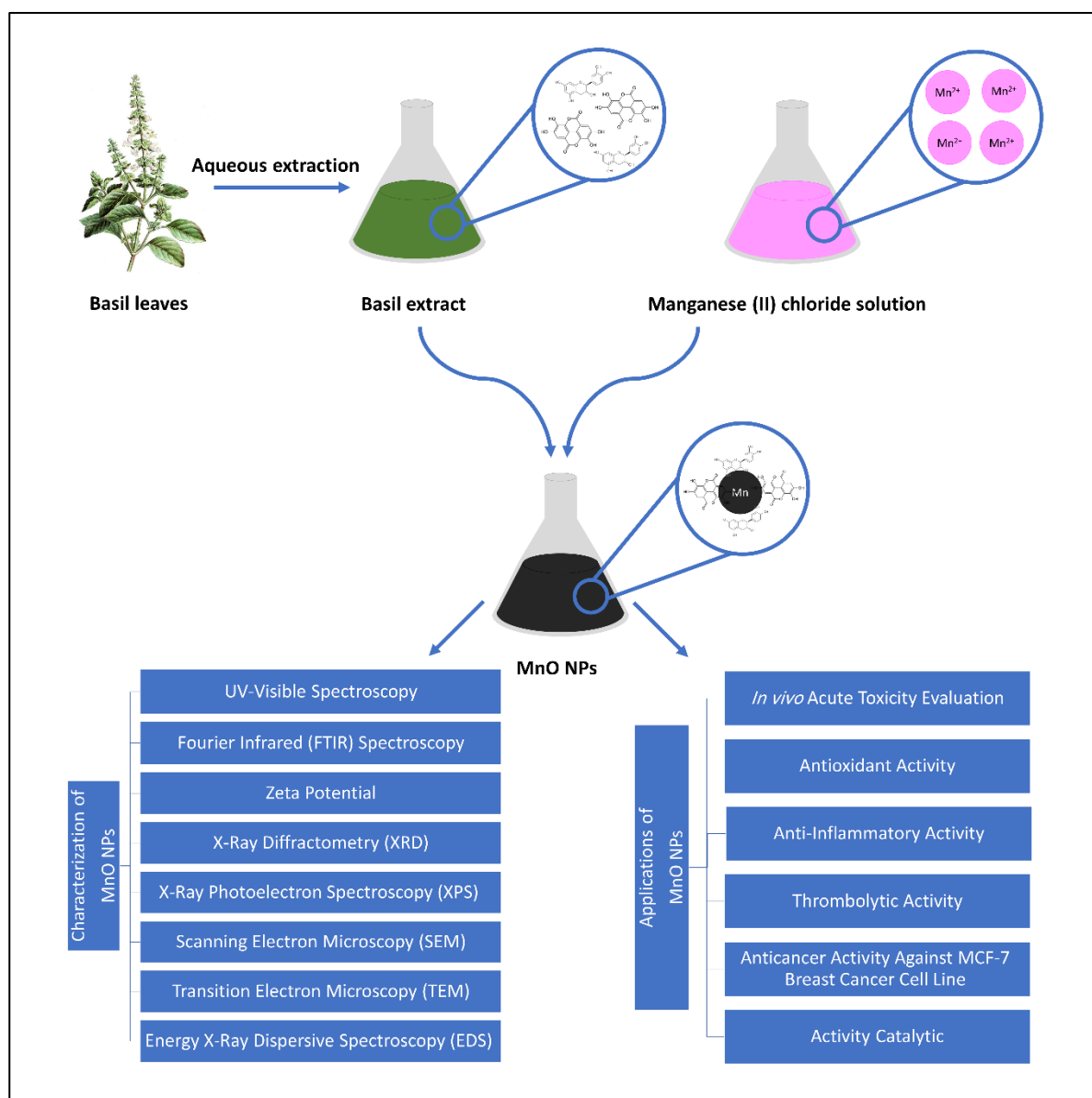


Figure 18: Experimental design of MnO NPs *in vitro* essays

II.1.2.2. Characterization of MnO NPs

II.1.2.2.1. UV-Visible Spectroscopy

To confirm the production and assess the optical properties (λ_{max} and λ_{min}) of the MnO NPs, the sample underwent analysis using a UV-visible spectrophotometer (JENWAY 6705) across a wavelength spectrum of 200 to 800 nm.

II.1.2.2.2. Fourier Infrared (FTIR) Spectroscopy

Fourier transform infrared spectroscopy (Agilent Technologies) was employed to identify the functional groups of MnO NPs. The analysis was conducted within a wavenumber frequency range of 4000 to 400 cm^{-1} , utilizing a resolution of 16 cm^{-1} .

II.1.2.2.3. Zeta Potential

The zeta potential of synthesized MnO NPs powder in water was determined by zeta sizer (NICOMP™ 380 ZLS).

II.1.2.2.4. X-Ray Diffractometry (XRD)

A PROTO® AXRD Benchtop X-ray diffractometer was employed to analyze the MnO NPs, determining their chemical formula, crystallite structure, lattice parameters, crystallite size, and degree of crystallinity. The XRD analysis was conducted over a 2θ range of 10-80° using Cu K α radiation ($\lambda=1.540593$ Å). The Debye-Scherrer equation, $D = k \lambda / \beta \cos \theta$, was applied to calculate the crystallite size. This equation incorporates the Scherrer constant (0.9-1), X-ray wavelength (1.540593 Å), full-width half maximum (FWHM), and Bragg angle (θ), with the latter two expressed in radians. The lattice parameters a, b, and c were determined using the equation $1/(d^2)=[(h^2+k^2)/a^2]+(l^2/c^2)$, where d represents the interplanar spacing and h, k, and l are the Miller indices.

II.1.2.2.5. X-Ray Photoelectron Spectroscopy (XPS)

II.1.2.2.6. Scanning and Transmission Electron Microscopy (SEM and TEM)

II.1.2.2.7. Energy X-Ray Dispersive Spectroscopy (EDS)

II.1.3. Applications of *Ocimum basilicum* L. and MnO NPs

II.1.3.1. Antioxidant Activity

II.1.3.1.1. DPPH Free-Radical Scavenging Activity

The ability of samples to neutralize the 1,1-diphenyl-2-picrylhydrazyl DPPH radical was used to assess their antioxidant properties. Various concentrations of basil extract, MnO NPs, ascorbic acid, BHT and BHA were prepared. One milliliter of samples or antioxidant standards (serving as positive control reference compounds) was combined with one milliliter of DPPH in methanol (0.1 mM). A negative control was prepared without antioxidants. After a 30-minute incubation period, the mixture's absorbance was measured at 517 nm using a UV-visible spectrophotometer (Singh, Singh, Saini, & Rao, 2008).

The following formula is used to calculate DPPH free-radical scavenging activity values:

$$\text{Inhibition percentage \%} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100 \quad (2)$$

II.1.3.1.2. Ferric Reducing Antioxidant Power FRAP

The reducing power of plant extract or nanoparticles was evaluated using the method outlined by (Rahman, Islam, Biswas, & Khurshid Alam, 2015). Various concentrations of basil extract, MnO NPs, ascorbic acid, BHT and BHA were prepared. Each test tube contained 0.25 mL of antioxidant standards or samples, 0.625 mL of potassium buffer solution (0.2 M, pH=6.6), and 0.625 mL of 1% potassium ferricyanide [$\text{K}_3\text{Fe}(\text{CN})_6$] solution. The reaction mixtures were then incubated in a water bath at 50 °C for 20 minutes to complete the reaction. Subsequently, 0.625 mL of 10% trichloroacetic acid (TCA) solution was added to each test tube. The entire mixture underwent centrifugation at 3000 rpm for ten minutes. Following this, 1.8% supernatant, 1.8% distilled water, and 0.36 mL of 0.1% ferric chloride (FeCl_3) solution

were combined. A negative control was prepared without antioxidants. The absorbance of the resulting solution was measured at 700 nm.

The calculation of ferric reducing antioxidant power (FRAP) values is performed as follows:

$$\text{Inhibition percentage \%} = 100 - \left(\frac{A_{\text{control}}}{A_{\text{sample}}} \right) \times 100 \quad (3)$$

II.1.3.2. Anti-Inflammatory Activity

II.1.3.2.1. BSA Anti-Denaturation Assay

The study examined how basil extract and MnO NPs could reduce inflammation by inhibiting protein denaturation. The experiment involved incubating 0.65 ml of 1% bovine serum albumin solution with 0.65 ml of various sample concentrations (5-25-50-100-150-200 µg/ml) at room temperature for 30 minutes. Subsequently, 0.01 ml of concentrated HCl was added, and the mixture was heated at 72 °C for 30 minutes. After cooling for ten minutes, the absorbance was measured at 660 nm. Diclofenac[®] served as the standard treatment (Djouadi & Derouiche, 2021).

The percentage inhibition of protein denaturation was determined using a specific formula:

$$\text{Inhibition percentage \%} = \left(\frac{A_{\text{sample}} - A_{\text{control}}}{A_{\text{sample}}} \right) \times 100 \quad (4)$$

II.1.3.2.2. RBC Membrane Stabilization Assay

The hemolysis assay was conducted following the protocol outlined by (Vinjamuri, Afshan, Shekar, & Saraswathi, 2015). Blood was collected in EDTA-containing tubes to prevent clotting, then centrifuged at 1000 rpm and 40 °C for 10 minutes. The supernatant was carefully extracted. The erythrocytes were then subjected to three additional 5-minute washes using 1X PBS (pH 7.4). The washed erythrocytes were stored at 4 °C and utilized within 6 hours for the hemolysis assay. A mixture of 0.05 mL of diluted erythrocyte suspension (0.1 mL erythrocytes + 0.9 mL 1XPBS) was combined with 0.1 mL of test samples (basil extract or MnO NPs) or standard. A 0.1 mL of 1XPBS served as the negative control. The reaction mixture was incubated at 37 °C in a water bath for 60 minutes. Subsequently, 0.85 mL of 1X PBS was added to bring the reaction mixture volume to 1 mL. The mixture was then centrifuged at 300 rpm for three minutes to separate the supernatant. The hemoglobin content in the

supernatant was quantified using a spectrophotometer at 540 nm. Diclofenac® was employed as the standard.

The formula for calculating the percentage inhibition of hemolysis remained unchanged:

$$Inhibition\ percentage\ \% = 100 - \left(\frac{A_{sample}}{A_{control}} \right) \times 100 \quad (5)$$

II.1.3.3. Thrombolytic Activity (Clot Lysis)

The thrombolytic activity of *Ocimum basilicum* L. and MnO NPs were assessed using the protocol described by (Anguraj, Michael, Sugumaran, Madhusudhanan, & Sivaraman, 2024). Empty Eppendorf tubes were weighed, then filled with 20, 40, and 60 µL of blood and incubated at 37 °C for 30 minutes to form clots. The clot weight (A) was calculated by subtracting the initial tube weight from the final weight after clot formation. Subsequently, 100 µL of samples at concentrations of 500 and 1000 µg/mL were added to the tubes and incubated at 37 °C for 90 minutes. The non-lyzed clot weight (B) was determined by subtracting the initial tube weight from the final weight of the remaining clot. Aspegic® served as a positive control.

The thrombolytic activity was calculated using the following equation:

$$Thrombolysis\ percentage\ \% = \left(\frac{A - B}{A} \right) \times 100 \quad (6)$$

II.1.3.4. Anticancer Activity Against MCF-7 Breast Cancer Cell Line

II.1.3.5. Catalytic Activity (Dye Degradation)

II.1.3.5.1. Photocatalytic Activity

The UV-visible absorption technique was employed to evaluate the photocatalytic performance of MnO NPs in degrading Congo red, following a method similar to that described by (Rasheed et al., 2021), with slight modifications. An aqueous solution of Congo red (10 mg/L) was prepared in a 100 mL volume. A UV-Vis spectrophotometer was used to measure the absorbance of the solution without nanoparticles (blank samples). MnO NPs (10 mg) were introduced into 100 mL of the 10 mg/L dye solution and stirred for 30 minutes in darkness to achieve adsorption/desorption equilibrium. Subsequently, the solution was exposed to a compact fluorescent light, and its absorbance was measured at 495 nm using a spectrophotometer at 30-minute intervals.

The following formula was used to calculate the dye degradation percentage of MnO NPs:

$$\text{Degradation percentage \%} = \left(\frac{A - B}{A} \right) \times 100 \quad (8)$$

Where A and B indicate the dye solutions absorbance without catalyst and with catalyst, respectively.

II.1.3.5.2. Sonocatalytic Activity

The sonocatalytic activity assessment was conducted following the methodology of (Chauhan et al., 2020). To investigate the sonocatalytic performance of MnO NPs and elucidate the dye (Congo red) degradation process, experiments were carried out at ambient temperature. The suspension (Congo red + MnO NPs) was agitated in darkness for 10 minutes prior to ultrasonic wave exposure, ensuring adsorption-desorption equilibrium of the dye on the MnO NPs. This step allowed for accurate measurement of adsorption effects during sonocatalysis. Subsequently, the mixture was subjected to ultrasonic irradiation in a digital ultrasonic cleaner for a specified duration. Following sonication, MnO NPs were removed from the suspension via centrifugation. The extent of degradation was determined by measuring absorbance changes

of the dye using a UV-Vis spectrophotometer at its peak absorbance wavelength (495 nm) at regular intervals.

The percentage of dye degradation was calculated using the following equation:

$$\text{Degradation percentage \%} = \left(\frac{A - B}{A} \right) \times 100 \quad (9)$$

Where A and B indicate the dye solutions absorbance without catalyst and with catalyst, respectively.

II.1.4. *In Vitro* Essays of Liposomes and Artificial Intelligence

II.1.4.1. Biosynthesis of Liposomes

The thin film hydration method, also known as the Bangham method, is employed for liposome preparation. This process involves dissolving lipids in ethanol, an organic solvent, followed by solvent evaporation under vacuum at 45-60°C to create a thin lipid film. Subsequently, the film is hydrated in an aqueous medium with continuous agitation for up to 2 hours at 60-70°C. This hydration step results in the formation of spherical, enclosed liposomes (Andra, Pammi, Bhatraju, & Ruddaraju, 2022).

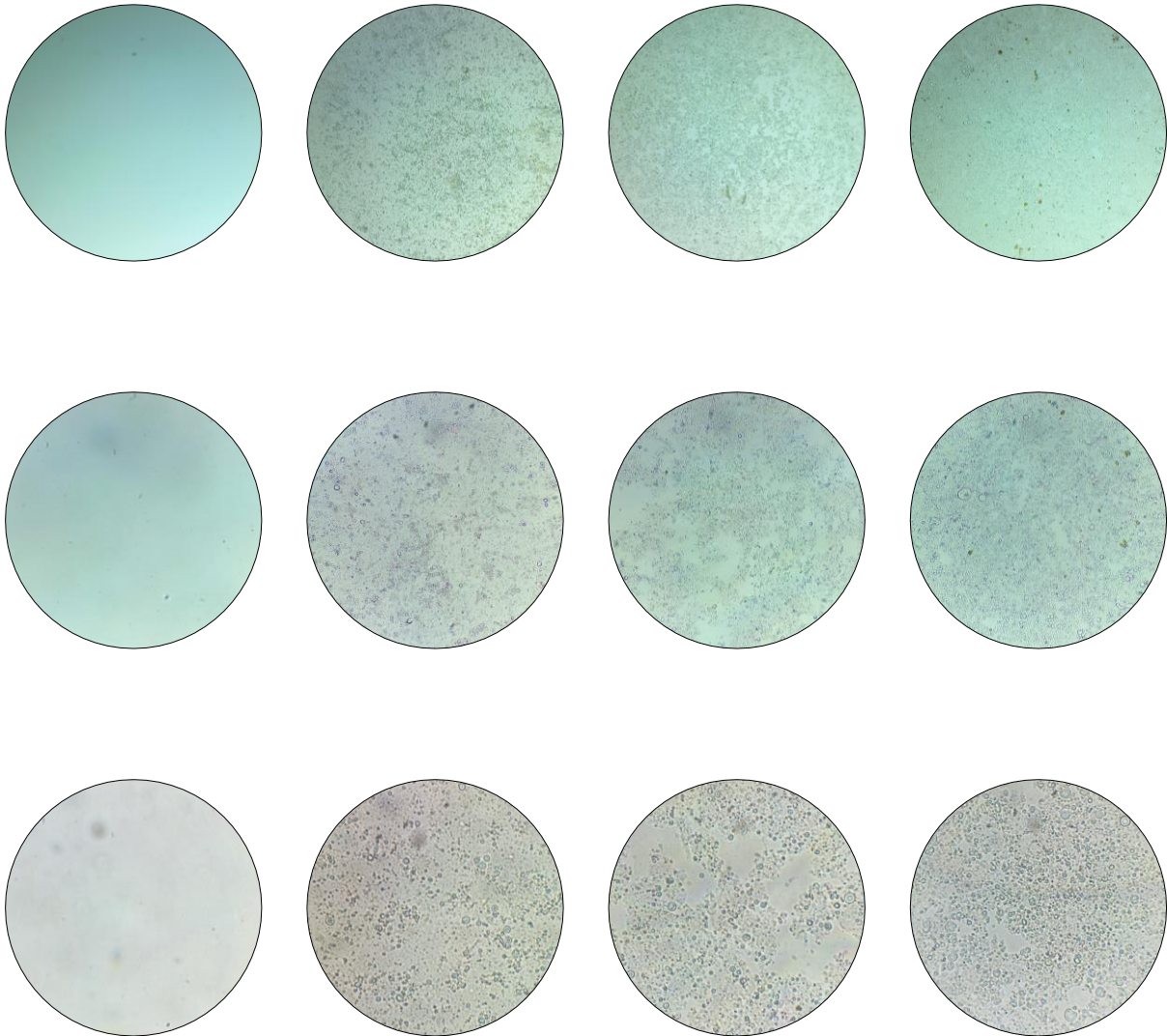
II.1.4.2. Liposomes Bioconjugation by Orcinol and Glutamine

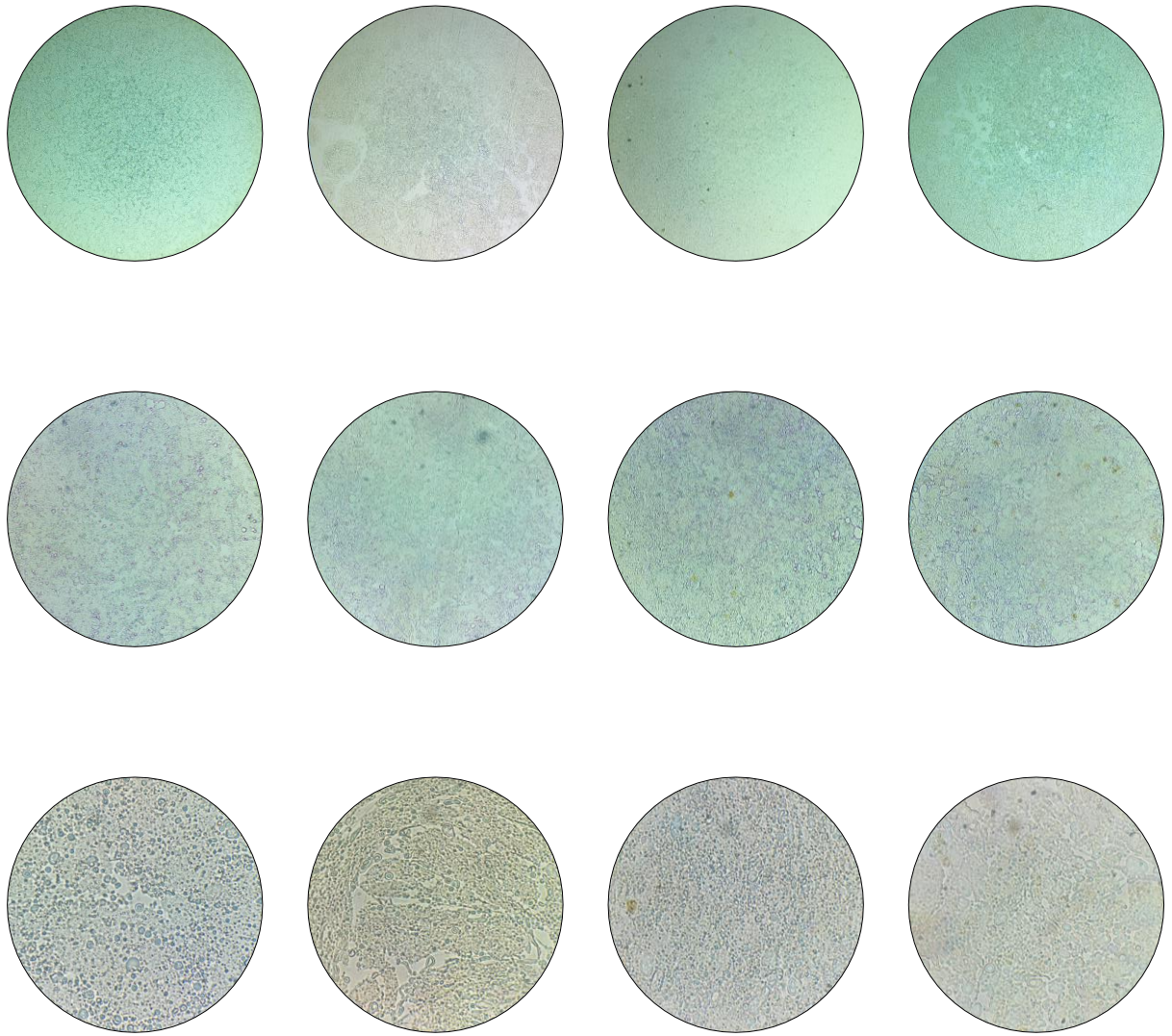
II.1.4.3. Characterization of Liposomes Using Optical Microscope

An optical microscope (OPTIKA® MICROSCOPE ITALY) was used to analyze the liposomes at different magnifications: 10 x 10, 40 x 10, and 100 x 10. To conduct the structural analysis, a thin layer of liposomes was spread on a glass slide and covered with a lamella.

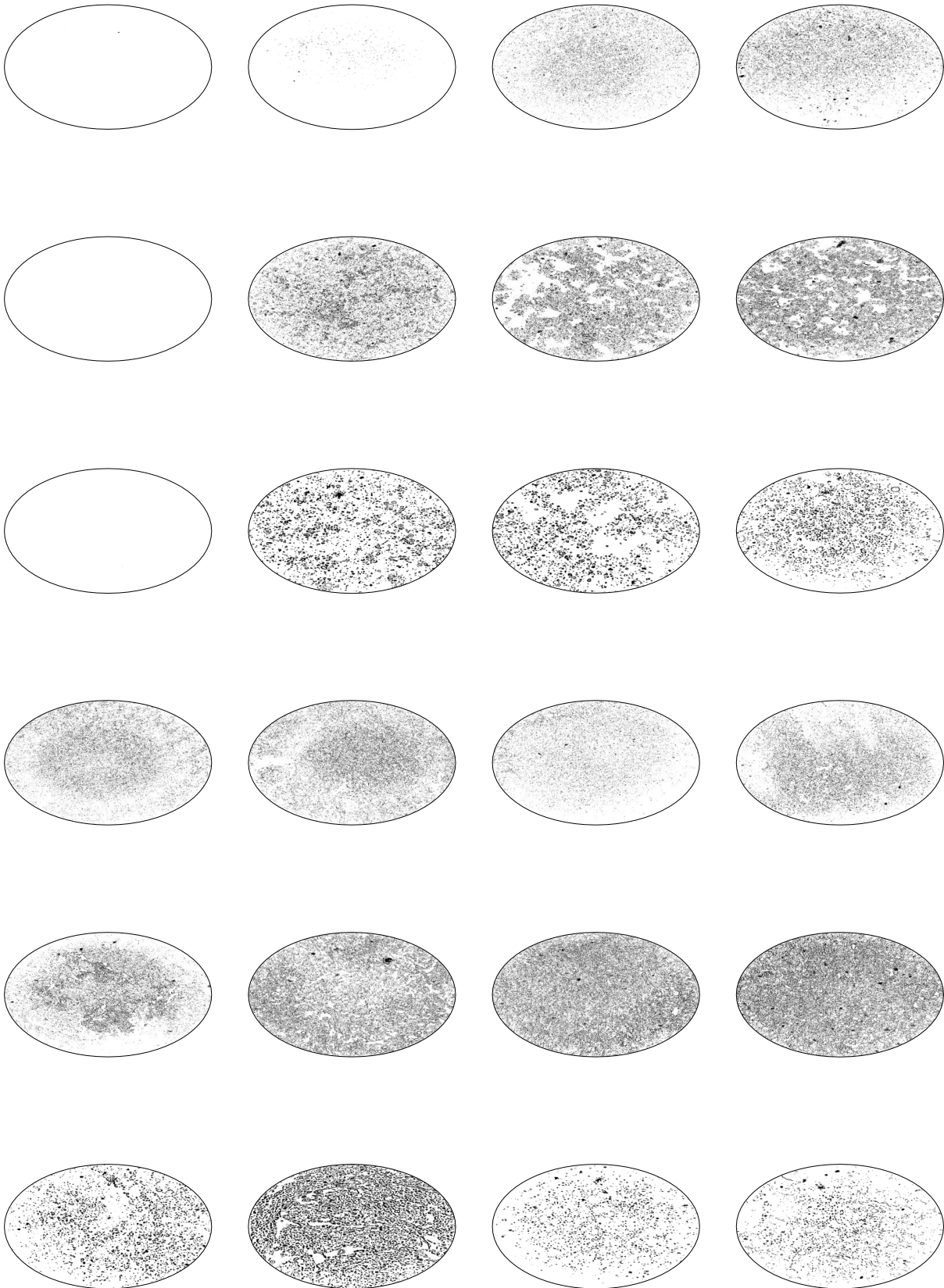
II.1.4.4. MobileNet Models as a Tool for Liposomes Classification

II.1.4.4.1. Data Set Collection





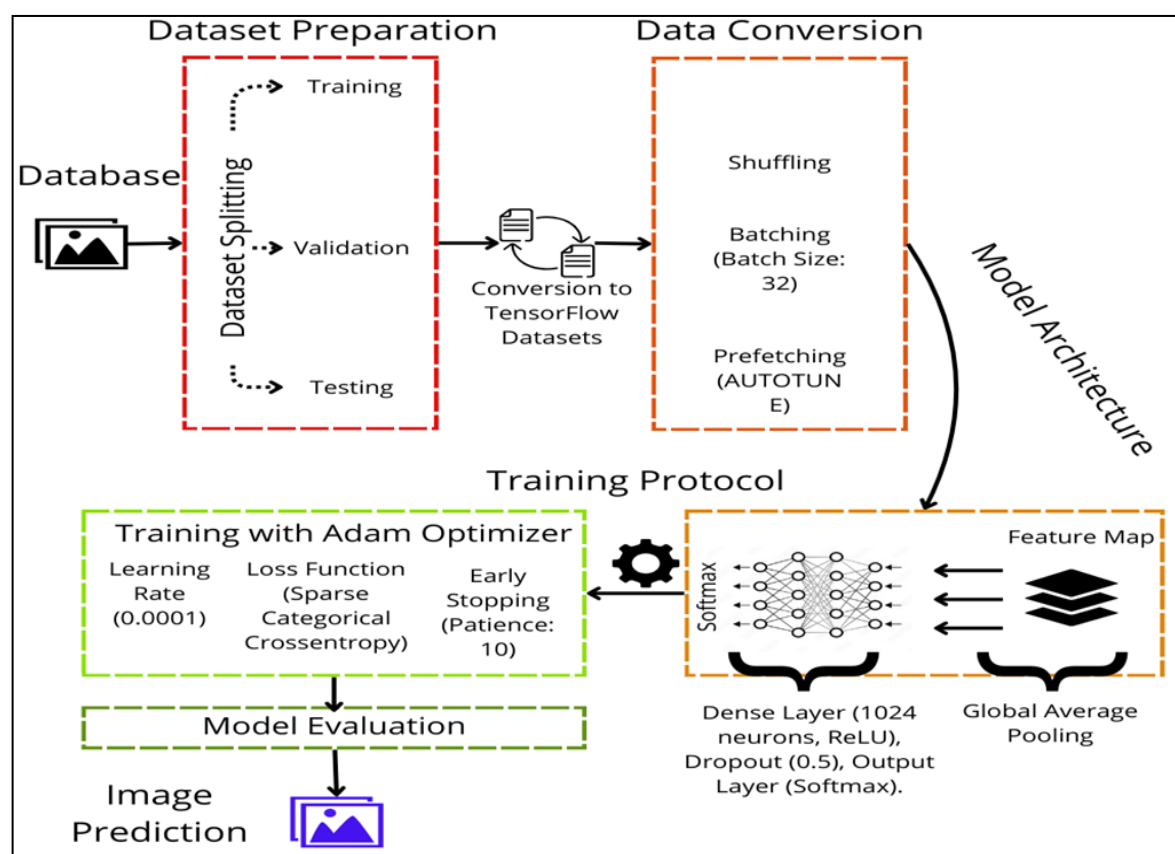
II.1.4.4.2. Image Pre-Processing



II.1.4.4.3. Training process and MobileNet architecture

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II.2. *In Vivo* Study

II.2.1. *In Vivo* Acute Toxicity Evaluation of Basil Extract

In vivo acute toxicity study was conducted using healthy Wistar albino rats. The experiment involved three groups, each containing two rats, which were given a single oral

dose of *Ocimum basilicum* L. aqueous extract. The dosages administered were control, 2000 mg/kg, and 5000 mg/kg of the rat's body weight. Over a 24-hour period, the rats' behavior and mortality were monitored (Kaouachi & Derouiche, 2018).

II.2.2. In Vivo Acute Toxicity Evaluation of MnO NPs

The acute toxicity study was conducted using the OECD guideline for testing of chemicals (n° 423): Acute Oral Toxicity – Acute Toxic Class Method. The study involved 09 healthy male Wistar albino rats, aged 8-12 weeks and weighing 212.44 ± 5.38 g, which were separated into three groups of three rats each (n=3). The animals underwent a 12-hour fasting period before dosing. Post-fasting, the rats were weighed, and the test substance was administered (each group received a single intraperitoneal injection of MnO NPs at doses of "Control, 25 and 50 mg/Kg of the rat's body weight"). Food was withheld for an additional 3-4 hours after substance administration. Rats were monitored individually following dosing, with observations made at least once during the initial 30 minutes, periodically over the first 24 hours (particularly during the first 4 hours), and daily thereafter for a total of 14 days. The animals' individual weights were recorded shortly before test substance administration and at least weekly thereafter. All observations and body weights were systematically documented, with individual records maintained for each animal (OECD, 2001).

II.2.3. Step-through Passive Avoidance Test (Passive Avoidance Learning Test)

II.2.3.1. Passive Avoidance Training

II.2.3.2. Retention Test

II.2.4. Body Weight Gain

The body weight gain was calculated using the following formula:

$$\text{Body Weight Gain } \left(\frac{g}{\text{day}}\right) = \left(\frac{\text{Final body weight (g)} - \text{Initial body weight (g)}}{\text{Days}}\right) \quad (10)$$

II.2.5. Relative Organ Weight

To calculate a relative organ weight, (Mossa, Swelam, & Mohafrash, 2015) utilized the following formula:

$$\text{Relative organ weight \%} = \left(\frac{\text{Organ weight (g)}}{\text{Body weight (g)}}\right) \times 100 \quad (11)$$

II.2.6. Biochemical and Hematological Markers

The assessment of albumin, renal markers, lipid profile and enzymatic activity was conducted utilizing commercially available assay kits (Spinreact) while the levels of VLDL, LDL and atherogenic index were calculated following the formula utilized by (FB & Barisi, 2016). Hematological markers were analyzed using a hematology autoanalyzer (Sysmex).

$$\text{VLDL level} = \left(\frac{TG}{5}\right) \quad (12)$$

$$\text{LDL level} = \text{Total cholesterol} - (\text{HDL} + \text{VLDL}) \quad (13)$$

$$\text{Atherogenic index} = \left(\frac{\text{Total cholesterol} - \text{HDL}}{\text{HDL}}\right) \times 100 \quad (14)$$

II.2.7. Oxidative Stress Parameters

II.2.7.1. Preparation of Homogenates

1 g of organ tissues was homogenized in a 9 mL tris buffer saline solution at pH 7.4. The homogenates underwent centrifugation at 4000 x g for 20 minutes at 4°C, and the supernatant was obtained for assessing oxidative stress parameters (Boulaares et al., 2024).

II.2.7.2. Reduced Glutathione (GSH) Levels

The measurement of GSH levels was conducted using the methodology outlined by Ellman (Weckbecker & Cory, 1988), which relies on the formation of a yellow color when DTNB is introduced to substances containing sulfhydryl groups. The process involved combining 0.8 mL of tissue homogenate with 0.2 mL of 0.25% salicylic acid, followed by centrifugation at 2500 g for 15 minutes. Subsequently, 0.5 mL of the resulting supernatant was mixed with 0.025 mL of 0.01 M DTNB and 1 mL TBS (pH 7.4). The absorbance was then measured at 412 nm. GSH concentrations were reported in nmol/mg of tissue, calculated using a specific equation.

$$GSH \text{ (nmol/mg of tissue)} = \frac{(OD \times 1 \times 1.525)}{13133 \times 0.8 \times 0.5 \times \text{mg of tissue}} \quad (15)$$

II.2.7.3. Malondialdehyde (MDA) Levels

The measurement of MDA in the samples was conducted using the colorimetric method described by (Yagi, 1976). This technique measures the optical density of a pink complex formed when TBA reactive substances condense with MDA in an acidic, heated environment in the presence of TBA. The process involved combining 200 µL of homogenate samples with 800 µL of TBA reagent in a sealed glass test tube. The mixture was then heated in a water bath at 100 °C for 15 minutes, followed by cooling in cold water for 30 minutes. After releasing the gases produced during the reaction, the solutions were centrifuged at 3000 rpm for 5 minutes. The supernatant's optical density (OD) was then measured at $\lambda = 532$ nm using a Jenway spectrophotometer. The concentration of thiobarbituric acid reactive substances (TBARS) was determined using the molecular extinction coefficient of MDA ($\epsilon = 1,53.105 \text{ M}^{-1} \cdot \text{cm}^{-1}$) and the following equation:

$$MDA \text{ (nmol/mg of tissue)} = \frac{OD \text{ sample}}{1.53 \times 10^5 \times \text{mg of tissue}} \quad (16)$$

II.2.7.4. Superoxide Dismutase (SOD) Activity

This study employed the SOD activity measurement technique developed by (Beauchamp & Fridovich, 1971). The procedure involved combining 50 μL of sample with 1000 μL (0.1 mM, 13 mM), 1800 μL phosphate buffer solution (50 mM; pH 7.8), and 100 μL of NBT (75 μM) in test tubes. After a 5-minute incubation at 25°C, 50 μL of riboflavin (2 μM) was added, followed by a 20-minute incubation in a light box. The optical density was then measured at 560 nm. SOD activity was expressed in Unit/mg of tissue, with the percentage inhibition of NBT reduction by SOD calculated using the following equation:

$$\text{Inhibition percentage (\%)} = \frac{OD \text{ blanc} - OD \text{ sample}}{OD \text{ blanc}} \times 100 \quad (17)$$

Where 1 Unit of SOD corresponds to 50% inhibition.

II.2.7.5. Glutathione Peroxidase (GPx) Activity

The glutathione peroxidase (GPx) activity was measured using the method described by (Flohé & Günzler, 1984). This technique relies on the reduction of hydrogen peroxide (H_2O_2) in the presence of reduced glutathione (GSH), which is converted to GSSG through GPx activity. The procedure involved combining 200 μL of homogenate with 400 μL of GSH (0.1 mM) and 200 μL of TBS buffer solution (Tris 50 mM; NaCl 150 mM; pH 7.4), followed by a 5-minute incubation at 25 °C in a water bath. Subsequently, 200 μL of H_2O_2 (1.3 mM) was introduced to initiate the reaction, which was allowed to proceed for 10 minutes. The reaction was then halted by adding 1000 μL of TCA (1%) and incubating for 30 minutes. The resulting mixture was centrifuged at 3000 rpm for 10 minutes, after which 480 μL of the supernatant was mixed with 2200 μL of TBS buffer solution and 320 μL of DTNB (1 mM). Following a 5-minute incubation, the optical density (OD) was measured at $\lambda = 412$ nm. GPx enzymatic activity was expressed in $\mu\text{mol GSH/mg}$ of tissue and calculated using an equation where 0.4 represents the concentration of GSH.

$$GPx (\mu\text{mol GSH/mg of tissue}) = \left[\left(\frac{OD \text{ sample} - OD \text{ blanc}}{OD \text{ blanc}} \right) \times 0.4 \right] \times 5/\text{mg of tissue} \quad (18)$$

II.2.7.6. Glutathione-S-Transferase (GSTs) Activity

The measurement of GST activity was conducted using spectrophotometry, following the method described by (Habig, Pabst, & Jakoby, 1974). This technique is based on the formation of a complex between GSH and CDNB, a GST substrate. The procedure involved combining 50 μL of CDNB (0.02M) with 850 μL and 830 μL of phosphate buffer in blank and test tubes, respectively. Subsequently, 100 μL of GSH (0.1M) was introduced to the mixture, followed by

the addition of 20µL of homogenate to the test tubes. Measurements were taken every minute for a duration of 5 minutes. The formation of the complex was observed through an increase in optical density at 340 nm. GST activity was expressed in units of nmol CDNB /min/mg tissue.

$$GSTs(nM /min/ mg \text{ of tissue}) = \frac{DO \text{ sample}/min - DO \text{ blanc}/min}{9.6 \times mg \text{ of tissue}} \quad (19)$$

II.2.7.7. Catalase (CAT) Activity

To assess the CAT activity, researchers utilized the technique developed by (Regoli & Principato, 1995). The measurement of catalase activity involves monitoring the reduction of H₂O₂ caused by the catalase present in the sample. The procedure requires combining 1 mL of phosphate buffer (0.1 M, pH 7.2), 0.975 mL of newly prepared H₂O₂ (0.091 M), and 0.025 mL of enzyme source (sample) in test tubes. Absorbance readings are taken at 560 nm at one-minute intervals for a total of 2 minutes. The enzyme activity is then determined in international units per minute per milligram of tissue (IU/min/mg tissue), using a specific formula:

$$CAT(UI/g) = \frac{\left(\left(\frac{2.3033}{T} \right) \times \left(\frac{\log A1}{\log A2} \right) \right)}{mg \text{ of tissue}} \quad (20)$$

II.2.8. Acetylcholinesterase (AChE) Activity

The method described by (Ellman, Courtney, Andres Jr, & Featherstone, 1961) was employed to measure AChE activity. The reaction mixture consisted of 50 µL acetylcholine (0.8 mM), 1000 µL phosphate buffer (100 mM, pH 7.5), and 50 µL DTNB (1.0 mM) to assess the rate of cholinergic hydrolysis. The homogenate (50 µL) was introduced to the pre-incubated solution, which had been shaken and maintained at 25 °C for 3 minutes. The AChE present in the tissue fraction catalyzes the breakdown of acetylthiocholine (ASCh) into acetate and thiocholine (SCh). The latter then interacts with 5-5'-Dithio-bis (2-nitrobenzoate) (DTNB) to produce a yellow TNB compound. The hydrolysis was monitored at λ= 412 nm every 3 minutes at 25 °C. The enzyme activity was expressed as µmol /min/ mg of tissue, calculated using the following equation:

$$AChE (nmol/min/mg \text{ tissue}) = \frac{\frac{\Delta DO}{\Delta t} \times 1691.18}{mg \text{ tissue}} \quad (21)$$

Δ OD / Δ t: variation optic density per minute.

II.2.9. Histological Study

After the rats were sacrificed, organ tissues were extracted and preserved in a 10% formaldehyde solution until slide preparation. The tissue samples underwent a series of processes, including toluene washing, paraffin embedding, and dehydration using an ethanol gradient. Subsequently, the samples were stained with hematoxylin and eosin. The prepared slides were then analyzed using a microscope equipped with a camera, allowing the captured images to be displayed on a computer screen.

II.2.10. Statistical Analysis

Statistical analysis of data was conducted using Minitab 19 software, specifically for performing ANOVA and the student's t-test for independent samples tests. The results are presented as mean values $\pm$ standard error, derived from the average of three replicates for each specific assay. P-value of less than 0.05 was used to assess statistical significance.

Chapter II

Results

I. Results

I.1. *In Vitro* Study

I.1.1. Phytochemical Study of *Ocimum basilicum* L.

I.1.1.1. Percentage Yield of Aqueous Plant Extract

The process of extracting aqueous solutions from the dried foliage of the basil plant, facilitated the attainment of a significant yield, as illustrated and detailed in Table 15.

Table 15: Percentage yield of crude extract

Plant species	Percentage yield (%)
<i>Ocimum basilicum</i> L.	19.063 ± 0.321

I.1.1.2. Qualitative Phytochemical Compounds

The aqueous extract exhibited a notable presence of a diverse array of phytochemical compounds, which include, phenols, flavonoids, catechic tannins, saponins, reducing sugars, alkaloids, and terpenes, as presented in Table 16.

Table 16: Phytochemical compounds of *Ocimum basilicum* L. aqueous extract

Phytochemical compounds	Test	Basil extract
Phenols	Ferric chloride test	+
Flavonoids	Magnesium test	+
Catechic tannins	Ferric chloride test	+
Saponins	Froth test	+
Reducing sugars	Fehling test	+
Alkaloids	Dragendorff's test	+
Terpenes	Salkowki's test	+

I.1.1.3. Quantitative Phytochemical Compounds

Analysis of the *Ocimum basilicum* L. extract, obtained using water as a solvent, revealed substantial levels of bioactive compounds. The extract contained notable concentrations of total phenols, flavonoids, and hydrolysable tannins, as illustrated in Figure 22.

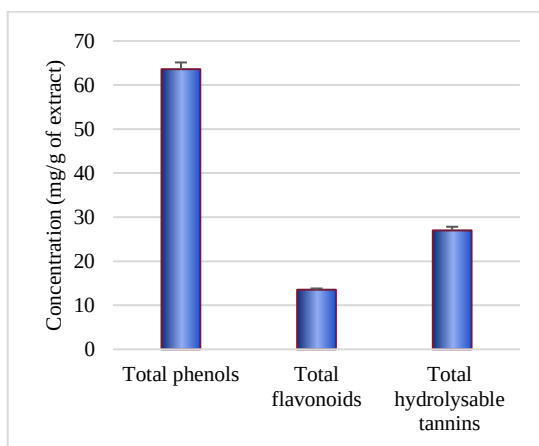


Figure 22: Total phenols, total flavonoids and total hydrolysable tannins concentrations in basil extract

I.1.1.4. HPLC-Q-TOF-MS Analysis for Phenolic Compounds

HPLC-Q-TOF-MS was employed to examine and detect phenolic compounds in the extract of *Ocimum basilicum* L. (Figure 23). Compound identification and characterization were performed by comparing retention time (RT), molar mass (M), and mass spectrometric (MS) data obtained using both negative and positive electron spray ionization modes (ESI-/ESI+). A total of 30 phenolic compounds were tentatively identified in the plant extract under positive and negative ionization conditions, as shown in Table 17. These compounds included flavonoids such as rutin, herbacetin, eupatorin, and baicalin; phenolic acids like rosmarinic acid and caffeic acid; and coumarins such as 3-hydroxycoumarin and aesculin, among others.

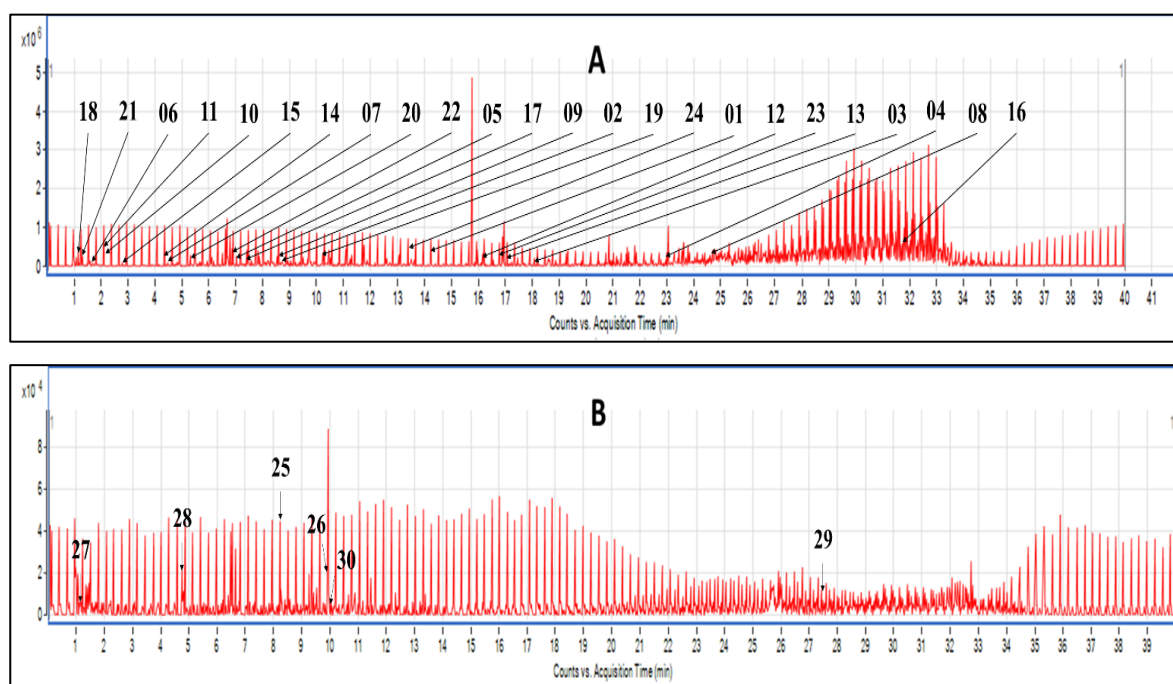


Figure 23: HPLC-Q-TOF-MS chromatogram of (A) positive and (B) negative phenolic compounds from *Ocimum basilicum* L.

Table 17: Characterization of phytochemicals found in extract of *Ocimum basilicum* L. by HPLC-Q-TOF-MS

No.	Name	Formula	RT (min)	Mass	Polarity	m/z	Diff (DB, ppm)
01	Sertraline	C ₁₇ H ₁₇ Cl ₂ N	14.253	305.0741	Positive	306.0814	-1.01
02	Rutin	C ₂₇ H ₃₀ O ₁₆	8.618	610.1523	Positive	611.1595	1.83
03	Phytosphingosine	C ₁₈ H ₃₉ NO ₃	18.093	317.2909	Positive	318.2982	6.66
04	Phthalic acid Mono- 2-ethylhexyl Ester	C ₁₆ H ₂₂ O ₄	22.948	278.1521	Positive	279.1594	-1
05	Ofloxacin	C ₁₈ H ₂₀ FN ₃ O ₄	7.026	361.1435	Positive	362.1508	0.84
06	L-Tyrosine	C ₉ H ₁₁ NO ₃	1.673	181.0749	Positive	182.0822	-5.6
07	Indole	C ₈ H ₇ N	4.53	117.057	Positive	118.0642	7.63
08	Himbacine	C ₂₂ H ₃₅ NO ₂	24.642	345.2636	Positive	346.2708	9.3
09	Herbacetin	C ₁₅ H ₁₀ O ₇	8.607	302.0415	Positive	303.0487	3.95
10	Guanosine	C ₁₀ H ₁₃ N ₅ O ₅	2.057	283.0919	Positive	284.0992	-0.73
11	Guanine	C ₅ H ₅ N ₅ O	2.046	151.0498	Positive	152.0571	-2.73
12	Eupatorin	C ₁₈ H ₁₆ O ₇	16.105	344.0889	Positive	345.0962	2.09
13	Eupatorin	C ₁₈ H ₁₆ O ₇	17.076	344.0889	Positive	345.0962	2.09
14	D-Tryptophan	C ₁₁ H ₁₂ N ₂ O ₂	4.485	204.0897	Positive	205.0969	1.06
15	DL-Phenylalanine	C ₉ H ₁₁ NO ₂	2.791	165.0792	Positive	166.0865	-1.4
16	Dioctyl phthalate	C ₂₄ H ₃₈ O ₄	31.711	390.2774	Positive	391.2847	-0.98
17	Chaetochromin	C ₃₀ H ₂₆ O ₁₀	7.421	546.15	Positive	547.1572	4.85
18	Betaine	C ₅ H ₁₂ NO ₂	1.21	118.0865	Positive	118.086	2.24
19	Baicalin	C ₂₁ H ₁₈ O ₁₁	10.199	446.0847	Positive	447.0919	0.53
20	Aesculin	C ₁₅ H ₁₆ O ₉	5.276	340.0787	Positive	341.0859	2.27
21	Adenosine	C ₁₀ H ₁₃ N ₅ O ₄	1.369	267.097	Positive	268.1043	-0.93
22	1-Indanone	C ₉ H ₈ O	6.958	132.0573	Positive	133.0646	1.41
23	19(R)-hydroxy-PGE2	C ₂₀ H ₃₂ O ₆	16.726	368.2158	Positive	391.205	11.12
24	11-deoxy-PGF1β	C ₂₀ H ₃₆ O ₄	13.452	340.2604	Positive	363.2496	2.78
25	Rutin	C ₂₇ H ₃₀ O ₁₆	8.257	610.1543	Negative	609.147	-1.52
26	Rosmarinate	C ₁₈ H ₁₆ O ₈	9.939	360.0858	Negative	359.0786	-3.64
27	Galactonic acid	C ₆ H ₁₂ O ₇	1.174	196.0599	Negative	195.0526	-8.01
28	Caffeic Acid	C ₉ H ₈ O ₄	4.767	180.0436	Negative	179.0363	-7.49
29	Betulinic Acid	C ₃₀ H ₄₈ O ₃	27.516	456.3609	Negative	455.3536	-1.25
30	3-Hydroxycoumarin	C ₉ H ₆ O ₃	9.985	162.0331	Negative	161.0258	-8.45

I.1.1.5. LC-ESI-MS Analysis for Phenolic Acids and Flavonoids

The LC-ESI-MS analysis of *Ocimum basilicum* L. extract, utilizing both qualitative and quantitative methods, revealed the presence of diverse phenolic acids and flavonoids. These compounds were identified based on their retention times and concentrations, including: quinic acid (1,582 min/ 5150,230 ppm), protocachucic acid (1,602 min/ 379,584 ppm), rosmarinic acid (1,959 min/ 3158,123 ppm), apigenin-7-o-glucoside (2,144 min/ 647,021 ppm), o-coumaric acid (2,393 min/ 277,711 ppm), luteolin (2,749 min/ 513,742 ppm), naringenin (3,463 min/ 26,925 ppm), apigenin (3,509 min/ 698,637 ppm), and cirsiolol (3,683 min/ 59,428 ppm) (Figure 24/ Table 18).

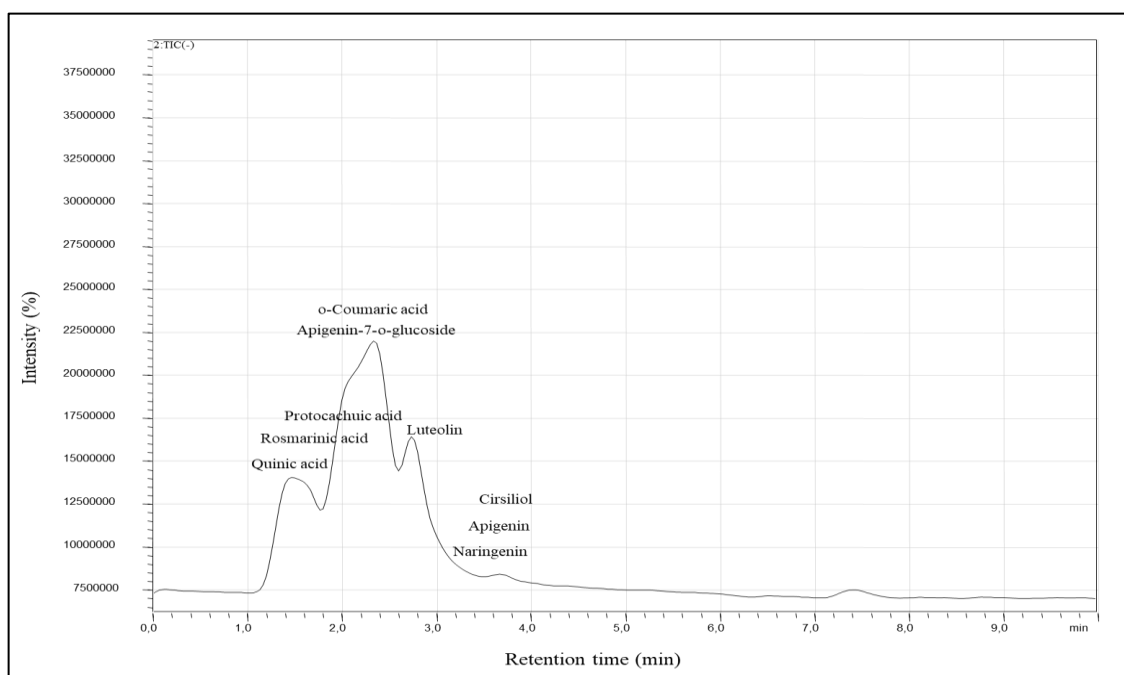


Figure 24: Chromatogram of phenolic acid found in *Ocimum basilicum* L. extract

Table 18: Phenolic compounds found by LC-ESI-MS analysis in basil extract

No.	Name	Formula	RT (min)	m/z	Conc. (ppm)
01	Quinic acid	C ₇ H ₁₂ O ₆	1,582	191,00	5150,230
02	Protocachucic acid	C ₇ H ₆ O	1,602	153,00	379,584
03	o-Coumaric acid	C ₉ H ₈ O ₃	2,393	163,00	277,711
04	Apigenin-7-o-glucoside	C ₂₁ H ₂₀ O ₁₀	2,144	431,00	647,021
05	Rosmarinic acid	C ₁₈ H ₁₆ O ₈	1,959	359,00	3158,123
06	Naringenin	C ₁₅ H ₁₂ O ₅	3,463	271,00	26,925
07	Apigenin	C ₁₅ H ₁₀ O ₅	3,509	269,00	698,637
08	Luteolin	C ₁₅ H ₁₀ O ₆	2,749	285,00	513,742
09	Cirsiolol	C ₁₇ H ₁₄ O ₇	3,683	329,00	59,428

I.1.1.6. GC-MS Analysis for Volatile Compounds

The analysis of volatile compounds in *Ocimum basilicum* L. extract was performed using GC-MS chromatography (Figure 25). This technique identified approximately 147 components (Table 19). The GC-MS analysis revealed several chemical constituents present in the aqueous extract at concentrations exceeding 0.5%. These included propanoic acid, 2-hydroxy-, ethyl ester (3.503%), butanoic acid, 4-hydroxy- (1.657%), cyclopentasiloxane, decamethyl- (1.346%), cyclotetrasiloxane, octamethyl- (0.983%), heptadecane, 2,6,10,15-tetramethyl- (0.785%), bis(tert-butyldimethylsilyl) 2,3-bis((tert-butyldimethylsilyl)oxy)fumarate (0.703%), cyclononasiloxane, octadecamethyl- (0.672%), phthalic acid, 8-bromooctyl isobutyl ester (0.655%), 3-isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy) tetrasiloxane (0.535%), and cyclononasiloxane, octadecamethyl- (0.506%).

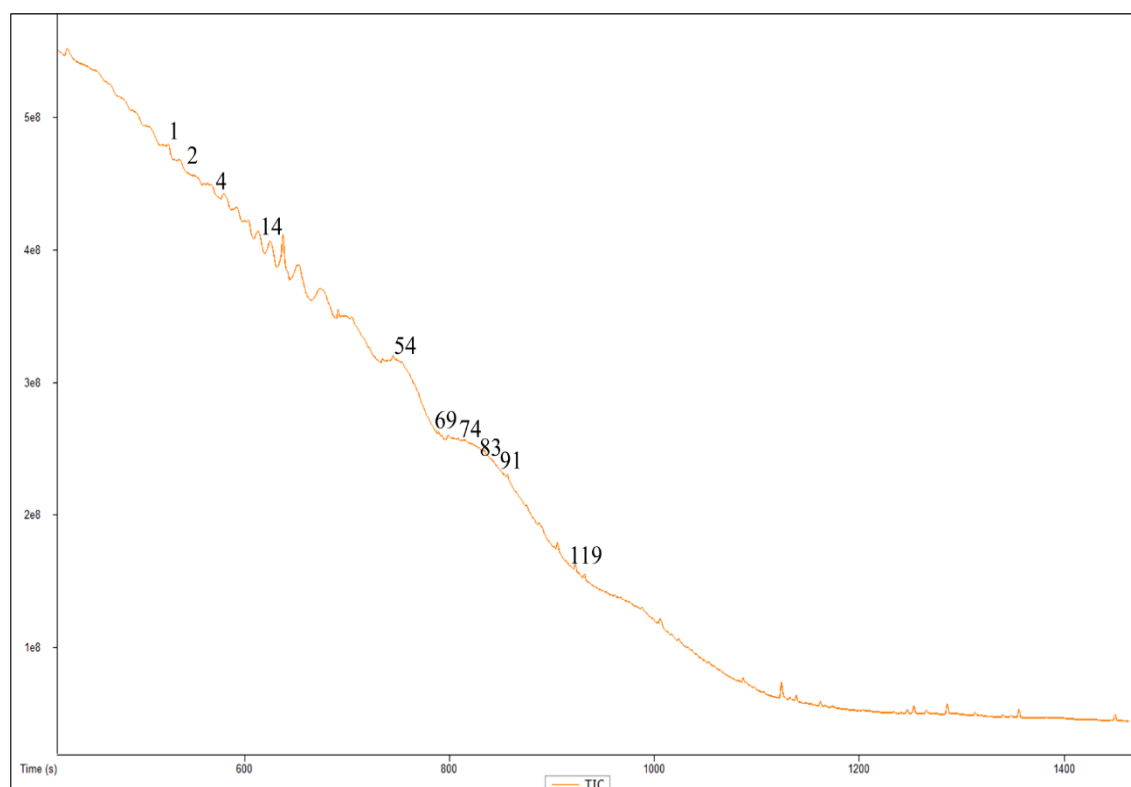


Figure 25: GC-MS spectrum of volatile compounds present in basil leaves extract

Table 19: Volatile compounds found by GC-MS analysis in basil extract

No.	Name	Formula	RT (s)	Area (%)
1	Propanoic acid, 2-hydroxy-, ethyl ester	C ₅ H ₁₀ O ₃	289.405	3.503
2	Butanoic acid, 4-hydroxy-	C ₄ H ₈ O ₃	427.257	1.657
3	Lycorenan-7-one,9,10-dimethoxy-1-methyl-	C ₁₈ H ₂₁ NO ₄	430.676	0.006
4	Cyclotetrasiloxane, octamethyl-	C ₈ H ₂₄ O ₄ Si ₄	525.997	0.983
5	2-Propanol, 1-(2-methoxypropoxy)-	C ₇ H ₁₆ O ₃	544.928	0.038

6	Benzene, 1-methyl-3-(1-methylethyl)-	C ₁₀ H ₁₄	558.773	0.056
7	1-Hexanol, 2-ethyl-	C ₈ H ₁₈ O	560.792	0.144
8	Cyclohexene, 4-ethenyl-1,4-dimethyl-	C ₁₀ H ₁₆	563.79	0.071
9	Benzyl alcohol	C ₇ H ₈ O	567.088	0.119
10	Benzeneacetaldehyde	C ₈ H ₈ O	578.822	0.237
11	Linalyl acetate	C ₁₂ H ₂₀ O ₂	637.621	0.45
12	Nonanal	C ₉ H ₁₈ O	642.028	0.096
13	1,5,7-Octatrien-3-ol, 3,7-dimethyl-	C ₁₀ H ₁₆ O	642.329	0.074
14	Cyclopentasiloxane, decamethyl-	C ₁₀ H ₃₀ O ₅ Si ₅	691.471	1.346
15	Octanoic acid	C ₈ H ₁₆ O ₂	705.073	0.214
16	1,5,5-Trimethyl-6-methylene-cyclohexene	C ₁₀ H ₁₆	734.686	0.075
17	Benzene, 1-methoxy-4-(1-propenyl)-, (Z)-	C ₁₀ H ₁₂ O	741.029	0.099
18	(3S,4R,5R,6R)-4,5-Bis(hydroxymethyl)-3,6-dimethylcyclohexene	C ₁₀ H ₁₈ O ₂	744.729	0.048
19	Benzothiazole	C ₇ H ₅ NS	770.123	0.118
20	1,4-Benzenedicarboxaldehyde	C ₈ H ₆ O ₂	776.346	0.015
21	Carvone	C ₁₀ H ₁₄ O	786.8	0.019
22	(+)-3-Carene	C ₁₀ H ₁₆	792.754	0.055
23	Nonanoic acid	C ₉ H ₁₈ O ₂	798.399	0.136
24	1H-Indene-4-carboxaldehyde, 2,3-dihydro-	C ₁₀ H ₁₀ O	813.245	0.045
25	Tetrasiloxane, 3,5-diethoxy-1,1,1,7,7,7-hexamethyl-3,5-bis(trimethylsiloxy)-	C ₁₆ H ₄₆ O ₇ Si ₆	820.45	0.015
26	1-[p-Chlorophenyl]-3-[4-[[2-(diisopropylamino) ethyl] amino]-6-methyl-2-pyrimidinyl]-guanidine	C ₂₀ H ₃₀ ClN ₇	839.416	0.021
27	Pentadecane	C ₁₅ H ₃₂	858.385	0.026
28	Pentanoic acid, 2,2,4-trimethyl-3-hydroxy-, isobutyl ester	C ₁₂ H ₂₄ O ₃	888.138	0.163
29	Hexadecane	C ₁₆ H ₃₄	922.883	0.221
30	Dodecanal	C ₁₂ H ₂₄ O	931.63	0.096
31	Caryophyllene	C ₁₅ H ₂₄	953.638	0.285
32	Phthalic acid, 4-fluoro-2-nitrophenyl methyl ester	C ₁₅ H ₁₀ FNO ₆	974.316	0.02

33	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	C ₁₄ H ₂₀ O ₂	988.069	0.124
34	Ethanone, 1-(6,6-dimethylbicyclo [3.1.0] hex-2-en-2-yl)-	C ₁₀ H ₁₄ O	999.114	0.027
35	Decyl octyl ether	C ₁₈ H ₃₈ O	1013.58	0.036
36	Oxirane, dodecyl-	C ₁₄ H ₂₈ O	1017.11	0.034
37	Butylated Hydroxytoluene	C ₁₅ H ₂₄ O	1023.99	0.111
38	1H-Indene, 1-methyl-3-propyl-	C ₁₃ H ₁₆	1026.14	0.006
39	Lilial	C ₁₄ H ₂₀ O	1036.3	0.038
40	Benzene, (1-butylhexyl)-	C ₁₆ H ₂₆	1041.35	0.072
41	Undecane, 5-methyl-	C ₁₂ H ₂₆	1044.53	0.015
42	3-[(4-Fluoroanilino) carbonyl]-1,2,2-trimethylcyclopentanecarboxylic acid	C ₁₆ H ₂₀ FNO ₃	1045.61	0.007
43	Tetradecane, 3-methyl-	C ₁₅ H ₃₂	1057.89	0.023
44	Oxirane, tetramethyl-	C ₆ H ₁₂ O	1085.53	0.003
45	Hexadecane	C ₁₆ H ₃₄	1086.64	0.167
46	Diethyl Phthalate	C ₁₂ H ₁₄ O ₄	1087.54	0.093
47	Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester	C ₁₆ H ₃₀ O ₄	1089.69	0.166
48	Hexestrol, O-trifluoroacetyl-	C ₂₀ H ₂₁ F ₃ O ₃	1096.71	0.006
49	Benzene, (1-pentylhexyl)-	C ₁₇ H ₂₈	1115.07	0.025
50	Benzene, (1-butylheptyl)-	C ₁₇ H ₂₈	1117.68	0.05
51	Phenol, 2,6-bis(1,1-dimethylethyl)-4-(1-methylpropyl)-	C ₁₈ H ₃₀ O	1121.14	0.029
52	Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester	C ₁₃ H ₂₂ O ₃	1132.63	0.137
53	Octane, 1,1'-oxybis-	C ₁₆ H ₃₄ O	1135.59	0.042
54	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	C ₁₈ H ₅₂ O ₇ Si ₇	1138.39	0.535
55	Nonane, 3,7-dimethyl-	C ₁₁ H ₂₄	1140.85	0.015
56	Decane, 1-chloro-	C ₁₀ H ₂₁ Cl	1146.63	0.011
57	Salicylic acid, isopropyl ether, isopropyl ester	C ₁₃ H ₁₈ O ₃	1152.88	0.026
58	Decyl acrylate	C ₁₃ H ₂₄ O ₂	1157.24	0.019

59	1-(2-Aminopropoxy)-2-methoxyethane	C ₆ H ₁₅ NO ₂	1163.35	0.001
60	Pentadecane, 2,6,10,14-tetramethyl-	C ₁₉ H ₄₀	1166.7	0.06
61	Hydrazinecarboxamide	CH ₅ N ₃ O	1167.88	0.005
62	2-(4,5-Dihydroxy-2-methylphenyl)-4-hydroxy-6-methoxybenzoic acid, 4TMS	C ₂₇ H ₄₆ O ₆ Si ₄	1172.97	0.142
63	Phenethyl isocyanate	C ₉ H ₉ NO	1191.03	0.021
64	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	1203.52	0.036
65	Hexadecane, 2,6,10,14-tetramethyl-	C ₂₀ H ₄₂	1240.94	0.045
66	Benzene, (1-methylnonadecyl)-	C ₂₆ H ₄₆	1243.15	0.016
67	Salicylic acid, 1-methylpropyl ester	C ₁₁ H ₁₄ O ₃	1245.87	0.021
68	Pentadecanal-	C ₁₅ H ₃₀ O	1246.84	0.063
69	Bis(tert-butyldimethylsilyl) 2,3-bis ((tert butyldimethylsilyl) oxy) fumarate	C ₂₈ H ₆₀ O ₆ Si ₄	1253.32	0.703
70	Nickel tetracarbonyl	C ₄ NiO ₄	1264.2	0
71	Isoamyl laurate	C ₁₇ H ₃₄ O ₂	1265.09	0.188
72	Dodecanoic acid, 1,1-dimethylpropyl ester	C ₁₇ H ₃₄ O ₂	1267.65	0.034
73	4-Fluorobenzylamine, N, N-dibutyl-	C ₁₅ H ₂₄ FN	1275.68	0.009
74	Phthalic acid, 8-bromooctyl isobutyl ester	C ₂₀ H ₂₉ BrO ₄	1285.64	0.655
75	Semioxamazide	C ₂ H ₅ N ₃ O ₂	1288.89	0.002
76	Sulfurous acid, 2-ethylhexyl nonyl ester	C ₁₇ H ₃₆ O ₃ S	1301.97	0.028
77	Hydrazinecarboxamide	CH ₅ N ₃ O	1309.44	0.007
78	2-(Diethylamino) ethyl 4-amino-2-hydroxybenzoate	C ₁₃ H ₂₀ N ₂ O ₃	1312.77	0.404
79	Pentadecanoic acid, 14-methyl-, methyl ester	C ₁₇ H ₃₄ O ₂	1319.53	0.049
80	Nickel tetracarbonyl	C ₄ NiO ₄	1324.45	0
81	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	1340.11	0.057
82	1,4-Dibutyl benzene-1,4-dicarboxylate	C ₁₆ H ₂₂ O ₄	1348.04	0.128
83	Cyclononasiloxane, octadecamethyl-	C ₁₈ H ₅₄ O ₉ Si ₉	1355.76	0.672
84	Decane, 6-ethyl-2-methyl-	C ₁₃ H ₂₈	1367.14	0.019
85	Hydrazinecarboxamide	CH ₅ N ₃ O	1391.66	0.001
86	Piceatannol, 4TMS	C ₂₆ H ₄₄ O ₄ Si ₄	1395.26	0.324
87	Hydrazinecarboxamide	CH ₅ N ₃ O	1406.69	0
88	Hydrazinecarboxamide	CH ₅ N ₃ O	1430.48	0.005

89	(1-Methoxy-pentyl)-cyclopropane	C ₉ H ₁₈ O	1436.74	0.009
90	Pentadecanoic acid, 14-methyl-, methyl ester	C ₁₇ H ₃₄ O ₂	1446.12	0.035
91	Cyclononasiloxane, octadecamethyl-	C ₁₈ H ₅₄ O ₉ Si ₉	1449.57	0.506
92	5-Hexyl-5-methyloxolan-2-one	C ₁₁ H ₂₀ O ₂	1457.25	0.004
93	Hydrazinecarboxamide	CH ₅ N ₃ O	1482.2	0.001
94	Benzoic acid, tetradecyl ester	C ₂₁ H ₃₄ O ₂	1494.32	0.028
95	Semicarbazide	CH ₅ N ₃ O	1500.05	0.002
96	Nickel tetracarbonyl	C ₄ NiO ₄	1509.8	0.005
97	Hydrazinecarboxamide	CH ₅ N ₃ O	1511.62	0.001
98	Cyclononasiloxane, octadecamethyl-	C ₁₈ H ₅₄ O ₉ Si ₉	1534.56	0.364
99	Hydrazinecarboxamide	CH ₅ N ₃ O	1540.15	0.001
100	Hydrazinecarboxamide	CH ₅ N ₃ O	1553.07	0.001
101	Benzoic acid, tridecyl ester	C ₂₀ H ₃₂ O ₂	1553.75	0.036
102	Semicarbazide	CH ₅ N ₃ O	1573.77	0.001
103	Semicarbazide	CH ₅ N ₃ O	1577.54	0.002
104	2-Hexanamine	C ₆ H ₁₅ N	1580.48	0.005
105	Benzoic acid, hexadecyl ester	C ₂₃ H ₃₈ O ₂	1610.8	0.036
106	Cyclononasiloxane, octadecamethyl-	C ₁₈ H ₅₄ O ₉ Si ₉	1612.91	0.29
107	Semicarbazide	CH ₅ N ₃ O	1621.61	0.002
108	L-Alanine, α -N-methyl-N-benzyl-, methyl ester	C ₁₂ H ₁₇ NO ₂	1637.98	0.02
109	Semicarbazide	CH ₅ N ₃ O	1645.43	0.007
110	Carbonic acid, bis(2-ethylhexyl) ester	C ₁₇ H ₃₄ O ₃	1656.43	0.017
111	Semicarbazide	CH ₅ N ₃ O	1664.98	0.001
112	Benzoic acid, pentadecyl ester	C ₂₂ H ₃₆ O ₂	1665.58	0.031
113	Benzyldiethyl-(2,6-xylylcarbamoylemethyl)- ammonium benzoate	C ₂₈ H ₃₄ N ₂ O ₃	1680.52	0.086
114	Phthalic acid, 2-ethylhexyl pentadecyl ester	C ₃₁ H ₅₂ O ₄	1682.77	0.031
115	Cyclononasiloxane, octadecamethyl-	C ₁₈ H ₅₄ O ₉ Si ₉	1686.3	0.274
116	Sulfurous acid, 2-ethylhexyl hexyl ester	C ₁₄ H ₃₀ O ₃ S	1712.83	0.04
117	Vinyl 2-ethylhexanoate	C ₁₀ H ₁₈ O ₂	1717.17	0.175
118	dl-Alanyl-l-alanine	C ₆ H ₁₂ N ₂ O ₃	1730.68	0.003
119	Heptadecane, 2,6,10,15-tetramethyl-	C ₂₁ H ₄₄	1732.27	0.785
120	Hydrazinecarboxamide	CH ₅ N ₃ O	1733.46	0.004

121	Hydrazinecarboxamide	CH ₅ N ₃ O	1754.55	0.001
122	Cyclononasiloxane, octadecamethyl-	C ₁₈ H ₅₄ O ₉ Si ₉	1757.18	0.304
123	Carbonic acid, bis(2-ethylhexyl) ester	C ₁₇ H ₃₄ O ₃	1758.38	0.011
124	Semicarbazide	CH ₅ N ₃ O	1760.82	0.001
125	Hydrazinecarboxamide	CH ₅ N ₃ O	1768.11	0.001
126	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	C ₂₄ H ₃₈ O ₄	1785.34	0.041
127	Semicarbazide	CH ₅ N ₃ O	1805.8	0.001
128	Benzenemethanol, α -(1-aminoethyl)-	C ₉ H ₁₃ NO	1833.93	0.013
129	Squalene	C ₃₀ H ₅₀	1835.62	0.108
130	Semicarbazide	CH ₅ N ₃ O	1836.47	0.001
131	Cyclononasiloxane, octadecamethyl-	C ₁₈ H ₅₄ O ₉ Si ₉	1839.37	0.28
132	2-Hydroxybenzene-1,3-dicarboxylic acid, trimethylsilyl ether, bis(trimethylsilyl) ester	C ₁₇ H ₃₀ O ₅ Si ₃	1850.8	0.311
133	Semicarbazide	CH ₅ N ₃ O	1863.63	0.001
134	Hydrazinecarboxamide	CH ₅ N ₃ O	1880.66	0.002
135	Hydrazinecarboxamide	CH ₅ N ₃ O	1882.03	0.001
136	Hydrazinecarboxamide	CH ₅ N ₃ O	1891.75	0.001
137	Semicarbazide	CH ₅ N ₃ O	1893.9	0.005
138	Benzenemethanol, α -(1-aminoethyl)-	C ₉ H ₁₃ NO	1904.71	0.003
139	Hydrazinecarboxamide	CH ₅ N ₃ O	1909.88	0.001
140	Benzenemethanol, α -(1-aminoethyl)-	C ₉ H ₁₃ NO	1911.59	0.001
141	Semicarbazide	CH ₅ N ₃ O	1917.4	0.001
142	Semicarbazide	CH ₅ N ₃ O	1927.25	0.001
143	Semicarbazide	CH ₅ N ₃ O	1932.07	0.001
144	Cyclononasiloxane, octadecamethyl-	C ₁₈ H ₅₄ O ₉ Si ₉	1941.29	0.485
145	Semicarbazide	CH ₅ N ₃ O	1945.74	0.001
146	Semicarbazide	CH ₅ N ₃ O	1946.04	0.001
147	Semicarbazide	CH ₅ N ₃ O	1958.37	0.001

I.1.1.7. Trace Metal Distribution in *Ocimum basilicum* L.

Sweet basil (*Ocimum basilicum* L.) is rich in various essential mineral nutrients. These nutrients encompass both essentials major minerals, including sodium, potassium, calcium, and magnesium, and essentials minor minerals, such as copper, zinc, iron, and manganese (Table 20).

Table 20: Trace metal distribution in *Ocimum basilicum* L.

	Minerals	Concentration ($\mu\text{g/g}$ of dried basil leaves)
Essentials major minerals	Sodium Na	804
	Potassium K	5348
	Calcium Ca	31392
	Magnesium Mg	6950
Essentials minor minerals	Copper Cu	0.32
	Zinc Zn	0.15
	Iron Fe	483
	Manganese Mn	0.56

I.1.2. Biosynthesis and Charactrization of MnO NPs

I.1.2.1. Biosynthesis of MnO NPs (Visual observation)

Conversely, the color transformation from a golden yellow to a deep brown at different time intervals indicates the formation of MnO NPs using basil extract (Figure 26). This process occurs after the reduction of manganese ions (Mn^{+2}) by phytochemicals present in basil under specific conditions, including temperature, pH, and reaction duration.

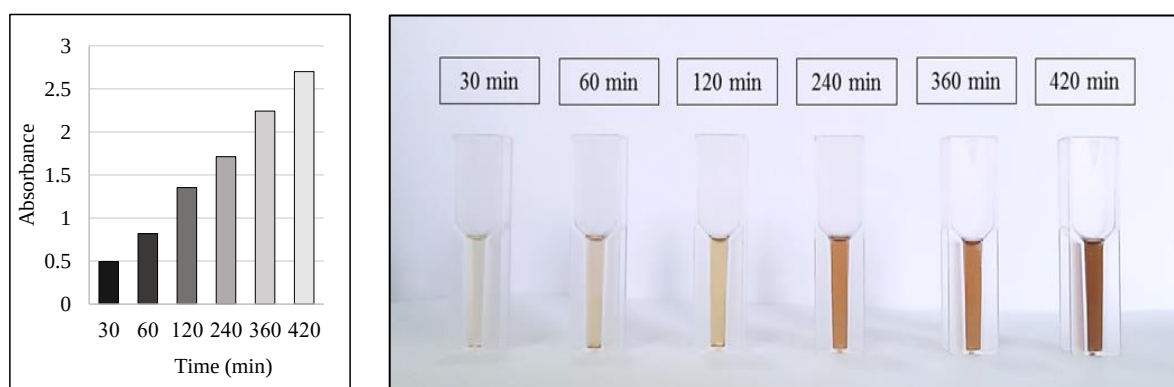


Figure 26: Biosynthesis of MnO NPs using basil extract at different time

I.1.2.2. Characterization of MnO NPs

I.1.2.2.1. UV-Visible (UV-Vis) Spectroscopy

UV-Vis spectroscopy examination revealed a broad, bell-shaped spectrum with a distinct absorption maximum at 405 nm wavelength. This finding strongly suggests the successful production and generation of MnO NPs, as illustrated in Figure 27.

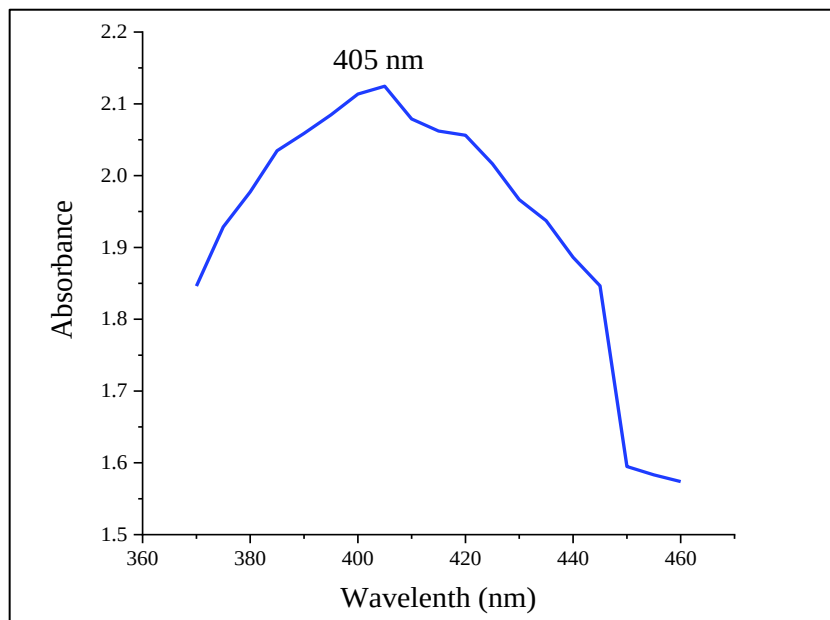


Figure 27: UV-Vis spectrum of MnO NPs

I.1.2.2.2. Fourier Infrared (FTIR) Spectroscopy

The FTIR analysis revealed a characteristic Mn-O transmittance band at 470 and 522 cm^{-1} . The spectrum also exhibited several peaks around 3200, 2900, 1550, 1380, and 1028 cm^{-1} , potentially corresponding to functional groups of active components from the basil extract coating the MnO NPs (Figure 28).

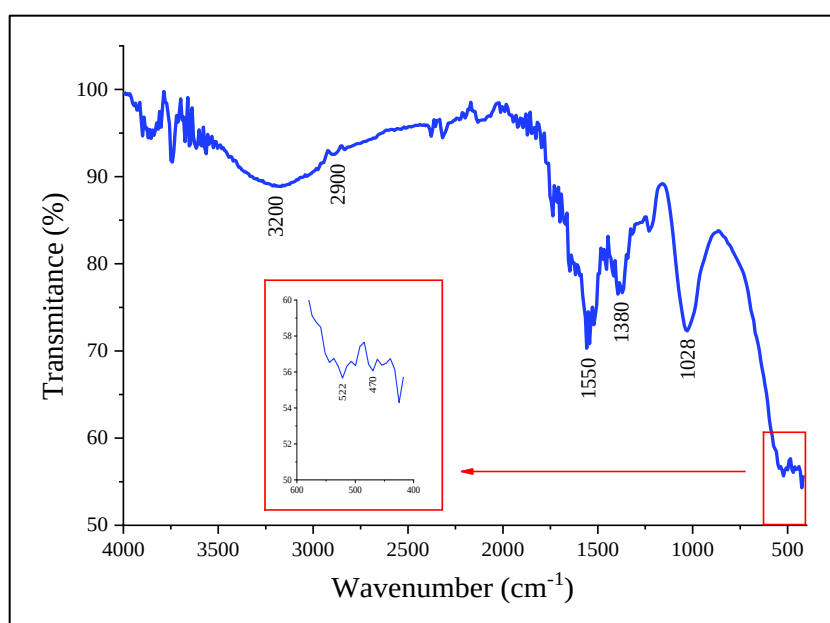
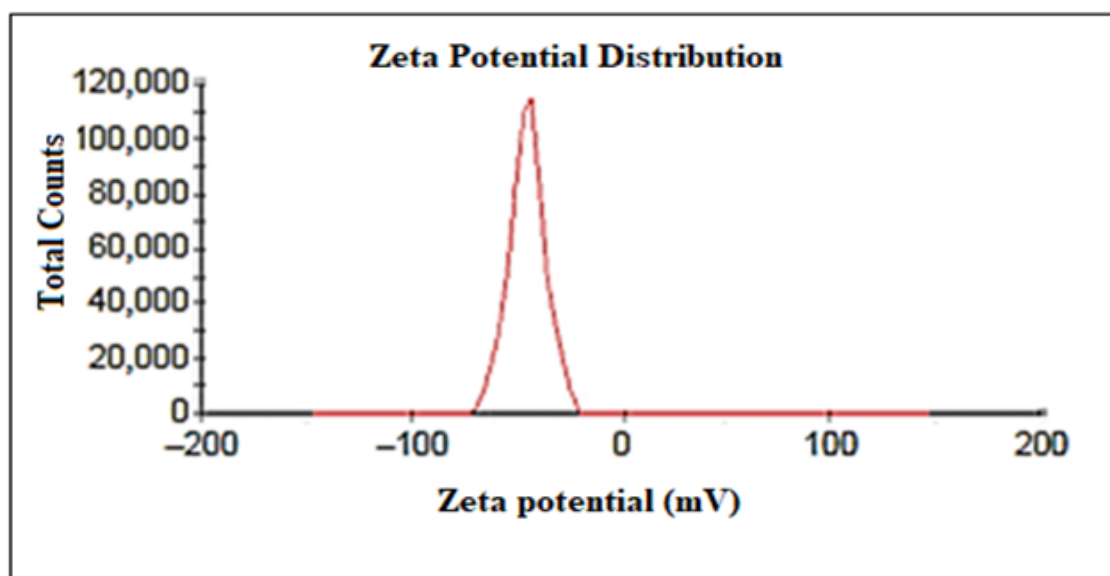


Figure 28: FTIR spectrum of MnO NPs

I.1.2.2.3. Zeta Potential



I.1.2.2.4. X-Ray Diffractometry (XRD)

The XRD analysis, which aligned with the JCPDS card N° 00-001-1127 database, revealed that the manganese oxide nanoparticles had a chemical composition of Mn_3O_4 and exhibited a tetragonal crystallite structure. The particles displayed lattice parameters of $a = b = 6.202 \text{ \AA} \neq c = 8.162 \text{ \AA}$, with a crystallite size (D) of 13.4 nm and 23.09% crystallinity. The diffraction pattern showed peaks at 18.02° , 29.07° , 32.66° , 36.11° , 38.42° , 44.40° , 55.88° , 60.20° , and 64.62° , corresponding to the crystallite planes (101), (112), (103), (211), (004), (220), (303), (224), and (314) h , k , and l , respectively (Figure 30).

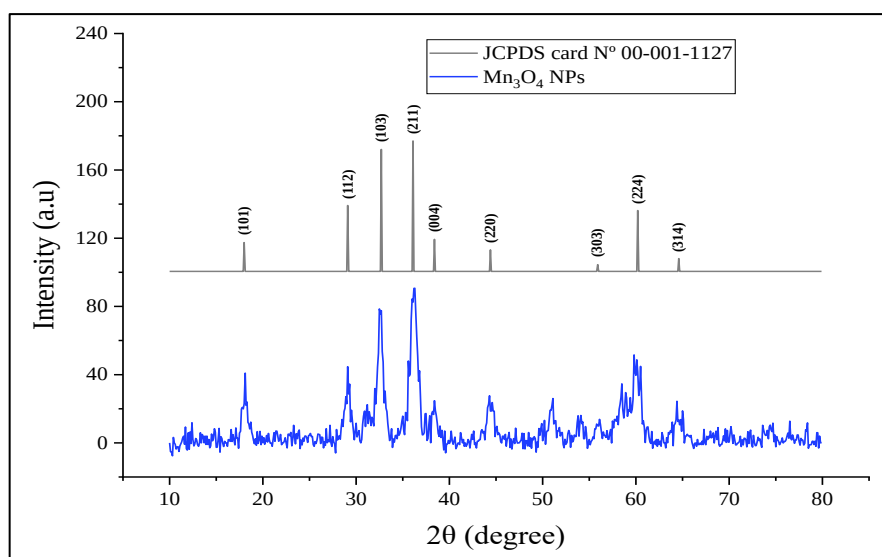
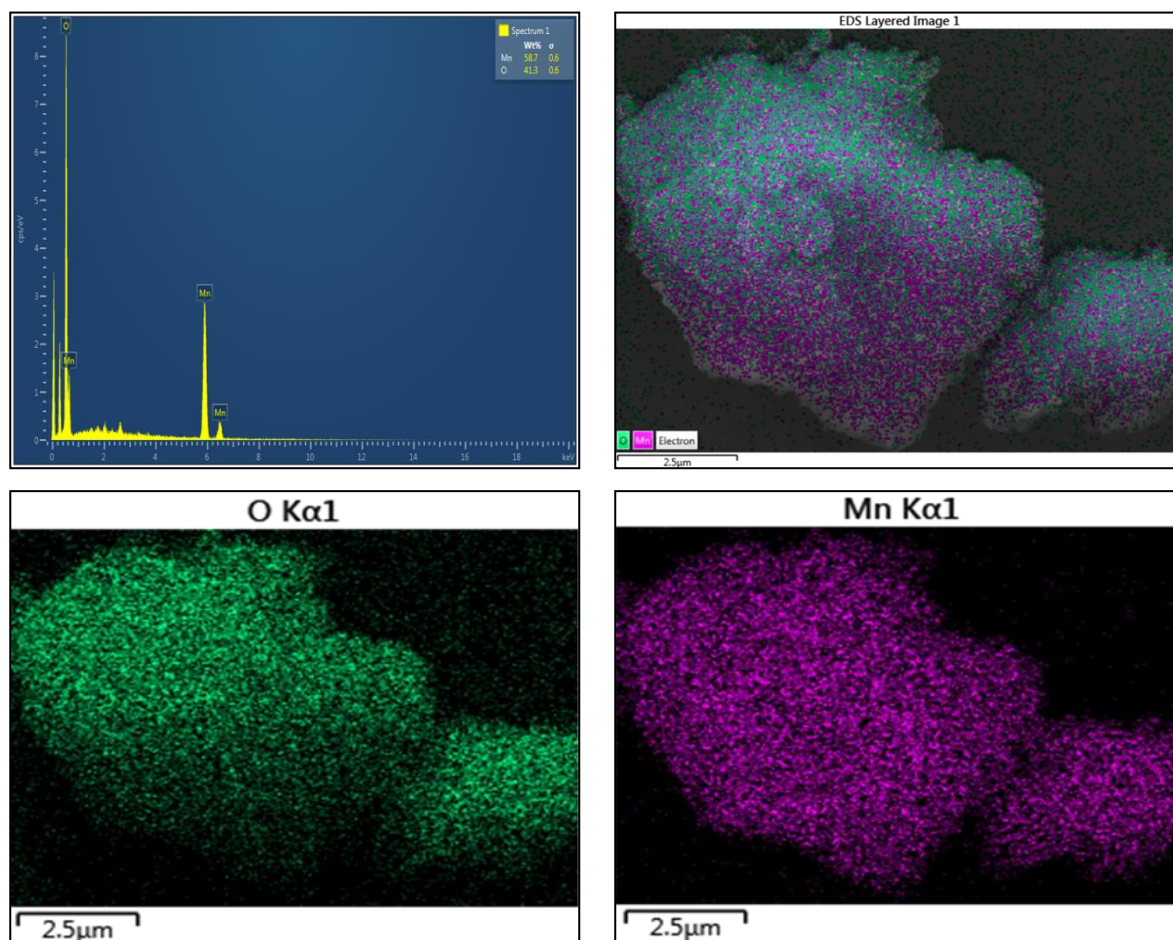


Figure 30: XRD patterns of MnO NPs

I.1.2.2.5. Energy X-Ray Dispersive Spectroscopy (EDS)



I.1.2.2.6. Scanning and Transmission Electron Microscopy (SEM and TEM)

High-resolution imaging of MnO NPs can be achieved using scanning and transmission electron microscopes (SEM and TEM), enabling detailed analysis of their dimensions, form, and composition. As shown in Figure 32 A/B, the SEM and TEM images offer a comprehensive view of the MnO NPs, revealing their consistent distribution and uniform spherical shape. Analysis of these images determined that the MnO NPs have an average diameter of 6.52 ± 0.88 nanometers, confirming their nanoscale dimensions, as depicted in Figure 32 C.

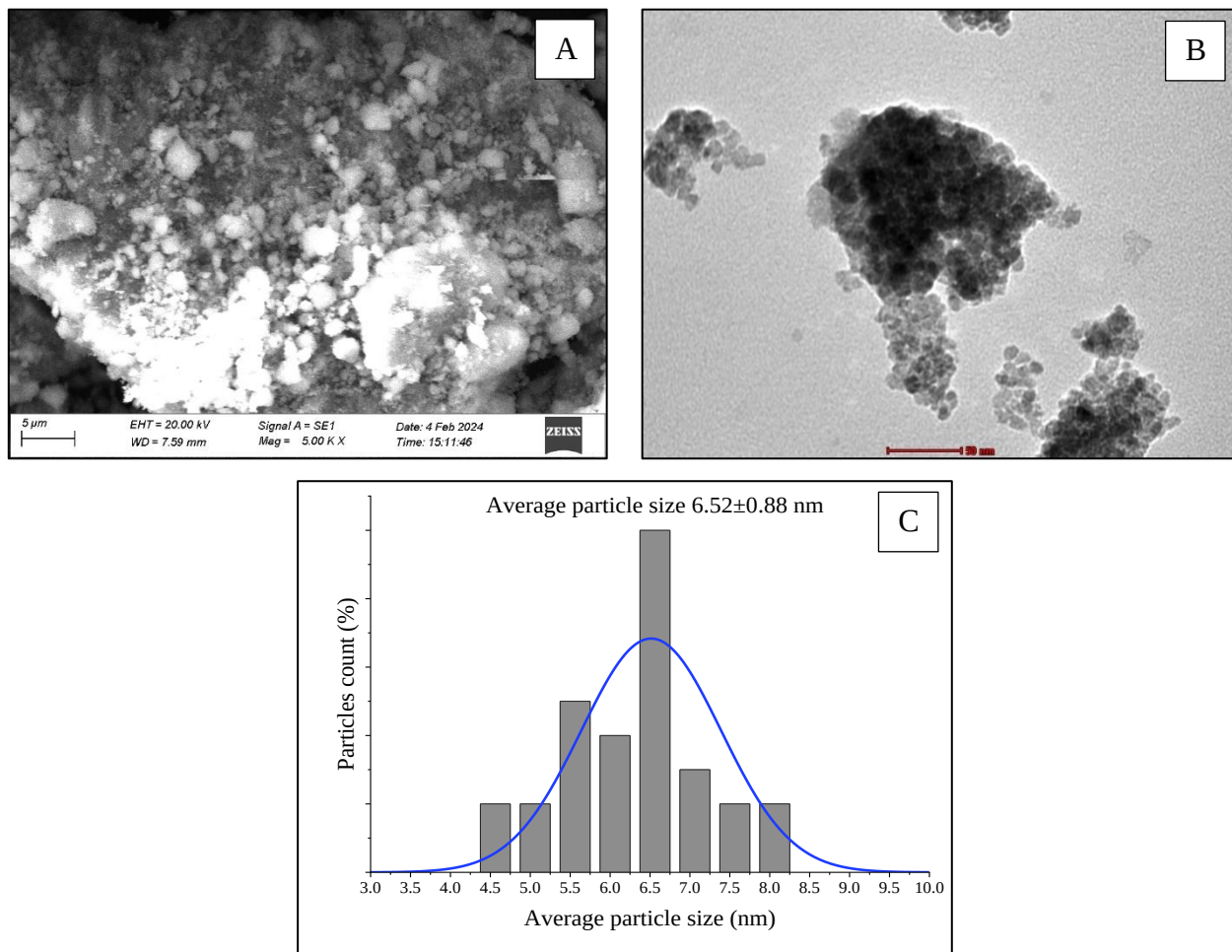
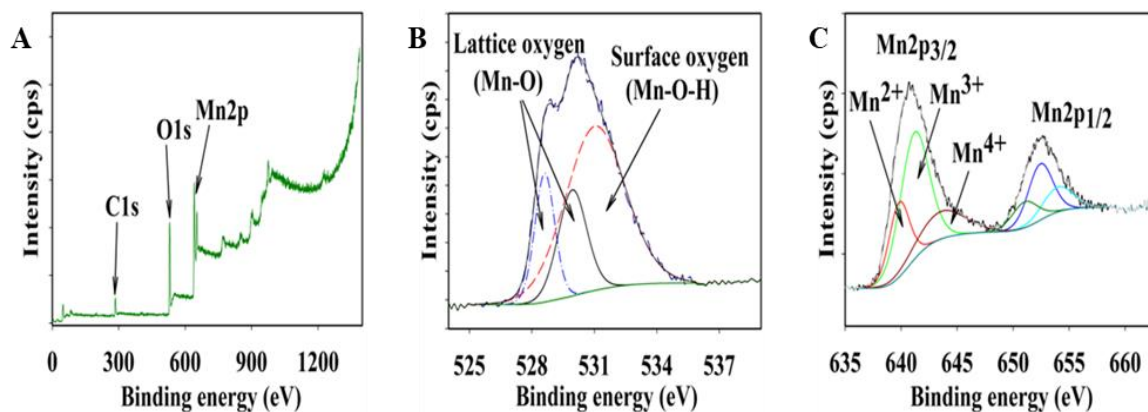


Figure 32: SEM and TEM image of MnO NPs and their average particle size

I.1.2.2.7. X-Ray Photoelectron Spectroscopy (XPS)



I.1.3. Biological Applications of *Ocimum basilicum* L. and MnO NPs

I.1.3.1. Anti-Oxidant Activity

This research assesses *in vitro* antioxidant activity, utilizing both DPPH free radical scavenging capacity measurements and ferric reducing antioxidant power (FRAP) analysis. The results of this investigation clearly demonstrated the potent antioxidant properties of the water-based extract from *Ocimum basilicum* L. and the MnO NPs. These findings were particularly noteworthy when compared to antioxidant standards, including ascorbic acid (AA), butylated hydroxytoluene (BHT), and β -hydroxy acid (BHA). This plant showed the highest FRAP, while its DPPH free radical scavenging capacity of MnO NPs was the highest, as illustrated in Figure 34.

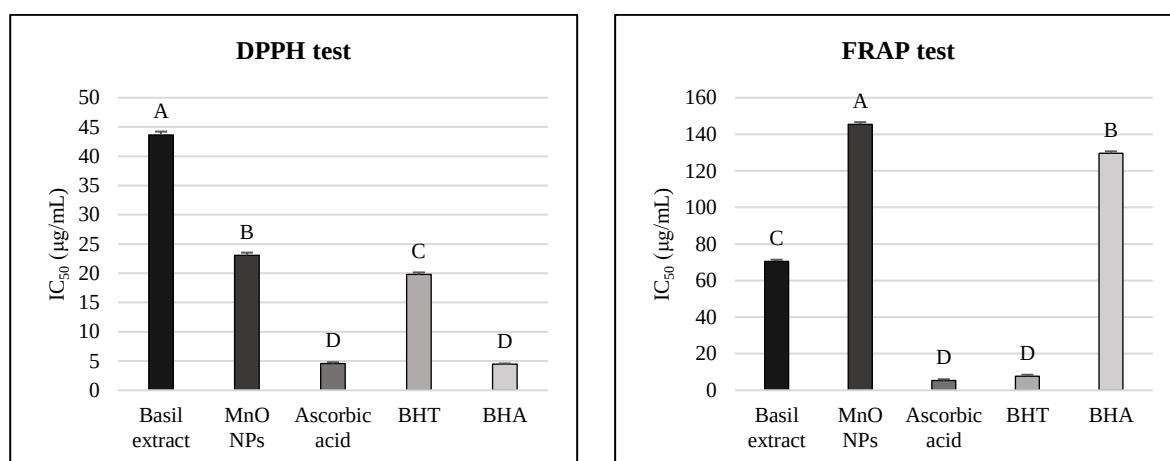


Figure 34: IC₅₀ values for antioxidant activity of basil extract, MnO NPs and different standards

I.1.3.2. Anti-Inflammatory Activity

This work evaluates *in vitro* inflammatory activity, using both protein anti-denaturation release and red blood cell membrane stabilization methods. These comprehensive tests revealed

that basil aqueous extract and MnO NPs have ability to protect against inflammation, especially when contrasted with the widely recognized anti-inflammatory drug, diclofenac® (Figure 35). The findings from these assays provided insights into the relative effectiveness of these substances in combating inflammatory processes.

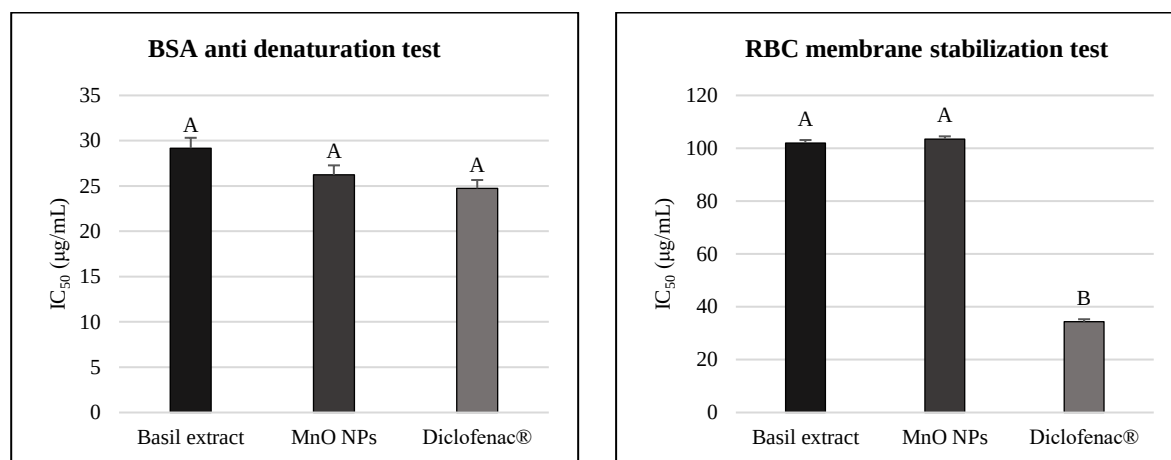


Figure 35: IC₅₀ values for anti-inflammatory activity (protein anti-denaturation and RBC membrane stabilization assays) of basil extract, MnO NPs and diclofenac®

I.1.3.3. Thrombolytic Activity (Clot Lysis)

Figure 34 displayed the thrombolysis activity results, demonstrating how the *Ocimum basilicum* L. extract and MnO NPs protect against clot formation compared to Aspegic®, a widely used synthetic thrombolysis drug. The findings indicate that both the MnO NPs and the plant extract exhibit significantly higher thrombolytic activity than the standard (Aspegic®) at concentrations of 0.05% and 0.1% across various blood volumes by 93.33% and 94.73% respectively (Table 21/ Figure 36).

Table 21: Thrombolysis activity (clot lysis) of basil extract, MnO NPs and Aspegic®

Volume of Blood	Basil		MnO NPs		Aspegic®	
	0.5 mg/mL	1 mg/mL	0.5 mg/mL	1 mg/mL	0.5 mg/mL	1 mg/mL
20 µL	85.00±1.23***	93.33±2.65###	87.50±0.12***	94.73±1.45###	76.190	70.588
40 µL	66.66±1.21***	64.28±0.28#	61.90±2.02***	64.10±0.37#	52.778	60.976
60 µL	63.79±0.18*	68.85±4.92##	67.21±0.96**	65.07±2.29#	61.905	63.492

The (mean ± SE) is used to express values:

* P<0.05, **P<0.01, ***P<0.001: compared to Aspegic (0.5mg/ml).

P<0.05, ## P<0.01, ### P<0.001: compared to Aspegic (1mg/ml).

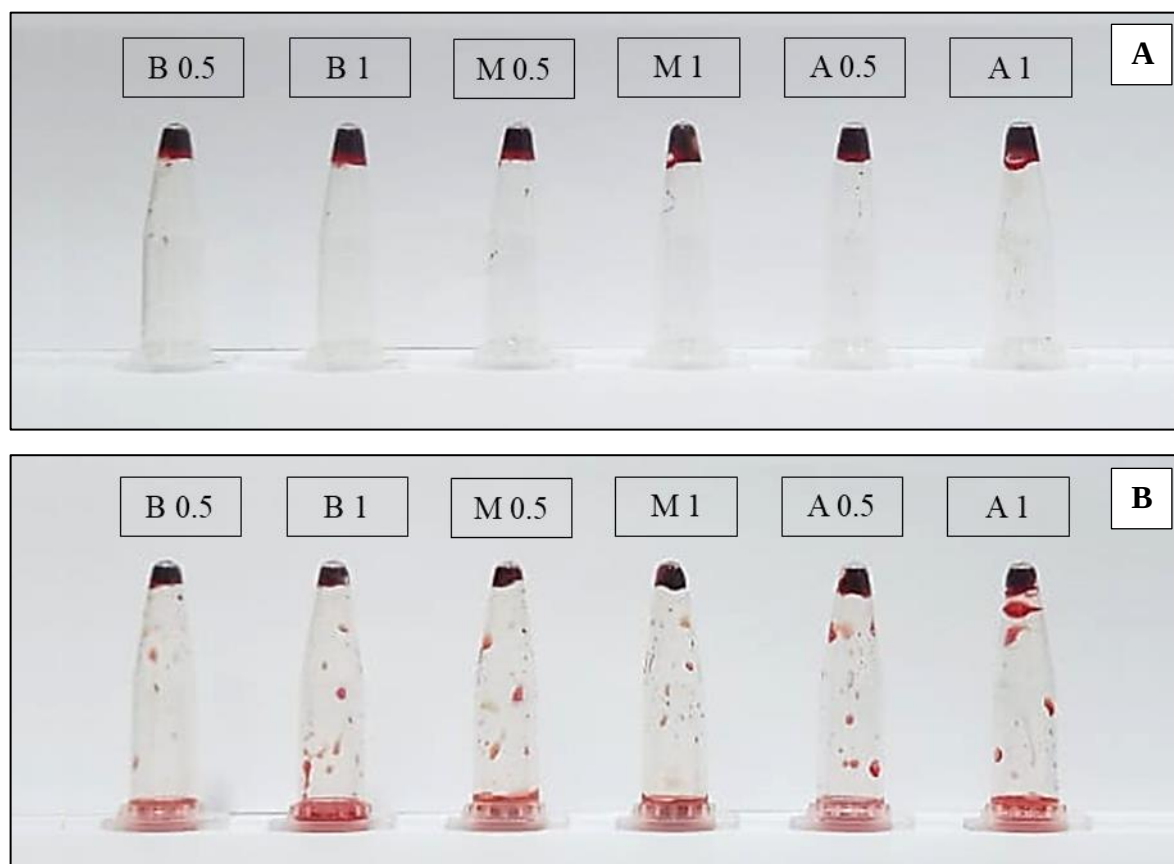
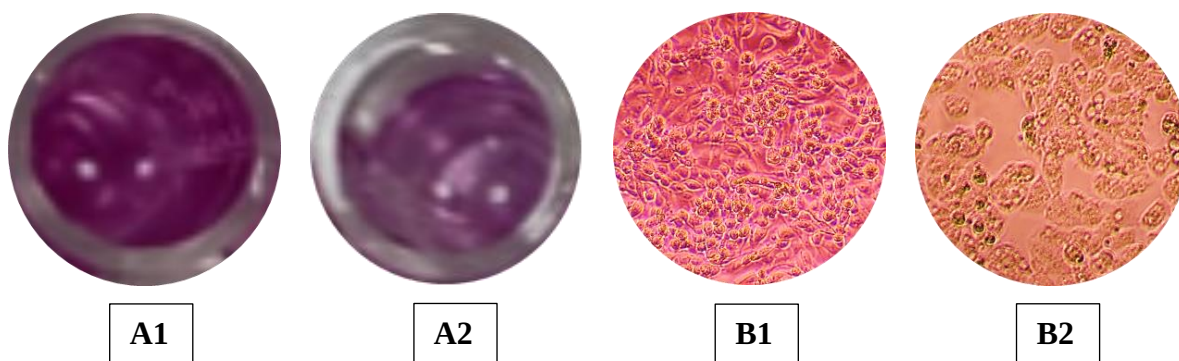
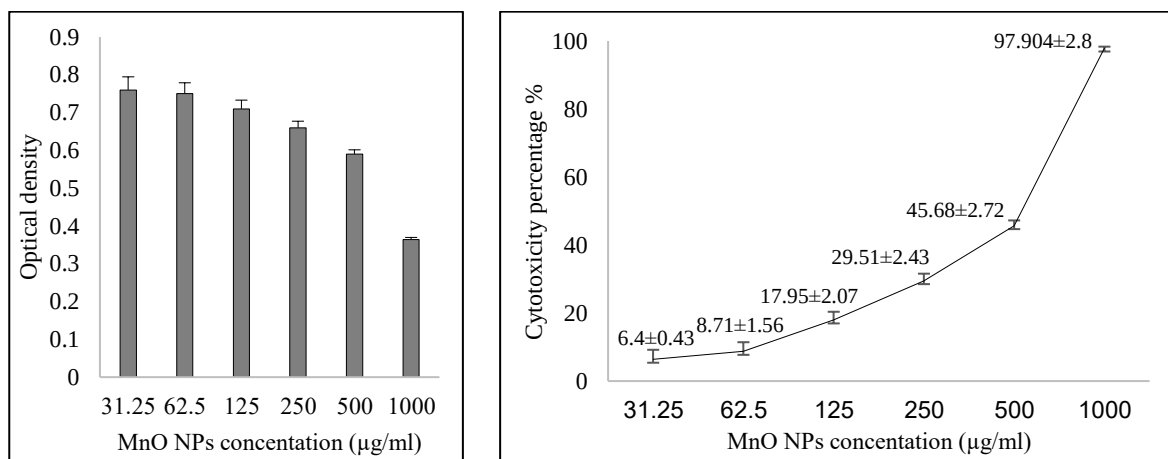


Figure 36: Blood clot lysis Before (A) and after (B) addition of *Ocimum basilicum* L. extract, MnO NPs and Aspegic®

I.1.3.4. Anticancer Activity Against MCF-7 Breast Cancer Cell Line



I.1.3.5. Catalytic Activity (Dye Degradation)

This research investigated the breakdown of MnO NPs using both photocatalytic and sonocatalytic techniques. The degradation of Congo red dye was visually monitored through color changes from deep to light red at specific intervals: 30, 60, 90, 120, 150, 180, 210, and 240 min. Results showed effectiveness rates of 70.18% for photocatalytic activity and 58.90% for sonocatalytic activity. A direct relationship was observed between the dye degradation percentage and the duration of exposure to light and ultrasound vibrations (Figure 39/40).

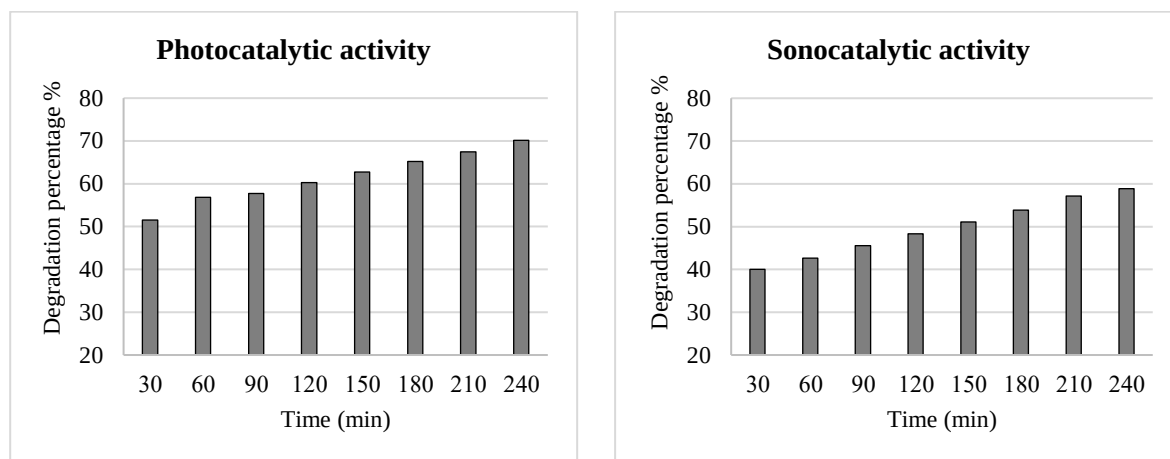


Figure 39: Photocatalytic and sonocatalytic activity of MnO NPs

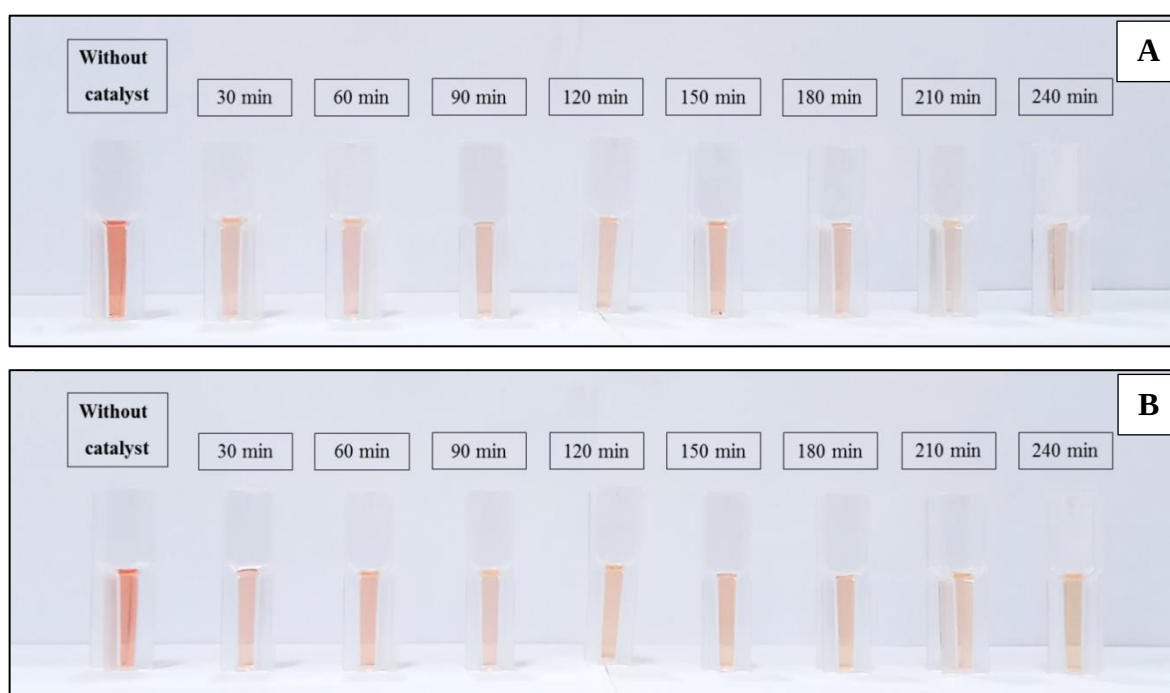


Figure 40: Congo red degradation using photocatalytic (A) and sonocatalytic (B) activities of MnO NPs

I.1.4. *In Vitro* Essays of Liposomes and Artificial Intelligence

I.1.4.1. Characterization of Liposomes Using Optical Microscope

Microscopic images revealed that liposomes containing *Ocimum basilicum* L. extract appeared as tiny, round vesicles (Figure 41).

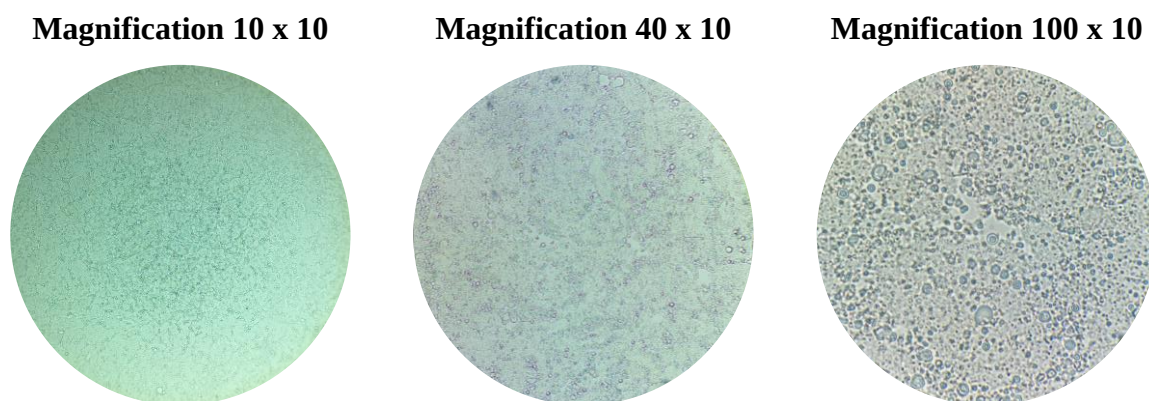
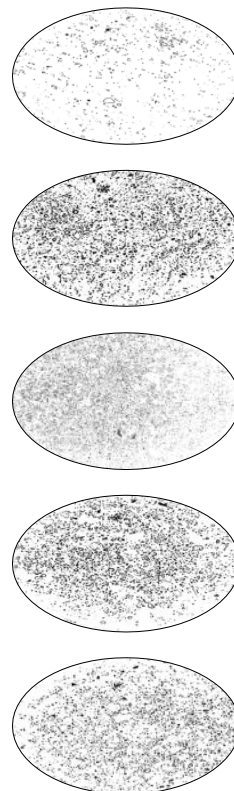
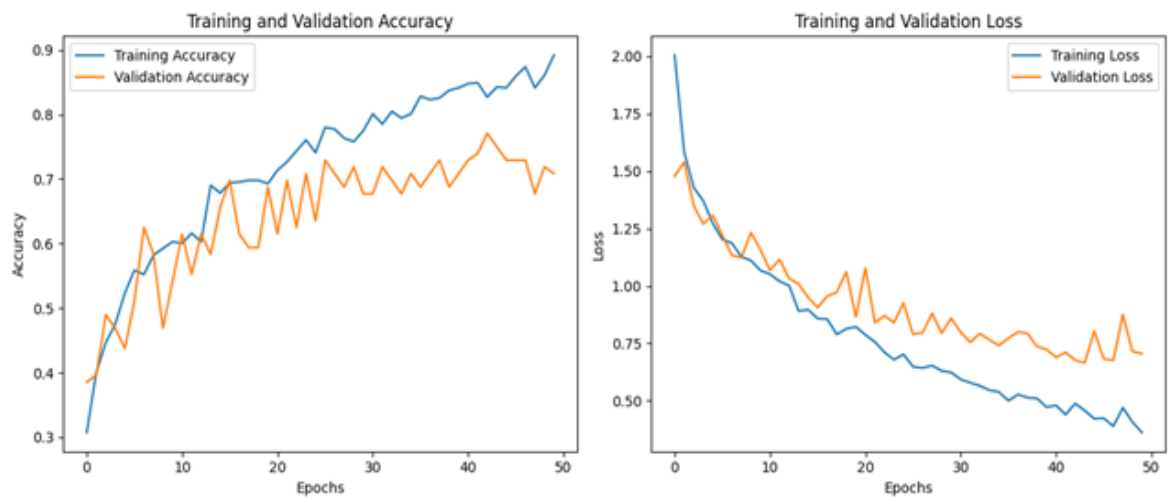


Figure 41: Basil extract-loaded liposomes as a novel drug delivery system (NDDS)

I.1.4.2. Liposomes Classification by MobileNet Models



I.2. In Vivo Study

I.2.1. In Vivo Acute Toxicity Evaluation of Basil Extract

In this study, Wistar albino rats were utilized to evaluate acute toxicity over a 24-hour period. The plant extract was administered to the rats at dosages of 2000 mg and 5000 mg per kilogram of body weight. Throughout the treatment duration, no deaths occurred, and other physiological parameters remained unaltered. These findings suggest that the aqueous extract of *Ocimum basilicum* L. did not exhibit toxic or adverse effects on the rats (Table 23).

Table 23: Effect of basil's extract on mortality and physiological parameters of Wistar rats

Parameter	Dose	Toxicity time				
		0 h	3 h	7 h	14 h	24 h
Mortality	Control	0 %	0 %	0 %	0 %	0 %
	2 g/ kg	0 %	0 %	0 %	0 %	0 %
	5 g/ kg	0 %	0 %	0 %	0 %	0 %
Eyes	Control	(n)	(n)	(n)	(n)	(n)
	2 g/ kg	(n)	(n)	(n)	(n)	(n)
	5 g/ kg	(n)	(n)	(n)	(n)	(n)
Sleep	Control	(n)	(n)	(n)	(n)	(n)
	2 g/ kg	(n)	(n)	(n)	(n)	(n)
	5 g/ kg	(n)	(n)	(n)	(n)	(n)
Diarrhea	Control	(n)	(n)	(n)	(n)	(n)
	2 g/ kg	(n)	(n)	(n)	(n)	(n)
	5 g/ kg	(n)	(n)	(n)	(n)	(n)

(n): normal / 0 %: none

I.2.2. *In Vivo* Acute Toxicity Evaluation of MnO NPs

Table 24: Mortality, physiological parameters and behavior observations after acute toxicity using MnO NPs

[illegible]

[illegible]

(n): normal / 0 %: none

I.2.3. Body Weight Gain

The findings revealed a notable reduction ($p<0.05$) in body weight gain for MTZ-treated rats compared to the control group. In contrast, there was a significant increase body weight gain in BE ($P<0.01$) and BE-LPs ($P<0.001$). However, no substantial difference ($p>0.05$) was observed in BE-MnO NPs-LPs when compared to the MTZ group (Figure 44).

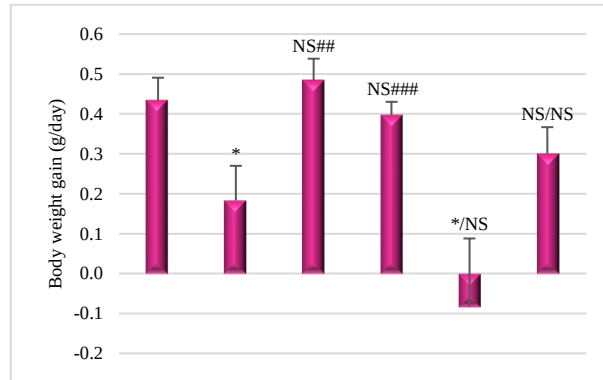


Figure 44: Body weight gain (g/d/rat) in control and experimental rats' groups

I.2.4. Relative Organs Weight

Compared to the control group, the MTZ group exhibited a notable increase in relative organ weights: liver and testes ($p<0.001$), kidneys ($p<0.01$), and lungs and heart ($p<0.05$). In contrast, treatment with BE, BE-LPs, MnO NPs, and BE-MnO NPs-LPs resulted in a significant reduction in all relative organ weights when compared to the MTZ group (Table 25).

Table 25: Relative organs weight in control and experimental groups

Paramaters						
Liver	2.227±0.006	2.681±0.02	2.506±0.040	2.537±0.049	3.163±0.075	2.788±0.025
		***	***/###	***/#	***/###	***/##
Lungs	0.665±0.005	2.138±0.503	0.741±0.028	0.717±0.025	0.990±0.110	0.778±0.002
		*	*/###	NS/###	*/###	***/###
Heart	0.254±0.003	0.324±0.023	0.254±0.007	0.266±0.004	0.298±0.004	0.306±0.001
		*	NS/###	*/###	***/##	***/###
Kidneys	0.478±0.010	0.541±0.013	0.487±0.006	0.517±0.017	0.638±0.011	0.562±0.007
		**	NS/###	NS/NS	***/###	***/#
Testes	0.906±0.031	1.187±0.021	1.138±0.014	1.085±0.043	1.033±0.059	0.755±0.150
		***	***/#	**/NS	NS/#	NS/#

Data are expressed as mean ± SD (n=6).

* $p<0.05$, ** $p<0.01$, *** $p<0.001$: significantly different from control group.

$p<0.05$, ## $p<0.01$, ### $p<0.001$: significantly different from metribuzin exposed group.

I.2.5. Step-through Passive Avoidance Test (passive avoidance learning)

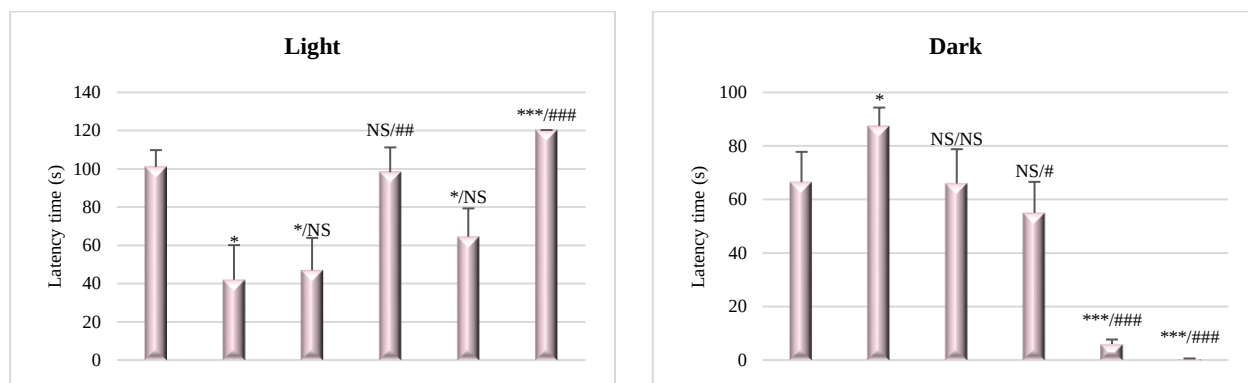


Figure 45: Passive avoidance learning across of control and experimental groups

Data are expressed as mean $\pm$ SD (n=6).

* p<0.05, **p<0.01, ***p<0.001: significantly different from control group.

p<0.05, ##p<0.01, ###p<0.001: significantly different from metribuzin exposed group.

I.2.6. Acetylcholinesterase (AChE) Activity

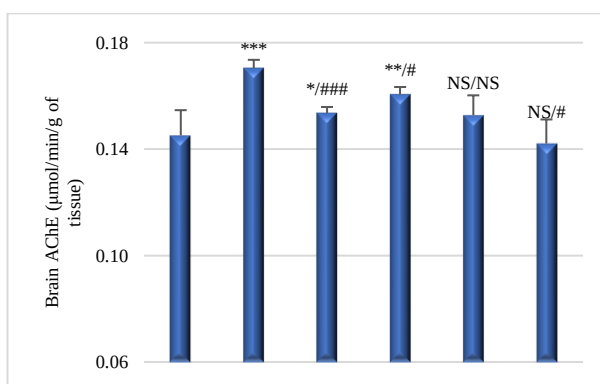


Figure 46: AChE activity in brain in control and experimental rats' groups

Data are expressed as mean $\pm$ SD (n=6).

* p<0.05, **p<0.01, ***p<0.001: significantly different from control group.

p<0.05, ##p<0.01, ###p<0.001: significantly different from metribuzin exposed group.

I.2.7. Hematological Markers

Analysis revealed that inflammation markers (WBC, Lymph, Mid, and Gran) were significantly elevated in the MTZ group compared to the control group ($P < 0.05$). In contrast, the BE, BE-LPs, and BE-MnO NPs-LPs groups exhibited a significant reduction ($P < 0.001$) in WBC, Mid, and Gran levels when compared to the MTZ group. The MnO NPs group showed a notable decrease in WBC ($P < 0.01$), Mid ($P < 0.001$), and Lymph ($P < 0.05$) levels, while Gran levels remained unchanged ($P > 0.05$) relative to the MTZ group. Furthermore, Lymph levels decreased significantly ($P < 0.01$) in the BE and BE-MnO NPs-LPs groups, with a very highly significant decrease ($P < 0.001$) observed in the BE-LPs group when compared to the MTZ group. Concerning erythrocyte line markers, our results showed a high significant increase ($P < 0.01$) in RBC and PLT, and a very high significant decrease ($P < 0.001$) in HGB, HCT, MCV and MCH in MTZ group as compared to control group simultaneously there was a significant amelioration in the levels of previous parameters in BE, BE-LPs, MnO NPs and BE-MnO NPs-LPs compared to MTZ group (Table 26).

Table 26: Leukocyte and erythrocyte line markers level in control and experimental groups

Parameter						
WBC (10⁹/L)	6.668±0.541	12.1±1.72 *	7.3±0.362 NS/###	7.667±0.379 */###	9.85±0.573 ***/##	6.068±0.62 NS/###
Lymph (10⁹/L)	3.667±0.306	5.65±0.666 *	4.1±0.286 NS/##	4.4±0.146 **/###	4.5±0.447 NS/#	3.5±0.407 NS/##
Mid (10⁹/L)	0.8±0.0447	1.575±0.286 *	0.775±0.075 NS/###	0.967±0.076 NS/###	1.1±0.0447 ***/###	0.7±0.0447 NS/###
Gran (10⁹/L)	1.933±0.223	4.368±0.822 *	2.425±0.111 **/###	1.833±0.067 NS/###	4.25±0.213 ***/NS	1.633±0.223 NS/###
RBC (10¹²/L)	7.787±0.184	8.31±0.128 **	8.05±0.0934 */#	8.052±0.105 NS/NS	8.655±0.127 ***/#	7.72±0.209 NS/#
HGB (g/L)	155.33±0.92	136.75±1.54 ***	146.25±0.83 ***/###	141.75±0.79 ***/###	138±1.67 ***/NS	130.67±1.28 ***/##
HCT (%)	42.2±0.567	37.65±0.197 ***	39.08±0.386 ***/#	38.26±0.429 ***/NS	37.867±0.52 ***/NS	35.87±0.496 ***/#
MCV (fL)	51.033±0.39	46.3±0.183 ***	49.075±0.47 **/##	47.825±0.22 ***/###	43.133±0.33 ***/###	47.95±0.604 **/#
MCH (pg)	19.067±0.20	16.625±0.164 ***	18.25±0.133 **/###	17.38±0.221 ***/#	15.7±0.256 ***/#	17.6±0.179 ***/##
MCHC (g/L)	370.25±2.18	362.67±2.97 NS	369.6±2.93 NS/NS	365.4±3.86 NS/NS	363.67±3.51 NS/NS	364.33±2.08 */NS

PLT (10⁹/L)	760.3±38.6	858.8±28.7 **	808±6.55 ***/###	944±9.36 ***/###	1040±173 NS/NS	606±51.2 */###
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Data are expressed as mean ± SD (n=6).

* p<0.05, **p<0.01, ***p<0.001: significantly different from control group.

p<0.05, ##p<0.01, ###p<0.001: significantly different from metribuzin exposed group.

I.2.8. Biochemical Parameters

I.2.8.1. Blood Glucose

The study findings demonstrated a statistically significant elevation in blood glucose (p<0.05) in the MTZ group compared to the control group. Conversely, the treatment with BE-LPs exhibited a very significant decrease (p<0.01) when compared to the MTZ group (Figure 47).

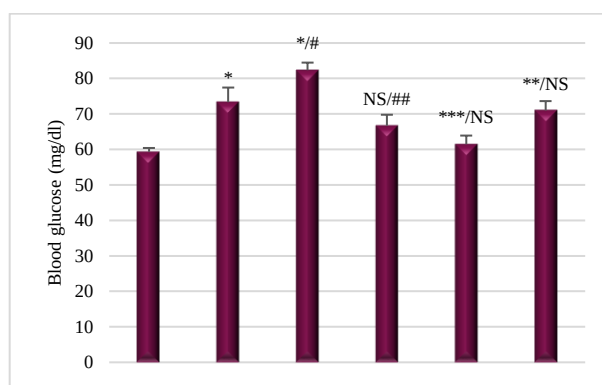


Figure 47: Blood glucose levels in control and experimental rats' groups

Data are expressed as mean ± SD (n=6).

* p<0.05, **p<0.01, ***p<0.001: significantly different from control group.

p<0.05, ##p<0.01, ###p<0.001: significantly different from metribuzin exposed group.

I.2.8.2. Albumin

The research results revealed a statistically significant reduction in albumin concentrations (p<0.05) among subjects in the MTZ group when compared to those in the control group. In contrast, the groups receiving treatment showed notable enhancements relative to the MTZ group (Figure 48).

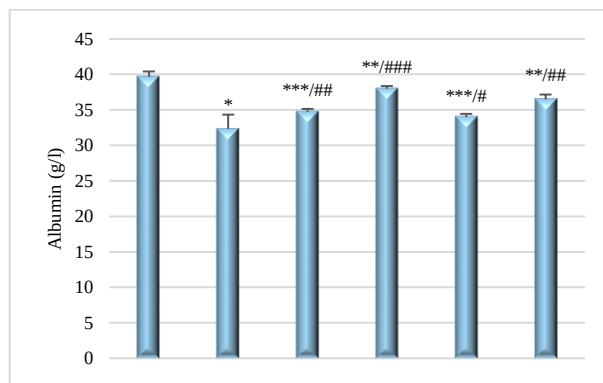


Figure 48: Albumin levels in control and experimental rats' groups

Data are expressed as mean $\pm$ SD (n=6).

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$: significantly different from control group.

$p < 0.05$, ## $p < 0.01$, ### $p < 0.001$: significantly different from metribuzin exposed group.

I.2.8.3. Renal Markers

The study findings demonstrated a statistically significant elevation in creatinine levels ($p < 0.01$), along with increases in urea, uric acid, and phosphorus concentrations ($p < 0.001$) in participants from the MTZ group compared to those in the control group. Conversely, the groups undergoing treatment exhibited substantial improvements when compared to the MTZ group (Figure 49).

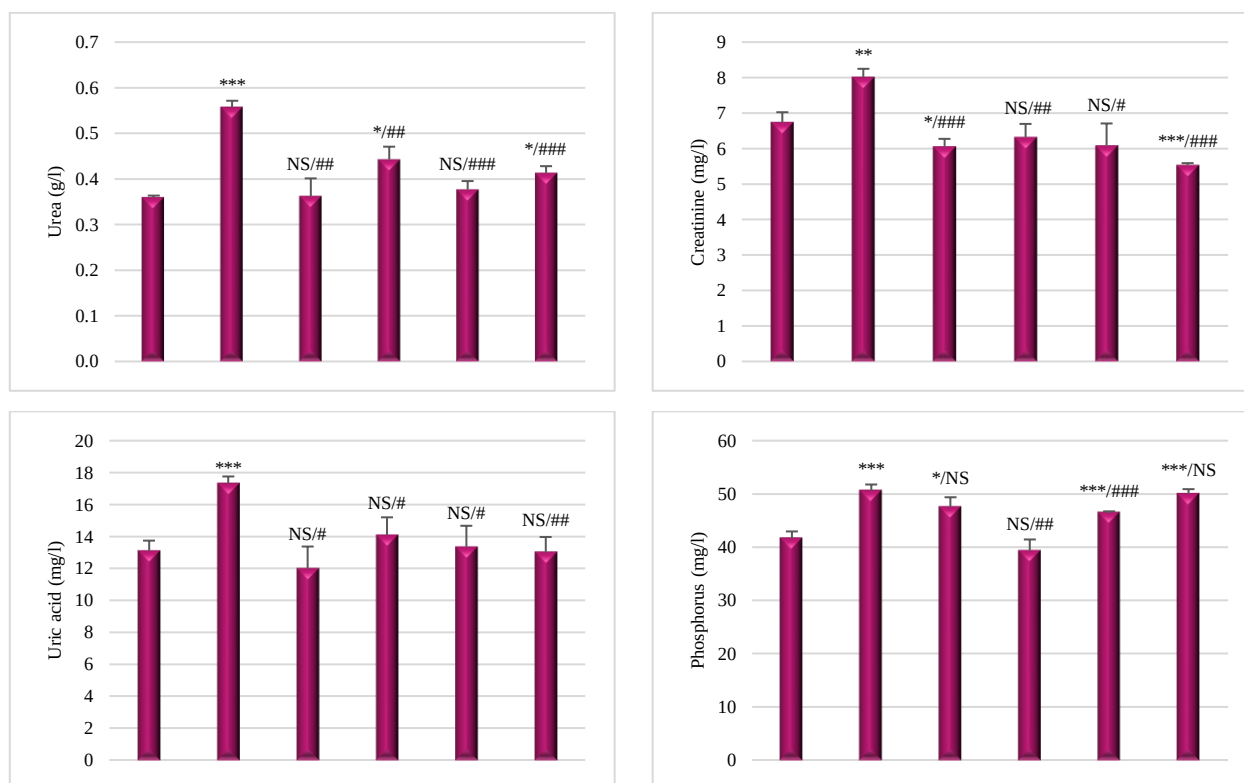


Figure 49: Renal markers levels in control and experimental rats' groups

Data are expressed as mean $\pm$ SD (n=6).

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$: significantly different from control group.

$p < 0.05$, ## $p < 0.01$, ### $p < 0.001$: significantly different from metribuzin exposed group.

I.2.8.4. Lipid Profile

Analysis of the lipid profile showed a significant elevation in cholesterol ($p<0.01$), triglyceride, VLDL, LDL and atherogenic index ($p<0.001$) levels with a significant diminution in HDL ($p<0.001$) levels within the MTZ group compared to the control. Conversely, the groups that were subjected to the various treatment protocols demonstrated a noteworthy yet partial enhancement in their lipid profile levels, particularly when evaluated and contrasted within the MTZ group (Figure 50).

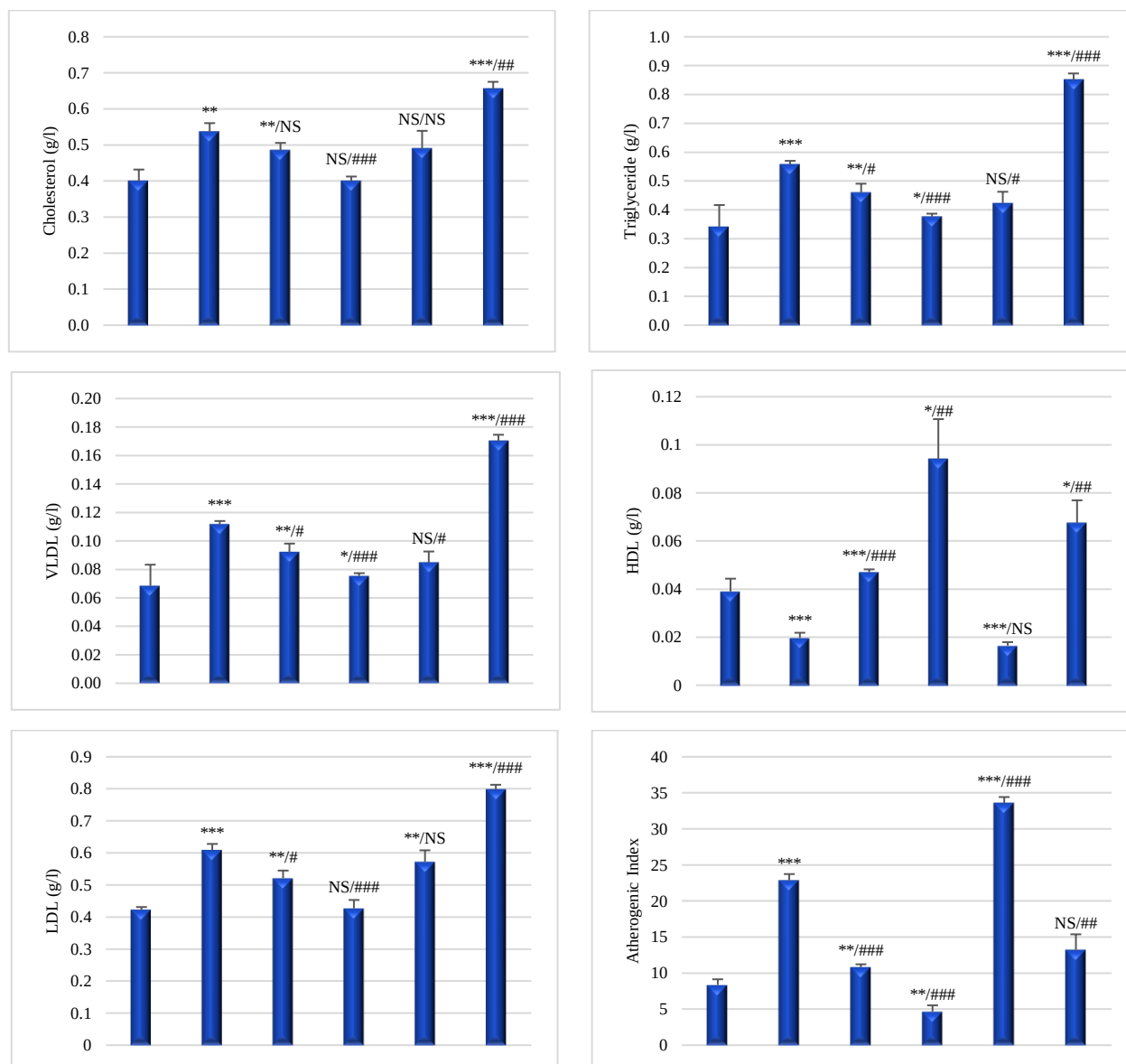


Figure 50: Lipid profile levels in control and experimental rats' groups

Data are expressed as mean $\pm$ SD (n=6).

* $p<0.05$, ** $p<0.01$, *** $p<0.001$: significantly different from control group.

$p<0.05$, ## $p<0.01$, ### $p<0.001$: significantly different from metribuzin exposed group.

I.2.8.5. Enzymatic Activity

The study findings demonstrated a statistically significant elevation in enzyme activity levels. Specifically, GOT and GGT showed an increase ($P<0.05$), GPT exhibited a rise ($P<0.01$), and ALP, CPK, and LDH displayed heightened activity ($P<0.001$) in the MTZ group compared to the control. Additionally, a significant reduction was observed in the treatment groups when compared to the MTZ group (Figure 51).

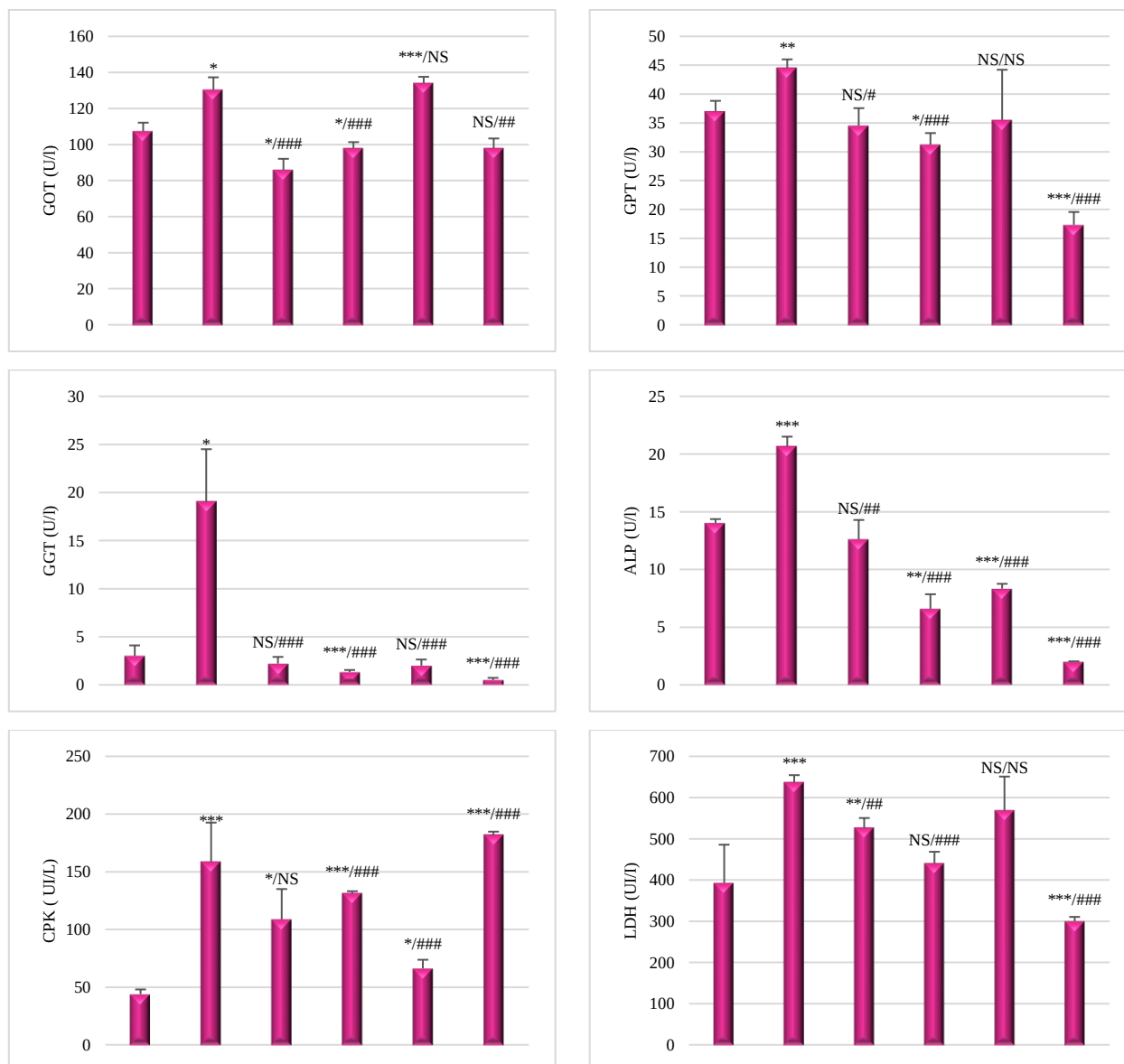


Figure 51: Enzymatic activity in control and experimental rats' groups

Data are expressed as mean $\pm$ SD (n=6).

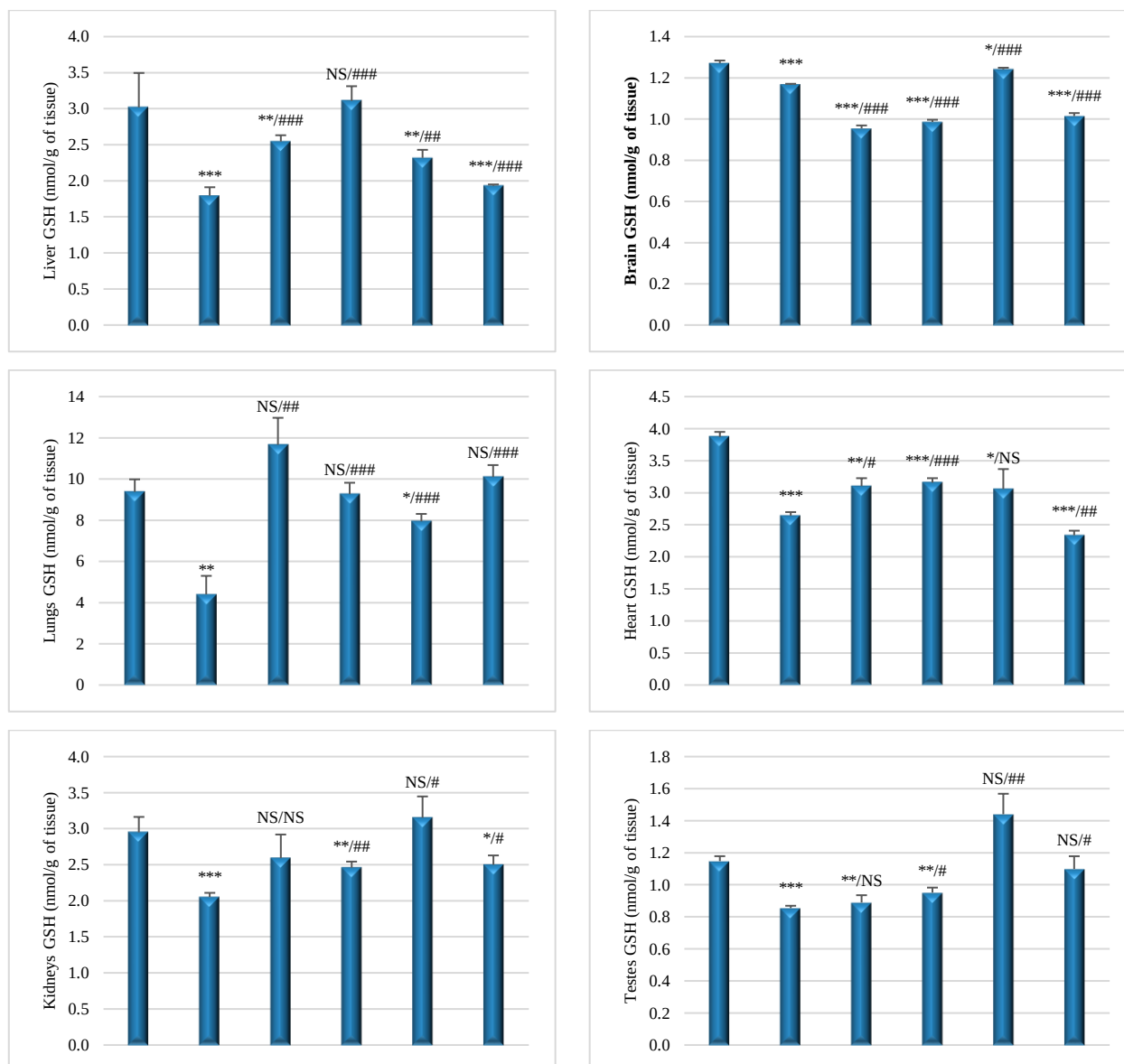
* $p<0.05$, ** $p<0.01$, *** $p<0.001$: significantly different from control group.

$p<0.05$, ## $p<0.01$, ### $p<0.001$: significantly different from metribuzin exposed group.

I.2.9. Oxidative Stress Parameters

I.2.9.1. Reduced Glutathione (GSH) Levels

The analysis of GSH levels revealed a very highly significant reduction ($P < 0.001$) across various organs, including the liver, brain, lungs, heart, kidneys, testes, and muscle in the MTZ group when compared to the control. Conversely, a notable enhancement in GSH levels was observed in the BE, BE-LPs, MnO NPs, and BE-MnO NPs-LPs groups relative to the MTZ group (Figure 52).



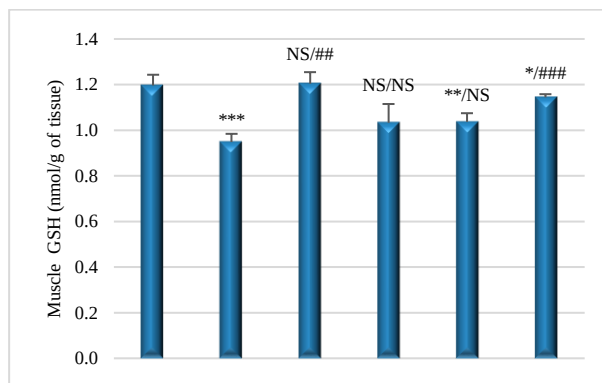


Figure 52: GSH levels in control and experimental rats' groups in various organs

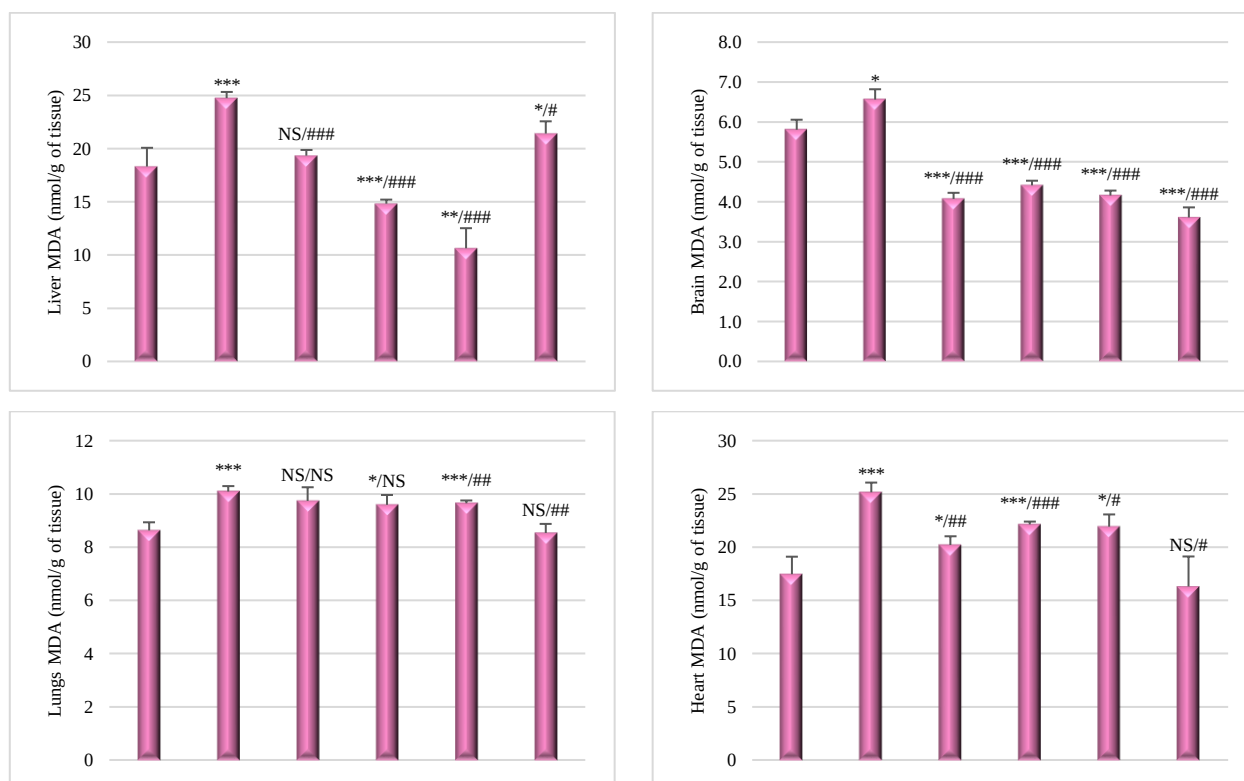
Data are expressed as mean $\pm$ SD (n=6).

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$: significantly different from control group.

$p < 0.05$, ## $p < 0.01$, ### $p < 0.001$: significantly different from metribuzin exposed group.

1.2.9.2. Malondialdehyde (MDA) Levels

Examination of MDA concentrations showed a very highly significant elevation ($P < 0.001$) in the liver, lungs, and heart tissues, along with a highly significant increase ($P < 0.01$) in brain, kidneys, testes, and muscle samples of the MTZ group compared to the control. In contrast, the BE, BE-LPs, MnO NPs, and BE-MnO NPs-LPs groups exhibited a substantial reduction in MDA levels when compared to the MTZ group (Figure 53).



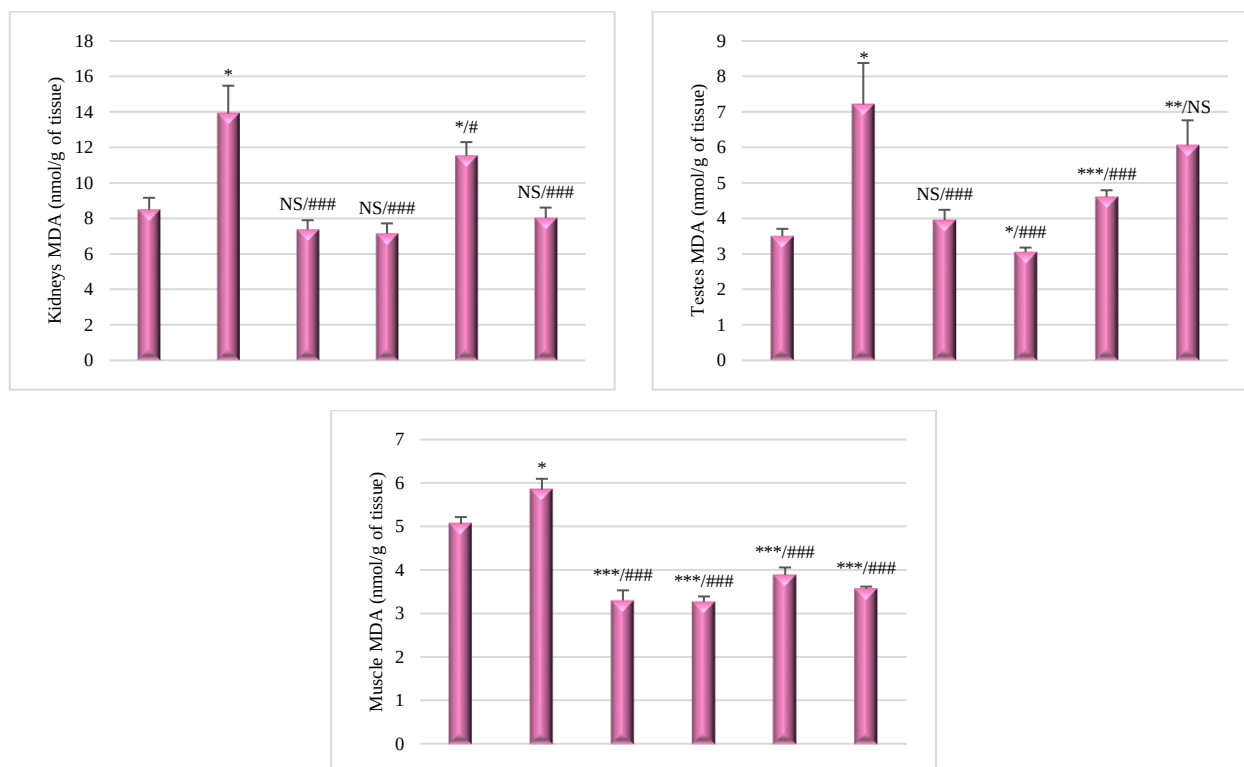


Figure 53: MDA levels in control and experimental rats' groups in various organs

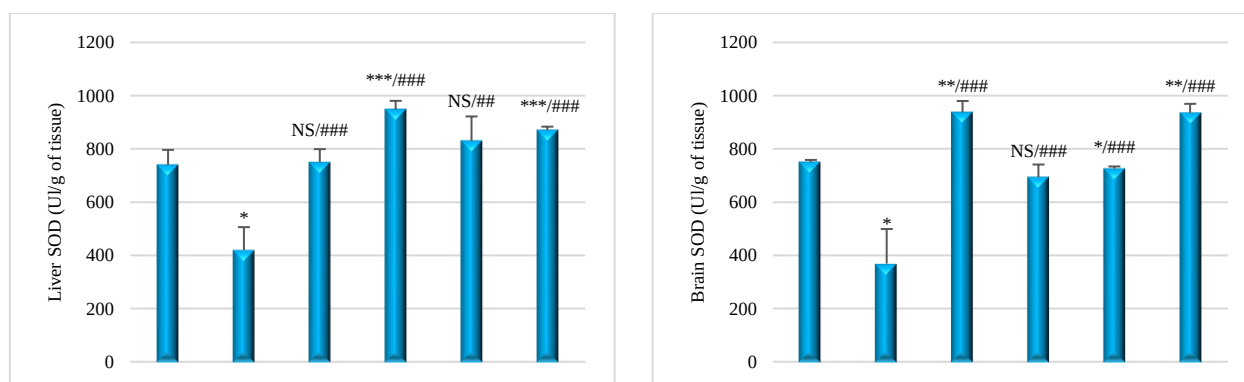
Data are expressed as mean $\pm$ SD (n=6).

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$: significantly different from control group.

$p < 0.05$, ## $p < 0.01$, ### $p < 0.001$: significantly different from metribuzin exposed group.

I.2.9.3. Superoxide Dismutase (SOD) Activity

Analysis of SOD activity revealed a notable reduction in various tissue samples, including the liver, brain, lungs, heart, kidneys, testes, and muscles, of the MTZ group when compared to the control. Conversely, the BE, BE-LPs, MnO NPs, and BE-MnO NPs-LPs groups demonstrated a marked increase in SOD activity relative to the MTZ group (Figure 54).



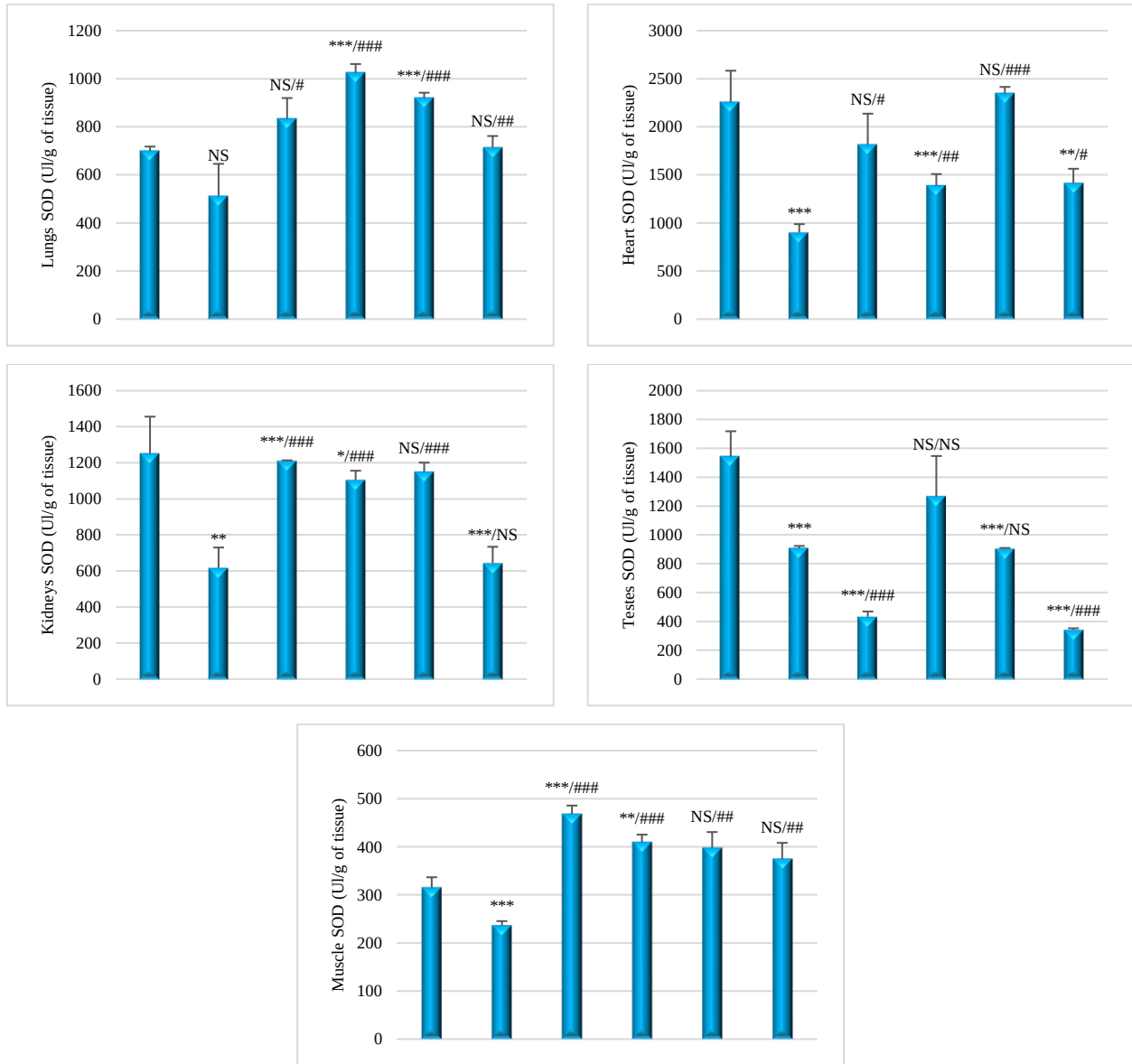


Figure 54: SOD activity in control and experimental rats' groups in various organs

Data are expressed as mean $\pm$ SD (n=6).

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$: significantly different from control group.

$p < 0.05$, ## $p < 0.01$, ### $p < 0.001$: significantly different from metribuzin exposed group.

I.2.9.4. Glutathione Peroxydase (GPx) Activity

Examination of GPx activity showed a significant decrease in multiple organs, including the liver, brain, lungs, heart, kidneys, testes, and muscles, in the MTZ group compared to the control. In contrast, the groups treated with BE, BE-LPs, MnO NPs, and BE-MnO NPs-LPs exhibited a substantial increase in GPx activity when compared to the MTZ group (Figure 55).

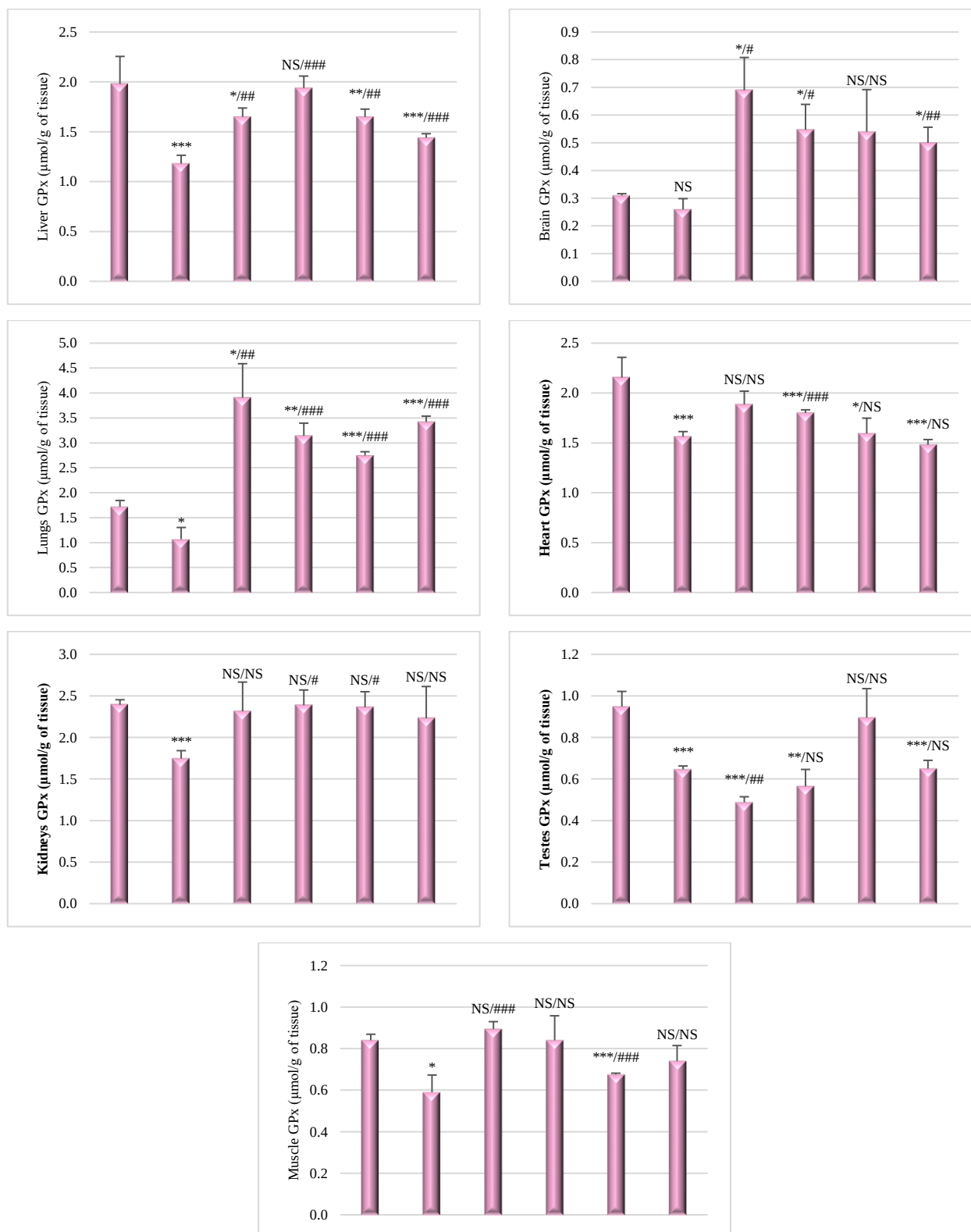


Figure 55: GPx activity in control and experimental rats' groups in various organs

Data are expressed as mean $\pm$ SD (n=6).

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$: significantly different from control group.

$p < 0.05$, ## $p < 0.01$, ### $p < 0.001$: significantly different from metribuzin exposed group.

I.2.9.5. Glutathione-S-Transferase (GSTs) Activity

Analysis of GSTs activity revealed a notable increase across various organs in the MTZ group compared to the control. Specifically, significant elevations were observed in the liver, brain, and muscles ($p < 0.001$), lungs ($p < 0.01$), as well as the heart, kidneys, and testes ($p < 0.05$). Conversely, groups administered BE, BE-LPs, MnO NPs, and BE-MnO NPs-LPs demonstrated a marked reduction in GSTs activity when juxtaposed with the MTZ group (Figure 56).

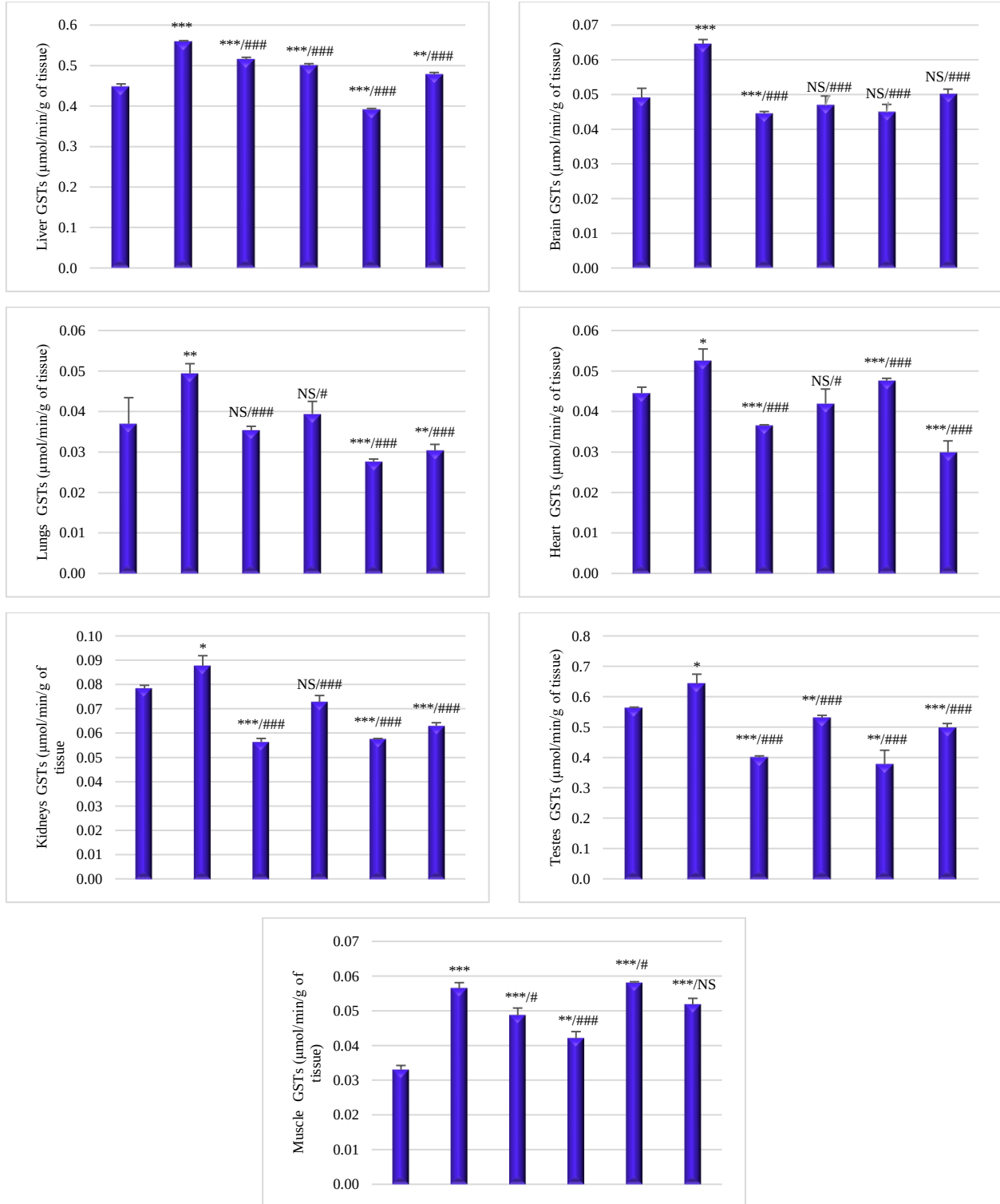


Figure 56: GSTs activity in control and experimental rats' groups in various organs

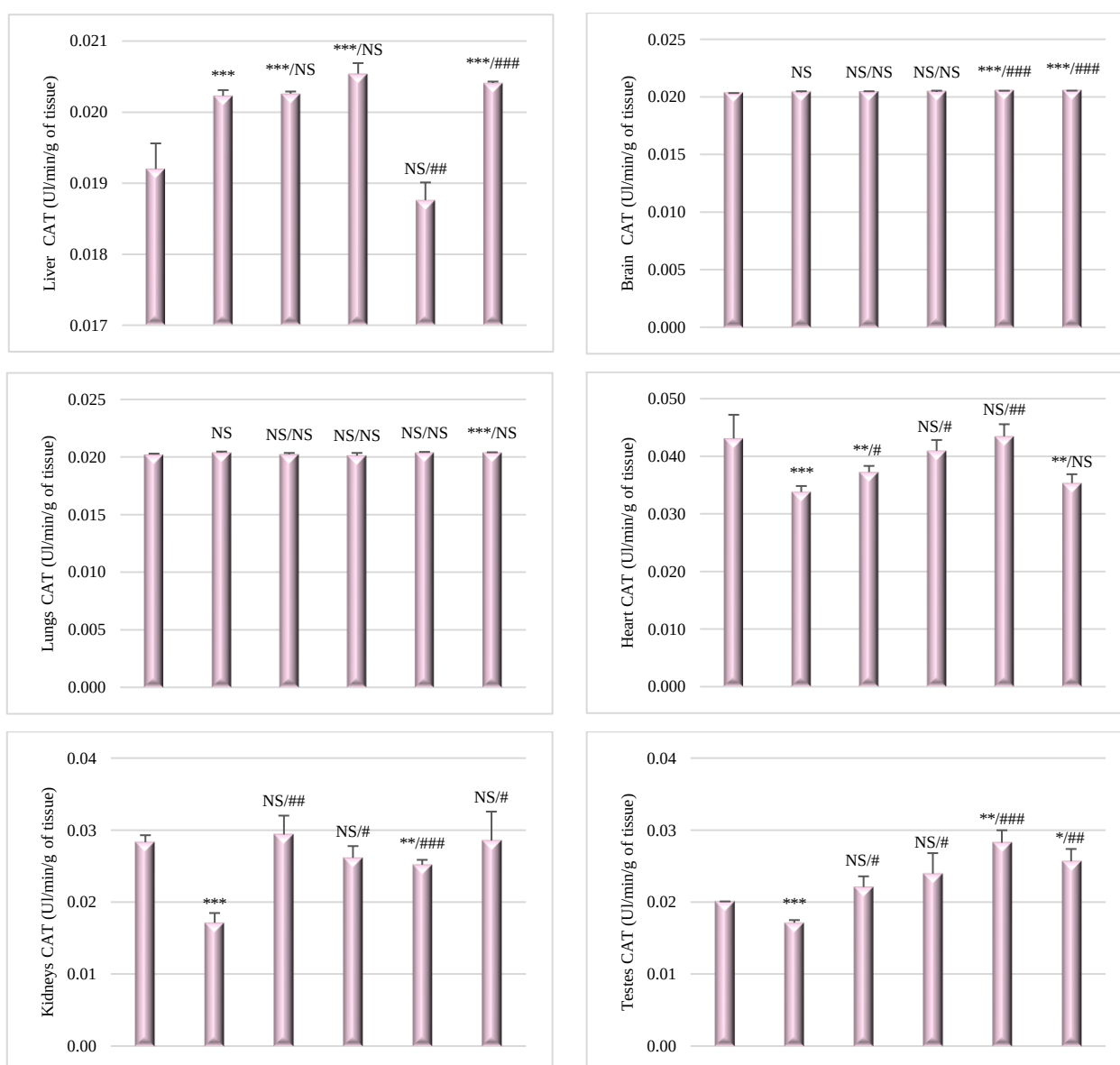
Data are expressed as mean $\pm$ SD (n=6).

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$: significantly different from control group.

$p < 0.05$, ## $p < 0.01$, ### $p < 0.001$: significantly different from metribuzin exposed group.

I.2.9.6. Catalase (CAT) Activity

Examination of catalase (CAT) activity showed a significant change across different organs in the MTZ group when compared to the control. A substantial decrease in CAT activity was noted in the heart, kidneys, testes, and muscles, while a considerable increase was observed in the liver CAT activity of the MTZ group relative to the control. In contrast, groups treated with BE, BE-LPs, MnO NPs, and BE-MnO NPs-LPs exhibited a notable partial recovery in CAT activity when compared to the MTZ group (Figure 57).



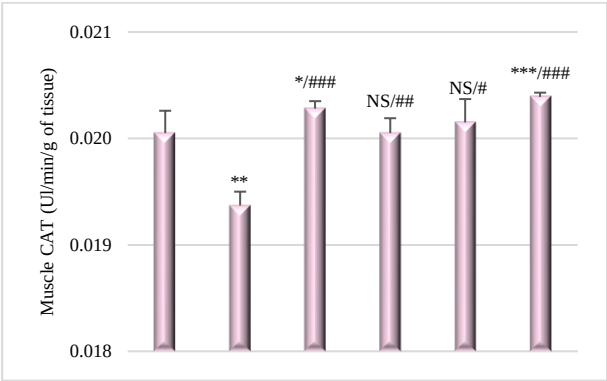


Figure 57: CAT activity in control and experimental rats' groups in various organs

Data are expressed as mean ± SD (n=6).

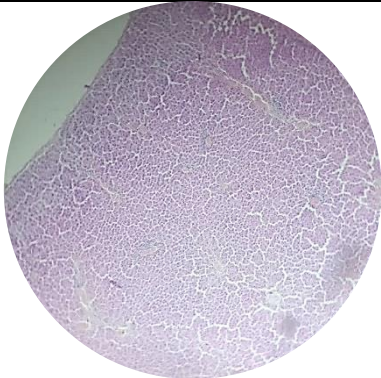
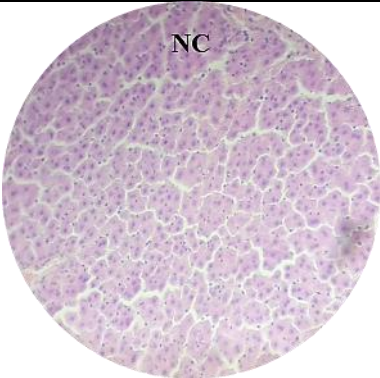
* p<0.05, **p<0.01, ***p<0.001: significantly different from control group.

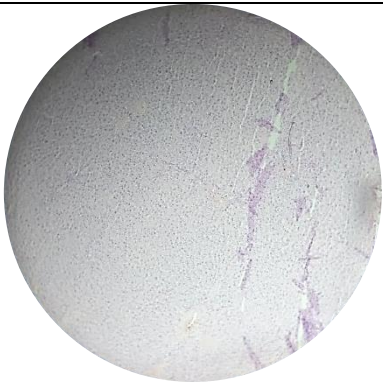
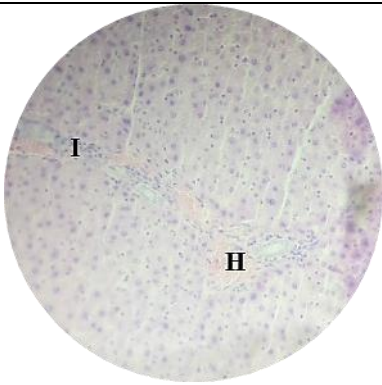
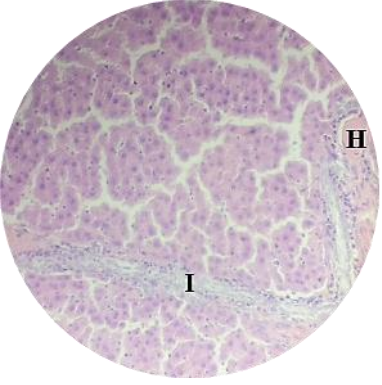
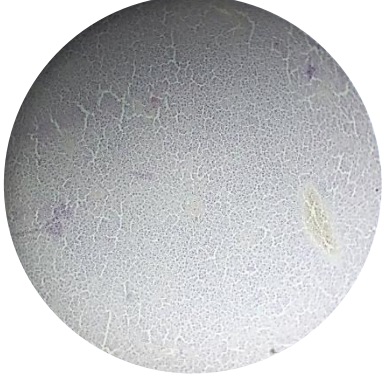
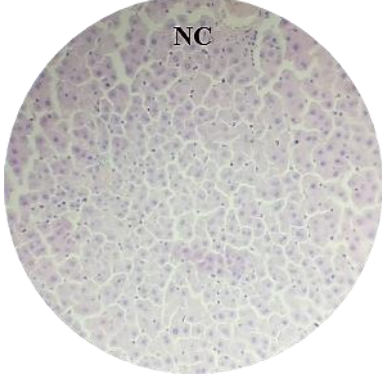
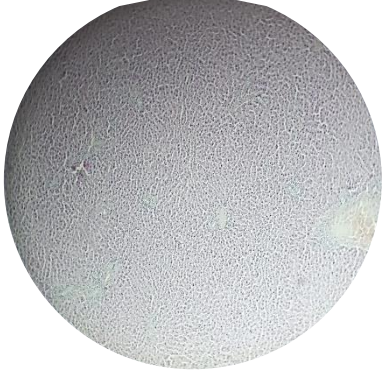
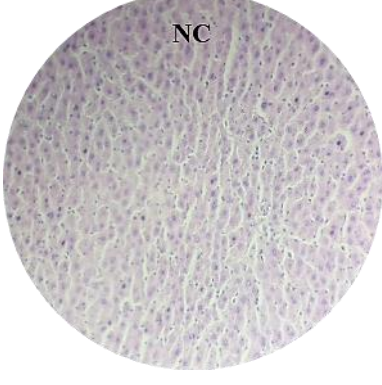
p<0.05, ##p<0.01, ###p<0.001: significantly different from metribuzin exposed group.

I.2.10. Histological Study

I.2.10.1. Photomicrographs of Liver Section

The control rat's liver section displayed normal histological features upon examination. In contrast, microscopic evaluation of liver tissue from rats treated with MTZ and BE showed various histological changes, including bleeding and inflammatory cell infiltration. However, the groups treated with BE-LPs, MnO NPs, and BE-MnO NPs-LPs exhibited significant improvement, with liver tissue appearing more comparable to that of the control group (Figure 58/Table 27).

	(A): 10 x 10	(B): 40 x 10
		

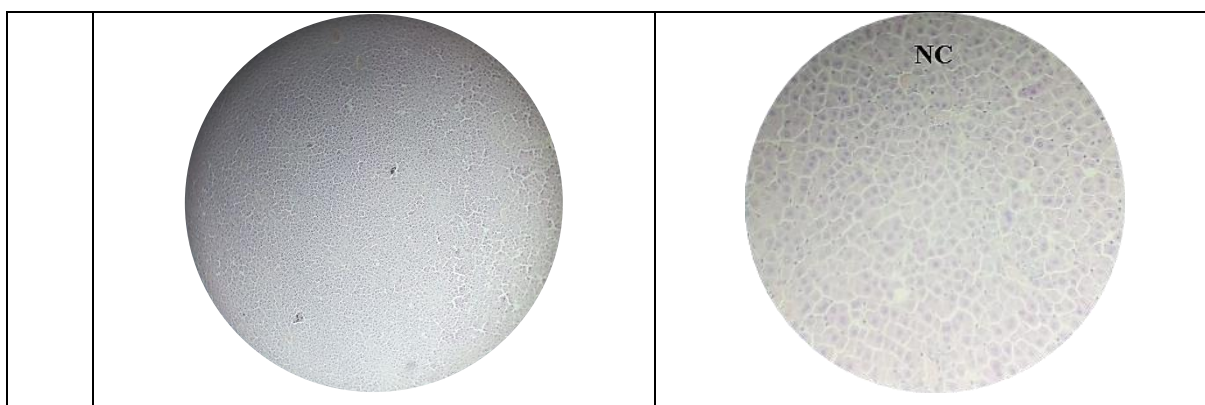


Figure 58: Histological photomicrographs of liver section of all experimental groups stained with hematoxylin and eosin (H&E), (A): x 40 and (B): x 100

Normal cells: **NC**; Hemorrhage: **H**; Inflammation: **I**; Vacuolization: **V**; Necrosis: **N**

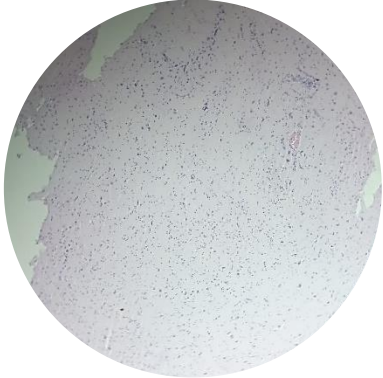
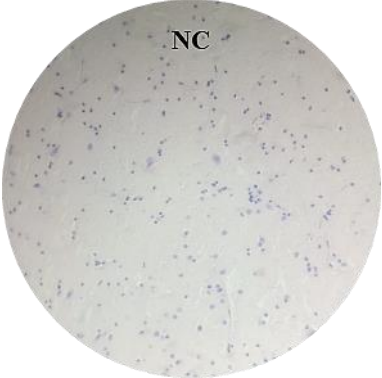
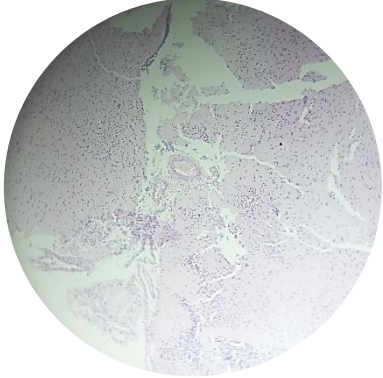
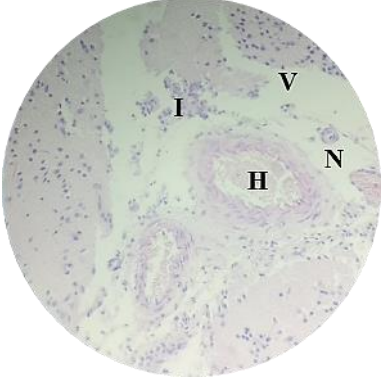
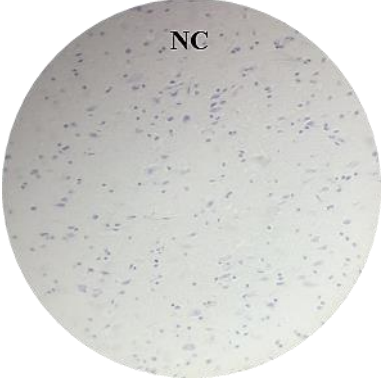
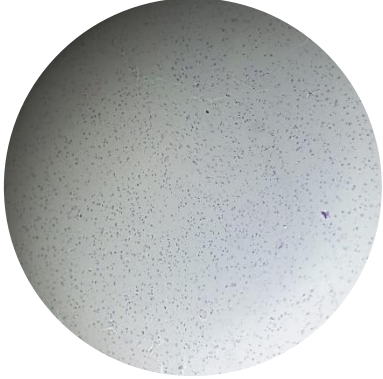
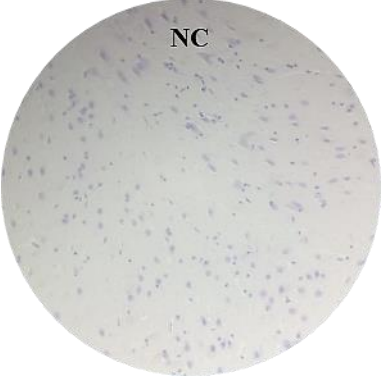
Table 27: Photomicrographs of liver section of control and experimental groups

Parameter						
Hemorrhage	-	+	-	-	-	-
Inflammation	-	+	-	-	-	-
Vacuolization	-	-	-	-	-	-
Necrosis	-	-	-	-	-	-

None (-); Moderate (+); Severe (++); Very severe (+++)

I.2.10.2. Photomicrographs of Brain Section

Normal histological structure was observed in the control rat brain section upon microscopic analysis. In contrast, the MTZ-treated rat heart tissues displayed various abnormalities, including bleeding, inflammatory responses, cell death, and the formation of vacuoles in cerebral cells. However, significant improvements were noted in the BE, BE-LPs MnO NPs, and BE-MnO NPs-LPs groups, with the brain tissue structure more closely resembling that of the control group (Figure 59/Table 28).

	(A): 10 x 10	(B): 40 x 10
		
		
		
		

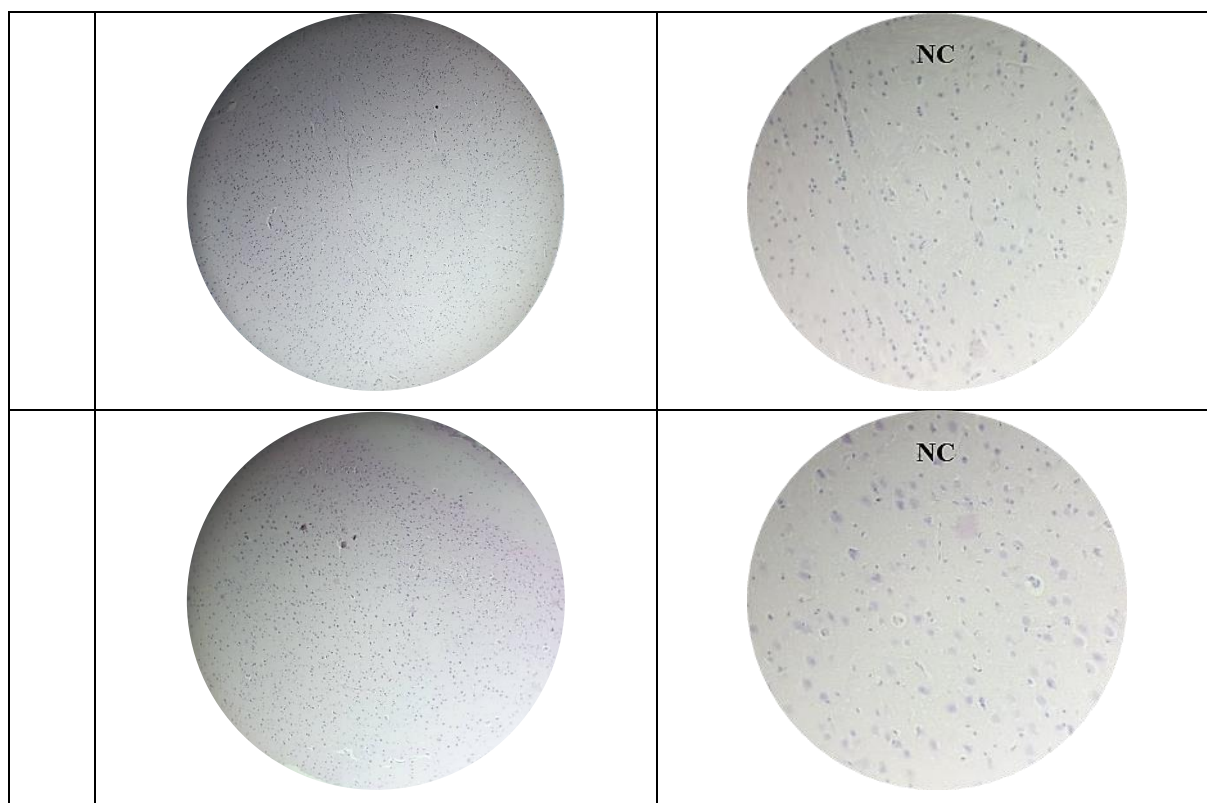


Figure 59: Histological photomicrographs of brain section of all experimental groups stained with hematoxylin and eosin (H&E), (A): x 40 and (B): x 100

Normal cells: **NC**; Hemorrhage: **H**; Inflammation: **I**; Vacuolization: **V**; Necrosis: **N**

Table 28: Photomicrographs of brain section of control and experimental groups

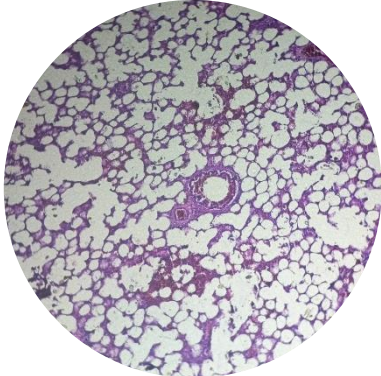
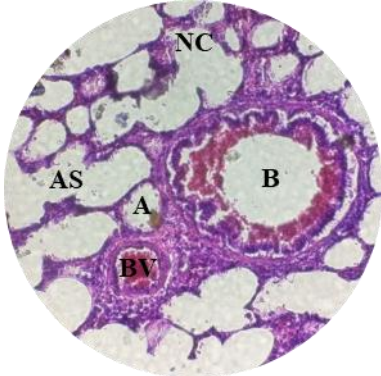
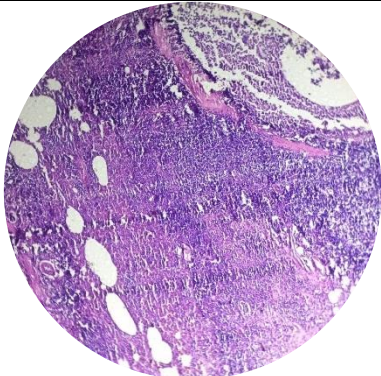
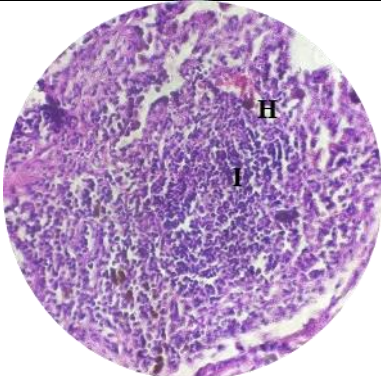
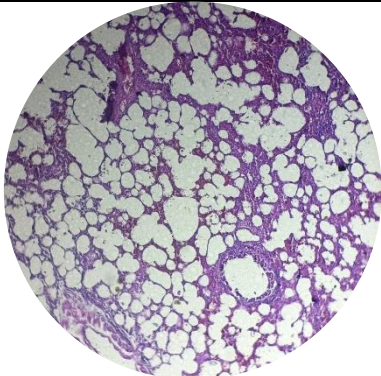
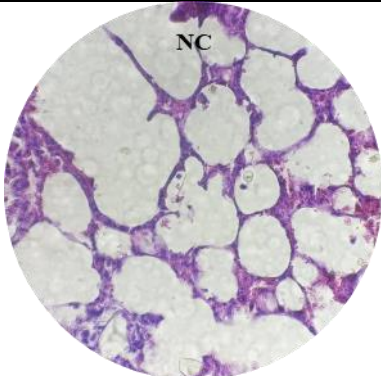
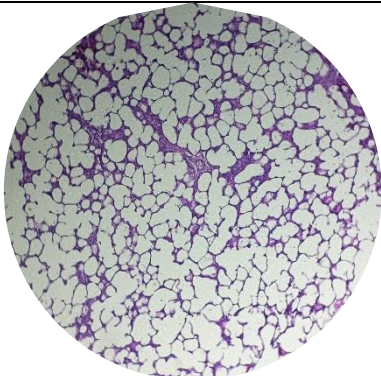
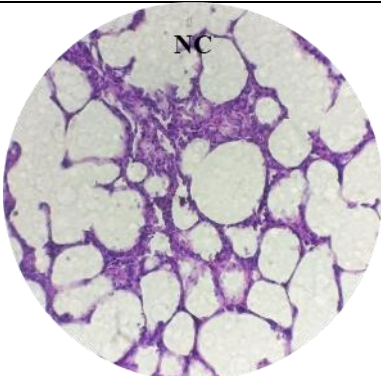
Parameter						
Hemorrhage	-	+	-	-	-	-
Inflammation	-	+	-	-	-	-
Vacuolization	-	+++	-	-	-	-
Necrosis	-	+++	-	-	-	-

None (-); Moderate (+); Severe (++); Very severe (+++)

I.2.10.3. Photomicrographs of Lungs Section

Examination of the control rat's lung section revealed normal histological structure, characterized by thin septa between alveoli. Microscopic analysis of the lung tissue from the MTZ-treated group exhibited various histological alterations, including thickened interalveolar septa and regions where air spaces were completely obstructed. These areas showed signs of hemorrhage and infiltration of inflammatory cells within the significantly thickened septa. In contrast, the BE, BE-LPs and BE-MnO NPs-LPs groups demonstrated a substantial improvement, with lung tissue appearing more similar to that of the control group. The MnO

NPs group displayed partial recovery in lung tissue, with some alveoli remaining collapsed while others were dilated and ruptured (Figure 60/Table 29).

	(A): 10 x 10	(B): 40 x 10
		
		
		
		

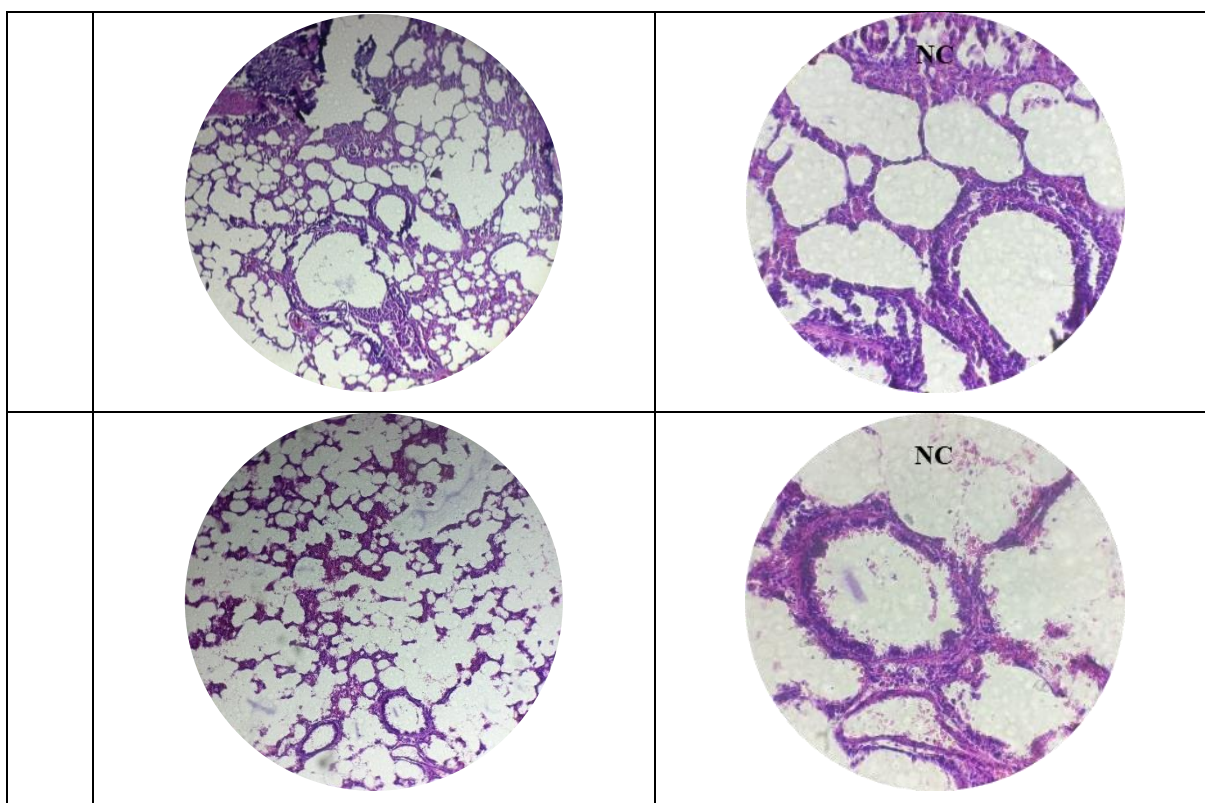


Figure 60: Histological photomicrographs of lungs section of all experimental groups stained with hematoxylin and eosin (H&E), (A): x 40 and (B): x 100

Normal cells: **NC**; Hemorrhage: **H**; Inflammation: **I**; Vacuolization: **V**; Necrosis: **N**
Bronchiole: **B**; Alveolus: **A**; Blood vessels: **BV**; Alveolar sacs: **AS**

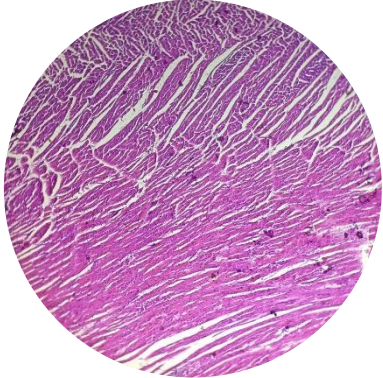
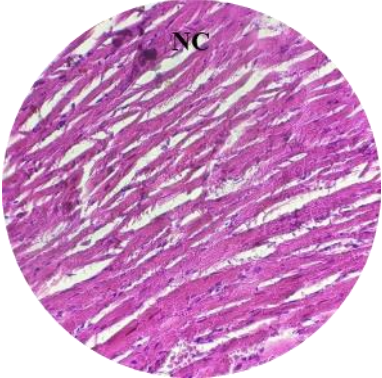
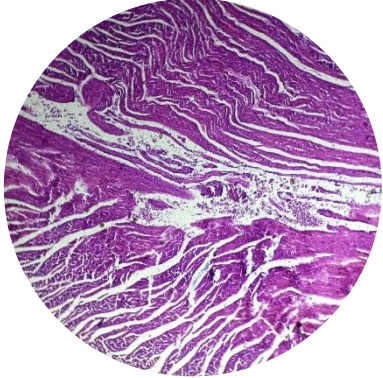
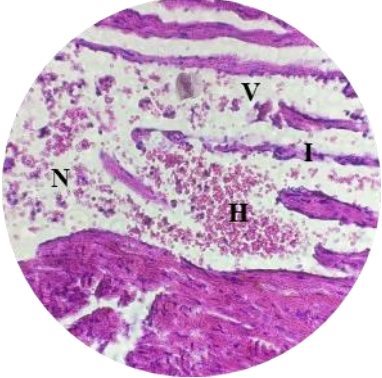
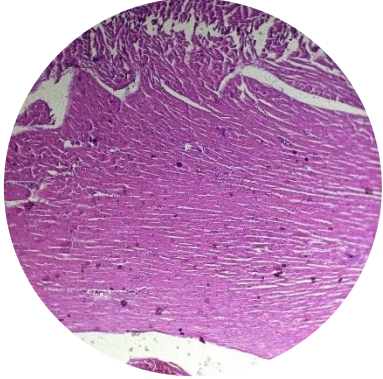
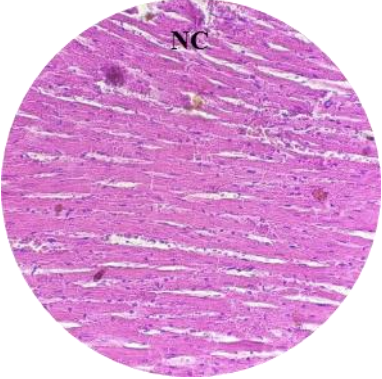
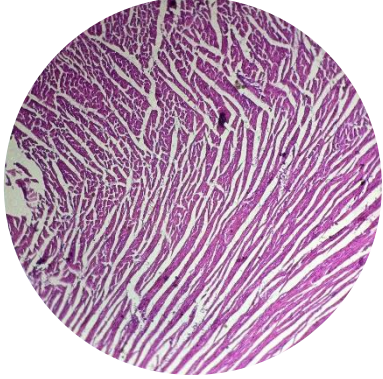
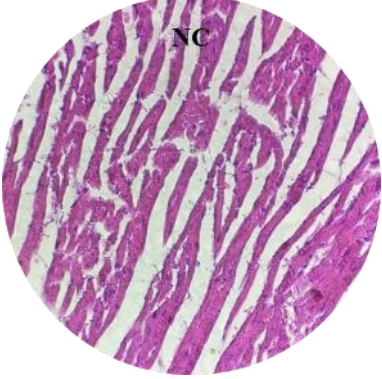
Table 29: Photomicrographs of lungs section of control and experimental groups

Parameter						
Hemorrhage	-	++	-	-	-	-
Inflammation	-	+++	-	-	-	-
Vacuolization	-	-	-	-	-	-
Necrosis	-	-	-	-	-	-

None (-); Moderate (+); Severe (++); Very severe (+++)

I.2.10.4. Photomicrographs of Heart Section

Microscopic examination of control rat heart tissues revealed normal myofibrillar structure and cells. In contrast, MTZ-treated rat heart tissues exhibited muscle fiber abnormalities, including hemorrhage, inflammation, necrosis, and vacuolization of cardiomyocytes. However, the BE, BE-LPs MnO NPs, and BE-MnO NPs-LPs groups showed significant improvement, with heart tissue morphology more closely resembling that of the control group (Figure 61/Table 30).

	(A): x 40	(B): x 100
		
		
		
		

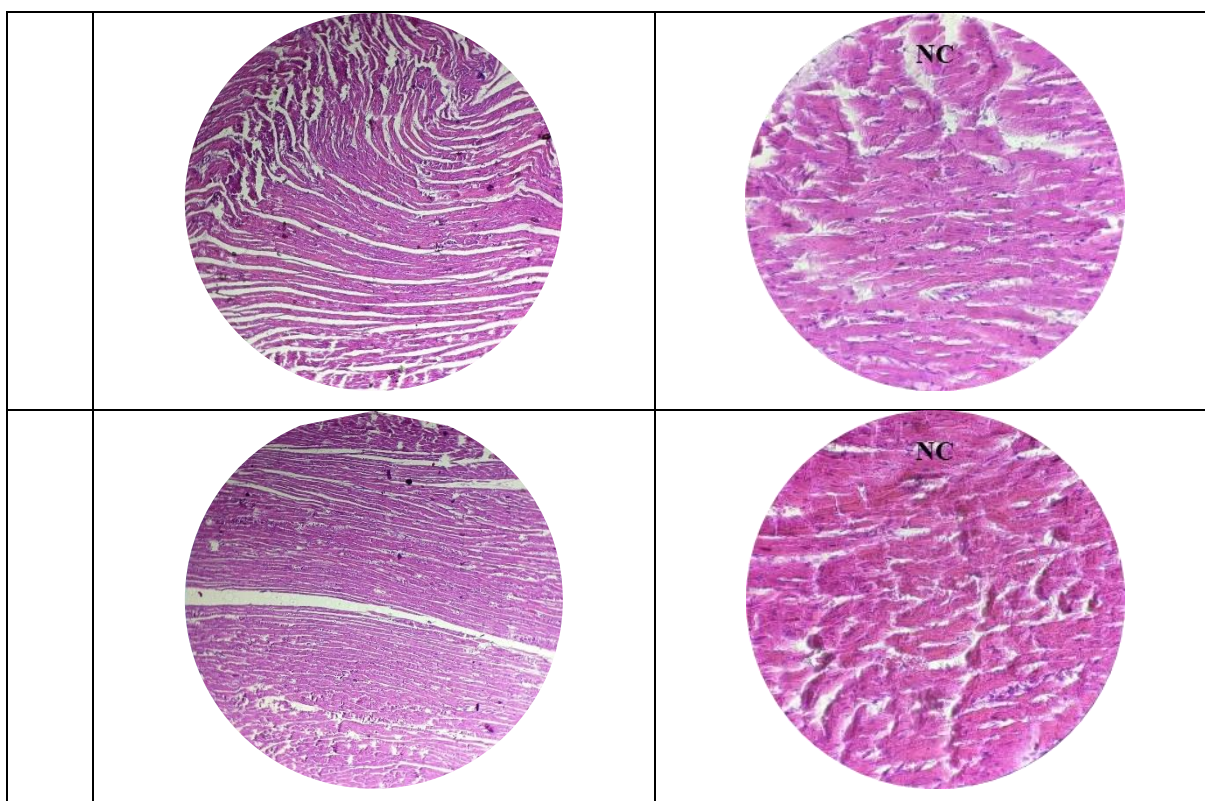


Figure 61: Histological photomicrographs of heart section of all experimental groups stained with hematoxylin and eosin (H&E), (A): x 40 and (B): x 100

Normal cells: **NC**; Hemorrhage: **H**; Inflammation: **I**; Vacuolization: **V**; Necrosis: **N**

Table 30: Photomicrographs of heart section of control and experimental groups

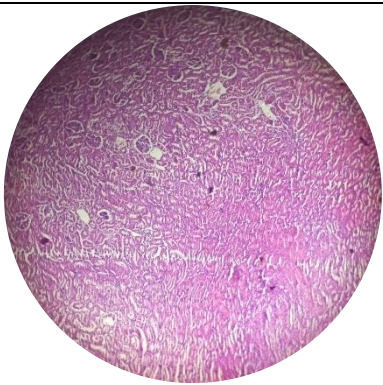
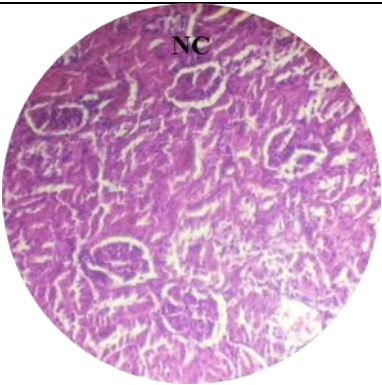
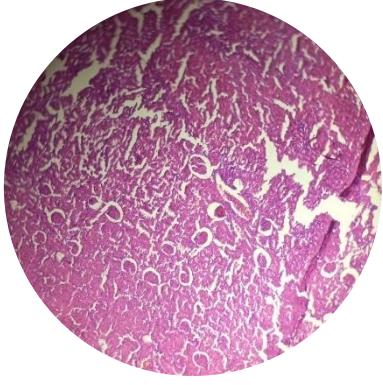
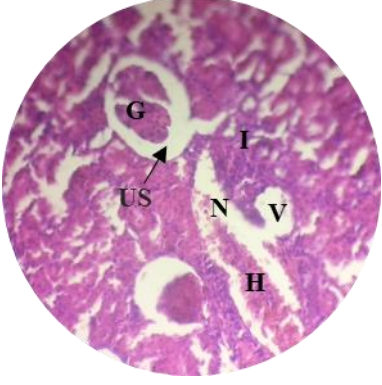
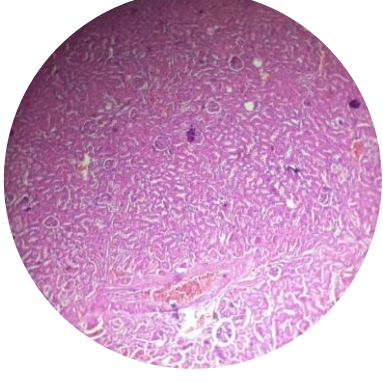
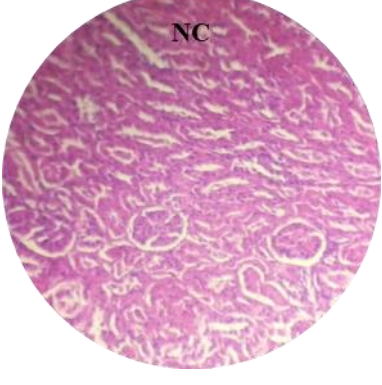
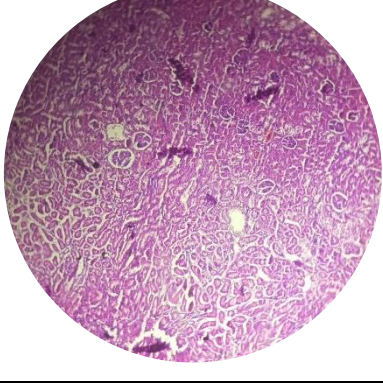
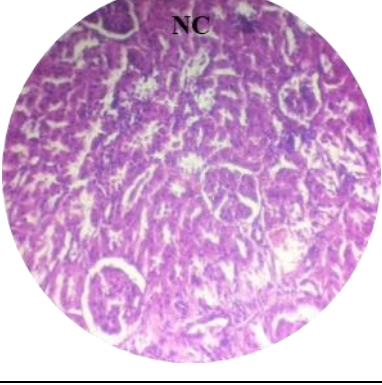
Parameter						
Hemorrhage	-	+++	-	-	-	-
Inflammation	-	++	-	-	-	-
Vacuolization	-	+++	-	-	-	-
Necrosis	-	+++	-	-	-	-

None (-); Moderate (+); Severe (++); Very severe (+++)

I.2.10.5. Photomicrographs of Kidneys Section

Microscopic analysis of the control group revealed normal kidneys tissue structure. In contrast, rats treated with MTZ displayed various renal tissue abnormalities, including destruction renal parenchyma, glomerular atrophy, bleeding, inflammatory responses, cell death, and formation of vacuoles. Notably, the groups treated with BE, BE-LPs MnO NPs, and BE-MnO NPs-LPs demonstrated considerable enhancement in kidneys histology, with tissue structure appearing more similar to that observed in the control group (Figure 62/Table 31).

	(A): 10 x 10	(B): 40 x 10
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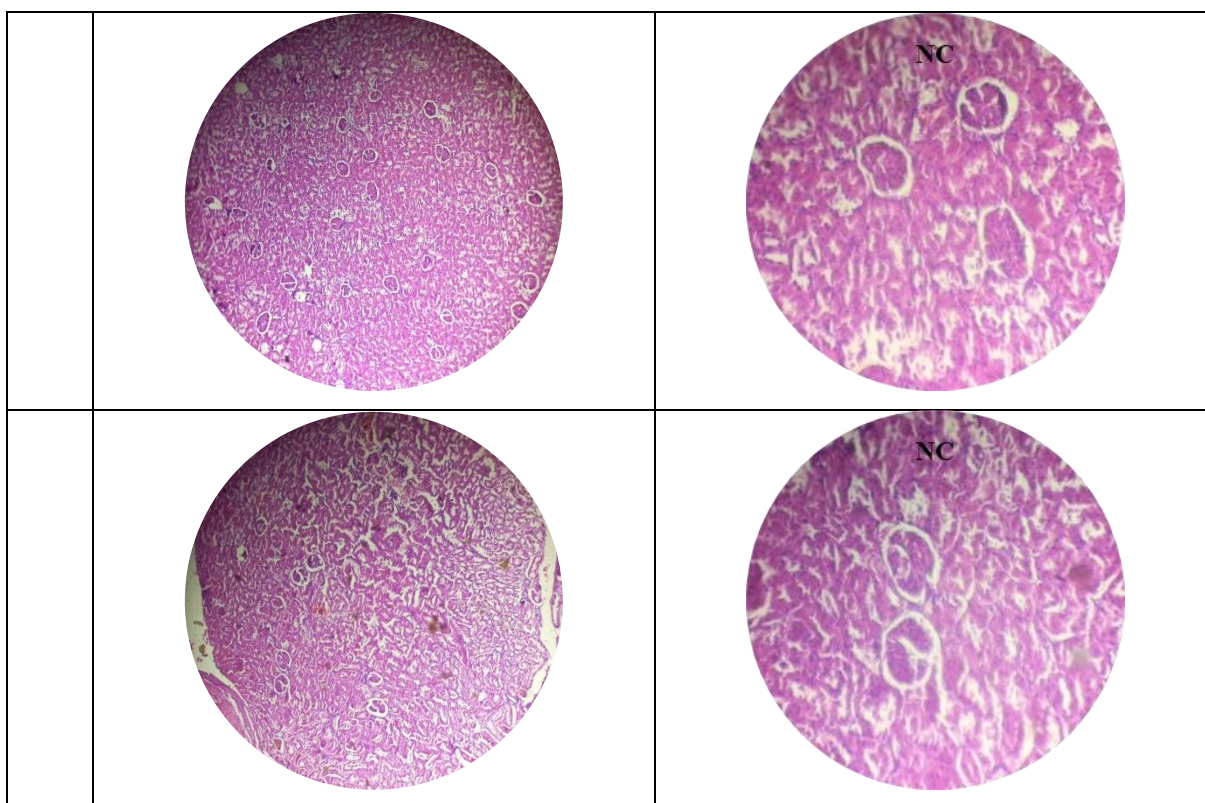


Figure 62: Histological photomicrographs of kidneys section of all experimental groups stained with hematoxylin and eosin (H&E), (A): x 40 and (B): x 100

Normal cells: **NC**; Hemorrhage: **H**; Inflammation: **I**; Vacuolization: **V**; Necrosis: **N**
Glomerulus **G**; Urinary space **US**

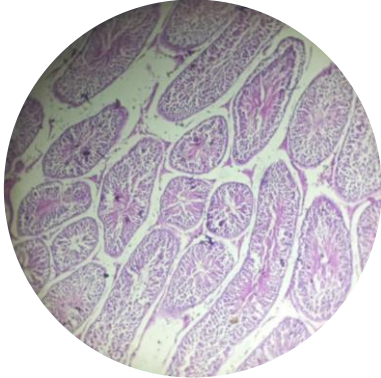
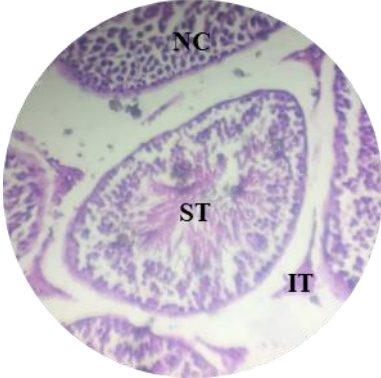
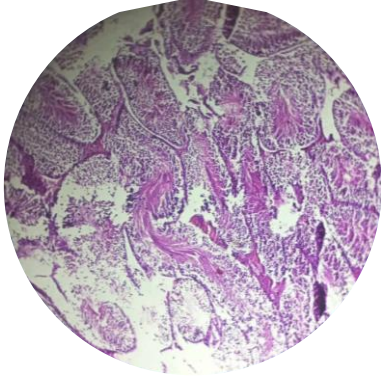
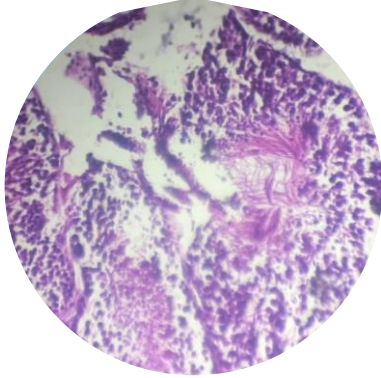
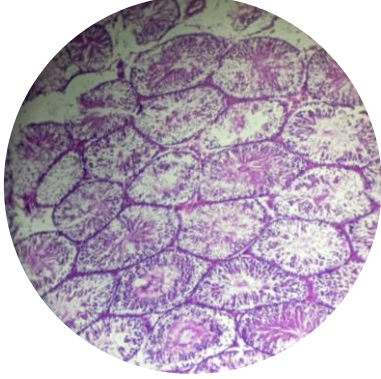
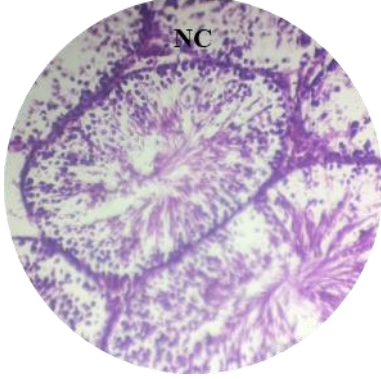
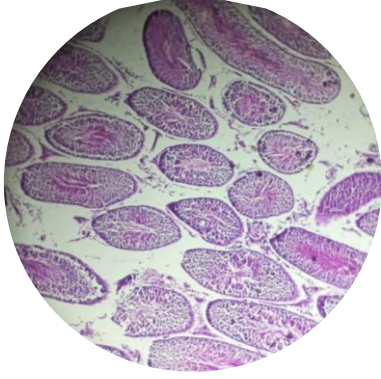
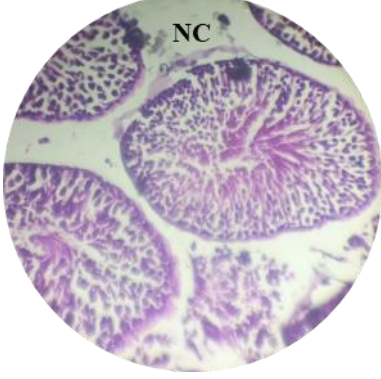
Table 31: Photomicrographs of kidneys section of control and experimental groups

Parameter						
Hemorrhage	-	++	-	-	-	-
Inflammation	-	++	-	-	-	-
Vacuolization	-	++	-	-	-	-
Necrosis	-	++	-	-	-	-

None (-); Moderate (+); Severe (++); Very severe (+++)

I.2.10.6. Photomicrographs of Testes Section

Analysis of the control rat's testicular section showed normal histological features, including properly arranged seminiferous tubules with regular interstitial spaces. Microscopic evaluation of testicular tissue from the MTZ-treated group revealed various structural changes, such as damaged seminiferous tubules and disrupted interstitial tissues. In comparison, the BE, BE-LPs, and BE-MnO NPs-LPs groups exhibited significant improvement, while the MnO NPs group showed only partial recovery, with some seminiferous tubules still collapsed and evidence of hemorrhage (Figure 63).

	(A): 10 x 10	(B): 40 x 10
		
		
		
		

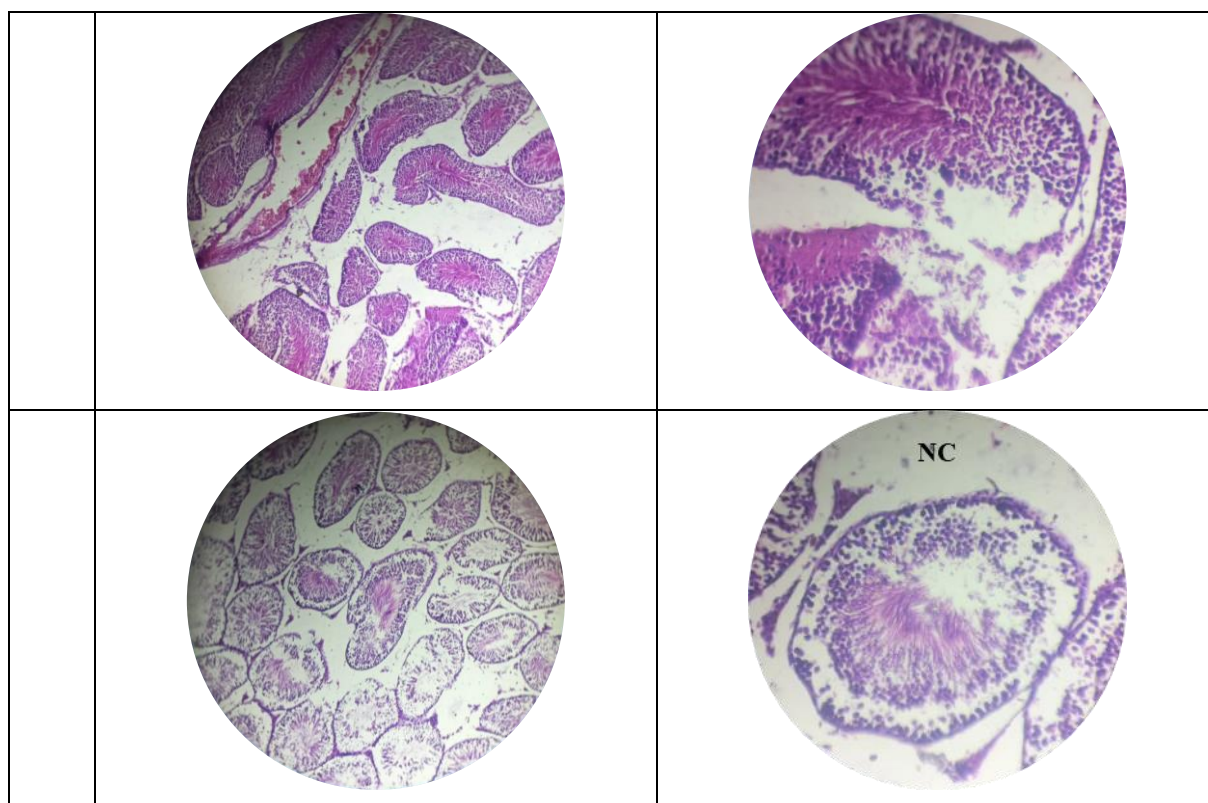


Figure 63: Histological photomicrographs of testes section of all experimental groups stained with hematoxylin and eosin (H&E), (A): x 40 and (B): x 100

Seminiferous tubule: **ST**; Interstitial tissue: **IT**

Chapter III

Discussion

I. Discussion

I.1. Qualitative and Quantitative Phytochemical Compounds

The aqueous extract from *Ocimum basilicum* L. leaves underwent comprehensive qualitative and quantitative phytochemical analyses to identify and measure its bioactive components. Our investigation uncovered the presence of various compounds, including phenols, flavonoids, catechic tannins, saponins, reducing sugars, alkaloids, and terpenes in the basil leaf extract. These findings align with a previous study that reported a wide range of compounds in *Ocimum basilicum* L., such as flavonoids, steroids, phenolics, catechic tannins, saponosides, carbohydrates, and alkaloids (Boulaares et al., 2024). The current study's phytochemical screening results are also consistent with those of Nadeem et al., who detected phenols, alkaloids, tannins, flavonoids, steroids, terpenoids, and glycosides in the water extract of basil leaves (Nadeem et al., 2022). Additionally, another study reported the presence of tannins, flavonoids, terpenoids, saponins, and reducing sugars in the aqueous extract of sweet basil leaves (Nguyen et al., 2021). Pushpalatha et al. conducted an initial screening of secondary metabolites in the aqueous extract of *Ocimum basilicum* L., revealing the presence of various phytochemicals including carbohydrates, tannins, saponins, flavonoids, anthocyanin, cardiac glycosides, terpenoids, triterpenoids, phenols, and steroids (Pushpalatha, Ponvel, & Narayanan, 2021). Studies indicate that the pharmacological potential of medicinal plants is attributed to their phytochemical constituents (Seema, Hindole, & Muddasir, 2016). Plant secondary metabolites, particularly phenolic and flavonoid compounds, exhibit a range of pharmacological effects, such as anti-allergic, antibacterial, antiviral, anti-inflammatory, antioxidant, anti-diabetic, anticancer, and neurodegenerative properties (Shoker, Raheema, & Shamkhi, 2021). The findings from the current study regarding the measurement of phytochemical compounds align with those reported by Nadeem et al. Their research on *Ocimum basilicum* L. aqueous extract revealed total phenolic content of 70.7 mg GAE/g, total flavonoids of 6.49 mg QE/g, and total tannins content of 13.3 mg GAE/g (Nadeem et al., 2022). These results are also consistent with a prior investigation, which determined that the total phenolic and flavonoid contents in *Ocimum basilicum* L. extracted using water as a solvent were $77,66 \pm 3,27$ mg GAE/g and $16,527 \pm 0,061$ mg of QE/g, respectively (Boulaares et al., 2024).

I.2. Chromatographic Methods Analysis Phytochemical Compounds

The phenolic constituents within the aqueous extract of *Ocimum basilicum* L. leaves were characterized utilizing the HPLC-Q-TOF-MS technique. In the present investigation, the evaluation of 30 phytochemical compounds derived from the *Ocimum basilicum* L. extract was performed through the application of HPLC-Q-TOF-MS. High-performance liquid chromatography quadrupole time-of-flight mass spectrometry (HPLC-Q-TOF-MS) represents a highly effective analytical approach characterized by elevated sensitivity, precision, resolution, and rapid data acquisition (He et al., 2018). The analysis conducted by Romano et al. indicated that the phenolic compounds present in basil predominantly consist of phenolic acids, including rosmarinic acid and caffeic acid, among others (Romano et al., 2022). Furthermore, Ullah et al. ascertained that the plant is abundant in bioactive components, encompassing phenolic compounds, caffeic acid, and flavonoids, notably rutin (Ullah, Naseem, Hussain, Sheikh, & Farooq, 2022). In addition, the high-performance liquid chromatographic findings reported by Lu et al. disclosed that rosmarinic acid, caffeic acid, and rutin are present in significant quantities in basil leaves (Lu, Gao, Chen, Charles, & Yu, 2014).

The phenolic acids and flavonoids present in the aqueous extract of *Ocimum basilicum* L. leaves were systematically identified and quantified utilizing the liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS) technique. According to the high-performance liquid chromatographic findings reported by Lu et al., luteolin acetylglucuronide, rosmarinic acid, and luteolin 5-O-glucoside were identified in substantial quantities within basil leaves (Lu et al., 2014). Moreover, the HPLC analysis executed by Romano et al. indicated that the phenolic compounds in basil predominantly consist of phenolic acids, particularly highlighting the presence of rosmarinic acid and other related compounds (Romano et al., 2022). In addition, Ullah et al. revealed that *Ocimum basilicum* L. exhibits a significant abundance of phenolic acids, which encompass gallic acid, as well as apigenin, which includes rutin (Ullah et al., 2022).

The volatile constituents present in the aqueous extract of *Ocimum basilicum* L. leaves were analyzed utilizing gas chromatography-mass spectrometry (GC-MS) methodology. In this investigation, the identification and characterization of 147 volatile compounds derived from basil aqueous extract were performed through the application of GC-MS. The identification of basil extracts was executed by Kaya and Keskin employing GC-MS, which revealed the existence of various bioactive compounds (Kaya & Keskin, 2020). The GC-MS analysis indicated that the compounds exceeding 4% identified in the aqueous extract by Ababutain included Propane, 3-chloro-1,1,14,5-Dichloro-1,3-dioxolan-2-one (49.87%), phenol (6.22%),

propane, 3-chloro-1,1,1-trifluoro (4.26%), and gamma-sitosterol (4.19%) (Ababutain, 2019). D'iaz-Maroto et al. in their research successfully identified several volatile components in basil extracts using GC/MS, with linalool being the predominant constituent (Díaz-Maroto, Sánchez Palomo, Castro, González Viñas, & Pérez-Coello, 2004). Numerous studies regarding the volatile composition of *Ocimum basilicum* L. have been undertaken (Calín-Sánchez, Lech, Szumny, Figiel, & Carbonell-Barrachina, 2012). Volatile organic compounds in plants, characterized by low molecular weight, exhibit enhanced chemical reactivity due to their functional groups, comprising hydroxy, α , β -unsaturated carbonyl, phenyl, alkoxyl, sulfhydryl, and ester groups, which confer significant polarity or electrophilic capacity to these molecules (Gong et al., 2023). The volatile constituents of basil, along with their antifungal, antibacterial, and antioxidant properties, have been subjected to extensive examination (Du et al., 2023). Prior research has demonstrated that the chemical profiles of volatile compounds from leaf tissues of a given plant genus are markedly diverse (Osei-Owusu et al., 2023).

I.3. Trace Metal Distribution in *Ocimum basilicum* L.

Ocimum basilicum L. contains a variety of essential mineral nutrients. These include major elements like sodium, potassium, calcium, and magnesium, as well as minor elements such as copper, zinc, iron, and manganese. The presence of these dietary minerals in sweet basil leaves has been corroborated by additional research (Roberto et al., 2021). Calcium intake is believed to help prevent osteoporosis and may contribute to the absence of negative effects related to gastric lesions. Sodium and potassium, two macronutrients, are essential for numerous metabolic functions in human body cells. These elements contribute to improved heart health, enhanced metabolism, reduced risk of sudden cardiac death, and regulation of body water content. Iron plays a crucial role in proper bodily function. Additionally, copper and zinc have been found to strengthen the antioxidant defense system (Chetehouna, Derouiche, Reggami, et al., 2024).

I.4. Biosynthesis and Characterization of MnO NPs

The green synthesis of MnO NPs using basil extract is evidenced by the transformation of the solution's color from golden yellow to dark brown. This color change serves as an initial visual confirmation of the plant extract's ability to form nanoparticles (Chetehouna et al., 2023). The shift in color during the bioreduction process demonstrates the phytochemicals present in the plant extract's role in reducing Mn^{+2} (Hasan, Salman, & Hafez, 2023). Additionally, this color alteration is attributed to the surface plasmon resonance effect of the nanoparticle (H. Lu

et al., 2022). Khan et al. noted that the color change in the metal ion solution acts as a visible indicator of MnO NPs synthesis (Kumar, Singh, Panwar, & Mehta, 2017). The exact mechanism for nanomaterial production using plant extracts remains unclear, largely due to the potential simultaneous involvement of various secondary metabolites in the extract solution (Wijaya et al., 2024). Plant extracts contain phytochemicals such as polyphenols, alkaloids, terpenoids, tannins, and alcohol, which serve as reducing and stabilizing agents for nanoparticles (Althobiti et al., 2023). These phytomolecules in leaves extract likely function as both reducing and capping agents during MnO NPs synthesis (Khan et al., 2020). Numerous research studies have reported similar green synthesis mechanisms for MnO NPs using different plant extracts (Amatya et al., 2021), (Amatya & Shrestha, 2021), (Usman Ahmed et al., 2020).

In theory, MnO NPs exhibit an absorption peak between 350-410 nm (Saod et al., 2022). When analyzed using UV-visible spectroscopy, the MnO NPs synthesized with basil extract demonstrated peak absorption at 405 nm. Research by Hoseinpour et al. revealed that biosynthesized MnO₂ NPs displayed maximum absorbance at 410 nm in UV-visible analysis (Hoseinpour, Souri, & Ghaemi, 2018). Similarly, Saod et al. reported that MnO NPs produced using green tea extract showed a UV-visible spectral peak at 410 nm (Saod et al., 2022).

The FTIR spectra of MnO NPs exhibited a transmittance band between 470 and 522 cm⁻¹, which falls within the range indicative of Mn-O bonds. Typically, metal-oxygen bond fingerprints show absorption bands in the 400-600 cm⁻¹ wavenumber range (Nayan, Jagadish, Abhilash, Namratha, & Srikantaswamy, 2019). Mylarappa et al.'s research revealed a notable absorption band at 538 cm⁻¹, potentially linked to the Mn-O stretching mode infrared domain (Mylarappa, Lakshmi, Mahesh, Nagaswarupa, & Raghavendra, 2016). Sahib & Naser identified characteristic peaks at 524 cm⁻¹ and 493 cm⁻¹, signifying the presence of Mn-O bonds (Sahib & Naser, 2021). In this study, the MnO NPs spectrum displayed several peaks at approximately 3200, 2900, 1550, 1350, and 1050 cm⁻¹. These peaks likely correspond to functional groups of active compounds from the basil extract coating the MnO NPs. FTIR has proven to be an effective technique for identifying functional groups that interact with metal particles and biomolecules, which facilitate Mn bioreduction and serve as capping and stabilizing agents (Ramesh et al., 2021). Saod et al. observed organic residues, including carboxyl and hydroxyl groups, on the surface of the synthesized MnO NPs (Saod et al., 2022). This observation accounts for the appearance of the peaks in the FTIR spectrum.

The measurement of zeta potential for MnO NPs created using basil extract revealed a value of about -42.260 ± 0.015 mV, indicating the electrical charge on the nanoparticles' surface. This surface charge significantly affects the nanoparticles' aggregation behavior,

reactivity, and toxicological properties (Wang, Hsu, Zhu, Tseng, & Hsu, 2013). Van der Waal's forces of attraction cause nanoparticles to tend to aggregate when they are dispersed in water. Nevertheless, if the particles become charged during dispersion, aggregation might be avoided if the electrostatic repulsive forces outweigh the Van der Waal's attractive forces (Ponticorvo et al., 2022). Zeta potential measurements are used to evaluate the surface characteristics of nanoparticles by characterizing the charge distribution on their surface (Mansouri, Mousavi, Attar, Saboury, & Falahati, 2018). Additionally, zeta potential can determine the aggregation state of nanoparticle solutions, as it relates to the electrostatic repulsion between particles based on their surface charge. The negative or positive charge on metal nanoparticles contributes to their long-term stability and prevents aggregation (Chetehouna, Derouiche, & Reggami, 2024). The development of hydroxyl groups on the particle surface during dispersion in water may be the cause of the negative zeta potential value. The adsorption of hydroxyl groups on the surface of particles is encouraged by raising the pH, which raises the zeta potential (Devadoss et al., 2023). In a similar study, Mn_3O_4 nanoparticles synthesized from *Ananas comosus* L. peel extract showed a zeta potential value indicating a negative surface charge (Asaikkutti, Bhavan, Vimala, Karthik, & Cheruparambath, 2016). This finding aligns with the results obtained in the current study.

XRD analysis confirmed the chemical formula of the manganese oxide nanoparticles as Mn_3O_4 , aligning with the JCPDS card N° 00-001-1127 database. They had a tetragonal crystallite structure, size (D) = 13.4 nm, 23.09 % crystallinity, and lattice parameters $a = b = 6.202 \text{ \AA} \neq c = 8.162 \text{ \AA}$. The XRD pattern revealed 9 distinctive peaks at 18.02° (101), 29.07° (112), 32.66° (103), 36.11° (211), 38.42° (004), 44.40° (220), 55.88° (303), 60.20° (224), and 64.62° (314). These findings align with those of Atique Ullah et al., who identified Mn_3O_4 (JCPDS card N° 00-001-1127) with a tetragonal crystal structure and planes (101), (112), (200), (103), (211), (004), (220), (105), (312), (321), (224), and (314) (Atique Ullah et al., 2017). Similarly, Zhang et al. reported that the primary diffraction peaks corresponding to (101), (112), (200), (103), (211), (004), (220), (105), (321), and (224) crystal planes were consistent with standard Mn_3O_4 data (Xiao Zhang, Yue, Zhang, Liu, & Zhou, 2020). Mn_3O_4 is characterized by prominent diffraction peaks at 2θ values of 28.91° , 31.08° , 32.47° , 36.49° , 36.59° , 38.19° , 44.53° , 50.68° , 58.38° , and 59.70° , with respective Miller indices of (112), (020), (013), (121), (022), (004), (220), (015), (231), and (224) (Jamil et al., 2018). The synthesized Mn_3O_4 NPs displayed a tetragonal structure with lattice constants $c = 0.9463 \text{ nm}$ and $a = 0.5746 \text{ nm}$, and an average crystallite size of 36 nm (Shaik et al., 2021).

XPS measurement was carried out with the aim to investigate the surface chemistry of the Mn_3O_4 greenly synthesized using basil extract. The survey spectrum of Mn_3O_4 displays core level carbon as C 1s, oxygen as O 1s and manganese as Mn 2p. It can be noted that no impurities are observed on the material surface. Concerning the high-resolution core level O 1s peak, it has broader and unsymmetrical shape and highest peak intensity was observed at BE value of ~ 530.1 eV. Three individual components with the BE values of 528.6 eV, 530.0 eV, and 531.0 eV were detected which can be assigned to lattice oxygen (Mn-O) and surface oxygen (Mn-O-H) (Ioffe, Bosch, Viveros, Sanchez, & Borodko, 1997; Chuanhua Li, Yu, Liu, & Kong, 2018; X. Tang, Li, & Hao, 2010). Regarding the core level Mn 2p peaks, two peaks located at BE value of 640.8 eV and 652.5 eV can be assigned to $\text{Mn}2p_{3/2}$ and $\text{Mn}2p_{1/2}$, due to spin orbit coupling nature. In addition, their binding energy difference is found to be 11.7 eV which is accordance with the literature value (Xie et al., 2017). Three individual components were observed for both $\text{Mn}2p_{3/2}$ and $\text{Mn}2p_{1/2}$ which can be assigned as the oxidation states of Mn_3O_4 . BE centered at 639.8 eV can be allocated as Mn^{2+} species, 641.2 can be noted as Mn^{3+} and 643.5 eV can be due to the presence Mn^{4+} state in our synthesized Mn_xO_x nanomaterial (H.-Y. Chen & Hsu, 2014). According to Moon et al., manganese dioxide nanoparticles exhibited Mn $2p_{3/2}$ and Mn $2p_{1/2}$ core level binding energies at 641.88 and 658.59 eV, respectively. The O1s scan revealed a binding energy of 529.69 eV, indicating the presence of O^{2-} in the sample (Moon et al., 2015). These findings align with our observations, supporting the successful synthesis of MnO NPs using basil extract.

SEM and TEM analyses were employed to examine the morphology and average particle size of MnO NPs. The results indicated that these nanoparticles were spherical and had an average size of 6.52 ± 0.88 nm. In a study by Lu et al., TEM imaging revealed that the synthesized MnO NPs were spherical and exhibited uniform dispersion (Lu et al., 2022). Srinivasa et al. reported that MnO_2 NPs had a rod-like shape and measured between 40 to 50 nm on average (Srinivasa et al., 2022). Research conducted by Moon et al. showed that the particles were predominantly spherical and small in size (Moon et al., 2015). The diameter of MnO_2 NPs ranged from 1 to 60 nm, with an average of 19.2 nm (Moon et al., 2015). Another investigation using SEM found that manganese oxide nanoparticles had diameters ranging from 30 to 40 nm (Sahib & Naser, 2021).

Energy-dispersive X-ray spectroscopy (EDS) constitutes an analytical technique that facilitates the characterization of the elemental composition of a given specimen (Dasgupta & Wahed, 2021). Furthermore, EDS serves as an analytical methodology utilized for the examination of elemental constituents to evaluate the chemical properties of various samples

The application of Energy-dispersive X-ray spectroscopy (EDS) substantiated the successful synthesis of manganese oxide (MnO) nanoparticles. The elemental analysis disclosed that the MnO NPs comprised 58.7% manganese and 41.3% oxygen. The most pronounced intensity peaks for manganese were detected at energy levels of 0.62 keV, 5.9 keV, and 6.47 keV, whereas the peak corresponding to oxygen was observed at 0.45 keV. In a separate investigation, maximum intensity peaks of 2.2 keV, 5.9 keV, and 6.2 keV for manganese were documented (Sahib & Naser, 2021). Jassal et al. reported that in manganese oxide (Mn₃O₄), the weight percentages for manganese and oxygen were determined to be 70.29% and 29.71%, respectively (Dubois-Deruy, Peugeot, Turkieh, & Pinet, 2020).

The comprehensive characterization of MnO NPs relies heavily on these analytical methods (UV-Vis, FTIR, Zeta potential, XRD, XPS, SEM, TEM and EDS). Such detailed analysis is crucial for understanding the nanoparticles' characteristics and evaluating their potential applications in various domains, including environmental and medical fields.

1.5. Anti-Oxidant Activity of *Ocimum basilicum* L. and MnO NPs

The study assessed *in vitro* antioxidant activity using two techniques: DPPH free radical scavenging capacity and ferric reducing antioxidant power (FRAP). The research found that MnO NPs and basil extract exhibited significant antioxidant potential. The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a commonly employed method for assessing antioxidants' ability to neutralize free radicals. During this test, DPPH, a stable free radical, undergoes a color transformation from purple to yellow upon accepting an electron or hydrogen radical from an antioxidant (Patel et al., 2024). On the other hand, the FRAP assay evaluates antioxidants' capacity to convert ferric ions to ferrous ions in a redox-linked colorimetric reaction. This method provides a direct measurement of antioxidants' ability to reduce oxidative species (Heckmann et al., 2024). *Ocimum basilicum* L. contains a wealth of secondary metabolites, including glycosides, tannins, phenolic compounds, saponins, flavonoids, and terpenes (Saba et al., 2016). Research has shown that various plant secondary metabolites, such as phenolic acids, flavonoids, terpenoids, alkaloids, and steroids, exhibit numerous pharmacological properties, including antioxidant activity (Osei Akoto, Acheampong, Boakye, Naazo, & Adomah, 2020). According to Lee and Scagel, basil demonstrates antioxidant potential due to its phenolic and aromatic compounds (Lee & Scagel, 2009); this is because the hydroxyl group found in phenolic substances can attach to hydroxy peroxide, superoxide, or hydroxyl radicals, thereby preventing these radicals from causing degenerative changes within the body (Adámek et al., 2021). Additionally, biogenic MnO₂ NPs have been found to possess antioxidant properties (Faisal et al., 2022). Research by Mahlangeni et al. revealed that MnO₂ nanoparticles

produced using *Cussonia zuluensis* strey displayed notable antioxidant properties, as evidenced by their DPPH free radical scavenging ability and ferric reducing antioxidant power (FRAP) analysis (Mahlangeni, Magura, Moodley, Baijnath, & Chenia, 2020). Similarly, a study conducted by Revathi et al. showed that green-synthesized MnO₂ was both biocompatible and effective in its antioxidant activity against DPPH free radicals (Revathi, Sankarganesh, Raja, Rajakanna, & Senthilkumar, 2023). Additionally, Li et al. found that MnO₂ nanoparticles possess the ability to consume excess H₂O₂ in situ and transform it into O₂, effectively reversing the process of excessive ROS generation (Li et al., 2021).

I.6. Anti-Inflammatory Activity of *Ocimum basilicum* L. and MnO NPs

The evaluation of *in vitro* anti-inflammatory activity was conducted using protein anti-denaturation and red blood cell membrane stabilization tests. Results confirmed the anti-inflammatory effects of basil extract and manganese oxide nanoparticles when compared to diclofenac[®]. The immune system's biological response, known as inflammation, can be triggered by various factors (Chen et al., 2018). The inflammatory process is characterized by increased protein denaturation and membrane changes (Gunathilake, Ranaweera, & Rupasinghe, 2018). To evaluate *in vitro* anti-inflammatory activities, researchers frequently employ protein denaturation and membrane stabilization assays (Sarveswaran, Jayasuriya, & Suresh, 2017). Numerous anti-inflammatory drugs function by inhibiting protein denaturation induced by heat (Kpemissi, Kantati, Veerapur, Eklu-Gadegbeku, & Hassan, 2023). In order to combat inflammation, it is crucial to identify effective anti-inflammatory substances (Derouiche, Chetehouna, Djouadi, et al., 2022). Research by Kamelnia et al. has demonstrated that basil and its derivatives possess anti-inflammatory properties, as evidenced in multiple studies (Kamelnia, Mohebbati, Kamelnia, El-Seedi, & Boskabady, 2023). The presence of compounds such as phenols, flavonoids, terpenoids, steroids, and glycosides in basil contributes to its anti-inflammatory effects, as reported in previous research (Osei Akoto et al., 2020). Additionally, various types of nanoparticles (NPs) have been found to exhibit potential anti-inflammatory characteristics (Wang et al., 2021). The inhibition of pro-inflammatory cytokines is regarded as a crucial process, given that cytokines amplify immune responses, and this mechanism is employed by nearly all nanoparticles (Agarwal, Nakara, & Shanmugam, 2019). Research conducted by Kumar et al. demonstrated that manganese dioxide nanoparticles shield cartilage from oxidative stress induced by inflammation (Kumar et al., 2019). We developed a theory suggesting that the phytochemical components found in basil extract and MnO NPs offer protection against *in vitro* protein denaturation and aid in stabilizing red blood cell membranes. This protective effect is typically achieved through particular interactions with proteins or

cellular membranes, which effectively prevent denaturation or potential harm to these biological structures.

I.7. Thrombolytic Activity (Clot Lysis) of *Ocimum basilicum* L. and MnO NPs

The results obtained from the investigation on thrombolysis activity have clearly demonstrated the superior efficacy of the extract derived from *Ocimum basilicum* L. and the MnO NPs in terms of safeguarding against the formation of blood clots. This protective effect was observed to be significantly more pronounced when compared to the commonly employed synthetic drug for thrombolysis, Aspegic[®], thereby indicating a promising alternative without any reported adverse effects. The natural process of blood clot formation serves to prevent excessive bleeding in response to injury ; however, when it occurs in an abnormal or excessive manner, it can lead to severe health complications (Zhang & Jiang, 2023). Thrombolysis is a medical procedure aimed at breaking down blood clots to restore proper blood circulation in affected areas. This treatment involves the administration of thrombolytic or fibrinolytic drugs, which function by breaking apart the proteins that hold blood clots together (Zhang & Jiang, 2023). Nanoparticles can be specifically designed to navigate the bloodstream more effectively and accumulate at clot sites, offering advantages over traditional thrombolytic agents administered without containment (Voros et al., 2015). In light of the increasing demand for precise and effective thrombosis treatments, scientists have created nanoparticles containing thrombolytic medications for use in thrombolysis (Zhang & Jiang, 2023). Over the past two decades, researchers have focused on identifying, developing, and producing plant-derived natural compounds with antiplatelet, anticoagulant, antithrombotic, and thrombolytic properties (Umesh, Sanjeevkumar, Hanumantappa, & Ramesh, 2014). Natural thrombolytic agents, often derived from plants, are generally considered less harmful and devoid of adverse effects compared to their synthetic counterparts (Muduli, Kumar Rout, & Kumar Prusty, 2022). *Ocimum basilicum* L., known for its rich content of biologically active compounds, possesses numerous pharmacological and therapeutic properties (Boulaares, Derouiche, & Niemann, 2024), making it a potential candidate for use as a thrombolytic agent. Research has shown that certain flavonoids can inhibit platelet aggregation, thereby preventing thrombus formation. Additionally, alkaloids have demonstrated antithrombotic effects by significantly breaking down blood clots and extending plasma recalcification time (Ogugofor, Njoku, Njoku, & Batiha, 2022). The observed clot lysis may result from either the combined action of multiple active compounds or the effects of individual components (Shivasharanappa & Londonkar, 2014).

I.8. Anticancer Activity of MnO NPs

Breast cancer stands as a leading cause of mortality in the United States and other nations (Umar et al., 2023). The MTT assay, which employs living cells, is used to assess *in vitro* mammalian cell growth, survival, proliferation, and the potential cytotoxicity of various substances (Srinivasa et al., 2022). Our study revealed that MnO NPs were capable of decreasing viability and inducing cytotoxicity in MCF-7 cells in a dose-dependent fashion over a 24-hour period. Research has shown that several metal oxide nanoparticles exhibit cytotoxic effects on cancer cells but not in healthy cells (Vinardell & Mitjans, 2015). Mahlangeni and Moodley reported that MnOx NPs demonstrated increased cytotoxic effects against A549 and HepG2 cancer cell lines at higher concentrations, but did not significantly affect HEK293 normal cells (Mahlangeni & Moodley, 2021). Research by Majani et al. showed that phyto-synthesized MnO₂ nanoparticles exhibit substantial cytotoxic effects on MCF-7 and MDA-MB-231 breast cancer cell lines, suggesting their potential use as an anticancer agent (Majani et al., 2023). Additionally, Khalilnejad et al. noted that MnO possesses numerous advantageous properties for cancer therapy (Khalilnejad, Mortezaazadeh, & Shayan, 2021). Findings from Feng and Yu's MTT assay revealed IC₅₀ values of 173 and 256 µg/mL for the nanoparticles, respectively. These concentrations represent the levels at which 50% of CAMA-1 and MCF-7 breast carcinoma cells in the culture medium are destroyed (Feng & Yu, 2024). The diminutive size of the nanoparticles facilitates easier penetration of the tumor cell membrane (Das et al., 2023). Nanoparticles with improved surface characteristics can more readily infiltrate tumor cells (Das et al., 2023). The primary mechanism by which nanoparticles induce cancer cell apoptosis is through reactive oxygen species, although other pathways exist. Inhibition of transcription, immunological interventions, site-specific cytotoxicity, up- and down-regulation of proteins, etc. are else action mechanisms of nanoparticles in apoptosis stimulation in cells of cancer (Mundekkad & Cho, 2022). Various nanoparticle types have been linked to cellular damage through oxidative stress, which is considered a common mechanism of action (Fahmy & Cormier, 2009). The harmful effects of oxidative stress can be observed on various cellular structures, including proteins, DNA, and other organelles, potentially leading to apoptosis, which is a form of programmed cell death (Benitha et al., 2023). Notably, cancer cells generally show elevated baseline levels of reactive oxygen species (ROS) compared to normal cells, due to an imbalance in oxidant and antioxidant levels (Nakamura & Takada, 2021). This phenomenon explains why MnO nanoparticles exhibit cytotoxic effects on the MCF-7 cell line without causing harm to healthy cells.

I.9. Catalytic Activity (Dye Degradation) of MnO NPs

Congo red, an anionic dye, is extremely toxic and carcinogenic (Naseem, Farooqi, Begum, & Irfan, 2018). The degradation of Congo red can be visually monitored over time as its color transitions from deep red to a lighter shade. The catalytic effectiveness of MnO NPs was evaluated using both photocatalytic and sonocatalytic methods. These nanoparticles exhibit a substantially increased surface area, which enhances their potential for efficient catalytic degradation of Congo red dye. The superior catalytic performance is linked to the nanoparticles' smaller size, allowing for more effective interaction with dye molecules and resulting in a significant reduction in dye concentration. Furthermore, the use of these nanoparticles in catalytic processes offers a considerable safety advantage due to their enhanced activity, ensuring a high level of protection against potential risks. The cost-effectiveness of MnO NPs in catalytic applications also adds to their appeal as a viable solution for remediating environments contaminated by dyes. Among advanced oxidation processes (AOPs), sonocatalysis and photocatalysis have attracted significant attention due to their eco-friendly nature and the absence of additional chemical requirements (Zarandona, Salazar, Insausti, Lanceros-Méndez, & Zhang, 2024). The synthesis of metal oxide-based photocatalysts has been successfully achieved using various plant extracts (Esnawan et al., 2024). Metal oxide nanoparticles (NPs) find applications in multiple areas, including the degradation of drugs and dyes, as well as wastewater treatment (Lanjwani, Tuzen, Khuhawar, & Saleh, 2024). The mechanism of dye degradation involves electron transfer from the nanoparticle to nearby oxygen species, biomolecules, or other organic compounds (Hendrix et al., 2023). Photocatalytic degradation offers several advantages when compared to conventional methods (Xu et al., 2023). The nanoparticles demonstrated potential photocatalytic activity in breaking down organic pollutant dyes (Krishna et al., 2022). This photocatalytic effect arises from the interaction between light and metallic nanoparticles (Ankita Ojha, 2020). Additionally, the chemical impact of ultrasonic (US) waves, resulting from acoustic cavitation, or the development, expansion, and collapse of bubbles in a liquid, contributes to the sonocatalytic degradation of harmful chemicals (Chauhan et al., 2020). Sonolysis, which involves breaking chemical bonds using ultrasound in aqueous solutions, may enhance the production of free radicals (Abdurahman, Abdullah, & Shoparwe, 2021). Research by Hoseinpour et al. showed that MnO₂ NPs, synthesized using an environmentally friendly method with *Yucca gloriosa*, exhibit photocatalytic properties for dye degradation under visible light conditions (Hoseinpour et al., 2018).

I.10. Biosynthesis and Characterization of Liposomes

Microscopic images revealed that liposomes containing *Ocimum basilicum* L. extract were tiny, round vesicles. These liposomes are vesicular structures with a colloidal nature, consisting of one or more bilayers of lipids enclosing an identical number of aqueous spaces (Teja, Navaneesh, Siddu, Manikanya, & Kumar, 2022). As nanoparticles, liposomes are commonly utilized as a delivery mechanism for various substances, including medications, enzymes, proteins, antioxidants, polyphenols, vitamins, flavors, and fragrances. This is due to their biodegradability, non-toxicity, and ability to encapsulate hydrophilic, amphiphilic, and lipophilic components (Jovanović et al., 2023). Additionally, encapsulation may potentially conceal strong odors and protect active ingredients from degradation (Ben-Fadhel, Maherani, Salmieri, & Lacroix, 2022). Encapsulation offers several key benefits, including enhanced stability of bioactive compounds during storage, protection against harsh environmental conditions and substances in the gastrointestinal tract or skin, improved bioavailability, modified active compound delivery, and extended product shelf life (Jovanović et al., 2023). Research by Caddeo et al. showcased the creation of PEG-modified liposomes for delivering naturally occurring resveratrol, resulting in small, spherical, unilamellar vesicles (Caddeo et al., 2018). In a separate study, Torres-Flores et al. utilized SEM to examine the morphological characteristics of liposomes, revealing that the samples exhibited a spherical form (Torres-Flores, Gonzalez-Horta, Vega-Cantu, Rodriguez, & Rodriguez-Garcia, 2020).

I.11. Liposomes Classification by MobileNet Models

Our model's assessment on the excluded test set resulted in an accuracy of 84.37% and a loss of 0.5543. Combining automation with AI enables the enhancement of specialized therapeutic nanoparticles for particular cell types and individuals (Egorov, Pieters, Korach-Rechtman, Shklover, & Schroeder, 2021). The development of nanoparticle systems can be greatly accelerated by utilizing computer algorithms and computational models that can predict the physico-chemical characteristics of nanosystems (Di Francesco, Boso, Moore, Schrefler, & Decuzzi, 2023). In the field of computer vision, CNNs have gained immense popularity among researchers and are commonly used for various tasks, including facial recognition, image classification, weather forecasting, and object detection (Bouguezzi et al., 2021). As a specific type of CNN, MobileNet offers a high recognition rate while requiring fewer computations and parameters, making it well-suited for use on embedded devices (Liao, Cai, Xu, & He, 2019). Currently, MobileNet is yielding positive results in the domain of image recognition (Zhu, Liu, Yang, & Wang, 2022). The convergence of Machine learning (ML) and computer vision

remains a fruitful area of contemporary research (Khan & Al-Habsi, 2020). Machine learning is utilized in computer vision to examine data from images (Mahadevkar et al., 2022). Cern et al.'s research has effectively employed Quantitative Structure-Property Relationship (QSPR) models in the design of liposomal drugs. This approach exhibited remarkable external validation accuracy, with findings indicating 81.8% accuracy using k-nearest Neighbors (KNN) and 92.3% using Iterative Stochastic Elimination (ISE), highlighting the dependability and efficacy of these models in forecasting drug encapsulation and release behaviors (Cern, Barenholz, Tropsha, & Goldblum, 2014). To our knowledge, this study represents the initial investigation into the use of MobileNet tools for categorizing liposomes as innovative drug delivery systems. Considering the lack of previous studies in this particular application, this groundbreaking work paves the way for new research directions and potential enhancements in drug delivery methodologies' effectiveness.

I.12. *In Vivo* Acute Toxicity Evaluation of *Ocimum basilicum* L. and MnO NPs

The findings from the acute toxicity assessment demonstrated that the oral administration of basil aqueous extract to rats at dosages of 2 and 5 g/kg did not elicit any signs of toxicity or mortality among the subjects. Acute toxicity assessments are characterized as single-dose evaluations that elucidate the manifestations of toxicity and the degree to which it impacts animal subjects (Benrahou et al., 2022). The primary objective of assessing the safety profile of any herbal remedy is to ascertain the nature and significance of any adverse effects and to determine the exposure threshold at which such effects are manifested (Ugwah-Oguejiofor et al., 2019). Given their prolonged use in human practices, it can be anticipated that plants utilized in traditional medicine exhibit minimal toxicity (Yuet Ping, Darah, Chen, Sreeramanan, & Sasidharan, 2013). The pharmacological efficacy of a medicinal plant is contingent upon the presence of its secondary metabolites (Sánchez-Ramos et al., 2021). In acute toxicity studies, certain plants have shown no observable toxic effects and a high degree of safety. Examples include *Portulaca oleracea* (Derouiche, Chetehouna, & Atoussi, 2022) and *Pinus halepensis* (Kaouachi & Derouiche, 2018).

A single-dose evaluation known as acute toxicity testing assesses the impact and indicators of toxicity on animals (Benrahou et al., 2022). The findings revealed that animals given MnO NPs exhibited no signs of toxicity at dosages of 25 and 50 mg/ kg. Motor activity has been widely utilized as a behavioral assessment in toxicology, pharmacology, and aging-neurobiology studies (Djouadi & Derouiche, 2021). In numerous instances, the biological properties (bioavailability, biocompatibility, cell internalization, antioxidant activity) of metal and metal oxide nanoparticles have been enhanced, and their toxicity reduced through plant

extract-mediated synthesis (Djamila, Eddine, Abderrhmane, Nassiba, & Barhoum, 2022). MnO NPs have become increasingly important in synthesis and production processes due to their reduced toxicity (Khan et al., 2020), with reports indicating safe and beneficial applications of green-synthesized manganese oxide nanoparticles. Various nanoparticles, including SeO NPs (Chetehouna et al., 2023), CuO NPs (Derouiche, Bouchoul, Bouchoul, Bya, & Abdemalek, 2022) and ZnO NPs (Djouadi & Derouiche, 2021), have shown no observable toxic effects and demonstrated a high degree of safety when subjected to thorough acute toxicity evaluations.

I.13. Body Weight Gain and Relative Organs Weight

The results demonstrated a significant decrease ($p < 0.05$) in body weight gain in rats treated with MTZ when compared to the control group. Changes in body weight are reliable indicators of an animal's overall health condition (Soren et al., 2019). These alterations can result from various factors, including modifications in growth (such as agents affecting growth hormone or somatostatin secretion), changes in hormonal balance (like agents influencing sex steroid secretion and subsequent maturation patterns), shifts in neurotransmitters that impact food intake (for instance, agents affecting central serotonergic or dopaminergic systems), decreased palatability of diets containing experimental compounds, or general systemic toxicity (Bailey, Zidell, & Perry, 2004). Additionally, weight loss may be attributed to anorexia caused by prolonged exposure to xenobiotics (Derouiche et al., 2019). Research by Chiali et al. demonstrated that metribuzin administration led to a notable decrease in body weight (Chiali et al., 2013).

Conversely, there was a marked increase in body weight gain in BE and BE-LPs relative to the MTZ group. Basil's health benefits are attributed to its high concentration of secondary metabolites, particularly phenolic and flavonoid compounds (Bensaid et al., 2022). The use of liposomes to encapsulate bioactive substances enables effective delivery to specific cells or tissues, maintaining therapeutic levels while minimizing the potential for overdose (Mureşan et al., 2021). Treatments incorporating both BE and BE-LPs appear to effectively promote the restoration of normal body weight gain by mitigating the harmful effects of metribuzin exposure, suggesting that these substances possess inherent protective and therapeutic qualities.

The MTZ group exhibited a notable rise in relative organ weights compared to the control group, while the treatment groups showed a significant reduction when contrasted with the MTZ group. These findings align with Kadeche et al.'s research, which demonstrated increased organs weight following metribuzin exposure (Kadeche et al., 2017). Organ weight alterations serve as highly sensitive indicators of early toxicity, particularly under controlled experimental

conditions (Haschek, Rousseaux, & Wallig, 2010). Rats subjected to subacute metribuzin in experiments likely experienced adverse effects, as evidenced by the substantial increase in both absolute and relative weights of various vital organs (Kadeche et al., 2016).

Liposomes, which encapsulate bioactive compounds, facilitate efficient delivery to cellular or tissue targets and help maintain therapeutic concentrations, thereby reducing the risk of overdose (Mureşan et al., 2021). The therapeutic interventions that incorporate both BE and BE-LPs seem to effectively facilitate the normalization of organ weight, a process that is achieved through the diminishment of the deleterious effects associated with metribuzin exposure, thereby implying that these aforementioned substances possess intrinsic protective and therapeutic properties.

Manganese (Mn) plays a crucial role as an essential micronutrient in the normal development of various organs (Balachandran et al., 2020). Research has shown that synthesizing nanoparticles using plant extracts can enhance their biological properties, such as bioavailability, biocompatibility, cell internalization, and antioxidant activity, while simultaneously reducing toxicity (Djamila et al., 2022). The decreased toxicity of MnO NPs has led to their increased significance in synthesis and production processes (Khan et al., 2020), effectively counteracting the organ hypertrophy caused by metribuzin.

I.14. Brain toxicity markers "Step-through Passive Avoidance Test (passive avoidance learning) and Acetylcholinesterase (AChE) Activity"

The study findings indicated that MTZ negatively impacted rat behavior, learning, and memory, while also increasing AChE levels. Human exposure to pesticides has been strongly linked to various severe health issues, including neurobehavioral disorders (Zeng et al., 2021). To evaluate neurotoxicity at a higher level, behavioral tests are frequently employed to assess the effects of pesticides on cognitive functions such as learning and memory (Authority et al., 2019). In rats, pesticides can lead to spatial learning and memory deficits, as well as neuronal death, through mechanisms involving synaptic damage and increased tau phosphorylation via PP-2A inhibition and GSK-3 β activation (Chen et al., 2012). Both human and animal studies have highlighted the central cholinergic system as a crucial component in learning and memory neural circuitry (Bali, Kaikai, Ba-M'hamed, & Bennis, 2019). Furthermore, long-term metribuzin exposure has been associated with a higher incidence of neurodegenerative diseases (Jyoti et al., 2021). Pesticides have been shown to affect acetylcholinesterase (AChE) activity, resulting in neurological system impacts and various neurotoxic effects (Pathak et al., 2022). AChE activity has been connected to genetic overexpression through oxidative stress (Chetehouna, Derouiche, & Atoussi, 2024). AChE can serve as a biomarker for neurological

conditions like Alzheimer's and Parkinson's diseases (Kumar, Sinha, Rajendra, & Sharma, 2023). Short-term memory is generally related to acetylcholine (ACh) levels, a key neurotransmitter in learning and memory processes. Changes in cholinergic activity are the primary neurochemical alterations observed in Alzheimer's disease (Chetehouna, Derouiche, & Atoussi, 2024).

Research results suggest that phyto and nanotherapy treatments can counteract the negative effects of MTZ on behavior and learning-memory. Herbal plant phytochemicals help maintain brain chemistry by altering the activity of key inhibitory neurotransmitter receptors (Sharma, Bindal, & Mohanty, 2022). Alkaloids have demonstrated superior effectiveness as protective agents in metabolic processes and frequently function as neurotransmitters and signaling systems (Aryal et al., 2022). Biological materials containing polyphenols, tannins, and flavonoids exhibit significant pain-relieving and cholinesterase-inhibiting properties. Furthermore, multiple studies have shown that plant extracts used in treating cognitive disorders often act as AChE inhibitors (Ncir et al., 2018). The active components of these herbs offer potential benefits beyond AChE inhibition, including modifying A β processing, safeguarding against apoptosis and oxidative stress, and providing anti-inflammatory effects (Jivad & Rabiei, 2014).

Liposomes remain a subject of significant interest due to their capacity to enhance bioavailability in the body and shield encapsulated substances while preserving their efficacy (Miere et al., 2021). The components enclosed within liposomal formulations are engineered to improve solubility, minimize side effects, extend drug release, target specific areas, safeguard drugs, and boost therapeutic effectiveness (Rahmi et al., 2022). Consequently, liposomes have demonstrated their utility in delivering plant-based compounds to combat systemic toxins (Kulabhusan et al., 2020).

Research suggests that Mn₃O₄'s ability to imitate the functions of various enzymes involved in oxidative stress regulation may make it a promising candidate for treating neurological disorders caused by oxidative stress, such as Parkinson's disease (Johnston, Du, Zborowski, & Kennedy, 2024). Observed behavioral alterations were linked to notably increased Mn concentrations in brain tissue, indicating that Mn successfully traversed the blood-brain barrier (Sobańska et al., 2021).

I.15. Hematological Markers

The MTZ group exhibited significantly elevated levels of inflammatory biomarkers in comparison to the control group. In contrast, the therapeutic group demonstrated a significant decrease in these biomarkers relative to the MTZ group. The lymphocyte count is an important

auxiliary diagnostic test as changes occur with systemic inflammation and other disease (Boulaares et al., 2020) (Boulaares & Derouiche, 2021). The immune system is a critical target when considering pesticide exposure (Medjdoub et al., 2011). Proposed mechanisms for pesticides' detrimental effects on immunity include their ability to either stimulate or suppress lymphocyte proliferation and cytokine production, as well as induce genetic damage and chromosomal abnormalities in cultured lymphocytes (Medjdoub et al., 2011). Pesticides can directly impact immune organs through oxidative stress or by altering regulatory signaling pathways. This can lead to the suppression of key immune system components, including esterases and serine hydrolases (Lopes-Ferreira et al., 2023).

Ocimum basilicum L., commonly known as basil, is often cited for its anti-inflammatory properties (Güez et al., 2017). The health advantages associated with basil are linked to its rich content of secondary metabolites, specifically phenolic and flavonoid compounds (Bensaid et al., 2022). The anti-inflammatory properties of basil extract can be ascribed to a complex interplay involving the inhibition of pro-inflammatory mediators alongside the enhancement of anti-inflammatory cytokines (Güez et al., 2017). Liposomal formulations exhibit extended-release properties and enhanced anti-inflammatory efficacy (Sogut et al., 2021).

Research has shown that various forms of nanoparticles can exert anti-inflammatory effects (Wang et al., 2021). These entities possess a high surface area to volume ratio, enabling them to have significant surface reactivity. This characteristic facilitates their interaction with biological membranes and enhances their physical transport within the membrane (Agarwal & Shanmugam, 2020). The improved penetration of nanoparticles in epithelial and inflammatory cells contributes to their enhanced efficacy and prolonged therapeutic effect. Furthermore, they demonstrate superior targeting of specific areas, such as inflammatory cells or tissues (Agarwal et al., 2019). The suppression of pro-inflammatory cytokines plays a vital role, as these molecules amplify immune responses and are the primary targets for most nanoparticles (Agarwal et al., 2019).

Our findings regarding erythrocyte line markers revealed a statistically significant elevation in RBC and PLT levels, accompanied by a highly significant reduction in HGB, HCT, MCV, and MCH values in the MTZ group when compared to the control group. Elevated levels of toxic substances can cause considerable harm to the hematopoietic system (Modra et al., 2008). Concerning erythrocyte line markers, the findings for hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) align with those reported by Kadeche et al. However, the outcomes for red blood cell count (RBC) and mean corpuscular hemoglobin concentration (MCHC) were found to be contrary to Kadeche et al.'s observations (Kadeche et al., 2017). The elevated red blood cell count may be to make up

for low blood oxygen levels in the body, which result from cardiac and pulmonic disorders induced by metribuzin. Platelets (PLT) also play a role in bridging innate and adaptive immune responses. Upon activation at sites of inflammation, they release substantial quantities of pro-inflammatory substances stored in their intracellular granules. This process enables communication, recruitment, and activation of leukocytes, endothelial cells, and immune-like cells, even at remote locations (Voudoukis, Karmiris, & Koutroubakis, 2014).

Simultaneously there was a significant amelioration in the levels of previous parameters in BE and BE-LPs compared to MTZ group. Saponins enhance blood cell production by promoting cell survival through the activation of FAK and Erk1/2, while also influencing cytokine production in the bone marrow (Chen et al., 2017). Research on *Ocimum basilicum* L. has utilized multiple analytical methods, uncovering a wide range of biologically active substances. Notably, phenolic compounds serve as important antioxidants and anti-inflammatory substances, helping to counteract the oxidative stress and inflammation that result from metribuzin-induced toxicity in different body organs.

Concurrently, a notable improvement was observed in the levels of previously measured parameters for MnO NPs when compared to the MTZ group. The formation of red blood cells and hemoglobin relies on the presence of manganese (Helbawi et al., 2024). The research findings suggest that MnO NPs, when given to rats, seemed to restore normal levels of erythrocyte line markers. This potential advantage arises from their unique characteristics, including heightened chemical reactivity, expansive surface area, and improved performance speed. They are also protective and therapeutic against the damage caused by metribuzin.

I.16. Biochemical Parameters

Changes in the levels of biochemical parameters are considered one of the most reliable general indicators of toxicity, thus playing a crucial role in toxicity assessment (Khorrami et al., 2019).

I.16.1. Blood Glucose

Our findings indicated that metribuzin administration leads to elevated blood glucose levels. In contrast, significant enhancements were observed in the treatment groups when compared to the MTZ group. This metribuzin-induced hyperglycemia has been previously observed in both rats and fish (Kadeche et al., 2016). Our results align with the work of Kadeche et al., who demonstrated that metribuzin exposure results in increased blood sugar concentrations (Kadeche et al., 2017). The rise in blood glucose following herbicide ingestion

may be attributed to disruptions in carbohydrate metabolism. This disruption could be caused by enhanced liver glycogen breakdown, triggered by increased adrenocorticotrophic and glucagon hormone activity, coupled with decreased insulin function or production (Khozimy, Ramadan, Shaldam, & Aly, 2017). Additionally, the elevated plasma glucose levels in treated rats might be explained by reduced glucose utilization and compromised tissue functionality (Chiali et al., 2013).

Research has shown that basil leaves possess antihyperglycemic and hepatoprotective properties. These effects are attributed to their ability to promote insulin release from the pancreas, suppress hepatic glucose production, and enhance glycogen synthesis (Widjaja, Rusdiana, & Savira, 2019). Flavonoids, one of the active compounds in basil, have been found to stimulate insulin secretion by facilitating the influx of Ca^{2+} ions through calcium channels (Rumengan et al., 2019). Additionally, phenolic acids have demonstrated efficacy in lowering glucose levels and exhibiting various anti-diabetic activities (Deka, Choudhury, & Dey, 2022). Another compound, tannin, functions as an antihyperglycemic agent by inhibiting intestinal glucose absorption and adipogenesis. It also acts as an antioxidant and aids in the regeneration of damaged pancreatic β cells (Rumengan et al., 2019). Saponins have been shown to alleviate high blood sugar levels through various proposed mechanisms. These include enhancing insulin responsiveness, boosting insulin signaling pathways, elevating plasma insulin concentrations, stimulating pancreatic insulin secretion, promoting glycogen synthesis, suppressing gluconeogenesis, blocking α -glucosidase activity, inhibiting the mRNA expression of glycogen phosphorylase and glucose-6 phosphatases, and enhancing Glut 4 expression (Shehadeh, Suaifan, & Abu-Odeh, 2021). Numerous investigations have explored nanocarrier formulations for plant-derived antidiabetic bioactive compounds and extracts. These formulations aim to address the limitations of plant-based products, such as their poor solubility, limited permeability, and low bioavailability (Zolkepli et al., 2022). Among various nanocarriers, liposomal vesicles stand out as exceptional options due to their similarity to cellular membranes and minimal toxicity (Abdelwahab, Abdelmohymen, Mostafa, Magdy, & Mazyed, 2023). Also, the components encapsulated within liposomal formulations are designed to enhance solubility, minimize adverse effects, prolong drug release, target specific regions, protect drugs, and improve therapeutic efficacy (Rahmi et al., 2022).

Studies have shown that nanoparticles composed of manganese oxide significantly influence glucose regulation in diabetic animal models (Ali et al., 2024). According to Adeyemi et al., the observed inhibitory effects of synthesized Mn_3O_4 NPs offer potential as an innovative diabetes treatment option (Adeyemi et al., 2022). The enzymes α -amylase and α -glucosidase facilitate carbohydrate breakdown, producing disaccharides and oligosaccharides in the

digestive system. This enzymatic process elevates blood glucose levels, triggering hyperglycemia in diabetic patients. The primary therapeutic strategy for managing hyperglycemia involves the use of drugs that specifically target these enzymes (Ramasubbu et al., 2023). The proposed mechanism for MnO₂ NPs' blood sugar-lowering effect is linked to the inhibition of α -amylase and α -glucosidase, two enzymes crucial in carbohydrate breakdown (Farhan & Mohammed, 2024). Research by Ahmad et al. demonstrated that green-synthesized manganese nanoparticles show promise as a potential diabetes treatment, with α -amylase and α -glucosidase assays yielding IC₅₀ values of 26.65 μ g/ml and 27.37 μ g/ml, respectively (Ahmad et al., 2024).

I.16.2. Renal Toxicity Markers

The assessment of renal function often involves measuring creatinine, urea, and uric acid levels (Djouadi & Derouiche, 2023). Also, chronic kidney disease is characterized by a decline in renal function, which is linked to imbalances in phosphorus regulation within the body (Birujete et al., 2023). The findings of our study indicated that metribuzin administration significantly elevated levels of creatinine, urea, uric acid, and phosphorus. Conversely, the treatment groups exhibited notable improvements when juxtaposed with the MTZ group. Pesticides have been shown to exert their toxic effects primarily on the kidneys (Ncir et al., 2018). Metribuzin's harmful impact on the kidneys is attributed to oxidative stress induction (Derouiche et al., 2019). The observed increase in serum urea and creatinine concentrations in treated male albino rats may be due to decreased glomerular filtration and impaired kidney tubule function (El-Damaty, Farrag, Rowayshed, & Fahmy, 2012). Our observations are consistent with those of Khozimy et al., who reported elevated blood renal parameter concentrations following metribuzin exposure (Khozimy et al., 2017).

Substances that can reduce the impact of nephrotoxic agents are known as nephroprotective agents (Chinnappan et al., 2019). Research has shown that kidney function and renal antioxidant enzyme levels can be enhanced through the use of basil leaf extract (EL-Hassanin, 2023). The therapeutic properties of plants are attributed to their bioactive phytochemical components, which elicit specific physiological responses in humans. These bioactive constituents, including alkaloids, phenolics, flavonoids, essential oils, tannins, and saponins, are typically responsible for the medicinal benefits of herbal plants (Alomar, 2020). Various analytical techniques have been employed to study *Ocimum basilicum* L., revealing a diverse array of bioactive compounds. Among these, phenols play a crucial role as antioxidants and anti-inflammatory agents, combating oxidative stress and inflammation caused by metribuzin-induced reductions in glomerular filtration and impaired kidney tubule function.

These formulations aim to address the limitations of plant-based products, such as their poor solubility, limited permeability, and low bioavailability (Zolkepli et al., 2022). Among various nanocarriers, liposomal vesicles stand out as exceptional options due to their similarity to cellular membranes and minimal toxicity (Abdelwahab et al., 2023). Also, the components encapsulated within liposomal formulations are designed to enhance solubility, minimize adverse effects, prolong drug release, target specific regions, protect drugs, and improve therapeutic efficacy (Rahmi et al., 2022).

Various manganese oxide nanoparticles (MnOx NPs), including MnO, MnO₂, Mn₂O₃, and the mixed-valence (II/III) Mn₃O₄, are utilized in biomedical applications (Johnston et al., 2024). Manganese is recognized for its role in immune function (Helbawi et al., 2024). Various types of nanoparticles have been found to exhibit potential anti-inflammatory characteristics (Wang et al., 2021). The inhibition of pro-inflammatory cytokines is regarded as a crucial process, given that cytokines amplify immune responses, and this mechanism is employed by nearly all nanoparticles (Agarwal et al., 2019). Research by Asaikkutti et al. demonstrated that Mn-oxide NP supplementation significantly enhanced antioxidant defense system activity and metabolic processes (Asaikkutti et al., 2016). The findings indicate that manganese oxide nanoparticles may provide nephroprotective effects. This potential benefit stems from their distinctive properties, such as increased chemical reactivity, large surface area, and enhanced performance speed. These nanoparticles might operate by regulating metribuzin-induced oxidative stress and inflammation, which are considered the main factors responsible for cell damage, decreased glomerular filtration, and impaired kidney tubule function. These effects ultimately result in elevated levels of creatinine, urea, uric acid, and phosphorus in the blood.

I.16.3. Lipid Profile

This study has noted an elevation in serum total cholesterol, triglycerides, VLDL, LDL and atherogenic index levels with a diminution in HDL levels following metribuzin exposure. It has been reported that metribuzin is most probably to impact lipids profiles (Kadeche et al., 2017). This alteration could stem from increased production or reduced breakdown, as previously documented for other pesticides (Chiali et al., 2013). The rise in serum cholesterol and triglycerides might be linked to enhanced cholesterol synthesis in the liver or liver damage leading to increased cell permeability. For triglycerides, the increase may be associated with inhibited lipolysis in liver triglycerides or blood lipoprotein (Khozimy et al., 2017). Comparable increases in cholesterol and triglyceride levels have been observed in other study examining metribuzin toxicity (Kadeche et al., 2016).

Notably, the study results indicate that administering BE and BE-LPs to rats appeared to normalize levels of serum total cholesterol, triglycerides, VLDL, LDL, HDL and atherogenic index. Numerous plant-based products and herbs have demonstrated lipid-lowering capabilities (Movahedian, Zolfaghari, Sajjadi, & Moknatjou, 2010). Herbal medicine's active compounds can reduce lipid levels through four mechanisms in lipid metabolism: inhibiting cholesterol absorption in enterocytes, decreasing cholesterol production, enhancing reverse cholesterol transport, and promoting hepatic cholesterol excretion (Ji et al., 2019). Various researchers have noted that certain secondary metabolites, including furanocoumarins, saponins, flavonoids, phenolic compounds, and triterpenoids, exhibit hypolipidemic effects (Movahedian et al., 2010). Nevertheless, these plant-based actives face several challenges, such as poor water or lipid solubility, limited permeation, lack of targeting specificity, instability in highly acidic environments, and liver metabolism, etc (Pandey et al., 2021). To address these issues, liposome technology has been applied to natural extracts, helping to overcome their highly hygroscopic structure, low solubility, poor bioavailability, instability, and high dose-induced toxicity (Choi et al., 2023).

Routine measurements include a comprehensive lipid profile, which encompasses total cholesterol, triglyceride, LDL-cholesterol (LDL-C) and HDL-cholesterol (HDL-C) levels (Dasgupta & Wahed, 2021). The research findings suggest that MnO NPs, when given to rats, seemed to restore normal levels of total cholesterol, triglycerides, VLDL, LDL, HDL and atherogenic index. This potential advantage arises from their unique characteristics, including heightened chemical reactivity, expansive surface area, and improved performance speed. These nanoparticles may function by managing metribuzin-induced oxidative stress and inflammation, which are believed to be the primary causes of damage to the liver, a key metabolic organ crucial for lipid metabolism. These processes ultimately lead to increased lipid concentrations in the bloodstream.

I.16.4. Enzymatic Activity

In a comparison between the MTZ group and the control group, significant increases were observed in the activity levels of GOT, GPT, GGT, ALP, CPK, and LDH. The treatment groups exhibited notable improvements when compared to the MTZ group. According to an extensive regulatory toxicology dataset, the liver is identified as metribuzin's primary target organ (Bomann, Tinwell, Jenkinson, & Kluxen, 2021). The most sensitive biomarkers directly associated with the extent of liver damage and toxicity are the activities of ALT, AST, and ALP enzymes (Kadeche et al., 2017). Additionally, blood levels of GGT can serve as a sensitive indicator of liver injury (Ho et al., 2022). Transaminases, which are crucial enzymes involved

in cytolysis, are active in the liver, heart, and muscles. These enzymes enter the serum during hepatic or muscular cytolysis. A significant increase in transaminases is noted in cases of toxic hepatitis-induced cytolysis (Derouiche et al., 2018). The observed elevation in AST and ALT enzyme activities in rats exposed to metribuzin can be attributed to the compound's ability to cause damage to liver cells (Chiali et al., 2013). When albino rats consumed metribuzin, it led to liver malfunction, evidenced by significantly increased ALT, AST, and ALP activities. These changes in hepatocyte function resulted in alterations to the liver membrane's permeability (Khozimy et al., 2017). Our findings align with those of Kadeche et al., who demonstrated that administering metribuzin to rats induced liver toxicity, as indicated by elevated levels of liver damage marker enzymes such as AST, ALT, and ALP (Kadeche et al., 2016). GOT, LDH, and CPK are the three main cardiac enzymes measured. These enzymes, found in heart muscle cells, can be detected in the bloodstream. When cardiac tissue experiences inflammation (myocarditis) or cell death (myocardial infarction) due to various causes, the levels of these enzymes increase (Zheng, Wang, Hou, & Zhang, 2021). Compared to other organs, the heart is more vulnerable to free radical damage because it contains a higher concentration of mitochondria and relatively lower levels of antioxidant enzymes (Boulaares, Derouiche, & Guemari, 2024).

Administration of BE and BE-LPs resulted in reduced activities of GOT, GPT, GGT, ALP, CPK, and LDH compared to the MTZ group. This effect may be attributed to the presence of phytochemical compounds with bioactive properties that have shown efficacy as cardio-protective agents (Boulaares et al., 2024). Polyphenols are recognized as natural substances with various potential health advantages that can lower the likelihood of cardiac and vascular conditions (Liao & Meng, 2023). Certain flavonoids exhibit notable therapeutic effects on liver damage caused by various factors, including chemicals (Wan & Jiang, 2018). The hepatoprotective activity of flavonoids is achieved through multiple mechanisms, such as preserving normal cell membrane fluidity and stability, reversibly inhibiting cytochrome P-450, promoting ribosomal RNA synthesis, decreasing lipid peroxidation levels, reducing DNA damage, and lowering protein carbonylation (Abou Baker, 2022).

Furthermore, research has shown that nanoparticles have been utilized in the treatment of cardiovascular diseases (Agarwal et al., 2019). The observed results suggest that manganese oxide nanoparticles could potentially offer hepatoprotective and cardioprotective benefits. This is due to their unique characteristics, including enhanced chemical reactivity, expansive surface area, and improved performance speed. These nanoparticles may function by modulating metribuzin-induced oxidative stress and inflammation, which are considered the primary

factors responsible for cellular damage and the subsequent release of cellular contents into the bloodstream.

I.17. Oxidative Stress Parameters

While many pharmaceutical treatments are used to address pesticide poisoning in humans, their application is limited due to associated side effects. Research has shown that the generation of oxidative stress is a key mechanism in pesticide-induced toxicity. As a result, there is increasing focus on discovering safe antioxidants and evaluating their potential to reduce the oxidative stress caused by pesticides (Zeng et al., 2021).

Metabolic and physiological changes, as well as various diseases in the body, can be attributed to oxidative stress, which is a significant contributing factor (Derouiche, 2020). Research on male rats has shown that MTZ induces oxidative stress, as evidenced by the assessment of oxidative stress biomarkers (SOD, GPx, GSTs, CAT, GSH, and MDA) in multiple organs, including the liver, brain, lungs, heart, kidneys, testes, and muscle. In comparison to the MTZ group, a notable improvement was observed in the treatment groups. Some pesticides have been found to increase the production of reactive oxygen species (ROS), leading to oxidative stress in non-target organisms (Kadeche et al., 2016). Additionally, metribuzin has been linked to the initiation of intracellular oxidative stress (Derouiche et al., 2018).

Pesticide toxicity is primarily attributed to the generation of oxygen-free radicals, which leads to increased production of reactive oxygen and nitrogen species. This results in enhanced lipid peroxidation across various tissues and a reduction in antioxidant capacity (Medithi et al., 2021). Consequently, lipid peroxidation has been proposed as a key molecular mechanism underlying pesticide-induced toxicity (Ali et al., 2014). This chemical process; lipid peroxidation; capable of disrupting biological membrane structure and function, occurs when free radicals attack lipids and is typically measured by MDA levels (Kadeche et al., 2017). Malondialdehyde (MDA), a byproduct of lipid peroxidation, serves as a biomarker for assessing oxidative stress in diverse biological samples (Cordiano et al., 2023). Research by Kadeche et al. demonstrated that metribuzin treatment significantly elevated MDA levels compared to the control in several organs, including the liver, kidney, brain, and testis (Kadeche et al., 2016).

Glutathione serves as the primary defense mechanism against oxidative stress, playing a crucial role in safeguarding cells from oxidative harm and maintaining redox homeostasis (Basil Ribeiro, 2023). The observed reduction in GSH levels was likely attributed to the generation of substantial amounts of peroxides under metribuzin's influence (Derouiche et al., 2019). Glutathione's capacity to neutralize hydroperoxides formed during metribuzin metabolism,

facilitated by glutathione peroxidase, results in extensive oxidation of glutathione to its oxidized form, leading to a disruption in the GSH/GSSG balance (Derouiche et al., 2018). Research by Chiali et al. demonstrated that liver GSH content decreased steadily as metribuzin doses increased (Chiali et al., 2013).

An imbalance between free radicals and the body's antioxidant enzymes results in oxidative stress (Alam, Khan, & Syed, 2024). Xenobiotics can hinder antioxidant defense mechanisms, including glutathione (GSH), catalase (CAT), glutathione transferase (GST), and superoxide dismutase (SOD), leading to excessive and premature production of free radicals. This process causes damage to macromolecules, such as DNA (Derouiche et al., 2020). The induction of oxidative stress by pesticides can result in free radical generation and modifications to antioxidant or oxygen free-radical scavenging enzymes, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (Gr), and glutathione transferase (GST) (Sengupta & Banerjee, 2014).

Substances known as antioxidants help prevent cell damage caused by oxidative stress through various mechanisms, including catalytic metal chelation, free radical interaction, and oxygen scavenging (Rameshrad et al., 2015). The phytochemical analyses conducted on *Ocimum basilicum* L., utilizing diverse methodologies, reveal the existence of various categories of bioactive compounds; such as phenols that serve a pivotal function as antioxidants in counteracting oxidative stress provoked by metribuzin. Basil has been shown to possess antioxidant qualities due to its phenolic and aromatic components (Lee & Scagel, 2009) because the hydroxyl group found in phenolic compounds attaches to radicals like superoxide, hydroxy peroxide, or hydroxyl radicals, thereby preventing these radicals from causing bodily degeneration (Adámek et al., 2021). Liposomal delivery systems containing antioxidant compounds such as polyphenols, vitamins, and essential oils enhance the antioxidant effectiveness of the constituent while also safeguarding the liposomal system from lipid peroxidation. The membrane structure of liposomes provides an isolated environment that protects entrapped extracts. For instance, oxygen-sensitive compounds achieve improved stabilization by limiting their interaction with oxygen and slowing down the release of extracts (Sogut et al., 2021).

Manganese is regarded as an optimal metal for combating oxidative stress (Aguirre & Culotta, 2012). It serves as a crucial component in Mn-superoxide dismutase (Mn-SOD), which plays a primary role in eliminating reactive oxygen species (ROS) during mitochondrial oxidative stress (Li & Yang, 2018). Nanoparticles (NPs) of metal oxides, particularly manganese dioxide, can effectively mimic the functions of antioxidant enzymes by catalyzing the decomposition of superoxide anions and hydrogen peroxide (Luo et al., 2021). Antioxidant

properties have been observed in biogenic manganese dioxide NPs (Faisal et al., 2022). According to Li et al., MnO₂ NPs can transform excess H₂O₂ in situ into O₂, reversing the process of excessive ROS generation (Li et al., 2021). Furthermore, MnO_x NPs have been suggested to exhibit enzyme-like activities, including those of oxidase, peroxidase, catalase, superoxide dismutase, and glutathione peroxidase (Jiang, Gray, Patel, Zheng, & Yin, 2020).

Research by Singh et al. demonstrated the remarkable capacity of Mn₃O₄ nanoparticles to display the activity of major antioxidant enzymes, such as catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) (N. Singh, Savanur, Srivastava, D'Silva, & Mugesh, 2019).

I.18. Histological Study

All prior evaluations, comprising the observed augmentation in the weight of various organs, the elevated levels of oxidative stress, the assessment of biochemical toxicity, and the inflammatory responses elucidated in the current study, were corroborated by histopathological examinations of organ tissues. Examination of the control rats revealed normal histological characteristics in their liver, brain, lungs, heart, kidneys, and testes. In comparison, the organ tissue samples from the MTZ-treated group demonstrated various structural alterations, including hemorrhage, inflammation, necrosis, and vacuolization. Notably, the treatment groups showed significant improvement, with their organ tissue structures closely approximating those of the control group. Studies using animal models have shown that metribuzin may cause harmful health effects, including changes in in tissue histological (Medjdoub et al., 2011). Additionally, individual pesticides have been shown to cause cellular damage through oxidative stress mechanisms, which encompass both programmed and unprogrammed cell death, damage to lipid membranes, disruption of metabolism, changes in signaling pathways, or impairment of tight junction function (Ilboudo et al., 2014). Exposure to pesticides can also trigger inflammatory responses in tissues and organs (Sajad et al., 2024). Furthermore, pesticides have been found to induce both apoptosis and necrosis (Prabhavathy Das, Pasha Shaik, & Jamil, 2006). These findings help explain the diverse histological changes observed in tissue samples from the group exposed to MTZ.

Ocimum basilicum L. is highly valued for its medicinal and therapeutic qualities, which stem from its rich content of biologically active compounds (Boulaares, Derouiche, & Niemann, 2024). This plant species demonstrates properties that make it effective in combating and preventing metribuzin-induced toxicity. Liposomes, due to their phospholipid composition mirroring cell membranes, are known for their biocompatibility, biodegradability, and lack of immunogenicity. Liposomal formulations are designed to encapsulate active ingredients,

thereby improving solubility, minimizing side effects, extending drug release, targeting specific areas, safeguarding drugs, and boosting therapeutic effectiveness (Rahmi et al., 2022). These formulations help reduce toxicity, enhance pharmacological effects, improve stability, promote distribution to tissue macrophages, enable controlled drug release, and shield against physical and chemical breakdown (Setia et al., 2024).

The improvements noted across all previously mentioned parameters are supported by the results obtained from examining histological tissue samples. Manganese dioxide nanoparticles can catalyze H_2O_2 , leading to O_2 production and Mn^{2+} release in both acidic environments and at physiological pH (7.4). This enables these nanoparticles to sustain oxygen generation and reduce oxidative stress, consequently lessening inflammation (Huang et al., 2023). Research by Kumar et al. revealed that manganese dioxide nanoparticles offer protective benefits to cartilage against inflammation-induced oxidative stress (Kumar et al., 2019). Moreover, Mn_3O_4 nanozyme has been utilized to shield living mice from ear inflammation caused by reactive oxygen species *in vivo* (Jiang et al., 2020).

*Conclusion
&
Perspectives*

Conclusion

The examination of the phytochemical constituents of the aqueous extract of *Ocimum basilicum* L. undertaken through various methodological approaches, demonstrated that this extract is rich in a multitude of bioactive compounds, including phenolic compounds and flavonoids, which confer significant therapeutic advantages. Also, these compounds are essential for the green synthesis of MnO NPs.

Green synthesis of manganese oxide nanoparticles was successfully performed with lower cost and low toxicity by the phytochemical components present in the plant extract to both reduce and stabilize the nanoparticles.

Various analytical techniques were employed to thoroughly characterize the nanoparticles produced using the basil extract. The results indicated that the MnO NPs exhibited a spherical shape with an average size of 6.52 ± 0.88 nm. Furthermore, the chemical analysis revealed that these nanoparticles were composed of Mn_3O_4 and possessed a tetragonal crystal structure.

Ocimum basilicum L. aqueous extract exhibits strong antioxidant, anti-inflammatory, and thrombolytic effects, indicating its high level of safety for use in pharmaceutical and medical applications.

MnO NPs produced through biosynthesis using *Ocimum basilicum* L. aqueous extract demonstrate potent antioxidant, anti-inflammatory, thrombolytic, anticancer and catalytic properties which, qualifying their high degree of safety for usage in the environmental, pharmacological and medicinal domains.

Liposomal drug delivery system of *Ocimum basilicum* L. extract regarded as promising and versatile drug carriers, liposomes offer enhanced characteristics, such as targeted delivery, controlled or sustained release, shielding drugs from degradation and elimination, improved therapeutic outcomes, and reduced adverse effects.

The integration of nanotechnology and pharmaceutical sciences with artificial intelligence facilitated the development of a trained model for identifying liposomes as a Novel Drug Delivery System (NDDS) and assessing the nature and concentration of active ingredients they contain, employing the MobileNet model recognized for its efficacy in image classification. The model's evaluation on a separate test set resulted in an accuracy of 84.37% and a loss value of 0.5543.

The deleterious effects associated with metribuzin toxicity are predominantly facilitated by reactive oxygen species (ROS), which possess the capability to interact with a multitude of biological macromolecules, resulting in acute toxicological manifestations across various organ

systems, including the hepatic, neural, pulmonary, cardiac, renal, reproductive, and muscular tissues.

Ocimum basilicum L. exhibits a wide range of active biological compounds. Notably, phenols and flavonoids serve as potent antioxidants and anti-inflammatory substances, helping to counteract toxicity and cellular damage resulting from metribuzin-induced oxidative stress and inflammation.

The therapeutic and protective benefits of MnO NPs extend to various organs, stemming from their distinctive properties such as increased chemical reactivity, large surface area, and enhanced performance speed. These nanoparticles potentially mitigate the harmful effects of metribuzin by regulating oxidative stress and inflammation, which are considered the main causes of cells damage.

Perspectives

Considering the significance of these findings, they pave the way for future experimental avenues and more comprehensive investigations that should enable us to precisely determine:

- ❖ Assessing the protective and therapeutic effects of liposome-encapsulated basil extract and MnO NPs in rats with cancer disease.
- ❖ Conducting additional, comprehensive and in-depth analyses to ascertain the impact of metribuzin and the associated treatments on DNA.
- ❖ Investigating safe compounds and methods without adverse effects to mitigate organ damage caused by pesticide exposure.
- ❖ Moving towards using nanotherapy and phytotherapy as alternatives to chemical medications for treating acute or chronic conditions.
- ❖ Highlighting the importance of conducting extensive research within the specialized domain concerning innovative methodologies and systems for the effective delivery of novel therapeutic agents such as liposomes.

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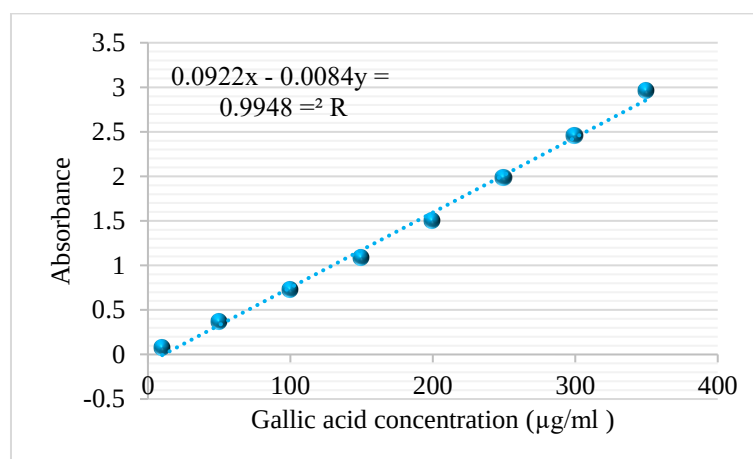
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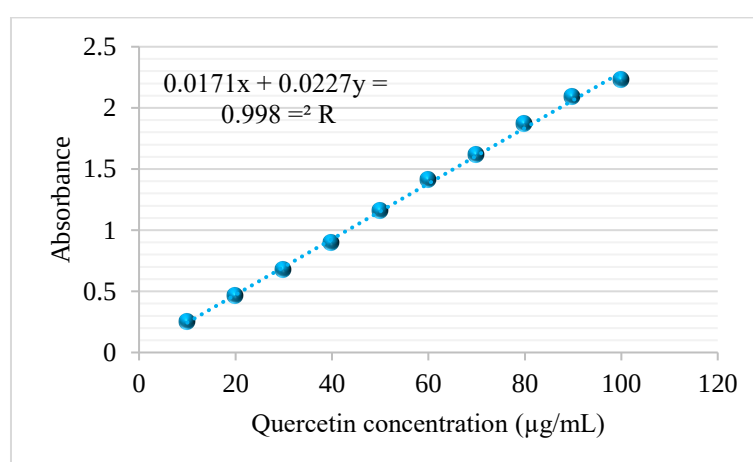
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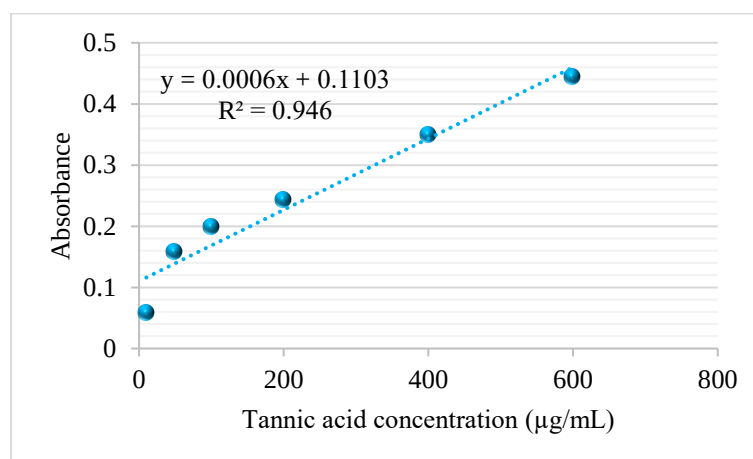
Annex



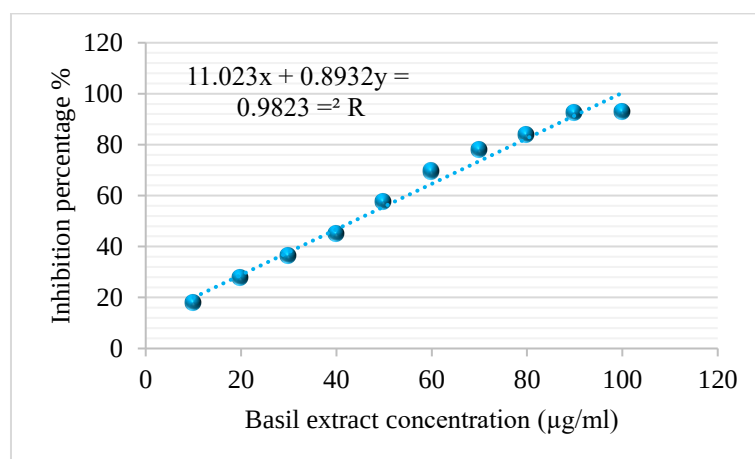
Annex 01: Gallic acid calibration curve for the quantitative determination of polyphenols



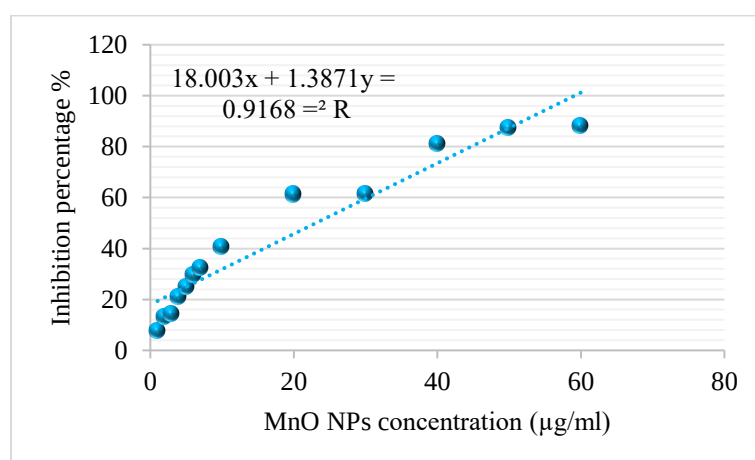
Annex 02: Quercetin calibration curve for the quantitative determination of flavonoids



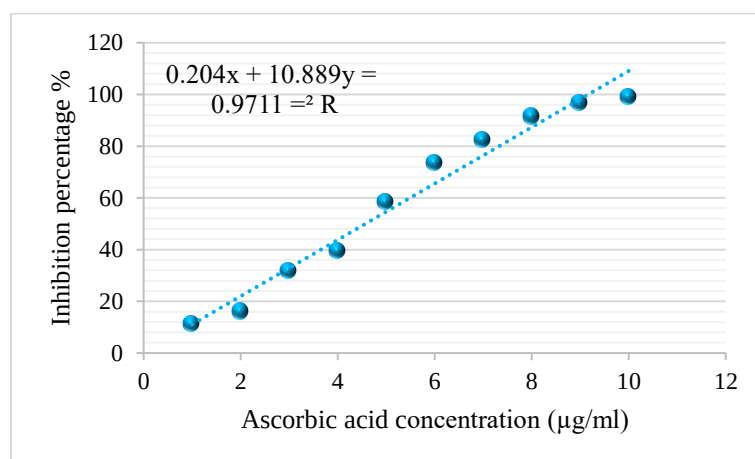
Annex 03: Tannic acid calibration curve for the quantitative determination of hydrolysable tannins



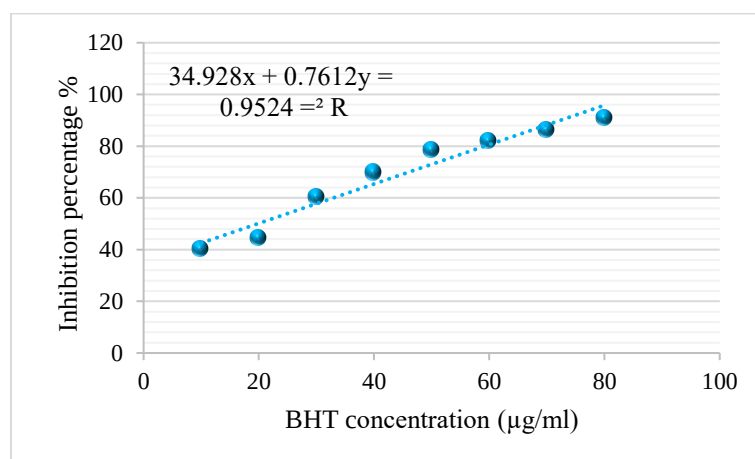
Annex 04: Inhibition percentage values of basil aqueous extract in DPPH test



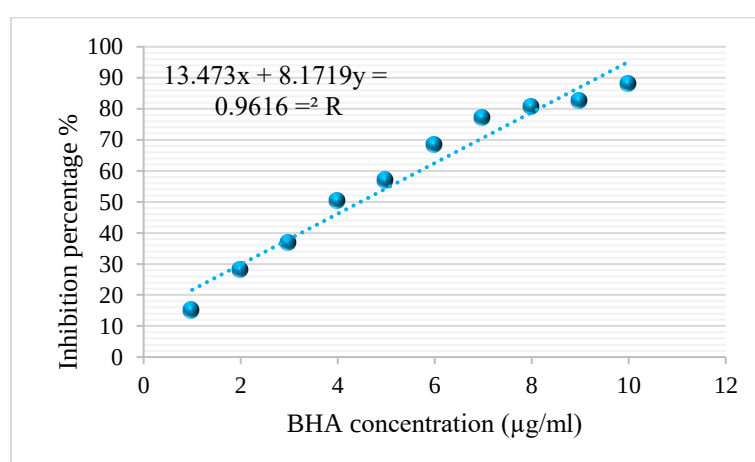
Annex 05: Inhibition percentage values of MnO NPs in DPPH test



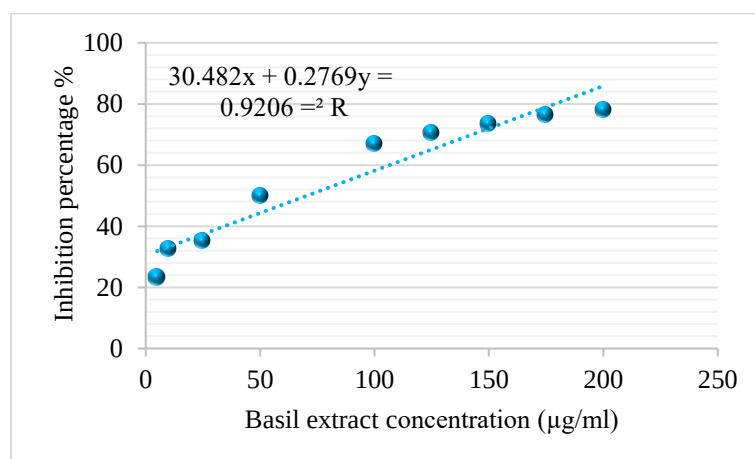
Annex 06: Inhibition percentage values of ascorbic acid in DPPH test



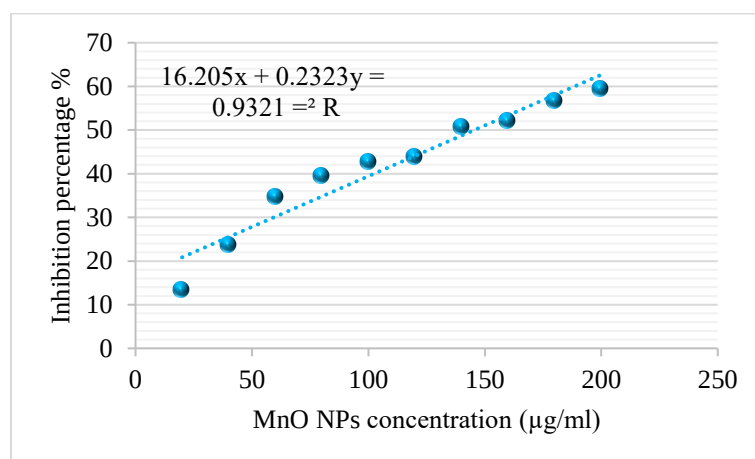
Annex 07: Inhibition percentage values of BHT in DPPH test



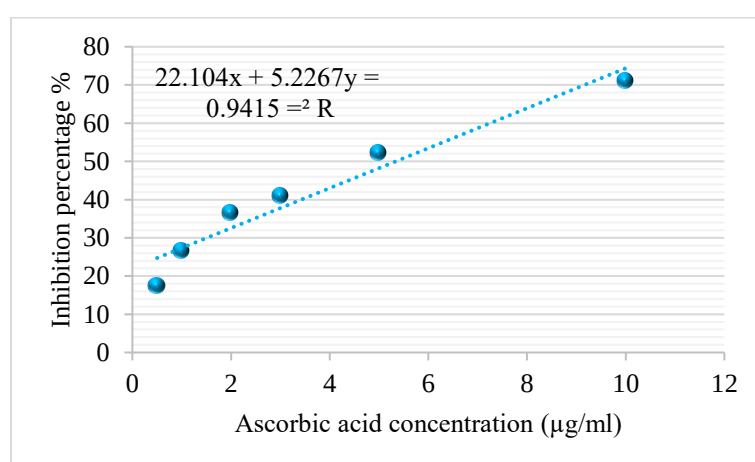
Annex 08: Inhibition percentage values of BHA in DPPH test



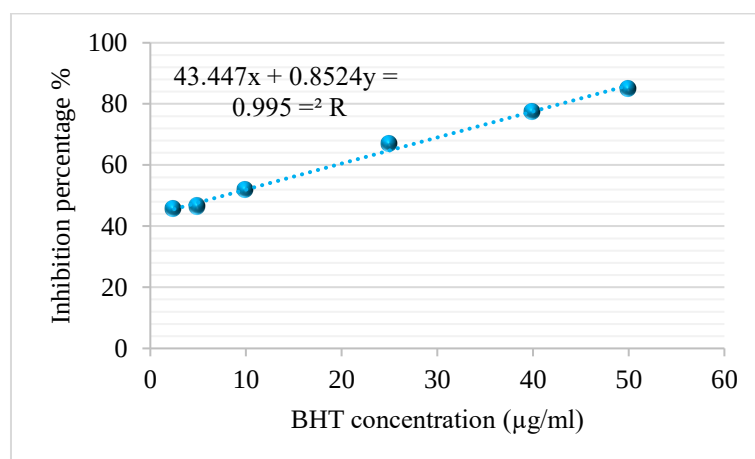
Annex 09: Inhibition percentage values of basil aqueous extract in FRAP test



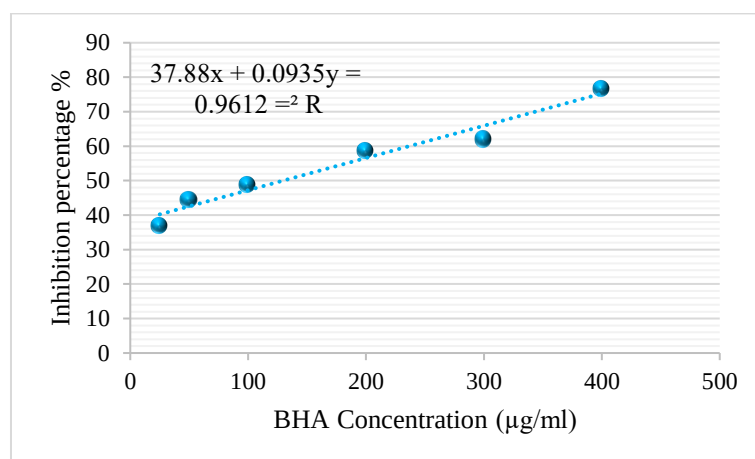
Annex 10: Inhibition percentage values of MnO NPs in FRAP test



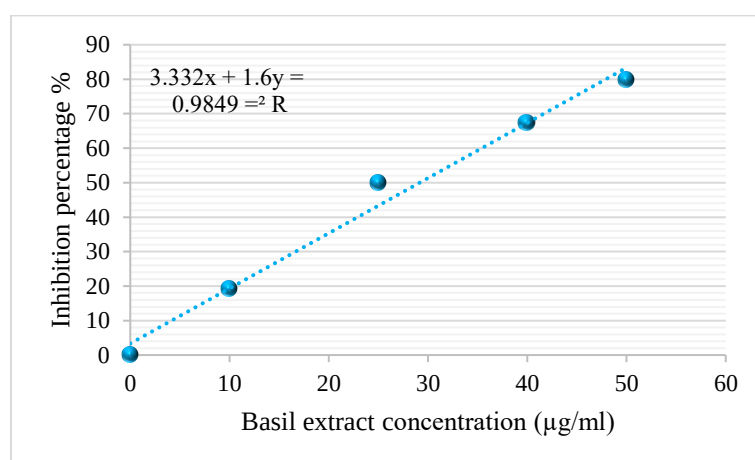
Annex 11: Inhibition percentage values of ascorbic acid in FRAP test



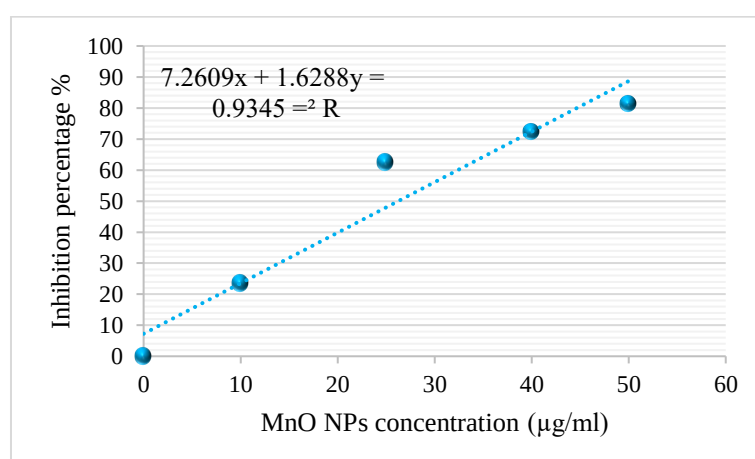
Annex 12: Inhibition percentage values of BHT in FRAP test



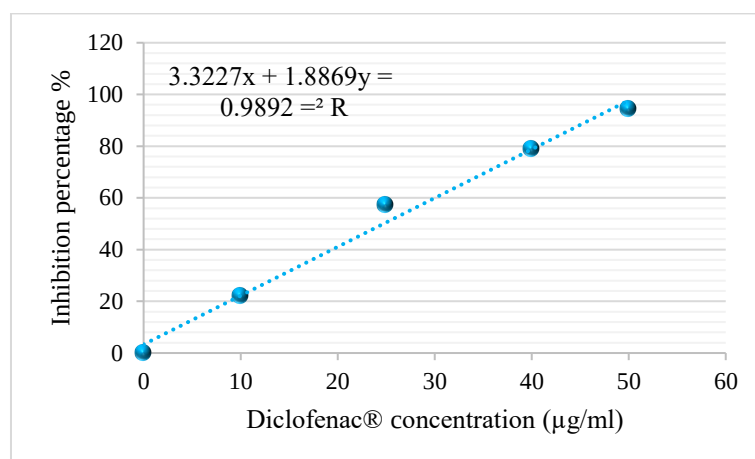
Annex 13: Inhibition percentage values of BHA in FRAP test



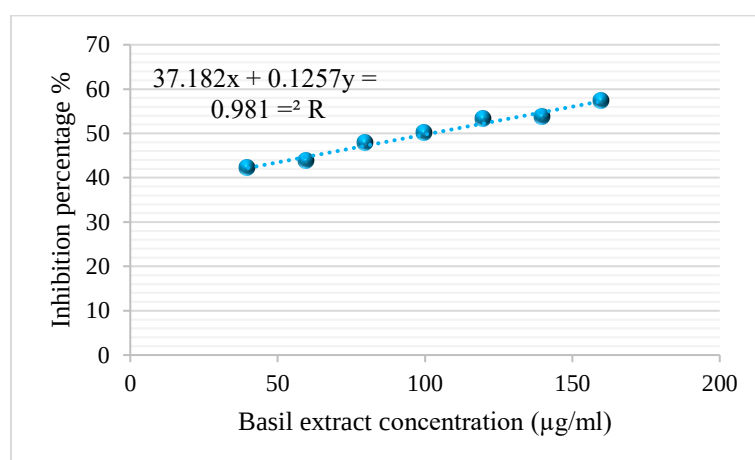
Annex 14: Inhibition percentage values of basil aqueous extract in BSA anti denaturation test



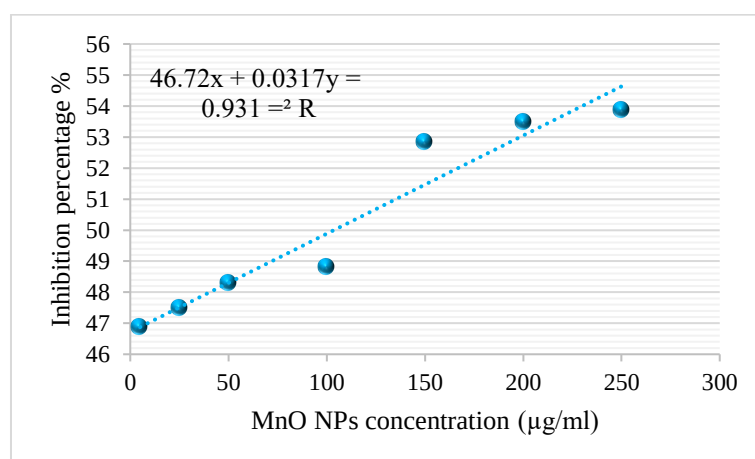
Annex 15: Inhibition percentage values of MnO NPs in BSA anti denaturation test



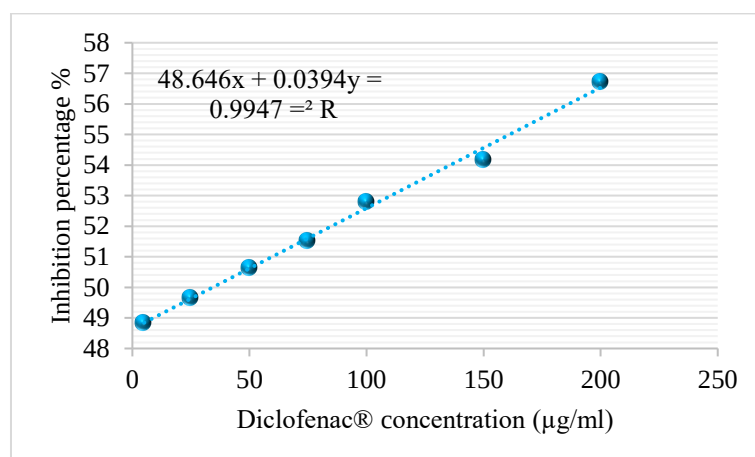
Annex 16: Inhibition percentage values of diclofenac® in BSA anti denaturation test



Annex 17: Inhibition percentage values of basil aqueous extract in RBC membrane stabilization test



Annex 18: Inhibition percentage values of MnO NPs in RBC membrane stabilization test



Annex 19: Inhibition percentage values of diclofenac® in RBC membrane stabilization test