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Theme

Formulation and Effectiveness Evaluation of U_{30}ACS as a New Therapeutic Approach to Alzheimer's Disease in Experimental Rats Model

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اهـــــــــــداء

لحظة لطالما انتظرتها كثيرا... أن أعيش واقعا بات حلما بعيدا بالأمس...متشبثا بأعماق نفسي... ألا و هو أن اتوج مسيرة دراستي و أكون ممن أضافوا رُبَّ قَطرة نافعة في بحر العلم الواسع...فلك الحمد **"ربِّي"** على ما أودعتني...و لك الحمد أن وفقتني على رفع هذا التحدي و أكرمتني بالصبر والثبات و سخرت لي من أجل هذا السبيل أحبتي...من عجزتْ أمامهم كل عبارات الشكر و الثناء و الاحترام...

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تَتدحرجُ حَبةُ رملٍ مُفعمةٍ بالأملْ ..

لا تُغريها إتجاهاتُ الرياح و لأ ينفعُ فيها تَصدُعَ الألمْ،

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Abstract

Our study aimed to investigate the impact of U_{30}ACS that combines phytotherapy by purslane and bio-nanotherapy by zinc and copper as a new therapeutic approach to physiological and biochemical alterations induced by Alzheimer disease (AD) in rats.

For this purpose, thirty males albino Wistar rats were randomly divided into 6 groups (n=5); healthy rats (Control), untreated Alzheimer rats (Exp Alzheimer), Alzheimer rats treated with colloidal solution (U_{30}ACS), Alzheimer rats treated with aqueous extract of *P. oleracea* (AEPo), Alzheimer rats treated with zinc nanoparticles (ZnNPs) and Alzheimer rats treated with copper nanoparticles (CuNPs). All types of treatments were given to rats orally for 21 days. Alzheimer in rats was induced by oral administration of $AlCl_3$ and D-galactose (200mg/kg) in each for 76 days. Various parameters as neurological, hematological, biochemical and oxidative stress markers were estimated. Histopathology of brain and liver tissues were observed. In in-vitro study on plant and nanoparticles, some quantitative, qualitative and characterization parameters were analyzed using standard protocols.

In vitro phytochemical test results revealed that purslane contains most of the principal active molecules, especially alkaloids of various kinds, such as trigonelline, sildenafil, indole and norharman. In vivo results, the Exp Alzheimer group showed an alteration in passive avoidance learning (PAL) and a significant increase in AChE activity ($P<0.01$) and protein levels ($P<0.001$). While, the antioxidant defend system in brain and liver was affected by increasing the MDA level and decreasing the GSH levels, GST and SOD activities compared to control. Furthermore, the hematological parameters revealed that Alzheimer disease induce an inflammation in rats by significant raise ($P<0.01$) of WBC, Monocyte, LYM and platelet ($P<0.001$) levels with a significant perturbation in lipid profile and biochemical parameters. In the other hand, histopathological analysis recorded an deep modification in brain and liver tissues of Alzheimer rats group compared to control. However, the treatment of Alzheimer rats by AEPo, ZnNPs and CuNPs ensured a partial amelioration and correction of the previous parameters. While the U_{30}ACS approach was the best treatment that it give, improvement results in most of the studied parameters.

We conclude that the use of purslane and bio-nanoparticles of zinc and copper seems to be the powerful limited of Alzheimer disease development, which opening the new horizons for the use of nanotherapy in medical approach. Where the new approach (U_{30}ACS) relying on phytotherapy and nanotechnology has proven highly effective against Alzheimer's symptoms, which shed a new lite and a new hopes for the possibility of treating Alzheimer’s patients.

Key words: Alzheimer disease, oxidative stress, *Portulaca oleracea*, U_{30}ACS, ZnNPs, CuNPs.

الملخص

تهدف دراستنا إلى تقييم تأثير العلاج بالنموذج (U_{30}ACS) والذي يجمع بين العلاج الطبيعي بواسطة نبات الرجلة *Portulaca oleracea* والعلاج بجزيئات النانو للزنك والنحاس كنموذج علاجي جديد ضد التغيرات الفيزيولوجية والبيوكيميائية المصاحبة لمرض الزهايمر عند الفئران.

لهذا الغرض، تم تقسيم ثلاثين جرذا ذكرًا من فصيلة ألبينو ويستار عشوائيًا إلى 6 مجموعات (العدد = 5)؛ الجرذان السليمة (الشاهدة)، جرذان مصابة بالزهايمر غير معالجة (Exp Alzheimer)، جرذان مصابة بالزهايمر معالجة بالنموذج (U_{30}ACS)، جرذان مصابة بالزهايمر معالجة بمستخلص مائي من *P. oleracea* (AEPo)، جرذان مصابة بالزهايمر معالجة بجزئيات الزنك النانوية (ZnNPs) و جرذان مصابة بالزهايمر معالجة بالجسيمات النانوية للنحاس (CuNPs). أعطيت جميع أنواع العلاجات للجرذان عن طريق الفم لمدة 21 يوما. تم تحفيز مرض الزهايمر عند الجرذان بواسطة مادتي ثلاثي كلوريد الألمنيوم والجلاكتوز بجرعة يومية 200ملغ/كغ لكل منهما عن طريق الفم لمدة 76 يومًا. وقد تم تقدير مختلف المعايير البيولوجية مثل معايير النقل العصبي، مكونات الدم، المعايير البيوكيميائية والاجهاد التاكسدي. كما تمت الملاحظة المجهرية لانسجة من المخ والكبد. بالنسبة للدراسة التحليلية لمستخلص النبتة و للجسيمات النانوية، قمنا بعض التحاليل الكمية والنوعية باستخدام الطرق الاعتيادية في التحليل.

كشفت نتائج الاختبارات الكمية والنوعية لمستخلص نبات الرجلة انه يحتوي على معظم الجزيئات النشطة الرئيسية ، وخاصة القلويدات مثل تريغونيلين، سيلدينافيل، إندول ونورهارمان. اما نتائج التجربة على الجرذان فان نتائج المجموعة المصابة بالزهايمر أظهرت تغييرًا في اختبار الذاكرة (PAL) وزيادة معنوية كبيرة ($P < 0.01$) في نشاط انزيم الاسيتيل كولين استيراز وايضا في مستويات البروتين ($P < 0.001$). في حين بينت هذه المجموعة خلل في نظام الدفاع المضاد للأكسدة في الدماغ والكبد بزيادة مستوى بيروكسيد الدهون وخفض مستويات الجلوتاثيون، وأنشطة كل من انزيمات نقل الجلوتاثيون (GST) وانزيم ديسميتاز عالي الاكسدة (SOD) وهذا مقارنة مع مجموعة الجرذان الشاهدة. علاوة على ذلك، كشفت معايير مكونات الدم أن مرض الزهايمر يصاحب استجابات التهابية عند الفئران وذلك بزيادة كبيرة ($P < 0.01$) لمستويات كريات الدم البيضاء و اللمفاويات والخلايا وحيدات الخلية والصفائح الدموية ($P < 0.001$) مع اضطراب كبير في المعايير البيوكيميائية ومعايير ايض الدهون. من ناحية أخرى، سجل التحليل النسيجي خللا عميقًا في أنسجة المخ والكبد لمجموعة الجرذان المصابة بالزهايمر مقارنة بالمجموعة الشاهدة. ومع ذلك، فإن نتائج علاج جرذان الزهايمر باستعمال المستخلص المائي لنبات الرجلة او جزيئات النانو للزنك او النحاس اظهرت تصحيح جزئي في معظم المعايير البيولوجية و التحاليل النسيجية السالفة الذكر. في حين أن نموذج U_{30}ACS كان الافضل من حيث النتائج المتحصل عليها حيث اظهرت الجرذان المعالجة به تحسنا كبيرا في مختلف الاعراض المصاحبة لمرض الزهايمر عند الجرذان.

نستنتج أن استخدام نبات الرجلة و الجسيمات النانوية الحيوية للزنك او النحاس له فعالية كبيرة في الحد من تطور مرض الزهايمر، وهو ما يشجع على استعمال النباتات وجزيئات النانو في النهج الطبي. حيث أثبت هذا العمل ان النموذج الجديد (U_{30}ACS) الذي يعتمد على العلاج الطبيعي المدعم بجزيئات النانو فعاليته العالية ضد أعراض مرض الزهايمر، وهو ما يفتح افاق وآمال جديدة في إمكانية علاج مرضى الزهايمر.

الكلمات الرئيسية: مرض الزهايمر، الإجهاد التأكسدي، نبات الرجلة، U_{30}ACS، النانو زنك، النانو نحاس.

ABBREVIATIONS LIST

ACh: Acetylcholine

AChE: Acetyl cholinesterase

AChEI: Acetyl cholinesterase inhibitors

AD: Alzheimer disease

AEPo: Aqueous extract *Portulaca oleracea*

AGE: Advanced glycation end

ApoE: Apolipoprotein E

APP: Amyloid precursor protein

Aβ: Amyloid beta

BACE1: Beta- site APP cleaving enzyme 1

BASO: Basophile

BBB: Blood brain barrier

BHT: Butylated hydroxytoluene

BPS: Phosphate buffer solution

BSA: Bovine serum albumin

bw: Body weight

CDNB: l-chloro-2,4-dinitrobenzene

CNS: Central nerveuse system

CuNPs : Copper nanoparticles

CuO NPs : Copper oxide nanoparticles

d: Dilution factor

D-gal: D-galactose

DPPH: 2,2-diphenyl-1-picrylhydrazyl

DTNB: 5,5'-dithiobis(2-nitrobenzoic acid)

DYRK1A: Dual specificity tyrosine-phosphorylation-regulated kinases activity

EDTA: Ethylenediaminetetra-acetic acid

EOAD: Early onset Alzheimer disease

Exp Alzheimer: Experimental Alzheimer

FRAP assay: Ferric reducing antioxidant power

FTIR: Fourier transform infrared spectroscopy

GPT: Glutamate pyruvate transaminase

GPx: Glutathione peroxidase

GSH: Reduced glutathione

GST: Glutathione-S-transferase

h: Hemorrhage

HBG: Hemoglobin

HDL-C: High-density lipoprotein cholesterol

i: Inflammation

IC50: Inhibition concentration 50

IL-1 β: Interleukin-1β

IL-6: Interleukin-6

IP: Inhibition percentage

LDL-C: Low-density lipoprotein cholesterol

LOAD: Late-onset Alzheimer disease

LPO: Lipid peroxidation

Lymph: Lymphocytes

mAChRs: Metabotropic muscarinic acetyl choline receptors

MAO: Monoamine oxidases

MAP: Microtubules associated proteins

MDA: Malondialdehyde

MNPs: Metallic nanoparticles

Mono: monocytes

mRNA: Messenger ribonucleic acid

n: Necrosis

nAChRs: Ionotropic nicotinic acetyl choline receptors

NBT: Nitro-blue tetrazolium chloride

NFTs: Neurofibrillary tangles

NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells

NPs: Nanoparticles

OD: Optical density

PAL: Passive avoidance learning

PLT: Platelet

PPAR-α: Peroxisome proliferator-activated receptor alpha

Prot: Protein

PSEN1: Presenilin 1

PSEN2: Presenilin 2

RAGE: Advanced glycation end receptor

RBC: Red blood cells

ROS: Reactive oxygen species

SEM: Scanning electron microscope

SEM: Standard error of the mean

SOD: Super oxide dismutase

STL: Step-through latency

TBA: Thiobarbituric acid

TBARS: Thiobarbituric acid reactive substances

TBS: Tris buffer saline

TC: Total cholesterol

TCA: Trichloroacetic acid

TDC: Time spent in the dark chamber

TG: Triglyceride

TNB: 2-nitro-5-mercocapturic acid

TNF-α: Tumor necrosis factor-α

U_{30}ACS: Ultrasonic technique for 30 min aqueous colloidal solution

UA: Uric acid

UHPLC: Ultra high performance liquid chromatography

UV-VIS: Ultraviolet-visible spectroscopy

vc: Vacuolar cytoplasm

VLDL-C: Very low-density lipoprotein cholesterol

WBC: White blood cell

XRD*:* X-ray powder diffraction

ZnNPs : Zinc nanoparticles

ZnO NPs : Zinc oxide nanoparticles

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SUMMARY

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Materials and methods

Results

Discussion

Introduction

Neurodegeneration occurs in various diseases affecting the central nervous system (CNS), the loss of specific populations of neurons related to the functional neuronal networks determines the clinical presentation of acute and chronic neurodegenerative diseases including the Alzheimer disease (AD) (Sairazi & Sirajudeen, 2020). Alzheimer disease is an age-related neurodegenerative disorder, which progressive by nature, where the symptoms of dementia continue to increase and quality of life deteriorates gradually over a number of years (Chiroma et al., 2018). The amyloid hypothesis proposes β-amyloid (Aβ) among the major pathological hallmark of AD of the disease and suggests that misfolding of the extracellular Aβ protein accumulated in senile plaques and the intracellular deposition of misfolded tau protein in neurofibrillary tangles cause memory loss, confusion and result in personality and cognitive decline over time (Chen et al., 2017). The disordered functioning of neurotransmitters in brain, including acetylcholine (Ach), also belongs to AD pathological (Zhang et al., 2016).

Many of recent landmarks in scientific research have shown that in human beings, oxidative stress is an important factor causing physiological alterations and various diseases in the body (Derouiche et al., 2019). The oxidative stress may be a crucial link in AD initiation and development. The imbalance between the generation and elimination of reactive oxygen species (ROS) can cause extensive and lasting damage in the central nervous system (CNS), which finally accelerates the occurrence and development of AD (Wei et al., 2017).

Alzheimer disease is a devastating neurodegenerative disorder with no cure and limited treatment solutions that are unable to target any of the suspected causes (Robinson et al., 2015). Current treatment strategies are aimed to reducing the symptoms and to slow down the progression of the disease (Cavalli et al., 2020).

The use of medicinal plants for the treatment of AD as an alternative therapy, which is a vital focus of researchers. The medicinal plants have been reported to enhance the memory and learning process that normally decline with AD. The phytochemicals have been clinically proven the significant potentials anti-AD by different mechanism of action (Ovais et al., 2018). Purslane (*Portulaca oleracea*) listed in the World Health Organization as one of the most used medicinal plants (Lim & Quah, 2007). It possesses a wide spectrum of pharmacological properties such as neuroprotective, antioxidant, anti-inflammatory, and anticancer activities (Zhou et al., 2015). These effects have been demonstrated to result from various active components.

The emergence of nano-medicine has enriched the knowledge and strategies of treating diseases, and especially some incurable diseases, such as neurodegenerative diseases. The application of nanoparticles in medicine is in the core of nano-medicine (Leng et al., 2018). The

nano-form offer to pass the blood brain barrier (BBB) more than the bulk one and that there is an inverse relationship between particle size and the ability to penetrate the BBB (Simko & Mattsson, 2014). Metallic nanoparticles (MNPs) have considerable interest owing to unique properties of nanoparticles (NPs) such as small size and the greater surface area to volume ratio (Yao et al., 2019). Zinc and copper known as potential metal involved in metabolic organism regulation, where the zinc oxide nanoparticle (ZnNPs) is considered as one of the most promising and novel magic materials because of its unique catalytic and antimicrobial properties as well as its low cost and extensive applications in diverse areas (Erjun et al., 2006). Also the copper nanoparticles (CuNPs), from their unique physical and chemical properties and the low cost of preparation, have been of great interest recently (Honary et al., 2012) copper nanoparticles have potential applications in diverse fields including biomedicine (Rubilar et al., 2013).

Due to the complexity of Alzheimer disease and inability researchers to obtain an appropriate and effective treatment, this allows us to try new treatment strategy including bio-nanotechnology.

In light of these data, the present study was carried out to investigate the two following complementary aspects:

- **The first part:** based on the in-vitro study; extraction of plant extract, preparation of zinc and copper nanoparticles, quantitative and qualitative characterization of these compounds and evaluation of their biological property.
- **The second part:** based on the in-vivo study for evaluation of the therapeutic efficiency of *P.oleracea* leave aqueous extract, ZnNPs, CuNPs and a novel approach (U_{30}ACS) against metabolic, physiological and histological alteration induced by experimental Alzheimer disease in rats.

First part

Bibliographic synthesis

I. Alzheimer disease

I.1. Definition

Alzheimer disease (AD) is the most common form of neurodegenerative dementia in the elderly. It refers to the neurodegenerative brain disorder regardless of clinical status (El Kadmiri et al., 2018). AD characterized by loss of neurons and synapses in the cerebral cortex and certain subcortical regions. This loss results in gross atrophy of the affected regions, including degeneration in the temporal lobe and parietal lobe, and parts of the frontal cortex and cingulate gyrus (Rani et al., 2016). Where the temporal lobe including hippocampus and adjacent amygdala is the starting point of the neurodegenerative process in this neurodegenerative disease (Coupé et al., 2019). In Alzheimer disease, the hippocampus is one of the first regions of the brain to suffer damage and therefore memory loss and disorientation are included among the early symptoms (Rani et al., 2016). The second structure of temporal lobe region is the amygdala; the atrophy of this structure with a rate of change less important than or similar to the hippocampal one (Coupé et al., 2019). Thus, this make the AD as CNS disorder by progressive deterioration of neurons resulting in loss of cognitive behavior, memory impairment, and disturbance in a daily routine activity (Agrawal et al., 2018).

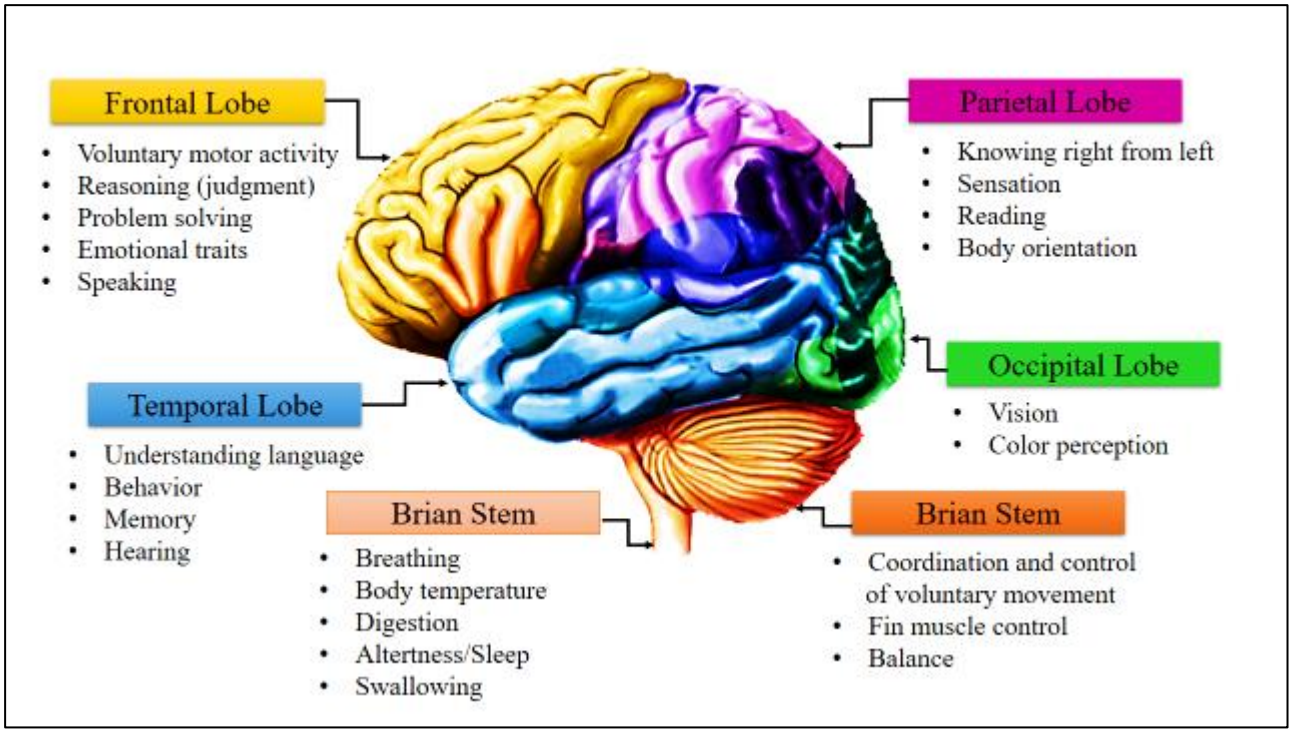


Figure01: Brain lobes fraction and function (Zhang, 2019)

I.2. Pathophysiology

Over the last twenty years, neuroimaging has increasingly contributed to major advances in Alzheimer's disease (AD), especially for the clinical diagnosis and to improve the understanding of the pathophysiological mechanisms of the disease (Perry et al., 2018).

At the macroscopic level, there is the atrophy of the cerebral cortex, hippocampus and amygdala, which in AD appears more sharply due to age. Microscopically it is possible to observe the formation of amyloid plaques, or senile plaques, accumulation of hyperphosphorylated Tau protein, which implies the formation of neurofibrillary tangles, and extensive neuronal loss (Singh et al., 2016).

Recent research showed other mechanisms are related to the formation of these AD markers, ranging from genetic imprint factors as family heritage, and mechanisms that involve apolipoprotein E, the mechanism of oxidation processes culminating in the neurodegeneration process (Dos Santos Picanço et al., 2018).

I.2.1. Genetic Mechanism

Molecular genetic investigation of multiple generations affected by a rare early onset form of Alzheimer disease (EOAD, <65 years) resulted in the identification of the amyloid precursor protein (APP), presenilin 1 (PSEN1) and presenilin 2 (PSEN2) genes as disease genes in AD. Pathogenic mutations in these genes converge to a general mechanism of increased amyloid beta (Aβ) accumulation. While a pathogenic mutation in APP, PSEN1 or PSEN2 is infrequently identified in late-onset Alzheimer disease (LOAD) patients, LOAD is typically considered multifactorial, with a strong polygenic component and an estimated heritability of up to 80%. The most well-known genetic risk factor for AD is the allele ε4 of apolipoprotein E (ApoE) (Verheijen & Sleegers, 2018). The mechanisms that correlate the presence of ApoE with AD are not well understood yet, however, it is being suggested that in such cases there is a decrease in clearance of Aβ in the brain (Dos Santos Picançoa et al., 2018).

I.2.2. Amyloid beta (Aβ) hypothesis

There are several theories, which have been proposed to explain the progression of Alzheimer disease with the amyloid cascade hypothesis remaining the most widely accepted. This hypothesis states that the primary neurologic insult is caused by toxic and soluble Aβ oligomers (Robinson et al., 2015). The transmembrane amyloid precursor protein (APP) is an integral membrane protein expressed in many tissues, especially in the synapses of neurons, which cleaved by β and γ secretases to produce Aβ monomers (Chen et al., 2017), which misfold to form toxic β-sheet oligomers and eventually form larger fibrils and plaques that known as senile

plaque. After the enzymatic processing of APP, Aβ monomers are free to misfold back onto themselves through side chain interactions producing a hairpin structure with residues in the central region and N-terminus forming intermolecular hydrogen bonds with other monomers. If amyloid burden can be reduced, it is speculated that it may be possible to slow the progression of Alzheimer's disease (Robinson et al., 2015).

I.2.3. Protein Tau hypothesis

Microtubules are essential in morphogenesis, cell division, and intracellular trafficking of organelles. Microtubule formation is stabilised by a protein family called Microtubules Associated Proteins (MAP). A major member of the MAP family is tau protein (Al-Hilaly et al., 2017). Hyperphosphorylation of Tau occurs during the course of AD, and is known to underlie the missorting of tau from a primarily axonal to a somatodendritic location in neurons. This alteration is thought to contribute not only to the deregulation of microtubule dynamics, but also to tau polymerization, aggregation and the formation of neurofibrillary tangles (NFTs) are a fundamental neuropathological hallmark of AD (Metaxas & Kempf, 2016).

I.2.4. Cholinergic hypothesis

Acetylcholine (ACh) is the neurotransmitter used by all cholinergic neurons, which exerts its physiological functions by activating either ionotropic nicotinic ACh receptors (nAChRs) or metabotropic muscarinic ACh receptors (mAChRs) (Jiang et al., 2014). Thus, it has a very important role in the peripheral and central nervous systems that are widely distributed. The cholinergic system is pointed as an important factor in many forms of dementia including AD, by its involving in critical physiological processes, such as attention, learning, memory, and sensory information (Ferreira-Vieira et al., 2016). The third important hallmark of A is cholinergic hypofunction that is closely linked to amyloid β and tau pathologies. In AD brains there are reduced choline acetyltransferase levels accompanied by decreased ACh synthesis, significant loss of cholinergic neurons, reduction in the numbers of postsynaptic neurons accessible to Ach, cholinergic neuronal and axonal abnormalities and reduction in nAChR levels (Jiang et al., 2014).

I.2.5. Oxidative stress

Oxidative stress, is a process increased in the brain with aging, is induced by an imbalance in the redox state, involving the generation of excess reactive oxygen species (ROS) or the dysfunction of the antioxidant system. Consequently, oxidative stress on nervous tissue may seriously damage the brain via several interacting mechanisms. The dysfunction mitochondrial mechanisms constitutes a risk for developing Alzheimer's disease (AD), when no efficient antioxidant system is available leads to neuron degeneration in AD are believed to be associated

with ROS generation, activation of mitochondrial permeability transition, excitotoxicity, impaired production of adenosine triphosphate and altered calcium homeostasis (Huang et al., 2016).

I.2.6. Inflammatory mechanism

Inflammation has been strongly implicated in the pathogenesis of Alzheimer disease, and associated with increased populations of activated microglia and astrocytes. Accordingly, multiple inflammatory stimuli can trigger the activation of microglia and astrocytes; these stimuli can be peripheral (e.g., systemic infections and peripheral chronic inflammation) or local (e.g., brain injury and the presence of various forms of Aβ and tau proteins). Activated microglia and astrocytes release neurotoxic proinflammatory cytokines such as interleukin-1β, tumor necrosis factor-α (TNF-α), and interleukin-6 (Ng et al., 2015). Production of these molecules can perpetuate a cycle of neuroinflammatory processes including amyloidosis, neuronal death, cortical thinning, reduced brain volume, infarcts and neurodegeneration (McGrattan et al., 2019).

I.3. Therapeutic approach for Alzheimer disease

There is no cure for AD; however, drug treatments are available to help relieve symptoms in several aspects of the disease, and researchers around the world are focusing on finding better treatments, preventive strategies, and ultimately cure. A variety of cellular mechanisms can lead to the generation of Alzheimer's disease (Singh et al., 2016).

I.3.1. Aβ- targeting therapies

Amyloid precursor protein (APP) is a transmembrane protein that has both cytoplasmic and extracellular cleavage products that are relevant in Alzheimer disease, APP is initially cleaved via α-secretase or β-secretase, which leads to normal or pathological products, respectively (Panek et al., 2017). Therefore, therapeutic targeting of this protein processing is an attractive target. Thus, both α-secretase agonists and β-secretase antagonists could act as Alzheimer therapeutics. Following α or β cleavage, γ-secretase cleavage yields extracellular p3 or Aβ peptides respectively, therefore inhibitors of β and γ secreatas could be one of the main targets for the development of therapeutics (Arbor et al., 2016).

I.3.2. Tau-targeting therapies

Although most drug development efforts so far have focused on Aβ, interest in tau as a drug target is currently growing. Several approaches are being employed which target various aspects of tau pathogenesis, including modulation of tau gene expression, modulation of posttranslational modifications, immunotherapy, and microtubule stabilization (Wu et al.,

2017). In addition, the block tau spreading, tau anti-aggregates, tau kinase inhibitors, and gene therapy approaches that inhibit tau production are also in development (Grundman, 2019).

I.3.3. Acetylcholinesterase inhibitors

The competitive inhibitors of the acetyl cholinesterase (AChE) that cleaved the Ach, which is a neurotransmitter molecule that associated with memory function, is the mostly wide used drugs to treat Alzheimer's disease (AD) patients (Ferreira-Vieira et al., 2016). The acetylcholinesterase inhibitors (AChEI) were developed as an effect of the cholinergic assumption of cognitive reduction. AChEI compounds are demonstrated for the symptomatic treatment of mild-to-moderate AD. They inhibit AChE, which is accountable for the separation of acetylcholine (ACh) (Turkan et al., 2019).

I.3.4. Complementary treatments applied to AD

The anti-inflammatory drugs act by reducing the inflammatory response in the brain tissue. It inhibits the activation of astrocytes in brain tissue and several other signaling cascades related to inflammation and oxidative stress. Thus, these activities favor the inhibition of the inflammatory processes associated with the physiology of AD and reduce the deleterious consequences of this disease (Dos Santos Picanço et al., 2018).

The aggregation of Aβ amyloid plaques and, consequently, the formation of reactive oxygen species (ROS), that are typical, for instance, of the first stages of AD (Piemontese, 2017). The ROS-mediated pathway is related to AD. It has been suggested that ROS levels are increased and oxidative stress occurs. For this reason, targeting ROS or ROS-related enzymes has been a therapeutic strategy in AD (Chun & Lee, 2018).

II. *Portulaca oleracea* L.

II. 1. Definition and localisation

Purslane (*Portulaca oleracea* L.) is an annual herbaceous plant belongs to the family Portulacaceae, it is cosmopolitan in distribution and occurring particularly in tropical and subtropical areas (Iranshahy et al., 2017). Purslane used as a potherb in Asian, central European and Mediterranean countries, which is utilized as one of medicinal plants and has been given term "Global Panacea", it also is popular as a traditional medicine in Algeria for the treatment of various diseases (Derouiche et al., 2017). *P. oleracea* is known to contain many active substances are also considered as sources of many dietary supplement (Derouiche et al., 2019). It has several pharmacological effects including; neuroprotective, hepatoprotective, antioxidant, anti-inflammatory and immunomodulatory effects (Khazdair et al., 2019).

II. 2. Botanical description and scientific classification

II. 2.1. Botanical description

Portulaca oleracea L. is considered invasive weed, which may reach 40 cm in hight (Chowdhary et al., 2013). Leaves are alternate, fleshy, obovate or spathulate with a cuneate base and obtuse apex, smooth and waxy on upper surface, margins are sometimes purple; sessile or indistinctly petiolate, 1–3 cm long, 0.5–1.5 cm wide. Flowers are solitary or clustered axillary or terminal, surrounded by 2 glabrous bracts; 2 unequal sepals, 5 glabrous yellow petals, stamens 6–15. Fruit are brown rounded capsule, 6–10 mm long, opening at top with lid. Seeds are numerous, small, 0.8 mm broad, reniform, and black in color (Alam et al., 2014).

II. 2.2. Taxonomy (Chugh et al., 2019)

Kingdom: Plantae
Subkingdom: Tracheobionta
Superdivision: Spermatophyta
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Caryophyllidae
Order: Caryophyllales
Family: Portulacaceae
Genus: Portulaca L.
Species: *Portulaca oleracea* L.

Figure 02: *Portulaca oleracea* L.

(Original photo)

II.3. Chemical composition

Purslane presents a variable chemical constituents mainly belong to flavonoids, alkaloids, terpenoids and organic acid and other classes of natural compounds including fatty acids, vitamins, and minerals (Sadhana & Deepali, 2017).

Flavonoids are one of the main active ingredients of purslane such as quercetin, kaempferol, myricetin, luteolin, and apigenin, (Andarwulan et al., 2015). Oleraceins A, Oleraceins B, Oleraceins C, Oleraceins D and Oleraceins E are exist in *P. oleracea*. Other alkaloids like dopa, dopamine and noradrenaline are also reported which are higher in leaves then in stem and seeds (Syed et al., 2016). *Portulaca oleracea* contains monoterpenes such as portulosides A and B, diterpenes as portulene, and βamyrin type triterpenoids (Zhou et al., 2015). It has high levels of tocopherols, vitamin C and some vitamins of complex-B (Petropoulos et al., 2016). Purslane has been demonstrated to be one of the major plant sources of omega-3 fatty acids, particularly α-linolenic acid (up to 30%) and other essential fatty acids such as palmitoleic, palmitic, linoleic, oleic, stearic eicosapentaenoic and docosahexaenoic acids (Iranshahy et al., 2017). Dietary minerals such as iron (Fe), zinc (Zn), potassium (K), boron (B), nitrogen (N), manganese (Mn), calcium (Ca), copper (Cu), magnesium (Mg) are the most abundant in purslane plant, whereas other minerals (i.e. phosphorus (P), sulfur (S), sodium (Na)) that exist in relative lower amounts, they also contribute to purslane valuable nutritional profile (Petropoulos et al., 2016).

II.4. Pharmacological propriety

Portulaca oleracea is considerable importance to the food industry and also possesses a wide spectrum of pharmacological properties, which are associated with its diverse chemical constituents, including flavonoids, alkaloids, polysaccharides, fatty acids, terpenoids, sterols, proteins, vitamins and minerals (Zhou et al., 2015).

Purslane has a distinguished antioxidant capacity, which is attributed to high vitamin A content along with ascorbic acid and various flavonoids and polyphenolics. These provide direct free radical scavenging activity and enhance the activity of many enzymes such as glutathione reductase, glutathione peroxidase, superoxide dismutase, and catalase (Amin et al., 2020). The possible neuroprotection mechanism could be related to its antioxidant activity (Martins et al., 2016). When the alkaloid plant extract significantly inhibited acetyl cholinesterase (AChE) activity, where the use of AChE inhibitors has been a promising treatment strategy for Alzheimer's disease (AD); therefore, purslane may be an effective agent for the prophylaxis and treatment of AD (Zhou et al., 2015).

P. oleracea has an anti-inflammatory activity, which inhibit the production of inflammatory mediators such as NO and pro-inflammatory cytokines including the interleukins IL-1β and IL-6. The purslane extracts in a dose- dependent manner significantly inhibited tumor necrosis factor (TNF-α) that induce intracellular reactive oxygen species (ROS) production (Khazdair et al., 2019).The immunomodulating activity of purslane mainly is related to the polysaccharides. They activated phagocytes that are involved in the first mechanism of innate defense and the process of inflammation and, as a result, stimulate the activity of the immune system (Rahimi et al., 2019)

As the oxidative stress and free radical formation have, a key role in propagation of remote organ in jury the hepatoprotective activity of purslane was linked to their anti-oxidant and anti-inflammatory activity (Askaripour et al., 2016). In addition, plant extract regulates the hepatic marker enzymes (Syed et al., 2016).

III. Nanoparticles

III. 1. Definition

Nanotechnology is one of the most promising technologies of the 21st century. It is the ability to observe measure, manipulate, assemble, control, and manufacture matter at the nanometer scale generally from 1 to 100 nm (Bayda et al., 2019). Although this is the most used definition, a size limitation of nanotechnology to a below 100-nm range seems to exclude numerous materials and devices, particularly in the pharmaceutical area. For that reason, some experts disagree with a rigid definition based on a sub-100 nm size (Silvestre et al., 2015).

III.2. Nanomedcine

Medical applications of nanotechnology (commonly known as nanomedicine) are expected to significantly improve disease diagnostic and therapeutic modalities and subsequently reduce health care costs. The use of nanotechnology in medicine has the potential to significantly improve human health and wellbeing due to highly accurate and sensitive diagnostic tests (Satalkar et al., 2015).

III.3. Metal nanoparticles

The metal nanoparticles exhibit many properties that are very useful or can be manipulated for numerous new scientific studies and multitudes of technological applications. Recently, there are advances in the use of metal nanoparticles in the study of biomolecular interactions, developing bioassays and biomedical devices, and various other biomedical applications such as immunodiagnostics, drug delivery, therapeutics, and gene transfer (Mei & Wu, 2017). Nanoparticles have great interest due to their extremely small size and large surface to volume ratio, which lead to differ in their property such as the biological one, compared to bulk of the same chemical composition (Iravani, 2011).

III.4. Zinc and copper nanoparticles

Transition metal oxides nanoparticles (NPs) have received the most attention in a variety of industrial applications such as cosmetics and medicine. Zinc and copper oxides (ZnO and CuO) NPs are the most common and attractive metal oxide NPs in industries (Rafiei et al., 2014).

III.4.1. Zinc oxide nanoparticles (ZnNPs)

The ZnNPs, characterized by its small size facilitates the absorption of zinc by the body, which make it attractive in the biomedical application (Jiang et al., 2018). Where zinc (Zn) is a

well-known as essential trace element that is highly distributed and plentiful, it plays an important role in many cellular functions including neurotransmission, gene regulation, structural maintenance, enzymatic activity and protect the blood-brain barrier (BBB) from oxidative damage and prevent the progress of neurodegenerative diseases. Thus, ZnO NPs have drawn the attention of many biotechnologists as it has useful properties with potentially unlimited therapeutic applications (Hafez & Gad, 2018).

III.4.2. Copper oxide nanoparticles (CuNPs)

According to the distinctive properties of copper, the copper oxide (CuO) based nanomaterials are gaining importance in biomedical sciences that includes both, diagnosis and therapy (Verma & Kumar, 2019). Thus, the research is increasingly carried out on replacement of standard Cu forms with nanoparticles (CuNPs), due to their very small size and specific surface area; it is assumed that, they may be differently utilized by the body than their macro counterparts. This suggests that they may be better absorbed in the body (Cholewińska et al., 2018). Moreover, copper is an essential trace element that is required as a cofactor for normal functioning of various metabolic enzymes. This property can be used to synthesize antitumor formulations that induce killing of tumor/diseased cells by altering the intracellular level of copper ions. Due to their nano size, they are easily accessible to the micrometer-sized human cellular entity and can readily interact with the biomolecules present on the cell surface and intracellularly (Verma & Kumar, 2019).

III.5. Green synthesize of nanometals

The green synthesis of nanoparticles is a novel way to synthesize nanoparticles by using biological sources. It is gaining attention and attracted the scientists from different fields due to its simplicity, and environmentally-friendly nature (Singha et al., 2014), especially in light of efforts to find greener methods of inorganic material synthesis (Philip, 2009), due to the growing need to develop environmentally benign technologies in material synthesis (Bar et al., 2009). The biosynthesis for obtaining nanoparticles using naturally occurring reagents such as vitamins, sugars, plant extracts, biodegradable polymers, and microorganisms as reductants and capping agents could be considered attractive for nanotechnology (Kharissova et al., 2013). Biosynthesis of nanoparticles is more unique and reliable not only because of its less toxicity as compared to some of physicochemical production methods but also because it can be used to produce large quantities of nanoparticles that have well shape and size. The biological synthesis approaches may actually produce nanoparticles of a better defined size and morphology as compared to some of other physicochemical methods of production (Khandel et al., 2018).

Second part

Experimental part

Materials and Methods

I. Materials

I.1. Plant material

The purslane (*Portulaca oleracea* L.) were collected in August from a village in Touggourt of Ouargla state, Algeria. The leaves were washed with distilled water, then dried at room temperature. The completely dried purslane leaves was powdered by using a mechanical grinder. The powder stored at room temperature in the airtight containers until the use.

Figure 03: *Portulaca oleracea* leaves **(original photo)**

I.2. Animals

In this study, thirty males albino Wistar rats at the age of 8 weeks old, weighting 131.28 ± 5.58 g, obtained from the Institute Pasteur of Algiers, and were housed in plastic cages at animal room of molecular and cellular biology department, of nature and life sciences faculty, in Echahid Hamma Lakhdar-El-Oued university, Algeria. With a temperature of 25 ± 2°C, the rat were given a free access to their standard diet and tap water during the study. The animals were adapted to an inverse 12:12 h light/dark cycle. All experimental procedures employed, as well as rat care and handling, were in accordance with guidelines provided by the local ethics.

Tableau 01: Standard diet composition (Southon et al., 1984)

Raw materials	Quantity (g/kg)	Percentage (%)
Maize	326	32.6
Cellulose	326	32.6
Protein	168	16.8
Sacrose	60	6
Vitamin	40	4
Minerals	40	4
Oil	40	4

I.2.1. Experimental Alzheimer induction

Alzheimer state was induced by oral administration 200 mg/kg b.w of both aluminum trichloride ($AlCl_3$) and D-galactose in drinking water during 76 days (Zhang et al., 2016 ; Debbache-Benaida et al., 2018). Therefore, treatment of $AlCl_3$ + D-gal to normal rats is usually considered as a model of Alzheimer. The co-administration of $AlCl_3$ and D-gal for one week could induce an ideal AD animal, which may produce neurodegenerative and neurobehavioral alterations similar to AD (Liaquat et al., 2017).

I.2.2. Experimental design

After two weeks of acclimatization, the animals were divided into six groups, 5 in each of, Alzheimer disease was induced by oral administration of 200 mg/kg b.w in drinking water for 76 days, the rats treated during 21 days as following:

Group 01 (Control): Healthy rats received distilled water.

Group 02 (Exp Alzheimer): Experimental Alzheimer rats received distilled water.

Group 03 (U_{30}ACS): Experimental Alzheimer rats treated orally by colloidal solution which contain the aqueous extract of *P. oleracea* (2g/Kg b.w/wk), ZnNPs (7mg/Kg b.w/wk) and CuNPs (3mg/kg b.w/wk).

Group 04 (AEPo): Experimental Alzheimer rats treated orally by aqueous extract of *P.oleracea* (2g/Kg b.w/wk).

Group 05 (ZnNPs): Experimental Alzheimer rats treated orally by zinc oxide nanoparticles (7mg/Kg b.w/wk).

Group 06 (CuNPs): Experimental Alzheimer rats treated orally by copper oxide nanoparticles (3mg/Kg b.w/wk).

The colloidal solution obtained by ultrasonic technique for 30 min. The doses of *P.oleracea* according to (Derouiche et al., 2019). But for the ZnNPs and the CuNPs were chosen according to our experimental study.

I.2.4. Sacrifice, Blood sampling and tissue collection

At the end of each treatment, and after 12 hours of fasting, animals were sacrified under slight anesthesia by Chloroform (94%) by inhalation; blood sampling was performed during the decapitation and blood samples were transferred into EDTA tubes to carried the hematological parameters and dry tubes previously labeled and numbered for each rat. The blood was separated by centrifugation for 3000 revolutions/min during 15 min, the obtained serum stored at -20°C until the use for biochemical analysis; fasting blood glucose level obtained by glucometer for each rat. Liver and brain were carefully removed, rinsed in NaCl 0.9% then weighed. Also, The organs stored in the freezer at -20°C until the preparation of homogenates for the determination of neurotransmitter and oxidative stress parameters.

I.3. Reagent and products

Hydrogen chloride (HCl), Methanol, Chloroform, Mayer reagent, Wagner reagent, Sulfuric acid, Fehling solution, Folin-Ciocalteu reagent (FCR), Magnesium (Mg), Sodium carbonate (Na_2CO_3), Aluminum trichloride ($AlCl_3$), galactose ($C_6H_{12}O_6$), Ferric chloride ($FeCl_3$), Potassium ferricyanide (K_3Fe), Zinc sulfate ($ZnSO_4$, $7H_2O$), Copper sulfate ($CuSO_4$, $5H_2O$), Bovine serum albumin (BSA), Gallic acid, Tris, Sodium chloride (NaCl) , Ammoniac, Nitric acid (HNO_3), Salicylic acid, Coomassie brilliant blue, Trichloroacetic acid (TCA), Thiobarbituric acid (TBA), Butylated hydroxytoluene (BHT), Phosphate buffer (Dipotassium phosphate K_2HPO_4 & Potassium dihydrogen phosphate KH_2PO_4), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), Reduced glutathione (GSH), l-chloro-2,4-dinitrobenzene (CDNB), Sodium hydroxide (NAOH), Ethylenediaminetetra-acetic Acid (EDTA), Methionine, Nitro-blue tetrazolium chloride (NBT), Riboflavin, Acetylcholine (Ach) and Ethanol.

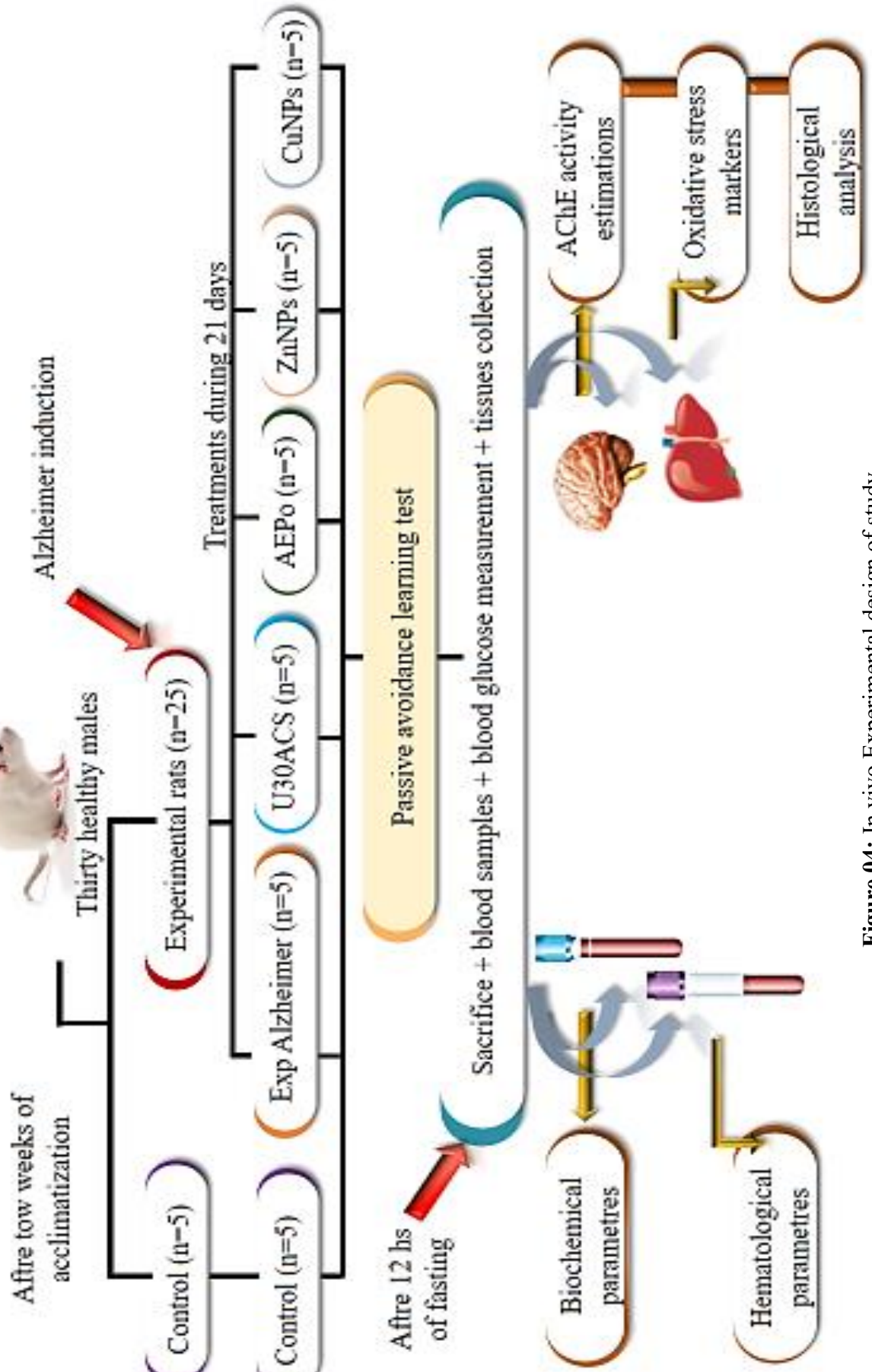


Figure 04: In vivo Experimental design of study

II. Methods

II.1. Aqueous extract preparation

The aqueous extract was prepared by adding 50 ml of distilled water to 5 g dry leaves powder of *Portulaca oleracea* L. at 50°C during 2 hours. After 24 h of maceration at room temperature, the mixture was filtered by Whatman paper then evaporated by using rotary evaporator (Derouiche et al., 2019), (figure 05).

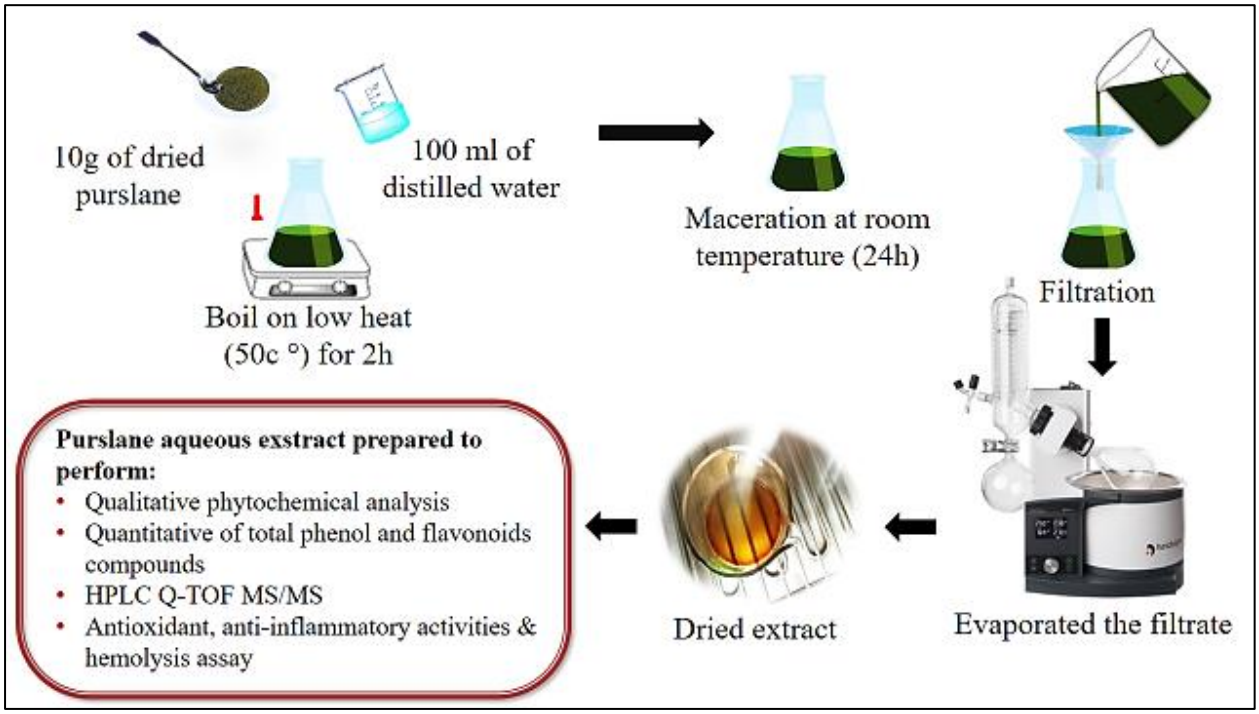


Figure 05: *P.oleracea* aqueous extract preparation

II.2. Green synthesis of nanoparticles

II.2.1. Green synthesis of zinc oxide ZnO nanoparticles

In order to prepare ZnNPs, as shown in figure 06, 200 ml of aqueous zinc sulfate was mixed with 20 ml of purslane leaves aqueous extract. The ions, which initiated the reaction, were afforded by the zinc sulfate in de-ionized water. The reaction mixture was incubated with constant stirring in the dark at 60 °C to avoid photo-catalysis. An observed off-white color marked the formation of ZnO NPs at the end of 24 h. The resultant product was further purified by centrifugation and washed in double-distilled water and ethanol, respectively, dried and kept in an amber-coloured sample bottle until use (Ezealisiji et al., 2019).

II.2.2. Green synthesis of copper oxide CuO nanoparticles

In order to prepare CuNPs, 1g of the copper sulfate was added into the defined amount of the prepared walnut aqueous extract (10mL) and the reaction solutions were mixed using a heater-stirred, adjusted at 500 rpm and 70°C, for 15 min. Finally, the samples were put in an electric furnace memmert adjusted at 200°C for 2 h. The obtained powders washed in double-distilled water and ethanol respectively, as farmed CuO NPs, were then used for further studies (Asemani & Anarjan, 2019), (figure 06).

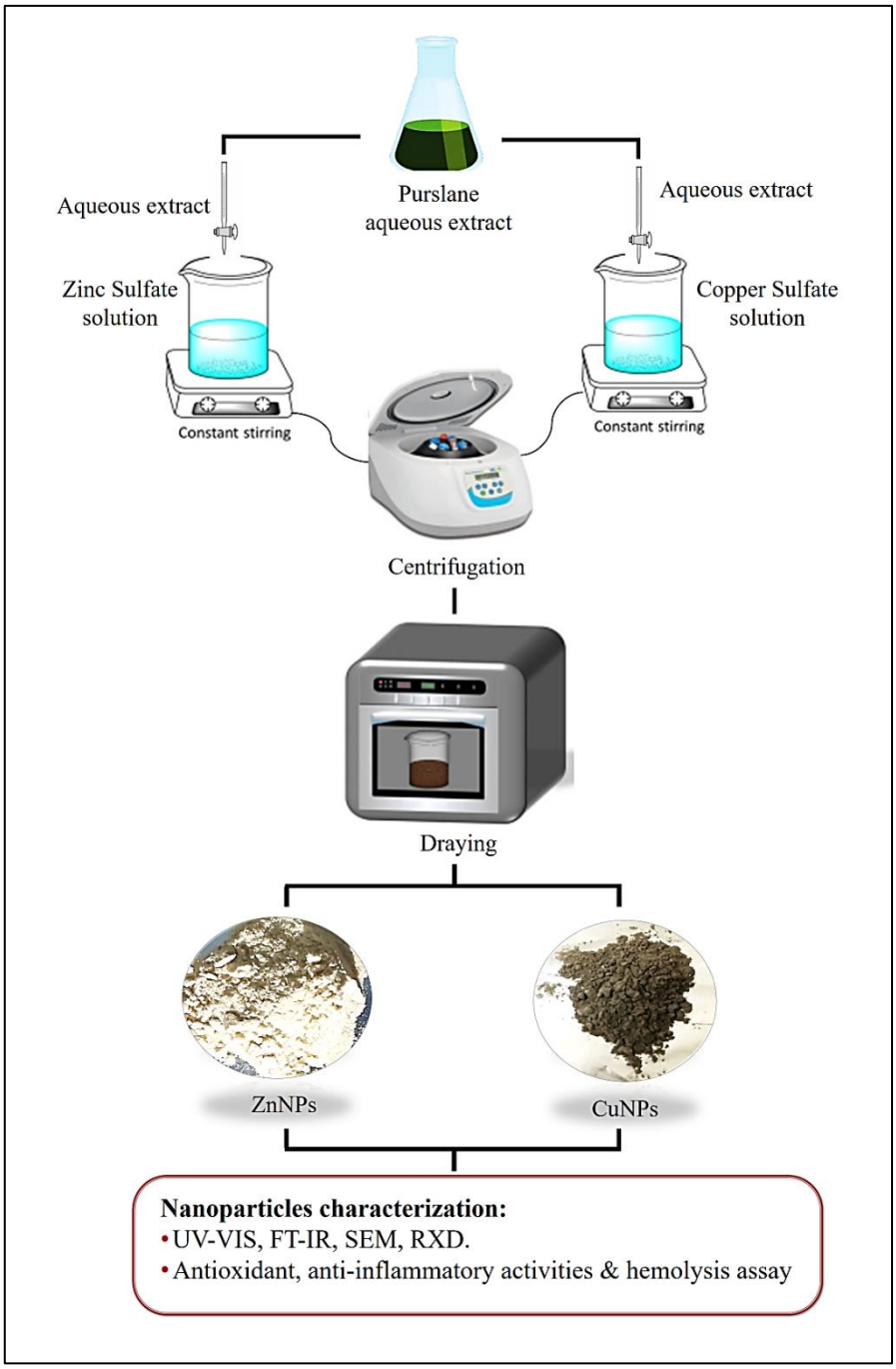


Figure 06: The green synthesis method of zinc and copper oxide nanoparticles

II.3. Phytochemical analysis

The phytochemical analysis were carried out on the aqueous extracts prepared from the plant by qualitative characterization method according to (Evans, 2009 ; Harborne, 1998 ; Wadood et al., 2013 ; Harborne, 1973)

II.3.1. Phenols

Introduce 5 ml of extract in a test tube, drops a few of natural 5% ferric chloride solution. A dark green color indicates the presence of phenolic compounds

II.3.2. Flavonoids

In a test tube, introduce 5ml of extract, 5ml of diluted ammoniac and 1ml of H_2SO_4. The appearance of a yellow color indicates the presence of flavonoids.

II.3.3. Alkaloids

1 ml of aqueous extract were treated with a few drops of hydrochloric acid then 1–3 drops of Wagner reagent were added. The appearance of brown precipitate reveals the presence of alkaloids in the sample.

II.3.4. Tannins

In a test tube, introduce 5 ml of extract and add 1 ml of a 2% aqueous solution of ferric chloride ($FeCl_3$). The presence of tannins was indicated by a greenish or bluish-blackish coloration.

II.3.5. Terpenoids

The formation of a reddish brown color indicates the presence of terpenoids, through the addition of chloroform (2ml) and concentrated sulfuric acid (3 ml) to 5 ml of plant extract.

II.3.6. Reducing compound

Add Fehling's liquor (1ml of reagent A and 1ml of reagent B) to the extract and incubate the whole in a boiling water bath, The appearance of a brick-red precipitate indicates the presence of reducing sugars.

II.3.7. Saponins

In a test tube, introduce 5ml of extract, mixed with 5ml of distilled water and with vigorous manual agitation. The formation of a steady foam indicates the presence of saponins.

II.3.8. Steroids

For 1ml of plant extract, add 0.5ml of acetic acid solution, followed by 0.5ml of concentrated H_2SO_4. If the solution does not give any green color, it proves the presence of unsaturated steroids. In a second tube, the same volume of H_2SO_4 was added. The presence of the red color indicates the presence of steroid derivatives.

II.4. Total phenols and flavonoids compounds

II.4.1. Total phenols

Determination of the total polyphenols was carried out according to the Folin-Ciocalteu (FC) method (Boizot & Charpentier, 2006); 100 µl of artichoke extract are mixed with 500µl of the FC reagent and 400 µl of Na_2CO_3 at 7.5% (w / v). The mixture is stirred and incubated in the dark and at room temperature for ten minutes and the absorbance is measured at 760 nm by a UV spectrophotometer. The results are expressed in mg gallic acid equivalent/ g of dry vegetable material with reference to the calibration curve of gallic acid. Calibration curve is carried out by gallic acid at different concentrations (20 - 40 - 60 - 80 - 100 - 120 µg/ml) under the same conditions and the same steps of the assay. The results are thus expressed in milligrams of gallic acid per gram of dry extract (mg of EAG / g). All measurements are repeated 3 times.

II.4.2. Total flavonoids

The determination of total flavonoids was carried out according to the method described by (Dehpour et al., 2009); 500 µl of each extract, 100 µl $AlCl_3$, 100 µl of 1 M sodium acetate and 2.8 ml of distilled water. The mixture is stirred and then incubated in the dark and at room temperature for 30 minutes. The blank is made by replacing the extract with 95% methanol and the absorbance is measured at 415 nm using a UV spectrophotometer. The results are expressed in mg equivalent quercetin / g of dry vegetable material with reference to the quercetin calibration curve. The quercetin calibration curve is performed by quercetin at different concentrations (20 - 40 - 60 - 80 - 100 – 120 µg/ml) under the same conditions and the same steps of the assay.

II.6. Characterization of ZnO and CuO nanoparticles

The characterization of ZnO and CuO NPs were identified by Ultraviolet-visible spectroscopy (UV-VIS), Fourier transform infrared spectroscopy (FTIR), Scanning electron microscope (SEM) and X-ray powder diffraction (XRD) analysis. The analysis were done by direct reading through JENWEY, Thermo Scientific iS5, PROTO AXRD Benchtop and Thermo Scientific Apreo S apparatus respectively.

II.7. Antioxidant activity (Ferric Reducing Antioxidant Power Assay "FRAP assay")

- **Principle**

The Antioxidants ability are determined by colorimetry. The ferric-tripyridyltriazine complex is reduced to the ferrous-tripyridyltriazine in presence of the antioxidants; the complex loses its yellow color to a dark blue. This coloration measured at 595 nm is proportional to the concentration of antioxidants present in the samples. The method is standardized by Trolox (Oyaizu, 1986).

- **Procedure**

l. Take 500µl of sample.
2. Add 1.25ml of the buffer solution (0.2 M, PH = 6.6).
3. Incubation during 20 min in a water bath at 50 ° C.
4. Add 1.25ml of the aqueous TCA solution (10%) to stop the reaction.
5. Centrifugation at 3000 rpm for 5 minutes.
6. 1.25 ml of supernatant are then mixed with 1.25 ml distilled water and 250 µl $FeCl_3$ (0.1%).
7. Reading at 700 nm against a blank.

The results expired by IC50, after calculating of the inhibition percentage values according to (Yazdani et al., 2019) as follows:

$$\text{IP } (\%) = 100 - \frac{\text{OD controle}}{\text{OD sample}} \times 100$$

II.8. Anti-inflammatory activity

- **Principal**

The anti-inflammatory activity is measured of protein denaturation inhibition in presence of the anti-inflammatory compound, which is studied through in vitro assay. The measured turbidity at 660 nm is proportional to the concentration of anti-inflammatory compound present in the sample (Vennila et al., 2018).

- **Procedure**

1. Add different concentrations (10–50 µg ml^{-1}) of the sample to bovine serum albumin (BSA) solution (1%).
2. Incubation during 30 min at room temperature.
3. The pH of the solution was adjusted to 2 using dropwise addition of concentrated HCl.
4. After incubation, the mixture is heated at 72 °C for 30 min.
5. The all tubes are cooled for 10 min.
6. The turbidity is measured at a wavelength of 660 nm. Diclofenac is used as standard.

The results expired by IC50, after calculating of inhibition percentage (IP) as follows:

$$\text{IP } (\%) = \text{A0} - \frac{\text{A1}}{\text{A0}} \times 100$$

II.9. Hemolysis assay

- **Principal**

The Hemolysis assay is done as described by (Vinjamuri et al., 2015) that determined the protective effect of the antioxidant compound presented in the sample against the membrane erythrocyte lysis which induced by 1XPBS. The detection of red blood cells (RBCs) membrane lysis by measuring the concentration of hemoglobin in blood plasma at 540 nm by spectrophotometer.

- **Procedure**

1. 5mL of blood was collected from healthy volunteers in the tubes containing 5.4 mg of EDTA to prevent coagulation.
2. The blood centrifuged at 1000 rpm for 10 min at 40C.
3. Plasma is removed carefully and the white buffy layer was completely removed by aspiration with a pipette with utmost care.
4. The erythrocytes were then washed for additional three times with 1X PBS, pH 7.4 for 5 min.
5. The Washed erythrocytes were stored at 4^{o}C and used within 6 h for the hemolysis assay.
6. Add 50 µL of 10 dilutions (100 µL Erythrocytes suspension and 900 µL 1XPBS) of erythrocytes suspension was mixed with 100 µL of test samples (20-80ng/mL), 100 µL of 1XPBS was used as a control.
7. Reaction mixture is incubated at 37^{0}C water bath for 60 min.
8. The volume of reaction mixture is made up to 1 mL by adding 850 µL of 1XPB.
9. The reaction mixture is centrifuged at 300 rpm for 3min.
10. The resulting hemoglobin in supernatant is measured at 540 nm by spectrophotometer to determine the concentration of hemoglobin.

The hemolysis inhibition percentage is calculated as follows:

$$\text{Hemolysis inhibition (\%)} = 100 - \frac{\text{OD sample}}{\text{OD control}} \times 100$$

II.10 Sub-acute toxicity tests

The test was performed using healthy albino rats of Wistar strain weighing between 154 and 181g. The animals were divided into three groups for each one of them tow rats, which administered 0,10 & 20 mg/kg b.w of CuO NPs, 0, 25 & 50 mg/kg b.w of ZnO NPs and 0, 2000 & 4000 mg/Kg b.w of aqueous extract of *P.oleracea* orally. Animals were observed after dosing at least once during the first 30 min, periodically during the first 24 h with special attention given during the first 4 h, and daily thereafter for a total of 14 consecutive days (Derouiche et al., 2017).

II.12. Hematological parameters

The determination of hematological parameters performed using fully Auto Blood Cell Counter (ERMA).

II.13. Biochemical parameters

The rates of uric acid (UA), Glutamate pyruvate transaminase (GPT), triglyceride (TG), total cholesterol (TC) and high density lipoprotein cholesterol (HDL-C) in the serum was determined by methods using commercial reagent kits Biomaghreb (Tunisia) (ref : uric acid-20091, triglyceride-20131, HDL-20113), Biosystems reagent kits (ref : total Cholesterol-12505) and for the enzyme was measured by another commercial reagent kits TECO DIAGNOSTICS (ref : GPT- A525: 04/2012)

II.14. Homogenates preparation

One gram of (brain and liver) tissue of all experimental group was grinding and homogenized in 9ml of buffer solution of Tris buffer saline (TBS, pH=7.4). Homogenates were centrifuged at (5000 rpm, 15min) at 4°C, and the obtained supernatant was conserved at -20°C (Derouiche et al., 2017).

II.15. Determination of tissue proteins

- **Principle**

The tissue proteins were determined according to a colorimetric method by a SHIMATZU type spectrophotometer using Coomassie blue as a reagent, which is reacted with the amine group (NH_2) of the proteins to form a blue complex. The appearance of the blue color reflects the degree of ionization of the acid medium and the intensity corresponds to the concentration of proteins. The absorption is measured at 595 nm (Bradford, 1976).

- **Preparation of Bradford's reagent**

- Dissolve 100 mg of Coomassie blue in 50 ml of ethanol (95%).
- Shake the mixture for 2 hours with a shaker away from light.
- Add 100 ml of orthophosphoric acid (H_3PO_4) (85%).
- Complete the volume to 1 liter with distilled water.
- Filter the solution obtained with filter paper.

Note: This reagent is stable for 2 weeks at 4°C.

- **Procedure**

l. Take 01 ml of the homogenate.
2. Add 5 ml of Coomassie blue.
3. Shake and let to stand for 5 minutes.
4. Read the optical densities against the blank at 595 nm.
5. Compare the protein concentration in the tissues studied.

The protein concentration is determined by comparison with a standard range of bovine serum albumin (0.1-0.2-0.4-0.6-0.8-1mg / ml) previously carried out under the same conditions.

II.16. Determination of Acetylcholinesterase (AChE) activity

AChE activity was determined by the method of (Ellman et al., 1961). Acetylcholine hydrolysis rate was measured in an incubation medium containing acetylcholine 0.8 mM, 100 mM phosphate buffer (pH 7.5) and 1.0 mM DTNB. Fifty microliters of supernatants were added to the reaction mixture and pre incubated for 3 min at 25 °C. The hydrolysis was monitored by the formation of the thiolate dianion of DTNB at 412 nm for 2–3 min (30 s intervals) at 25 °C. The results were expressed as mmol of hydrolyzed acetylcholine /min/ mg of protein.

II.17. Oxidative stress parameters

II.17.1. Determination of malondialdehyde (MDA) level

- **Principle**

The principle of this assay is based on the condensation of MDA in an acid medium and under heat with thiobarbituric acid. The reaction leads to the formation of a pink complex between two molecules of thiobarbituric acid which can therefore be measured by absorption spectrophotometry at 532nm (Yagi, 1976).

- **Reagent**

375 mg of TBA, 20g of TCA, 0.01g of BHT, 25 ml of 1 N HCL and 50 ml of distilled water were added to a beaker. The solution obtained was heated to 40°C in a water bath until the TBA was completely dissolved, then transferred to a 100 ml flask and the volume made up with distilled water to the gauge.

- **Procedure**

Pipette into the glass vise test tubes, 200 µl of sample, 800 µl of TBA reagent and close tightly. Heat the mixture in a water bath at 100°C for 15 minutes. Then cool in a cold water bath for 30 minutes, leaving the tubes open to allow the gases formed during the reaction to be removed. Centrifuge at 3000 rpm for 5 minutes and read the absorbance of the supernatant at 532 nm using a spectrophotometer.

- **Expression of results**

The concentration of thiobarbituric acid reactive substances (TBARS) was determined using the molecular extinction coefficient of MDA (å = $1{,}53.10^5$ $M^{-1}.cm^{-1}$). The results were expressed in nmol /mg of prot.

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

II.17.2. Determination of reduced glutathione (GSH) level

- **Principal**

The level of reduced glutathione is determined according to (Weckbecker & Cory, 1988) by measuring the optical density results from the formation of 2-nitro-5-mercocapturic acid (TNB) from the reduction of dithio-bis-2-nitrobenzoic acid (DTNB) ,which is called Ellman reagent with SH groups exist in GSH briefly.

- **Procedure**

1. 800µl of homogenate samples are add to 200µl of salicylic acid (0.25%).
2. The mixture was centrifuge at 1000 rpm for 5 min.
3. Take 500 ml of supernatant and mixed with 1000µl of tris buffer (tris 0.4mol, 0.02mol NaCl, ph = 8.9) and 25 µl of DTNB (0.01 mol/L).
4. Read the absorbance at λ= 412 nm after 5 min of incubation.

- **Expression of results**

The calculate of GSH concentration expressed in nanomoles per milligram of proteins (nmol/mg of prot) according to the following formula:

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

II.17.3. Determination of Super Oxide Dismutase (SOD) activity

- **Principal**

The assay method of SOD activity using the NBT by the superoxide anion ($O_2^{\cdot -}$), is used as a basis for detecting of presence of SOD by measuring the spectrophotometrically absorbance at 560 nm (Beauchamp & Fridovich, 1971).

- **Procedure**

Collect in tubes	Blank	Sample
EDTA-Met (0.1mM, 13mM)	1000µL	1000µL
Phosphate buffer (50Mm)	892,2µL	892,2µL
Sample	-	50
Phosphate buffer (50Mm)	1000µL	950µl
NBT (75µM)	85,2µL	85,2µL
Riboflavin (2µM)	22,6µL	22,6µL

- **Expression of results**

Inhibition percentage of NBT reduction by SOD

$$\text{SOD} = \frac{OD\ blanc - \text{OD sample}}{OD\ blanc} \times 100$$

II.17.4. Determination of Glutathione-S-transferase (GST) activity

- **Principle**

The method used in this study to measure the GSTs is that of (Habig et al., 1974); this one consists in causing the GSTs contained in the homogenate to act on a mixture (GSH + CDNB) at a temperature of 37 ° C. and at a pH of 6.5. The variation of optical density, due to the appearance of the GSH-CDNB complex, is measured for 1 minute for 5 minutes at a wavelength of 340 nm.

- **Procedure**

Reagents	Blank (µl)	Assay (µl)
Phosphate buffer (0.1M) ph 6.5	850	830
CDNB (0.02M)	50	50
GSH (0.1M)	100	100
Homogenate	-	20

- **Expression of results**

The activity of the GST expressed in nanomoles of CDNB per minute per milligram of proteins (nmol CDNB / min / mg prot) according to the following formula:

$$\text{GST (nM/min/mg of prot)} = \frac{\text{OD sample/min} - \text{OD blank/min}}{9.6 \times mg\ of\ prot}$$

II.18. Histopathological study of the brain and liver tissues

After the sacrifice of rats, brain and liver were removed and immersed for 48 h in a fixative solution (solution 4% formaldehyde, in phosphate buffer, pH＝7.6), dehydrated in ascending graded series of ethanol, cleaned with toluene, immersed in paraffin, and colored with hematoxylin and eosin. Histopathological evaluation was performed with a light microscope.

II.19. Statistical analysis

The results obtained are expressed as the mean ± standard error of the mean (Mean ± SEM). The analysis of the data was carried out by application of the Student's T test, which is based on the comparison between two means, using the MINITAB software (Version 13Fr) and EXCEL (Version 2019) which helps us to do the tests and the curves.

Results

I. In vitro assays

I.1. Phytochemical analysis

I.1.1. Qualitative and quantitative phytochemical analysis of *Portulaca oleracea L.*

I.1.1.1. Phytochemicals test

Our phytochemical results demonstrated that *P.oleracea* aqueous extract rich by different compounds such as phenols, alkaloids, flavonoids, terpenoids, saponins, and derived steroids. However, it was poured from tannins, reducing compounds and unsaturated steroids (table 02).

Table 02: Phytochemical assays of *Portulaca oleracea* aqueous extract

Phytochemical parameters	***Portulaca oleracea (L)***
Flavonoids	+
Tannins	-
Alkaloids	+++
Terpenoids	+
Saponins	+
Reducing compounds	-
Phenols	+++
Unsaturated steroids	-
Derived steroids	+

(+) presence ; (-) absent

I.1.1.2. Phenols and flavonoid compounds

The results presented in table 03 showed a richness from each of total phenols and flavonoids in purslane aqueous extract.

Table 03: Total phenols and flavonoids concentration in *P.oleracea* aqueous extract

Parameters	**Total phenols (mg EGA/g of extract)**	**Total Flavonoids (mg EQer/g of extract)**
Aqueous extract of *P.oleracea (L)*	38.2 ± 10.0	0.324 ± 0.006

I.1.1.3. I.2. Characterization of Zinc and Copper nanoparticles

I.2.1. UV–Vis spectroscopy

Figure 09 showed a maximum absorption of ZnNPs and CuNPs biosynthesized from aqueous extract of *P.oleracea* in the range from 200 to 250 and appeared peak at 300 nm respectively in UV-vis spectrums.

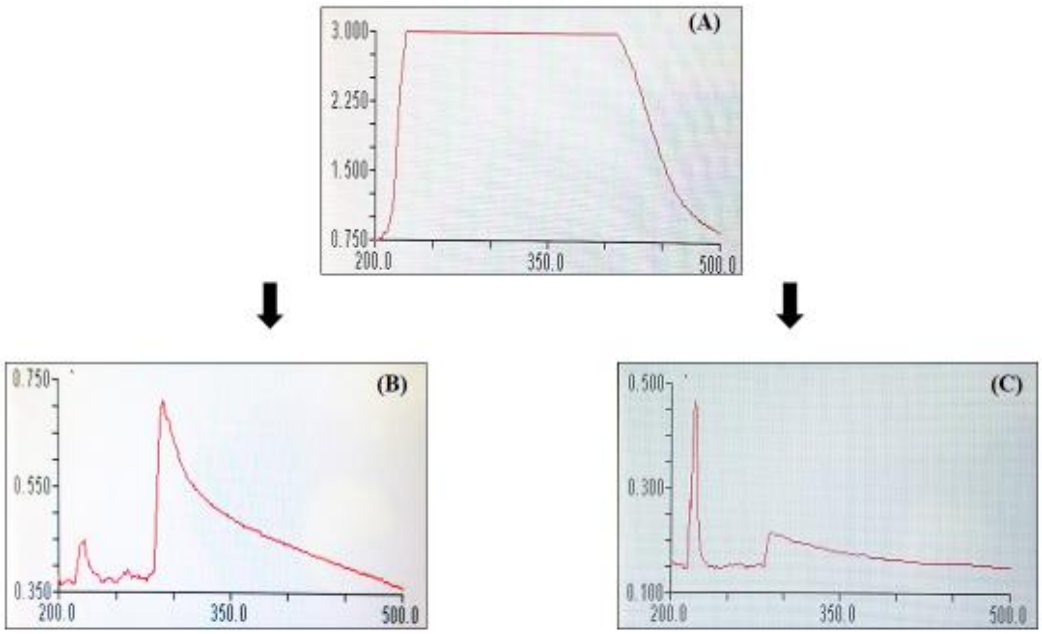


Figure 09: UV-Vis spectrums of purslane leaves aqueous extract (A), CuNPs (B) and ZnNPs (C)

I.2.2. FT-IR analysis

Figure 10 and 11 showed the photograph of the FTIR spectroscopy recorded for the prepared samples. From the figure 10 it was observed that, the presence peak in the rang 400-700 cm^{-1}corresponds to the O-Zn, compound. Nevertheless, from the figure 11, the energy band presented at about 509cm^{-1} corresponds to the O-Cu, compound.

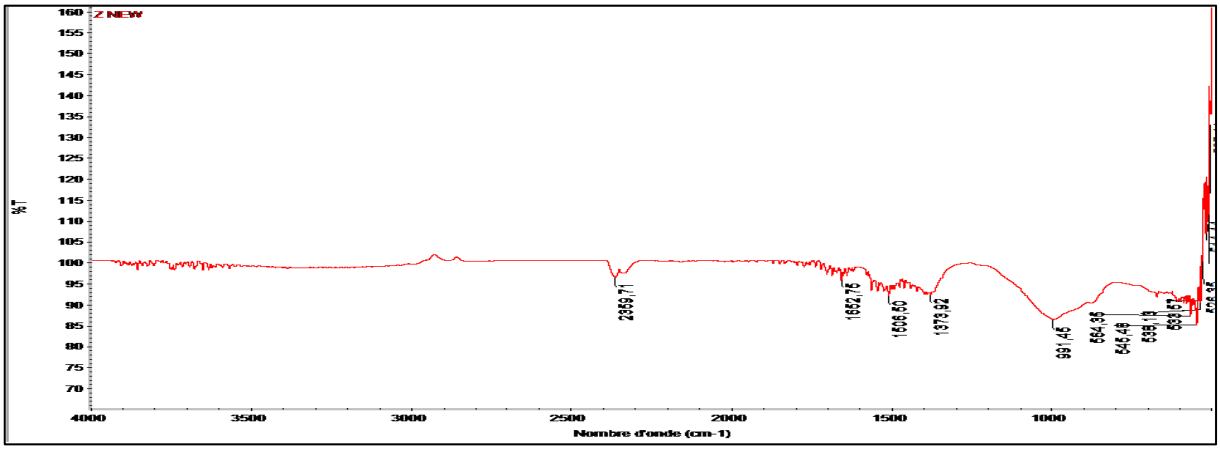


Figure 10: FT-IR spectrum of ZnNPs synthesized by *P.oleracea* aqueous extract

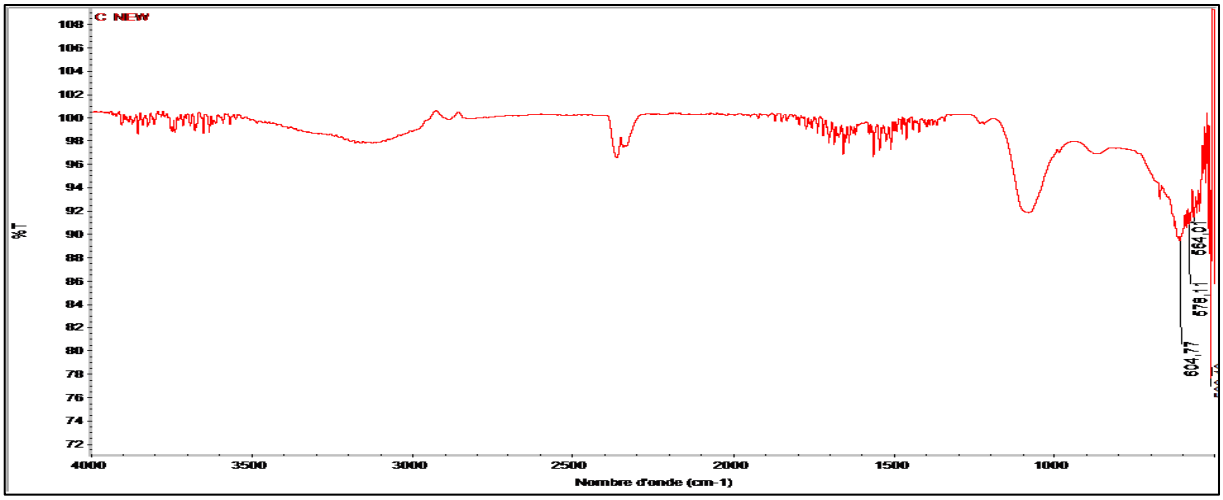


Figure 11: FTIR spectrum of powder CuNPs obtained from purslane aqueous extract

I.2.3. SEM analysis

Scanning electron microscopy image (figure12 and 13) showed that the biosynthesized ZnNPs and CuNPs powder by *P.oleracea* aqueous extract had a size relatively less than 37.5 and 45.94 nm subsequently.

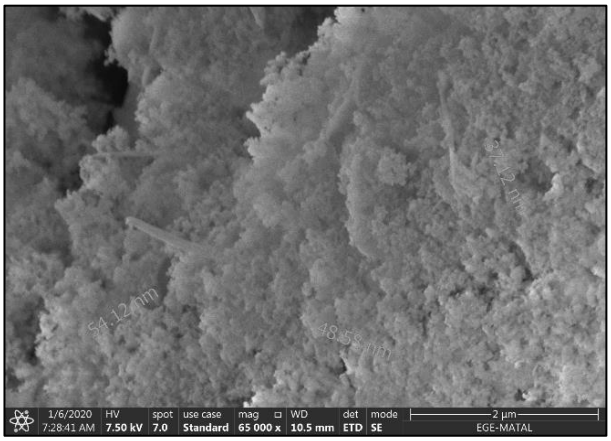


Figure 12: Scanning electron microscopy image of the biosynthesized Zn nanoparticles from purslane aqueous extract

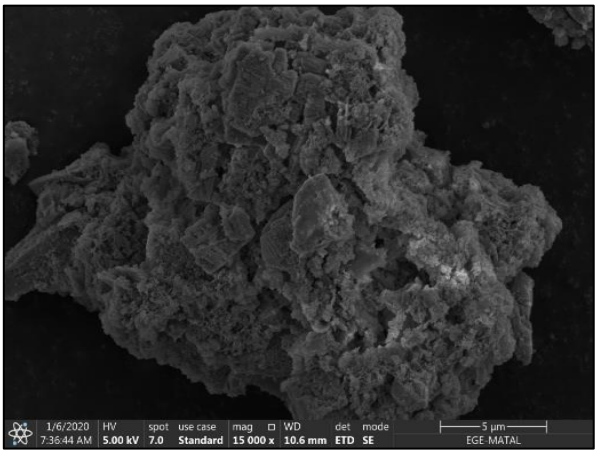


Figure 13: Scanning electron microscopy images of the biosynthesized CuNPs

1.2.4. XRD analysis

A detailed analysis of the X-ray diffraction diffractograms showed that the main components of test powder were ZnO and CuO nanoparticles as shown in figure 14. The peaks of ZnO appeared at 35.84°, 38.02°, 39.15°, 64.61° and 77.72° which are indexed as (*).The XRD analysis of CuO demonstered the peaks at 36.81°, 39.52°, 42.31°, 49.09°, 53.98°, 63.17°, 68.97°and 75.45° as what symbolized by (●). The results of XRD analysis are consistent with the Inorganic Cristal Structure Database, ICSD 01-75-1526 for zinc oxide, ICSD 01-089-5898 for copper oxide.

I.3. Biological Activities

I. 3.1. Antioxidant activity “Ferric reducing antioxidant power (FRAP)”

Figure 15 showed the antioxidant activity, which expressed by the inhibition concentration 50 level, the IC50 of ZnNPs was higher more than CuNPs level, which presented less than purslane aqueous extract level.

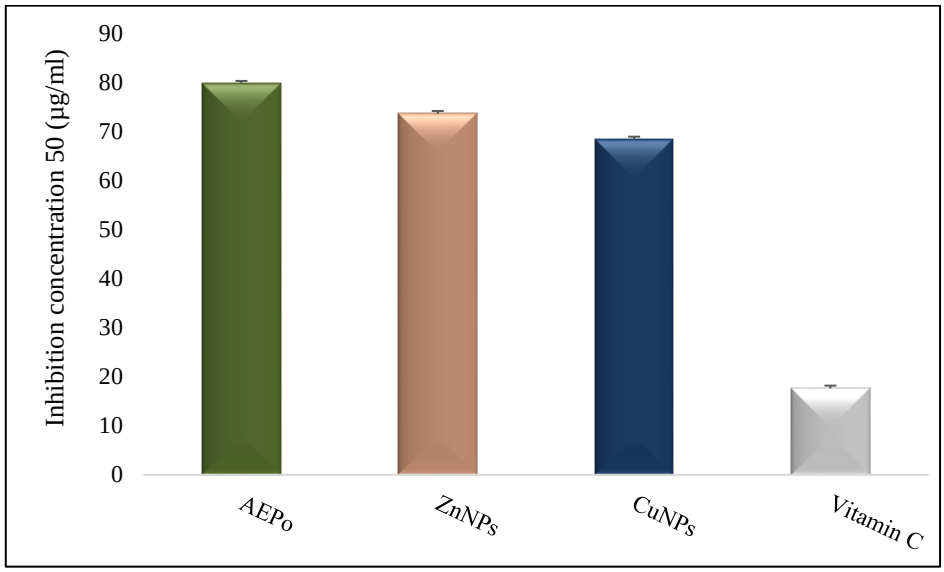


Figure 15: IC50 levels of AEPo, ZnNPs, CuNPs and Vitamin C

I.3.2. Anti-inflammatory activity

The results obtained in figure16, according to diclofenac level, the zinc oxide nanoparticles had a very high anti-inflammatory activity in comparison with aqueous extract and copper oxide nanoparticles.

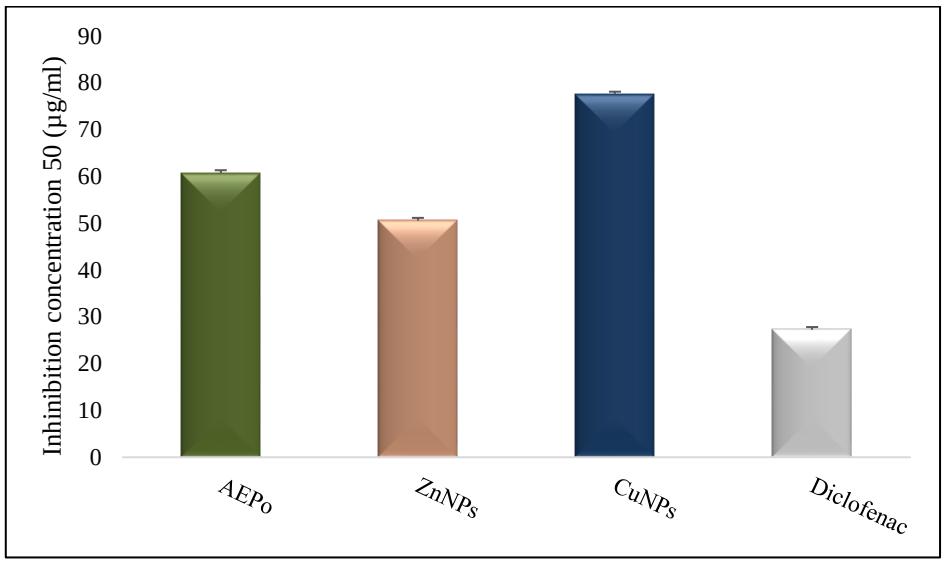


Figure 16: IC50 levels of anti-inflammatory activity of AEPo, ZnNPs, CuNPs and diclofenac

I.3.3. Hemolysis assay

As shown in figure 17, the greater protection percentage was showed in ZnNPs at the concentration 80 (mg/l) as compared to aqueous extract and CuNPs.

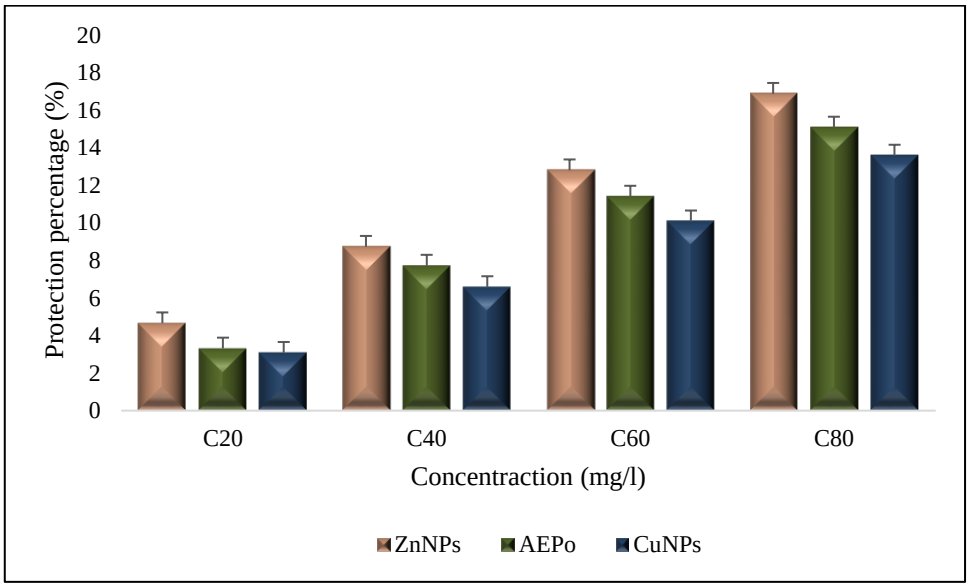


Figure17: Effects of ZnNPs, AEPo and CuNPs on red blood cells (RBCs) hemolysis

II. In vivo assays

II.1. Acute toxicity study

The results obtained from this test (table 05) showed that there were a normal physiological parameters during the experimental period as the eyes, sleep, movement and diarrhea of the albino Wister rats were treated by different dose from each of CuNPs 0, 10 and 20 mg/kg; ZnO NPs 0, 25 and 50 mg/kg either the purslane aqueous extract 0, 2000 and 4000 mg/Kg. In addition, there were no abnormal symptom or adverse effects and no mortality case were noted before 14 days.

Table 05: Sub-acute toxicity test of ZnNPs, CuNPs and aqueous extract of *P.oleracea* on physiological parameters of Wister albino rats

Parameters	0 h		3 h		24 h		Day- 7		Day-14	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
Dead rats	0	0	0	0	0	0	0	0	0	0
Eyes	N	N	N	N	N	N	N	N	N	N
Sleep	N	N	N	N	N	N	N	N	N	N
Movement	N	N	N	N	N	N	N	N	N	N
Diarrhea	N	N	N	N	N	N	N	N	N	N

N: Normal

II.2. Growth parameters

The results of gain body weight demonstrated a significant raise in Exp Alzheimer rats ($p<0.05$) when compared to controls rats, while a significantly lower ($p<0.001$) in U_{30}ACS, AEPo and CuNPs compared to Exp Alzheimer. The relative liver weight and food intake results

presented a significant increase (P<0.001) in experimental Alzheimer rats as compared to the control. However, there were a significant decrease in all experimental treatment groups with exception the AEPo in relative liver weight and CuNPs for food intake (table 06).

Table 06: Food intake, and relative liver weight in control and experimental rats

Parameters	Control (n=5)	Exp Alzheimer (n=5)	U_{30}ACS (n=5)	AEPo (n=5)	ZnNPs (n=5)	CuNPs (n=5)
Initial body weight (g)	188 ± 11.8	184.4 ±10.8	165.6 ± 6.82	178.8 ±11.2	177.8±10.3	237.3±10.9
Body weight gain (g/day)	0.094 ± 0.03	0.31 ± 0.06*	-0.12±0.33^{***c}	0.10 ± 0.004^{c}	0.34±0.08*	-0.91±0.52^{***c}
Relative liver Weight (g/100g b.w)	3.09 ± 0.07	3.42±0.06***	3.04±0.08^{b}	3.40±0.05***	3.03±0.09^{b}	2.75±0.05^{c}
Food intake (g/rat/day)	9.00±0,86	14.1±0.24***	8.50±0.44^{c}	11.00±1.13^{a}	8.7±0.82^{c}	13.2±0.71***

* p<0.05, ** p<0.01, *** p<0.001: significantly different from control group. a p<0.05, b p<0.01, c p<0.001 : significantly different from Exp Alzheimer group. Values are mean ± SEM, n=5

II.4. Determination of protein level

As shown in figure 20; in the brain, as compared to the control the experimental Alzheimer and ZnNPs groups presented a significant increase in protein levels ($P<0.001$). In the liver, also there were a significant increase in protein level of Alzheimer disease rats ($P<0.001$). However, a significant decrease for liver protein levels ($P<0.001$) in U_{30}ACS, AEPo, ZnNPs and CuNPs groups compared to Alzheimer animals. In addition, a significant decrease of brain protein concentration in U_{30}ACS ($P<0.001$) and CuNPs ($P<0.01$) rats as compared to Alzheimer group.

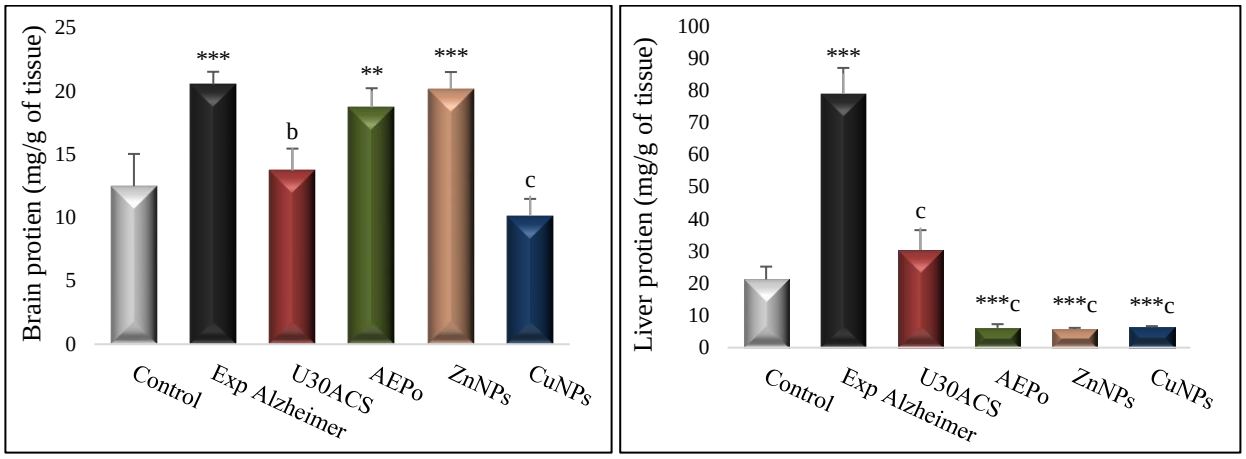


Figure 20: Brain and liver protein concentration in control and experimental groups

** p<0.01, *** p<0.001: significantly different from control group. b p<0.01, c p<0.001: significantly different from Exp Alzheimer group. Values are mean ± SEM, n=5

II.5. Determination of Acetyl cholinesterase activity

The results illustrated in figure 21 demonstrated that, there were a significant increase in acetyl cholinesterase (AChE) activity for the experimental Alzheimer animals (P<0.01) and the treated group by ZnNPs (P<0.05) compared to the control. However the U_{30}ACS and AEPo groups presented a very high significantly decrease in AChE activity (P<0.001) compared to control and Exp Alzheimer rats.

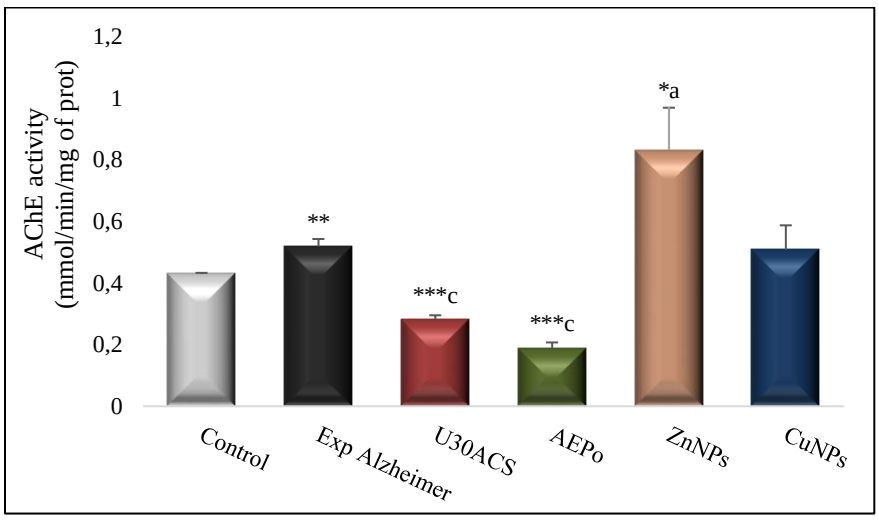


Figure 21: Acetyl cholinesterase activity in the control and experimental groups

** p<0.01, *** p<0.001: significantly different from control group. b p<0.01, c p<0.001: significantly different from Exp Alzheimer group. Values are mean ± SEM, n=5

II.6. Hematological parameters

Hematological parameters illustrated in table 07 showed that, there were a significant raise (P<0.01) of white blood cell (WBC), lymphocytes (Lymph), monocytes (Mono) and platelet levels

and no significant change of red blood cell (RBC), hemoglobin (HBG) and basophile (BASO) in Alzheimer disease rats compared to the control . In the other hand all experimental treatment rats showed significantly reverse change (P<0.001) in WBC. Whether treatment with ZnO and CuO significantly decreased (P<0.001) Lymph, Mono levels, however Platelet levels was significantly decline (P<0.001) by ZnO NPs. Additionally, the treatment by AEPo were significantly reduced (P<0.01) the Lymph, Mono and Platelet levels as compared with Alzheimer group.

Table 07: Erythrocyte and Leukocyte line in blood of control and experimental groups

Parameters	Control (n=5)	Exp Alzheimer (n=5)	U_{30}ACS (n=5)	AEPo (n=5)	ZnNPs (n=5)	CuNPs (n=5)
Red blood cell (10^6/µl)	10.01 ± 0.08	10.07± 0.11	10.18 ± 0.30	10.27 ± 0.19	9.77±0.04^{***c}	10.11±0.21
Hemoglobin (g/dl)	15.27±0.24	15.66 ± 0.2	15.88± 0.27	15.20 ± 0.42	14.87 ± 0.11	15.16±0.46
Platelet (10^3/µl)	959 ± 29.4	1020.3 ± 5.1***	1029.6 ±24.6*	775,8 ± 65.1^{*b}	915.5 ±5.48^{***c}	951 ± 36.3
White blood cell (10^3/µl)	4.84 ± 0.255	7.01 ±0.63**	6,43 ± 0,13^{***c}	5,61 ± 0,26^{*c}	4,23 ±0,36^{c}	4,07± 0,33^{*c}
Lymphocytes (10^3/µl)	2.60 ± 0.23	5.000 ± 0.56**	4,46± 0,32***	3,89 ± 0,25^{***b}	2,86± 0,20^{c}	2,54 ± 0,28^{c}
Monocytes (10^3/µl)	0.06 ± 0.009	0,25 ± 0,04***	0,23± 0,03***	0,13±0,03^{*b}	0.09 ± 0.02^{c}	0.08±0.01^{c}
Basophyle (10^3/µl)	0.0025±0.001	0.004 ± 0.001	0.01±0.00^{***c}	0.008±0.001^{**a}	0.002± 0.001	0.004± 0.001

* p<0.05, ** p<0.01, *** p<0.001: significantly different from control group. a p<0.05, b p<0.01, c p<0.001: significantly different from Exp Alzheimer group. Values are mean ± SEM, n=5

II.7. Lipid profile

Our results obtained concerning the lipid profile (figure 22) indicated that there were a significant increase (P<0.001) of total cholesterol level, and a significant decrease of TG (P<0.05), VLDL-C (P<0.05) levels while no significantly variation in HDL-C and LDL-C levels in experimental Alzheimer rats as compared to the control. Comparison with Alzheimer group, results revealed that CuNPs treatment induced a very significant change (P<0.001) when it decreased in the TG & HDL-C and increased the total cholesterol levels which reversed by AEPo treatment with (P<0.05), however the LDL-C level were significantly increased by minor change in each of U_{30}ACS, ZnNPs and CuNPs groups.

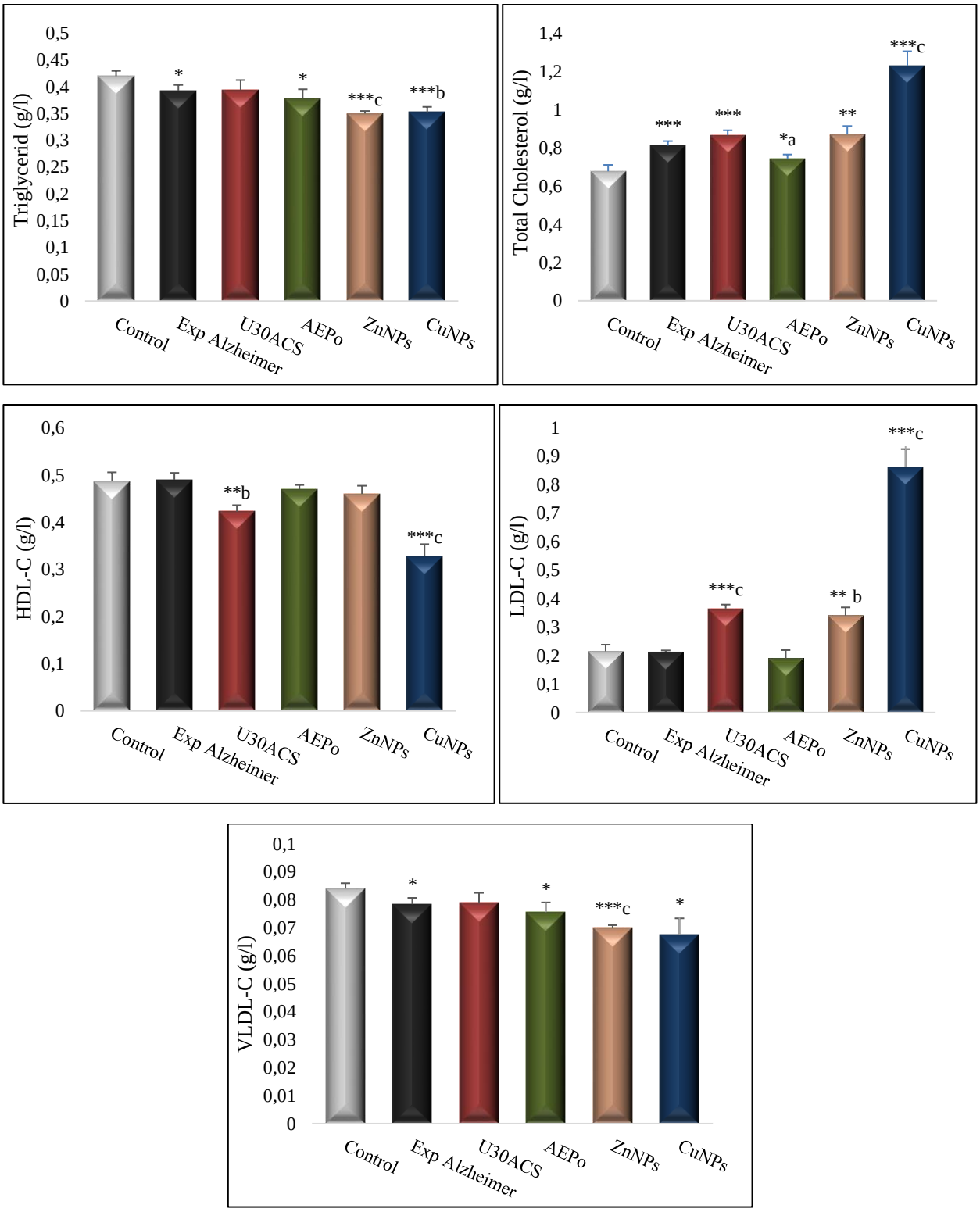


Figure 22: lipid profile levels in the control and experimental groups

* p<0.05, ** p<0.01, *** p<0.001: significantly different from control group. a p<0.05, b p<0.01, c p<0.001: significantly different from Exp Alzheimer group. Values are mean ± SEM, n=5

II.8. Biochemical parameters

As shown in figure 23 and 24; the obtained results show that the blood glucose and uric acid levels were not significantly affected (P>0.05), while glutamate pyruvate transaminase (GPT)

activity was significantly increased (P<0.001) in Alzheimer group as compared to the control. In the other hand, the results indicated a significant decrease in GPT activity (P<0.001), while there were some minor changes in blood glucose and uric acid levels when using various treatments compared to experimental Alzheimer group.

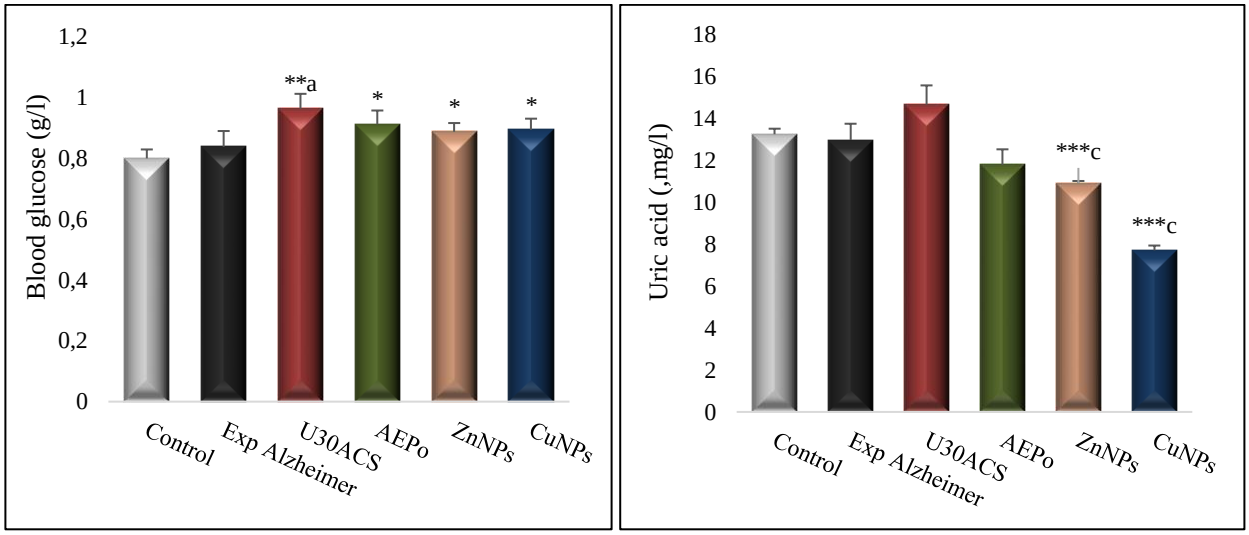


Figure 23: Blood glucose and uric acid concentrations in the control and experimental groups

* p<0.05, ** p<0.01, *** p<0.001: significantly different from control group. a p<0.05, c p<0.001: significantly different from Exp Alzheimer group. Values are mean ± SEM, n=5

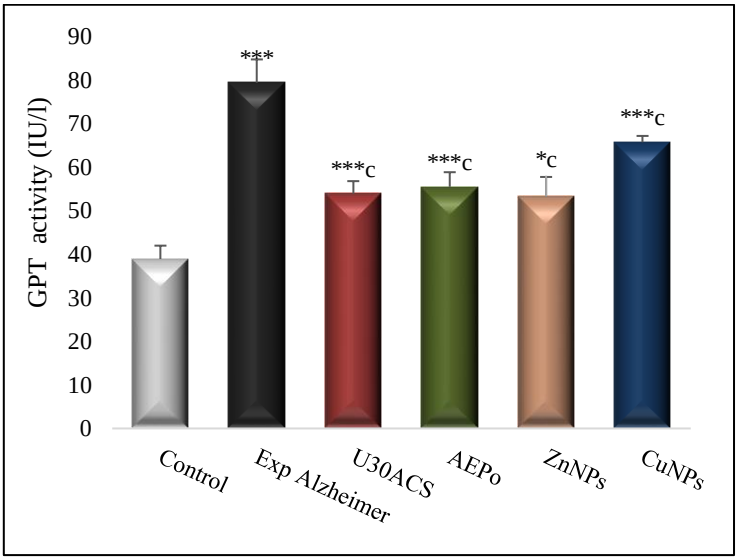


Figure 24: Glutamate pyruvate transaminase (GPT) activity in control and experimental groups

* p<0.05, *** p<0.001: significantly different from control group. c p<0.001: significantly different from Exp Alzheimer group. Values are mean ± SEM, n=5

II.9. Oxidative stress parameters

II.9.1. Malondialdehyde (MDA) level

In comparison to control group, the results demonstrated that there were a significant increase of MDA level in experimental Alzheimer group in brain (P<0.05) and liver (P<0.01)

organs. In addition as compared to Alzheimer rats, a significant decrease (P<0.001) of brain MDA level was observed in U_{30}ACS and AEPo groups, while the results indicated a significant increase with a minor change in the ZnNPs and CuNPs groups in both tissues (figure 25).

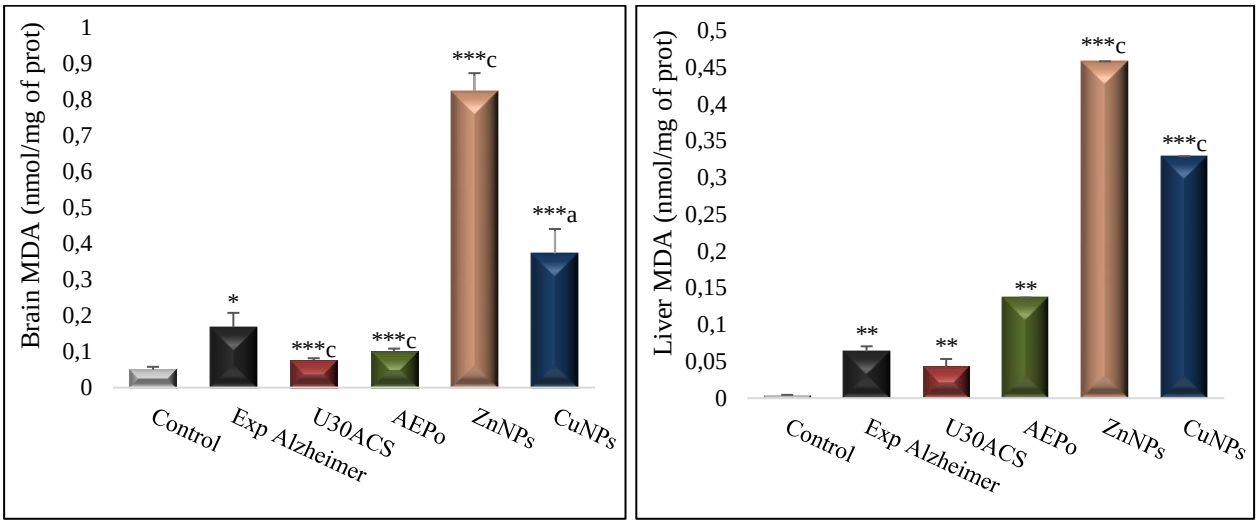


Figure 25: Brain MDA and liver MDA concentration in control and experimental groups

* p<0.05, ** p<0.01, *** p<0.001: significantly different from control group. a p<0.05, c p<0.001: significantly different from Exp Alzheimer group. Values are mean ± SEM, n=5

II.9.2. Reduced glutathione (GSH) level

Figure 26 presented the results of GSH concentration in the brain and liver for the experimental group, as compared to the control there were a significant decrease (P<0.001) of GSH level in the all groups except U_{30}ACS rats group (P>0.05). Furthermore, the liver GSH concentration of different treatments groups were significantly increased as compared to the experimental Alzheimer rats. In brain, only in U_{30}ACS and CuNPs groups we observed a significant increase (P<0.001 and P<0.05) of GSH level compared to Exp Alzheimer group.

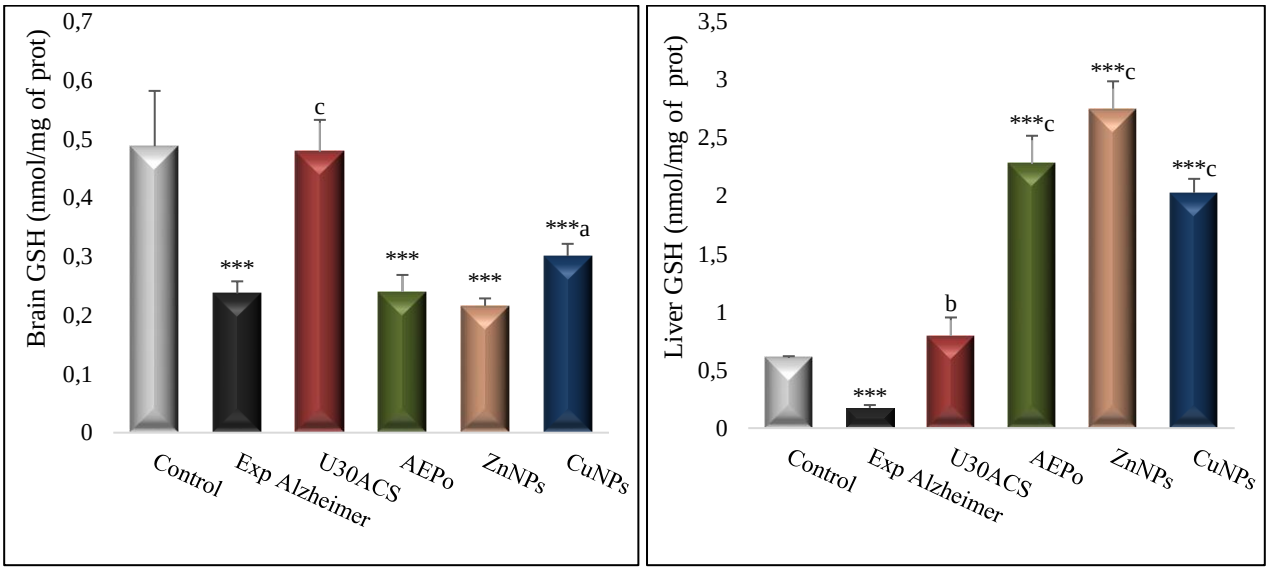


Figure 26: Brain GSH and liver GSH concentrations in control and experimental groups

*** p<0.001: significantly different from control group. a p<0.05, b p<0.01, c p<0.001: significantly different from Exp Alzheimer group. Values are mean ± SEM, n=5

II.9.3. Glutathione-S-Transferase (GST) activity

Figure 27 presented the cerebral and hepatic GST activities. In brain, the results clarified that no significant change in all experimental groups. In the liver, the data showed a significant decreased in experimental Alzheimer group (P<0.001) compared to the control, moreover results demonstrated also a significant increase of GST activity in ZnNPs (P<0.001), CuNPs (P<0.001) and U_{30}ACS (P<0.01) treatment groups compared to experimental Alzheimer animals.

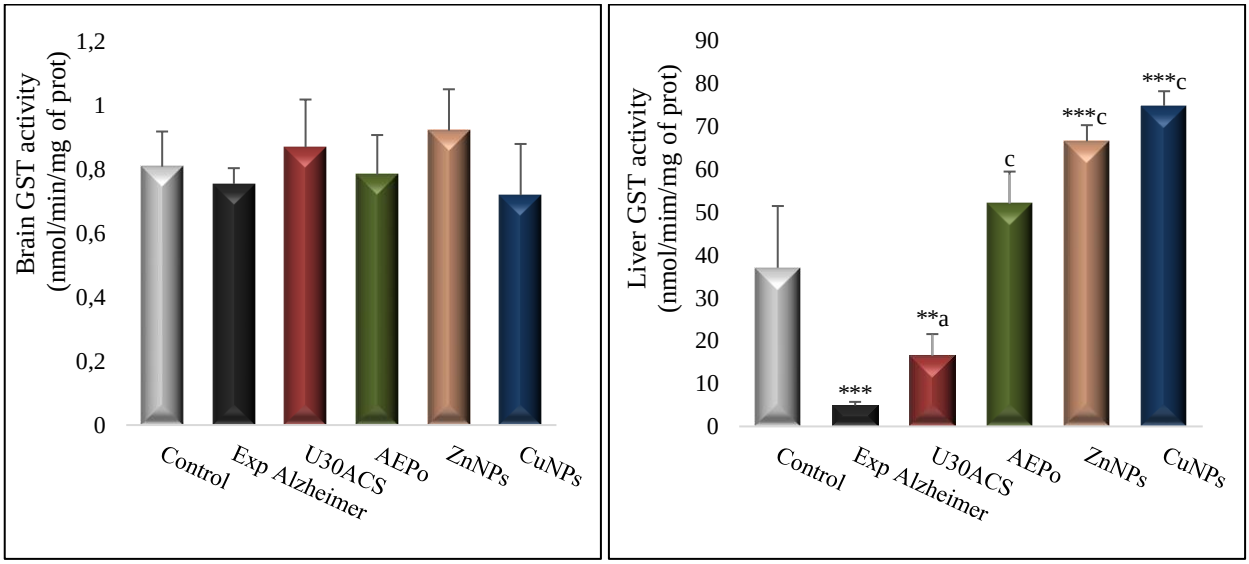


Figure 27: Brain GST and liver GST activities in control and experimental groups

** p<0.01, *** p<0.001: significantly different from control group. a p<0.05, c p<0.001: significantly different from Exp Alzheimer group. Values are mean ± SEM, n=5

II.9.4. Superoxide dismutase (SOD) activity

The histogram results of cerebral and hepatic SOD activities showed a significant decrease (P<0.001) in the experimental Alzheimer animals as compared to the control. As well, the treatment by U_{30}ACS and CuNPs induce a significant amelioration (P<0.001 and P<0.05) in the brain SOD activity. Furthermore, in liver organ SOD activity was significantly increased (P<0.01) in U_{30}ACS and (P<0.001) for the AEPo, ZnNPs and CuNPs groups in comparison to Alzheimer disease rats (figure 28).

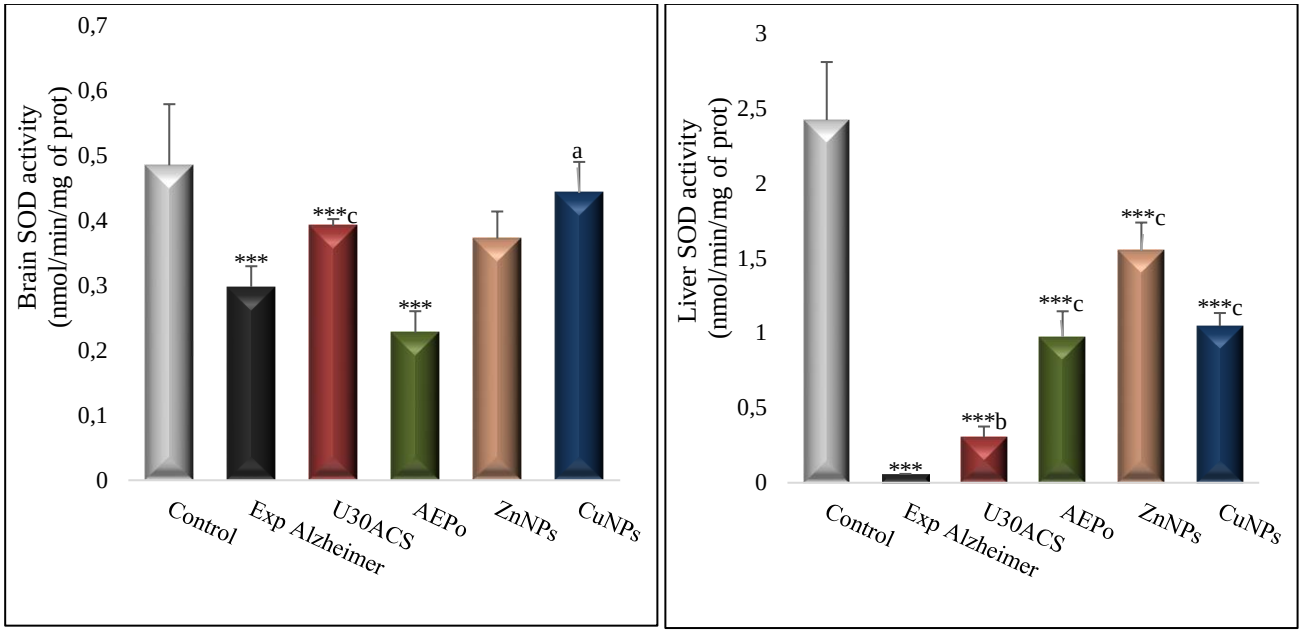


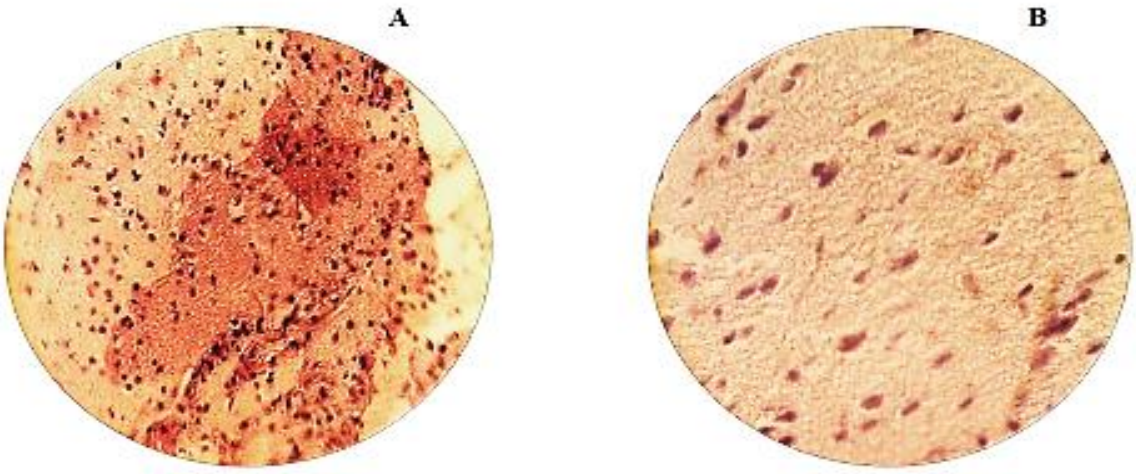
Figure 28: Brain SOD and liver SOD activities in control and experimental groups

*** p<0.001: significantly different from control group. a p<0.05, b p<0.01, c p<0.001: significantly different from Exp Alzheimer group. Values are mean ± SEM, n=5

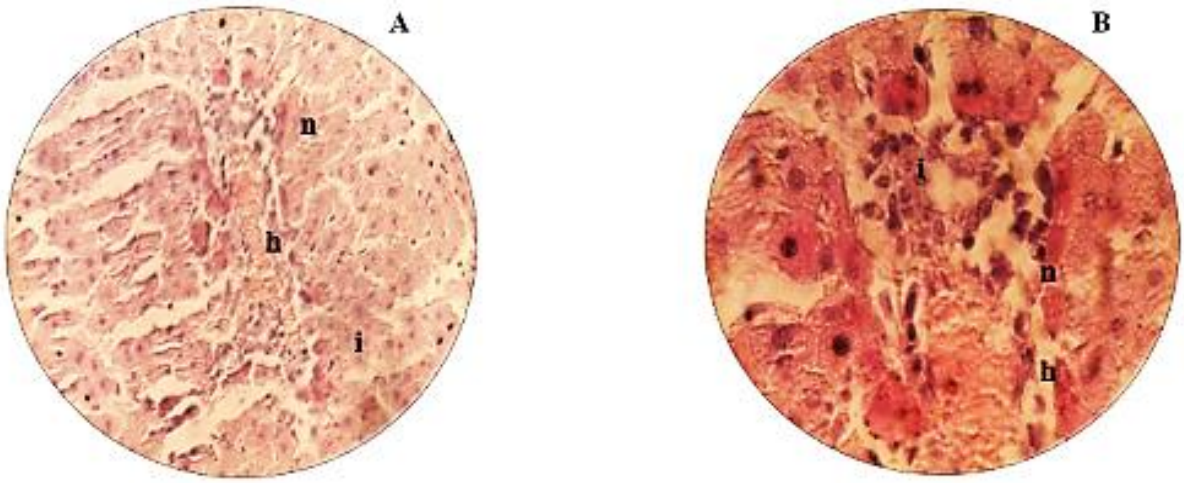
II.10. Histological analysis

Performed the histopathological studies to determine the severity of Exp alzheimer and neuroprotective effects of *P.oleracea* aqueous extract, copper and zinc oxide nanoparticles. Microscopic examination of the brain tissues representing normal histological structure in the control while a huge alteration from hemorrhagic, inflammatory to degeneration cells in brain tissue structure of Exp Alzheimer rats. Furthermore, the treatment by AEPo, CuNPs, ZnNPs and U_{30}ACS improved that these histological alterations had a structure not far from the control (figure 29).

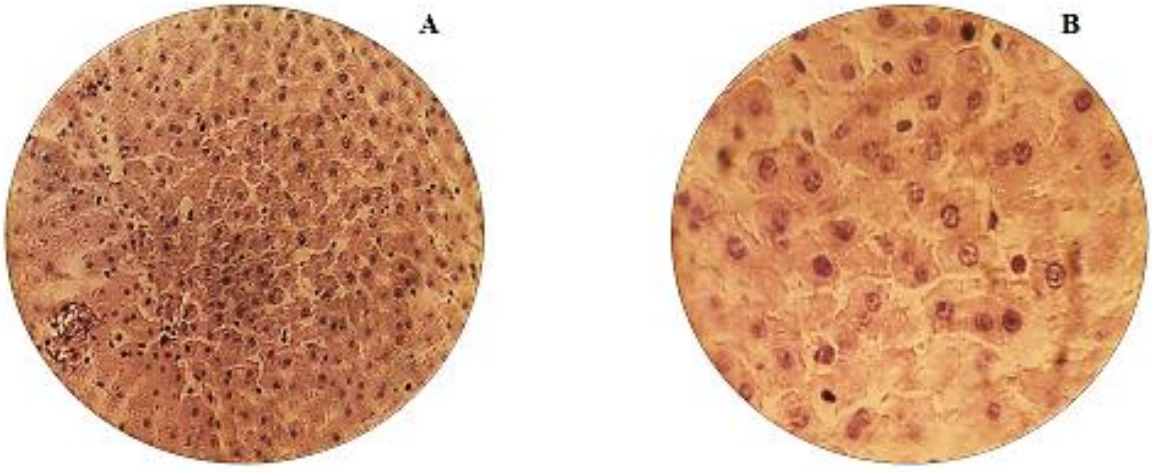
Control group



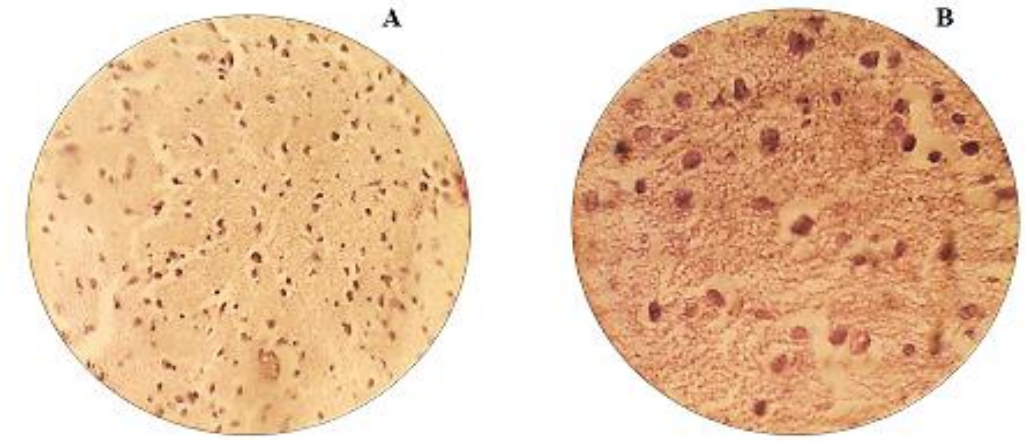
Experimental Alzheimer group



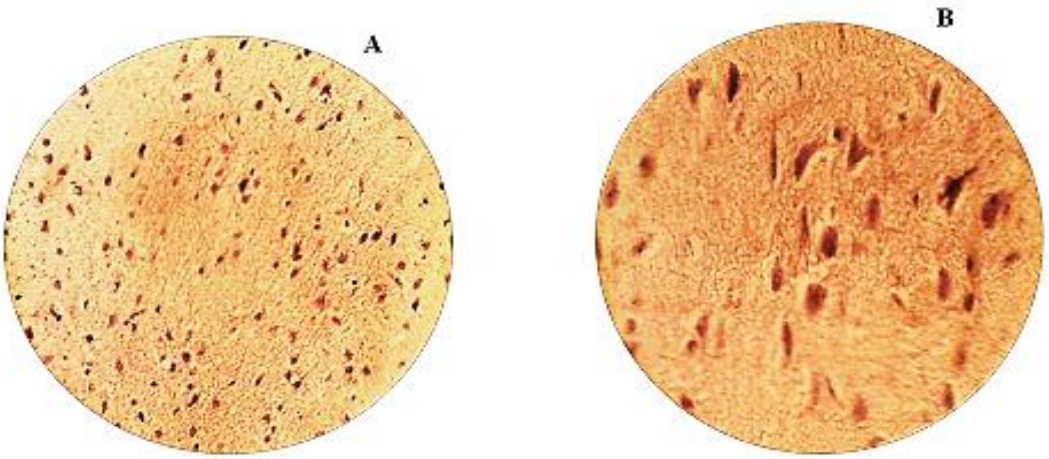
U$_{30}$ACS group



AEPo group



ZnNPs group



CuNPs group

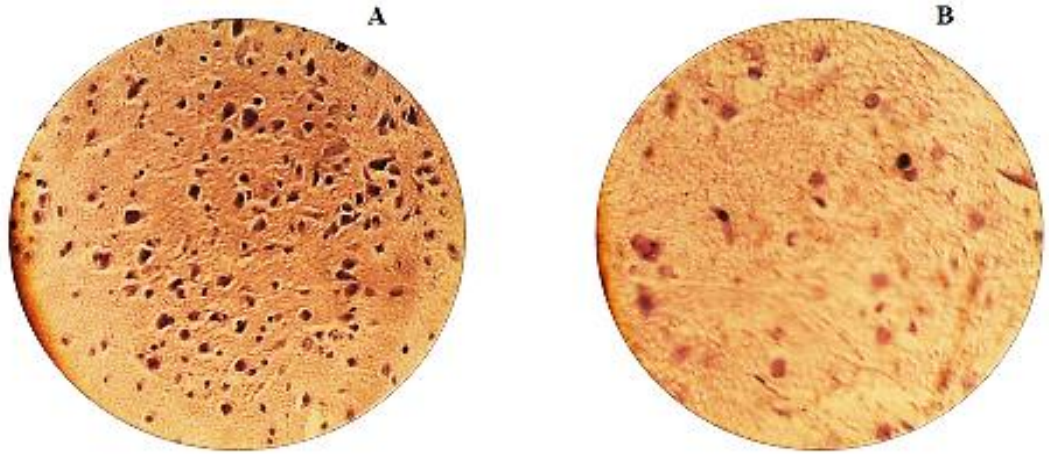
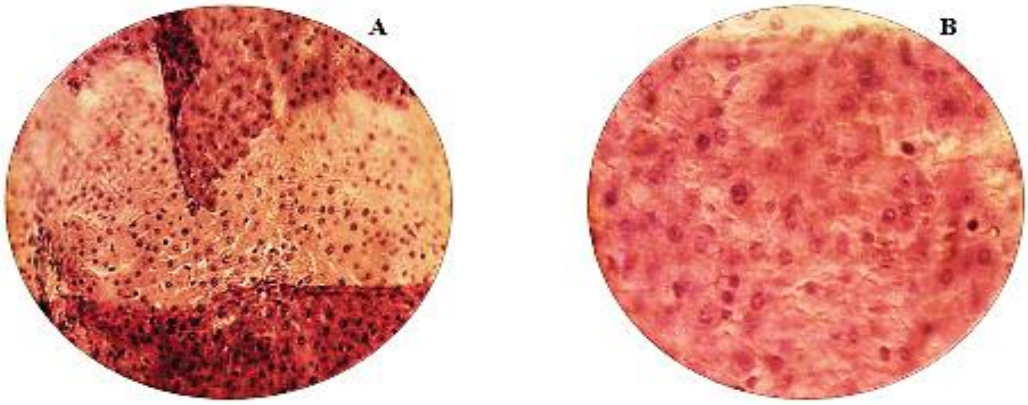


Figure 29: Photomicrographs of brain section of all experimental groups stained with hematoxylin and eosin (H&E), (A): x 40 and (B): x 100

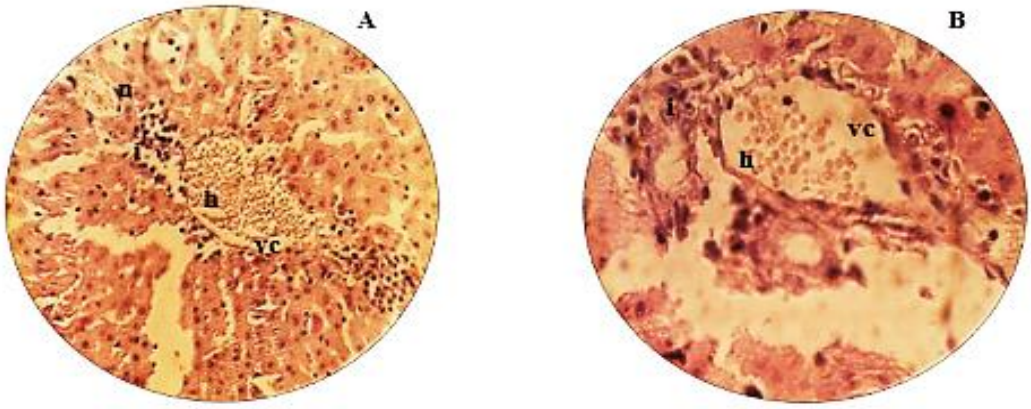
n: necrosis ; h: hemorrhage ; i: inflammation

Histological observations of the liver tissue section of control group showed normal structure of hepatocytes conversely in Exp Alzheimer rats, which showing hemorrhagic, necrosis, infiltration of inflammatory cells and vacuolization of cytoplasm. However, nearly normal liver structure was showed in U_{30} ACS and AEPo groups, whether a distracted liver architecture was appeared in CuNPs and ZnNPs groups (figure 30).

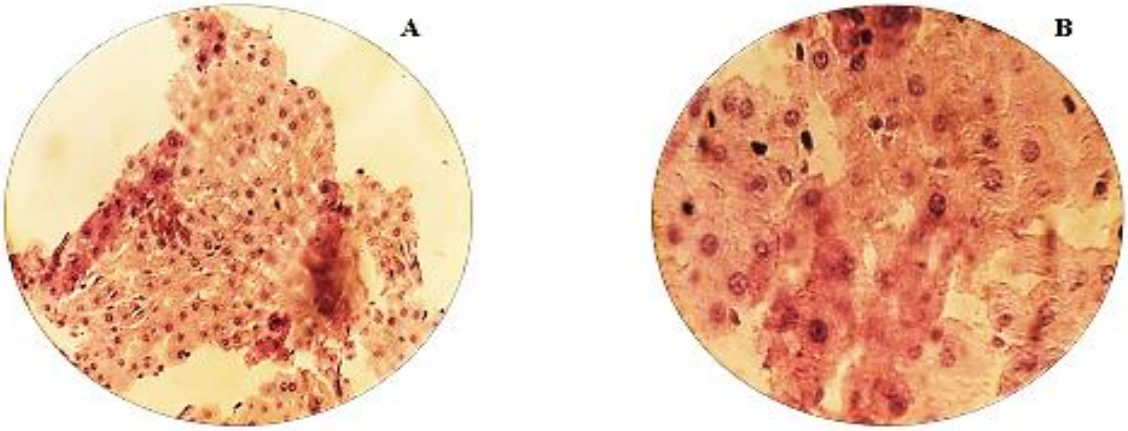
Control group



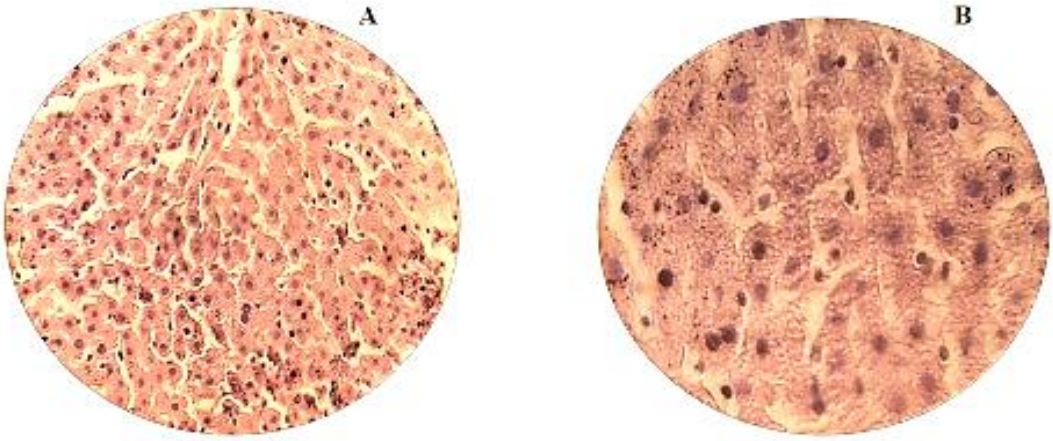
Experimental Alzheimer group



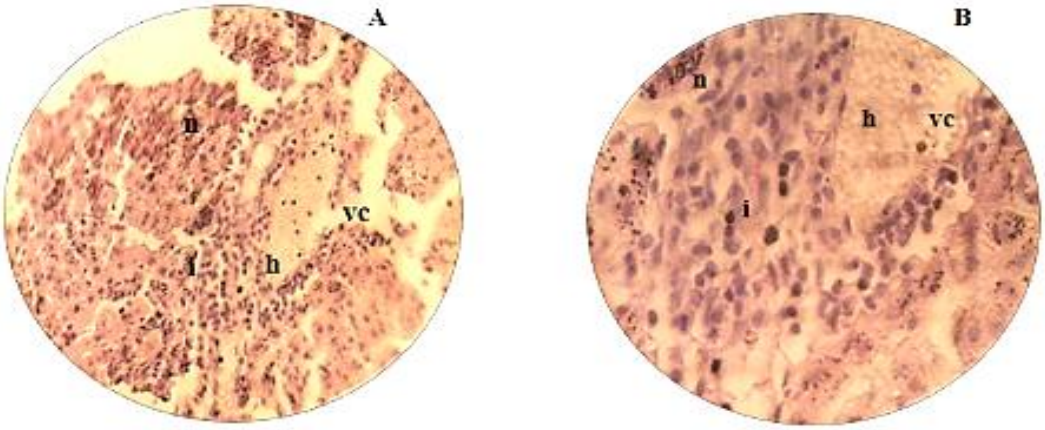
U_{30}ACS group



AEPo group



ZnNPs group



CuNPs group

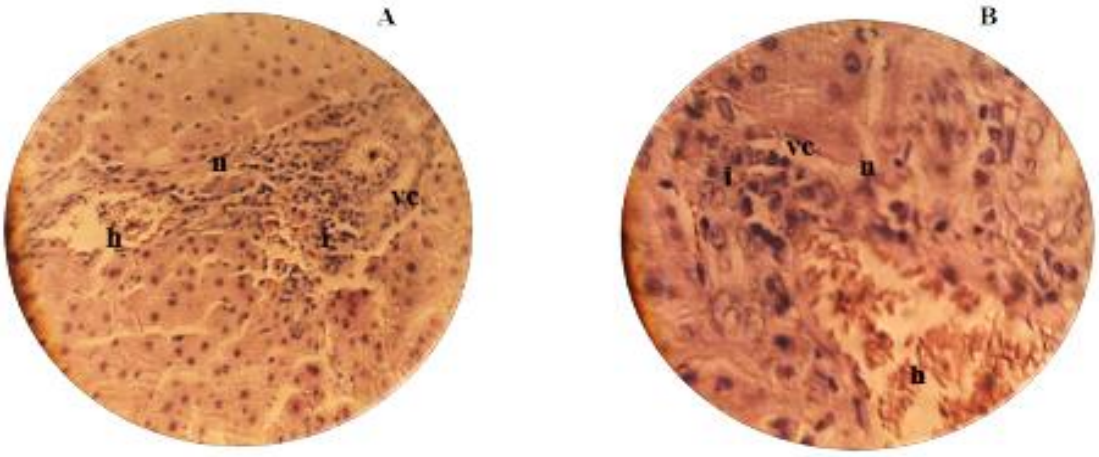


Figure 30: Photomicrographs of liver section of all experimental group stained with hematoxylin and eosin (H&E), (A): x40 and (B): x 100.

n: necrosis ; h: hemorrhage ; i: inflammation ; vc: vacuolar cytoplasm

Table 08: Grading of the histopathological changes in brain and liver sections of control, Exp Alzheimer and treated rats

Groups	organe	Haemorrhage	Inflammation	Necrosis	vacuolar cytoplasm
Control	**Brain**	-	-	-	-
	Liver	-	-	-	-
Exp Alzheimer	**Brain**	+++	+++	+++	-
	Liver	+++	+++	++	+++
U_{30}ACS	**Brain**	-	-	-	-
	Liver	-	-	-	-
AEPo	**Brain**	-	-	-	-
	Liver	-	+	+	+
ZnNPs	**Brain**	-	-	-	-
	Liver	+	+++	-	+
CuNPs	**Brain**	+	-	-	-
	Liver	+++	++	+	++

none (-) ; moderate (+) ; severe (++) ; very severe (+++)

Discussion

I. In vitro study

I.1. Qualitative and quantitative phytochemical analysis of *Portulaca oleracea* L.

Leaves are one of the highest sources of bioactive compounds accumulation as secondary metabolites, which identified in the test samples (Bhat & Al-Daihan, 2014). The phytochemical analysis of *Portulaca oleracea* aqueous extract revealed the presence of different phytochemical compounds such as phenols, flavonoids, alkaloids, terpenoids, saponins, and steroids.

The presence of these phyto-constituents in purslane are greatly determined the several pharmacological activities, such as the hepatoprotective and neuroprotective activities (Iranshahy et al., 2017). Among the phytochemicals compound phenols, flavonoids and terpenoids have been proven its great importance due to their role in immune defense activity (Prabu et al., 2018). Saponins also exhibited anti-inflammatory effects and a calming effect on nerves additionally to its immune regulation, blood sugar control, and blood pressure lowering activities (Zhao et al., 2018). Concerning the phytosterols in purslane, it has the hypolipidemic fonction (Tarish et al., 2019)

The phenolic compounds provide additional values and potential sources in biomedicines, due to their high antioxidant capacity that based on their chemical structures (Ge et al., 2019) through a membrane stabilizing activity by inhibiting the generation of reactive oxygen species (ROS) (Seif et al., 2019). According to (Iranshahy et al., 2017); the flavonoids compound in *P.oleracea* are the biologically active constituents, due to the presence of free -OH groups in its structure (Kaouachi & Derouiche, 2018), which gives its strong antioxidant and anti-inflammatory activity by free radical scavenging properties (El Atki et al., 2019).

I.2. Characterization of nanoparticles

To confirm the synthesis of ZnNPs and CuNPs we usually used spectroscopy UV-visible. Which is an important technique to determine the formation and stabilization of metal NPs in aqueous solution (Dhandapani et al., 2020). The results indicate that the sample absorb the radiation at 225 nm. Contrary to (Moharram et al., 2014), an appeared pic in 298 nm. While, there were a maximum absorbance of CuNPs at 300 nm as confirmed by the surface plasmon's resonance (SPR), which is defined as collective excitation of negatively charged ions (electrons) in a conduction band around the surface of synthesized copper nanoparticles. Electrons present in the nanoparticles were confined to have an exact vibration mode because of their particular shape and size (Sharma et al., 2019). This result was very close to other study while they used *R.tuberosa* leaf extract in biosynthesis of CuNPs and the distinct peak at 327nm (Vasantharaj et al., 2019) .

The FTIR analysis used in order to investigate the functional groups responsible to confirm the successful preparation and stabilization of nanoparticles (Fritea et al., 2017). From the spectrums the peak of ZnNPs presented in the rang 400-700 cm^{-1}, as what reported by (Sharma et al, 2015). Nevertheless, the energy band presented at about 509 cm^{-1} correspond to CuNPs, similar to what we found in study of (Ghidan et al., 2016).

Scanning electron microscopy image of the biosynthesized ZnO nanoparticles powder have a size relatively less than 37.5 nm, while the ZnO nanoparticle synthesized using *Melia azedarach leaf* with a size rang 33-96 nm (Dhandapani et al., 2020). However, CuNPs owned a size 45.94 nm, this result as reported by (Rajeshkumar & Rinitha, 2018), when the copper nanoparticles biosynthesized by *Persea americana* seed extract and the size range from 42 to 90 nm.

X-ray diffraction (XRD) analysis showed the presence of a large number of sharply pronounced reflections, which are indicative of the crystallinity of the synthesized particles structures (Shutov et al., 2017). The ecofriendly method of nanoparticles synthesis is confirmed by XRD technique as showing in study of (Elumalai & Velmurugan, 2015) and (Sankar et al., 2014) which used *Azadirachta indica* (L.) and *Carica papaya* for synthesing ZnO and CuO respectively.

I.3. Biological activities

From our results, we can say that purslane aqueous extract has anti-oxidant activity is attributed to its constituents, such as the vitamin C and flavonoids compounds (Derouiche et al., 2017), glutathione and the total phenolic content (Petropoulos et al., 2016). However, the nanoparticles have no known mechanism of action as an antioxidant (Tettey et al., 2019). The ZnNPs results was similar to what presented by (Safawo et al, 2018), while the ZnNPs synthesized

by *C.abyssinica* tuber extract has antioxidant activity that determined by DPPH assay. Furthermore, the CuNPs had an antioxidant activity, as reported by (Ljaz et al., 2017).

Extracting plant materials is the first major step towards testing the biological activities of this plant. When a whole extract is used, there is a good chance for synergism between active components that might be lost when each of these components is isolated such as the alkaloids in *P.olerecea*, which are discovered in several medicinal tests, including those for anti-inflammatory activity (Azab et al., 2016 ; Rafieian-Kopaei & Alesaeidi, 2016). The major finding of this study indicated that purslane aqueous extract showed a significant anti-inflammatory activity when compared to the diclofenac sodium as the positive control a similar finding in (Mubashir et al., 2011). The anti-inflammatory activity of ZnNPs as reported by (Nagajyothi et al., 2015), who reveled this activity in ZnNPs synthesized from *Polygala tenuifolia* root extract, by the reduce of the mRNA and protein expressions of interleukin 6 (IL-6) and tumour necrosis factor alpha (TNF- α) on polysaccharides induced inflammation in RAW 264.7 macrophage. Also, the copper nanoparticles have a good anti-inflammatory activity as what demonstrated by (Angajala et al., 2014).

Our results demonstrated the protective effect of purslane aqueous extract and the nanoparticles against Red Blood Cells (RBCs) lysis at different concentration by measuring the concentration of Hgb in blood plasma (Tran et al., 2018). The protective effect of ZnNPs is similar to what found by (Babu et al., 2017), whether the interaction of nanoparticles with red blood cells can be caused by their small size and their physicochemical properties (Dobrovolskaia et al., 2008). Additionally, the cooper nanoparticles exhibited a protective effect in contrary to what it mentioned by (Chen et al., 2013). Also, the results of aqueous extract of *P.oleracea* as confirmed by (Karimi et al., 2011) through the antioxidants, in particular the phenolic compounds are responsible for protecting RBCs against the hemolysis process by the phenolic hydrogen atom (Chiste et al., 2014).

II. In vivo discussion

Alzheimer's disease (AD), is an irreversible neurodegenerative disorder (Chiroma et al., 2019). The primary cause of AD is still unclear, but brain cholinergic dysfunction, oxidative stress, and decreased metabolic rate of neurons are the principle and the most reported pathogenic factors (Haider et al., 2020). Aluminum is considered as a contributing factor in the etiology of several neurodegenerative disorders like AD (Liaquat et al., 2017). while exerts its effect on axonal transport and synaptic structure leading to degeneration of cholinergic neurons presenting with learning impairments (Chiroma et al., 2018), the Al^{3+} and Fe^{3+} ions are similar in size, the

former occupies iron-binding sites in transferrin and circulates throughout the body. Al first enters into the endothelial cells of blood brain barrier and then into brain cells via specific high affinity receptors for transferrin (Rather et al., 2018). Transferrin receptor is postulated to be an access point for iron into brain from circulation. Binding of aluminum to transferring reduces binding of iron to protein resulting in an increased concentration of free iron in intracellular spaces can cause peroxidation of membrane lipids resulting in memory damage (Singh et al., 2014). Also, prolonged half-life of Al raises the risk of accumulation in all the regions of rat brain, particularly in hippocampus and cortex, which are the sites responsible for memory and learning (Rather et al., 2018). Therefore, D-gal is an accepted drug for accelerating ageing (Chiroma et al., 2018), the oxidation of D-gal causes free radical generations, which inhibit cellular defense mechanism and elevate lipid peroxidation (LPO) that may cause cellular damage and dysfunctioning of the central nervous system (Samad et al., 2019). In the other hand, D-gal may also alter the configuration of protein and peptide trough the combinations with their amine groups that cause an increase of advanced glycation end (AGE) product without enzymatic glycation (Chen et al., 2018). AGEs bound to their receptors (RAGE) are common thread in aging, neuroinflammation, and neurodegeneration such as AD, whereas the AGEs increase the expression of Aβ via ROS and RAGE acts as receptor for Aβ transport across the blood brain barrier that results in cerebral Aβ accumulation and plaques formation (Rehman et al., 2016). The used in combination of the D-gal and $AlCl_3$ on rats can enhanced and induce the neurodegeneration (Chiroma et al., 2018), and more striking changes in the expression of Aβ metabolism-associated molecules similar to AD (Luo et al., 2009). Such as deficits in cholinergic system, expression of high levels of amyloidogenic proteins, oxidative stress, and memory impairments (Chiroma et al., 2018).

II.1. Acute toxicity

The results distinctly indicate that the oral administration of AEPo, ZnNPs and CuNPs, did not produce any mortality or indication of toxicity, as evaluated by the behaviour of albino rats, this results confirmed by (Aljeboori et al., 2014), (Shanker et al.,2017) and (Kadammattil et al., 2018) respectively. Thus demonstrating the safe and therapeutic applications of these aqueous extract and green-synthesized nanoparticles.

II.2.Growth parameters

Our results showed that a marked the variation in gain body weight in rats may be explained by observed change in feed intake. According to (Olajide et al., 2017); the maintenance of a reduced or elevated body weight is associated with changes in cellular energy expenditure, wherein increased metabolic demands oppose the maintenance of a constant body weight. The

overexpression of Aβ in AD showed reduced NG2-glia (Nirzhor et al., 2018), which leads to primary leptin resistance increase body weight (Djogo et al., 2016). Study of (Pakdel et al., 2018) indicate that *P.oleracea* have lipolytic effects, whether its constituent are necessary as coenzymes for converting proteins, fat, and carbohydrates into ATP. Copper treatment inhibit lipid accumulation in the body by reducing the intramuscular fat content, hepatic fat content, and inhibiting their accumulation in adipocytes by improving oxidation of fatty acid, and by promoting the lipolysis through the activation of peroxisome proliferator-activated receptor alpha (PPAR-α) signaling in the liver, skeletal muscle, and adipose tissues. Whereas PPAR-α is known to regulate lipid metabolism through a ligand-dependent transcriptional activation of the expression of genes involved in the fatty acid oxidation pathway (Lei et al., 2017). Additionally, AEPo and CuNPs have a protective effect on NG2-glia through their impact on the resistance of the Aβ aggregation as consistent by (Li et al., 2017 ; Scheiber et al., 2014) respectively. Thus, these properties could be effective in losing weight. Collectively, our evidence strongly indicated that a proper combination of copper, zinc and natural anti-oxidants might have a positive effect on the reduction of gain body weight may recurrent to energetic and lipidic regulatory metabolisms through lipolytic activating pathway. Either by prevention of AD through the anti-amyloid therapy that have been the targets of new drug development (Huang et al., 2020). Moreover, relative organs weights were executively use as marker for organ toxicity (Mohafrash et al., 2017). In the present study, $AlCl_3$ caused increase in relative liver weight in treated-rats. Similar results have been indicated by the work of (Mahmoud & Elsoadaa, 2013), this elevation may be due to liver necrosis (Derouiche et al., 2017), according to the results of histological section. In consistent with some previously published reports (Lei et al., 2017; Kang et al., 2009) reveled that CuNPs and ZnNPs decreased the relative liver weight. Additionally to U_{30}ACS which presented the same effect for the relative liver weight. This means that CuNPs, ZnNPs and U_{30}ACS might play protective effect on aluminum induced liver toxicity in rats through increasing cell division as adaptive mechanism of liver to improve liver function (Mohafrash et al., 2017).

Short term memory is generally associated with the levels of acetylcholine (ACh) (Liaquat et al., 2017), which is the key neurotransmitter involved in the learning and memory processes and the alterations on the cholinergic activity is the main event in the neurochemical changes of AD (Rather et al., 2018). Acetyl cholinesterase (AChE) is the marker enzyme for the cholinergic activity present in the central nervous system catalyzes the hydrolysis of the ACh to choline. The inhibition of acetyl cholinesterase (AChE) has been one of the most used strategies for the treatment of Alzheimer's disease (Dall'Acqua, 2013). Data from our study showed a significantly increase of brain AChE activities for the Exp Alzheimer rats. AD causes disturbances in cholinergic neurotransmission, which may be associated with altered memory and learning processes (Thenmozhi et al., 2016), in the other hand the increase of AChE activities has been linked to the genetic overexpression through oxidative stress (Qu et al., 2015). *Portulaca oleracea* leaves aqueous extract significantly decreased the activity of AChE, which reflect to their alkaloids compound (Zhou et al., 2015), especially the indole and trigonelline has been considered as inhibitor of AChE (Hostettmann et al., 2006 ; Khalili et al., 2018). However, ZnNPs apparently increased the AChE activity (Navaei-Nigjeh et al., 2018). Thus, due to the combination effect of the compounds existed in U_{30}ACS approach, the activity of this enzyme is decreased, according to down-regulation of its expression or by affecting the stereometric active site.

II.4. Protein

The increase level of protein in Exp Alzheimer due to hyperphosphorylation and abnormal aggregation of tau protein as confirmed by (Chiroma et al., 2019), and an abnormally high expression of amyloidogenic proteins such as APP, β-secretase, γ-secretase and Aβ (Wei et al., 2017). Copper nanoparticles treatment improve this increase of proteins in Alzheimer group by several mechanisms including regulation of expression of APP protein synthesis, which encompasses APP promoter sequence region (Hung et al., 2009). In addition, copper regulates the activity of β-secretase-BACE1 in the amyloidogenic pathway by its localization in the binding site in the C-terminal domain of BACE1, which proves that the expression of mRNA of BACE1 is affected by the copper level. (Mathys et al., 2017). Furthermore, the tau phosphorylation can be modulated by copper (Voss et al., 2014), while tau was found to form Cu-complexes with one Cu(II) ion bound permonomer with a dissociation constant in the micromolar range Cu(II), having

an inhibiting effect on the in vitro aggregation of tau (Kaden et al., 2011). Moreover, the presence of the three compound together may enhanced the activity of ZnNPs and purslane, which may inhibit the aggregation of Aβ according to (Vilella et al., 2018) and (Li et al., 2017) respectively. A significant decrease of protein level is a beneficial result of U_{30}ACS, which make it possible to say that this new approach may be has the ability to suppression the aggregation of Aβ and tau protein. That probably by a specific interaction with it, or by inhibition of the enzymes that responsible of its synthesis or activate the specific proteolytic system. Also, the cleavage of APP by γ -secretase, is the final step in the generation of β-amyloid. So, the γ -secretase inhibitors may be a viable therapy for reducing Aβ in Alzheimer's disease (Anderson et al., 2005), which may it possible by Cu^{2+} and Zn^{2+} ions (Isaev et al., 2019).

Aluminum accumulates in all tissues of the body including liver (Cheng et al., 2014). Al significantly decreases antioxidant enzyme activity, which enhanced ROS generates that cause lipid peroxidation and oxidative damage to proteins (Yang et al., 2018), induced carbonylation, which is an irreversible and irreparable protein modification (Thanan et al., 2012). Which may be even resistant to mammalian proteases (Korovila et al., 2017), where resulting the high level of protein in Exp Alzheimer. As confirmed in the in vitro study that purslane, ZnNPs and CuNPs have the ability to protect the protein against damage, and their power to enhance the hepatic antioxidant system in vivo. This less to conclude that a high ability of these compounds to prevent the protein from the damage and their aggregation. Additionally, previous studies (Qiao et al., 2019 ; Jiang et al., 2018) revealed that the decrease of protein level may be due to the effects of *Portulaca oleracea* extract and ZnO-NPs on inflammatory cytokine, by suppressing the NO production as well as the related protein expressions of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). The advantageous results of U_{30}ACS approach may be reflect to its power to reduce the mechanisms of protein oxidation or by its ability to enhancing a cellular system to activate their preexisted protein in order to face the protein modification or stopping the production of free radical. Furthermore, it may be intervening in the anti-inflammatory side through reducing the production of pro-inflammatory cytokine. Which may be by reducing the nuclear translocation of the transcription factor, or it could be attributed to alterations of intracellular signaling.

II.5. Hematological parameters

Besides the brain's innate immune system, the resident microglia, peripheral macrophages (monocyte) as well as cells from the adaptive immune system (lymphocyte T), are increasingly recognized as being involved in AD pathology (Unger et al., 2018). Suggested that the abundance of leucocytes in general and lymphocyte in particular is a prominent response of body tissues

facing injurious impact of $AlCl_3$ (Saleh et al., 2018). Additionally, immuno imbalance occurs mainly due to oxidative damage, which is responsible for ROS generation by enhancing the inflammatory response (Dey et al., 2020). NF-κB is a transcriptional factor that plays a key role in activating a cascade of processes involved in the defense of the organism, where regulator for pro-inflammatory gene expression, by producing cytokines such as TNFα, IL-1β (Abhimannue et al., 2016). However, over-expression of NF-κB may also lead to subclinical chronic inflammation (Sawosz et al., 2010). When a cascade event like endothelial cell activation, lymphocyte-monocyte adhesion to activated endothelium, their transmigration into extra vascular space, leads to the production and subsequent release of pro-inflammatory cytokines and chemokines; the key mediators of inflammation (Abhimannue et al., 2016). PLT can also act as mediators between innate and adaptive immune systems. When it activated at inflammatory sites, they excrete large amounts of pro-inflammatory substances located in their intracellular granules, by which they crosstalk, recruit, and activate leukocytes and endothelial even at distant sites (Voudoukis et al., 2014). The significant effect of *P. oleracea* as an immunomodulator is mostly associated with its anti-inflammatory activity as previously reported (Catap et al., 2018). It is likely then, that the ameliorative property of purslane could be attributed to plant-derived alkaloids such as trigonelline, sildenafil and betaine may regulate NF-κB factor as what reported by (Khalili et al., 2018 ; Nunes et al., 2015 ; Go et al., 2005) respectively. While the ability of reduction TNFα and IL-1β pro-inflammatory cytokine could be by citric acid and indole as consistent with (Abdel-Salam et al., 2014 ; Da Silva Guerra et al., 2011) respectively. The nano minerals have more significant immunomodulatory effects than the conventional counterparts (Atef et al., 2016). The information obtained from (Jarosz et al., 2017) suggest that zinc supplementation may lead to down-regulation of the inflammatory cytokines (IL-1β and TNF-α) through up-regulation A20 to inhibit induced NF-κB activation. While the high level of copper may be led to inhibit this factor too (Iseki et al., 2000). The immunoregulated of WBC effect shown by U_{30}ACS approach, which probably due to its anti-inflammatory activity through the inhibition of cytokines either by decrease their synthesis or decrease their concentration in free active form or block their interaction with specific receptors.

II.6. Lipid profile

In this study $AlCl_3$ exposure caused a significant decrease in each of triglycerides (TG) confirmed by (Ali et al., 2018), while a significant increase presented in total cholesterol (TC) compared to the controls. The accumulation of $AlCl_3$ in the liver may lead to a disturbance of lipid metabolism and elevation in lipid profile. Also, $AlCl_3$ increased lipid peroxidation and significantly affect various membrane-bound enzymes loss of membrane integrity, which

altered lipid metabolism and were closely associated with hypercholesterolemia due to hepatic dysfunction (Al-Eisa et al., 2017). The results of this study showed that the purslane extract, reduce the levels of TC, due to its contains of phenolic alkaloids, which are substances that can inhibit cholesterol synthesis (Changizi-Ashtiyani et al., 2013). In addition, the administration of Zn has improved the health status of patients suffering from hyperlipidemias (Bayrami et al., 2019). Because a zinc can play an important role in enzymes involved in lipid metabolism (Derouiche & Kechrid, 2016). Furthermore, study of (Galhardi et al., 2004), indicate that the exposure of Cu may be increase the total cholesterol level. However, study of (Majewskia et al., 2019) signal that the CuNPs has not any significant change in TG level, this may enhance our obtained results.

II.7. Biochemical parameters

The soluble enzymes of blood serum have been considered as indicators of the hepatic dysfunction and damage (Al-Qayim et al., 2013). Thus, the significant raise of marker liver function enzyme (GPT) denoted in the Exp Alzheimer rats indicate the grate cellular damage of hepatocytes caused by $AlCl_3$/D-gal administration. The decrease of serum GPT in AEPo group results of hepatoprotective activity of the phytoconstituents like the flavonoids, triterpenoids, saponins and alkaloids, which presented in our extract (Anusha et al., 2011). Also, the protective effect of ZnO-NPs demonstrated by a significant decline in liver enzyme as confirmed by (Bashandy et al., 2018). In our study, the results revealed that the treatment by CuNPs decreased the level of serum GPT. However this result in contrary with (Mohammadyari et al., 2014), which find after the exposure to CuO at high concentration an increase in the level of GPT, while he conclude that the exposure of CuO nanoparticles in doses (<400ppm) is safe for biomedical application and has no side-effects. Consequently, the reversal of increased serum enzymes in toxic-induced liver damage may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity, which consistent by our in vitro results. The powerful protecting hepatocytes by U_{30}ACS approach presented when decreasing GPT level, which allows us to suggesting that this new approach may be has the power to prevent hepatic dysfunction by membrane stabilization or to be facing either to oxidative stress or to inflammatory mechanisms.

II.8. Oxidative stress

The Aβ promotes oxidative stress, which is known to play an important role in the pathogenesis linked to the etiology of Alzheimer's disease (Cheignon et al., 2018). In this study, the oxidative stress was highly remarked in Exp Alzheimer group by the decrease in SOD activity and GSH level. Meanwhile, MDA level were dramatically elevated, this results similar to what mentioned by (Resende et al., 2008). So, to investigate the effect of our compound on AD and as it known

that the blood brain barrier (BBB) is a highly complex structure that allows the passage of all essential molecules for functional brain activity. The applications of nanotechnology in AD, including the delivery of neuroprotections against oxidative stress, antiamyloid approaches, which could benefit from delivery beyond the BBB (Krol et al., 2012). The positive role of copper promotes the non-amyloidogenic processing of APP and thereby lowers the Aβ production (Kaden et al., 2011), by increased the secretion of α-cleaved, and hence decreases β-cleavage of APP (Mathys & white, 2017). Thus decreased the oxidative stress for the CuNPs rats. However, the ZnNPs group no demonstrate any improve in oxidative stress state and this is may be due to its lower concentration employed on our treatment, and this probably the same reason of the non-diminution of MDA level in CuNPs. Furthermore, AEPo mainly decreased lipid peroxidative damage. This antioxidant effect of purslane may be a result of its relatively rich content of known antioxidant vitamins such as vitamins C (Derouiche et al., 2017). Which is good antioxidant capable of preventing lipid peroxidation in tissue (Abdel-Moneim et al., 2013). Accumulating evidence (Demurtas et al 2019 ; Miralles et al., 2005) respectively, reported that the indole and norharman considered as an active antioxidant compounds, while the second one is a reversible inhibitor of the monoamine oxidases (MAO) activities, as well as protecting membranes, organelles and protein against oxidative damage. From all of this, we can say that the two nano-compound associated with an natural antioxidant power of plant in U_{30}ACs approach can give an ideal reduction, due to the presense of Cu/Zn as a cofactor of SOD enzymes. Which serves as a first line of the antioxidant defense system, which converts superoxide anion to hydrogen peroxide (H_2O_2) and oxygen (Li et al., 2017). Additionally, the Cu^{2+} and Zn^{2+} were shown to suppress the activity of γ secretases that, along with beta- site APP cleaving enzyme 1 (BACE1), are involved in the formation of Aβ (Isaev et al., 2019), which can contribute to a reduction in the formation of this peptide that may explicate the reduction of oxidative stress in this group.

Liver is the key organ regulating homeostasis in the body. Which plays a major role in detoxification and excretion of many endogenous and exogenous compounds, fight against disease, nutrient supply, energy production and reproduction. Because of its unique metabolism and relationship to the gastrointestinal tract, the liver is an important target for toxicity produced by drugs, xenobiotics and oxidative stress (Anusha et al., 2011). The role of aluminum in causing liver damage is receiving considerable attention, which accumulates more in the liver than in the brain, and may mediate Alzheimer's disease through liver toxicity by disturbing ATP and mitochondrial metabolism. It is known that Al induces liver dysfunction, one possible mechanism of Al-induced liver toxicity is induction of oxidative stress and disturbance of the intracellular

redox system (Cheng et al., 2014), which has been considered as a conjoint pathological mechanism for the initiation and progression of liver aging (Gao et al., 2018).

The sub-chronic exposure of $AlCl_3$ and D-gal induce hapatoxicity, which manifested by a remarkable oxidative stress. Several investigations reported that Al has the ability to potentiate iron-mediated LPO. This through replacing iron ions with Al and the subsequent increase in the amount of the free iron which have a strong catalytic power to generate highly reactive hydroxyl radicals from hydrogen peroxide through Fenton's reaction. These radicals are able to initiate LPO and cellular damage (Abdel-Wahab, 2012). In the other hand, D-gal is oxidized into hydrogen peroxide (H_2O_2) and aldehydes by galactose oxidase when it exceeds its normal level (yanar et al., 2011). The finding of the present study showed that the administration of $AlCl_3$ and D-gal induced a status of oxidant/antioxidant imbalance, as was indicated by (Abdel-wahab, 2012) and (Yu et al., 2015), the administration of aluminum chloride (200 mg/Kg) increased the MDA level with a concomitant depletion in GSH content and in SOD activity. Additionally, (Saleh et al., 2019) demonstrated that the D-gal treatment decrease the activity of GST in the liver. So Al and D-gal intake can be related to alteration in the activity of tissue antioxidant enzymes and promotion of oxidative stress. *P.oleracea* leaves extract improved the oxidative stress through enhancing the activities of SOD and GST, hepatic glutathione. Due to its anti-oxidant activity through its phytochemical compound (alkaloids, flavonoids, phenols continent) as discussed previously (in vitro parte). There is complex interaction between antioxidants and oxidants such as reactive oxygen species, which modulates the generation of oxidative stress (Derouiche et al., 2017). The glutathione obtained from the purslane is directly absorbed by the gastrointestinal tract, which can readily increase the antioxidant status (Sadeghi et al., 2016). The Antioxidant property of *P.olerecea* could be attributed to the endogenous GSH antioxidant balance against cellular oxidation, which protect the GSH related enzymes and in turn inhibits the oxidation of GSH which acts as a substrate for GPx in animal cells (Sudhakar et al., 2010). The treatment with ZnNPs can improve antioxidant activity. The mechanism of what could be the ZnNPs enter in the target cells where it dissociates and slowly but consistently released as Zn^{+2} ions (Hassan et al., 2019). It is well known that Zn is a powerful antioxidant metal; it is the core constituent of the antioxidant enzymes such as SOD (Abd El-Fatah et al., 2017). The activity of antioxidant, which then detoxify the free radicals. These factors protect cells from ROS damaging (Atef et al., 2016). The copper has an antioxidant role through involvement in maintaining antioxidant defenses, which may to ameliorate the state of oxidative stress (Buzadžić et al., 2002).The elevation in GSH after the treatment with NPs might further help in restoring normal cellular redox status (Hassan et al., 2019). Glutathione S-Transferase (GSTs) are affected

in the all experimental groups, GST plays an important role in the detoxification of toxic electrophiles by catalyzing the conjugation of these electrophiles with glutathione to form more water soluble compounds to protect against peroxidative damage (Sudhakar et al., 2010). The extensive damage induced by $AlCl_3$ and D-gal in the liver tissue and the low treatment doses of CuNPs and ZnNPs may be could not improve the hepatic lipid peroxidation. However, in the group treated by the U_{30}ACS approach, we noticed a significant improvement in of oxidative stress markers by decreasing lipid peroxidation and an increase in antioxidants, which gives the impression that this new approach has great effectiveness as an power antioxidant, either by its superior ability to limit the spread and production of free radicals or by activating it to the enzymatic antioxidants or it could also have a benefic effect in stimulating liver cells to increase the synthesis of antioxidants such as glutathione.

II.9. Histological analysis

Our histological results indicated that there is a necrosis, hemorrhage and infiltration of immune cells in brain section of Exp Alzheimer group, similar finding with (Syed Umesalma, 2015). Which revealed that AD mediates progressive alterations, including neurons degeneration, apoptotic changes and necrosis, which related to oxidative damage of brain cells via free radical production (Abd-Elghaffar et al., 2005), whereas the oxidative stress state showed in Exp Alzheimer rats may be confirmed this suggestion. The extracellular amyloid beta and tau protein play a significant role in neuroinflammatory processes, hence the vasculotropic, Aβ peptides that may cause cerebral hemorrhage (Bell & Zlokovic, 2009). Additionally, can affect the blood brain barrier (BBB) function by increasing vascular permeability to small molecules, plasma protein and finally enhancing migration of immune cells from periphery into the brain parenchyma (Majerova et al., 2019), which can explain the inflammatory cell infiltration in our histology results. The potent effect of AEPo, ZnNPs and CuNPs in AD was very clearly through the histological brain section. This may be translate by the potential anti-inflammatory and stabilization of cells membrane of each one, that may be refer to their reduction power as what demonstrate in in-vitro study. Without forgetting the strong effect of these compounds on the accumulation of Aβ and Tau protein, which is the main cause of the appearance of both oxidative stress and inflammation in the brain. Where many studies have shown in order that zinc, copper and their combination either, have the anti-amyloid activity (Hane et al., 2016 ; Scheiber et al., 2014 ; Bagheri et al., 2018). Moreover, the potential effect of *P.oleracea* enhanced by its alkaloids. Trigonelline and sildenafil in this species, which possibly contribute to its activity as confermed by (Makowska et al., 2014) that trigonelline is mostly positioned close to the imidazole ring of His13 of Aβ peptide , altering its structure and therefore inhibiting its aggregation, according to Docking Procedure.

Also, Sildenafil is successful in inhibiting neuroinflammation and is able to lower amyloid-β in aged mice models (Durães et al., 2018). Additionally, this both compound have an effect on tau aggregation through an apoptotic activity by inhibiting the pro-inflammatory as reported by (Khalili et al., 2018 ; Nunes et al., 2015) respectively. Where the same effect showed by citric acid (Abdel-Salam et al., 2014) and betaine (Xia et al., 2018). While, norharman may to alter the tau phosphorylation by inhibiting dual specificity tyrosine-phosphorylation-regulated kinases activity (DYRK1A) (Frost et al., 2011). The ameliorative effect of our new approach U_{30}ACS may appear distinctly in its histological section, whether the presence of the three compound together may enhance either its anti-inflammatory activities by inhibition the pro-inflammatory substances or inhibit the amyloidogenic activity and probably affect the BBB by decreasing vascular permeability.

The expected toxic effect of Al in liver is related to the generation of reactive oxygen species (ROS), which results in oxidative damage. That attributed to severe cell membranes damage, increased permeability of plasma membrane (Abdel-Wahab, 2012) and vascular alteration induced hemorrhage (Wolf & Wheeler, 2018). Results revealed that the distorted liver architecture marked vacuolization of hepatocytes and inflammatory cell infiltration, which are in agreement with (Hosseini et al., 2020). In addition, $AlCl_3$ induced a significant elevations in the levels of pro-inflammatory cytokines; including TNF-α that cause apoptosis degeneration of liver cells (Al Dera, 2016), the restoration of normal physiological state and reduction of histopathological alterations provoked by $AlCl_3$ was quite appreciable by AEPo. It can be attributed to the antiradical/ antioxidant efficacy, which significantly reduced the oxidative stress. These results suggest that, the derivatives indole (Da Silva Guerra et al., 2011) and betaine (Ahn et al., 2014) act as modulators of the immune system by decreasing cell migration and the production of pro-inflammatory cytokines. The protective effect of ZnNPs and CuNPs not clearly appear in histological section, this may refer to the strong liver injury induced by aluminum. Alternatively, the short period of treatment for the liver in order to restore the damaged cells, and their regeneration through the beneficial effect of these components as what confirmed in our results, by the amelioration of anti-oxidant system and decrease level of liver enzyme in blood. The hepatoprotective capacity of new approach U_{30}ACS may be by its affecting on several mechanisms and factors that might be contribute in the liver disorder. Either related by the different molecules and pathways, which included in oxidative or inflammatory system.

Conclusion

Alzheimer's disease (AD) is the most prevalent neurodegenerative disease and a major cause of death worldwide. So far, the therapeutic paradigm “one-compound-one-target” has failed, the disease is still incurable. Even entering into the brain is always a big challenge for the drug moieties acting on of central nervous system disorders, recently, the light is shed to the multi-target drugs due to their abilities to inhibit the factors currently widely recognised to be involved in AD, depending on to the obtained results we can be conclude that:

- ✓ The phytochemical findings demonstrate that *Portulaca oleracea* L. is rich source of phenols, flavonoids, alkaloids, saponins and terpenoids. analysis identify several important alkaloids as trigonelline, sildenafil, indole, betaine, citric acid and norharman. This allows us to discover a new source of bioactive molecules that have an important therapeutic effect against several pathologies.
- ✓ The green synthesis of ZnNPs and CuNPs using *P.oleracea* refer to their potential properties as natural stabilizers of nanoparticles, which characterized by different methods; UV-VIS spectroscopy, FT-IR spectroscopy and SEM analysis. Also we may be classify these compounds as one of the important biological and medical molecules that can used in various fields due to the powerful in vitro stabilization of cell membrane, anti-inflammatory and antioxidant activities.
- ✓ The acute toxicity study allow the safe and therapeutic applications of purslane aqueous extract and green-synthesized nanoparticles.
- ✓ Our treatment *P.oleracea*, ZnNPs, CuNPs and U_{30}ACS ameliorate the physiological state in our study, via the loss of body gain weight and the relative liver weight that clearly support the efficacy therapeutic compound against tissue alteration.
- ✓ The observed abnormalities of acetyl cholinesterase activity and learning memory function corrected partially by ZnNPs, CuNPs and U_{30}ACS treatment. Whereas the AEPo treatment is the best improver for the AChE activity which contribute the cholinergic cellular signalization as restoring the learning memory function.
- ✓ All treatments decreasing the protein level in each of brain and liver that demonstrate a protective effect on the carbonylic protein induced by oxidative stress, while U_{30}ACS and CuNPs act as anti-amyloidogenic and anti-tau in Alzheimer disease.
- ✓ Overall, we demonstrate that all our treatments ameliorate immunity defense system specially the nanotherapy shown a remarkable immunomodulatory of innate and adaptive lines. Which lead to the protective effect of these therapeutic types against the neuro and hepatoinflammatory action.

- ✓ The positive impact of AEPo, ZnNPs, CuNPs and U_{30}ACS approach on restoring some of lipid profile and biochemical markers that demonstrate the beneficial effect of treatment against the physiologic and metabolic alteration in several biological system related to our studying data.
- ✓ Oxidative stress state, which is the main cause of the AD is attenuating by *P.oleracea*, ZnNPs and CuNPs treatment that limit radical phenomena and repairing oxidative damage by reducing lipid peroxidation in brain and liver and improving the mobility of the antioxidant defense. Whereas, the new approach treatment (U_{30}ACS) present the bests therapeutic effect, that we can used it against pathologies associated with oxidative stress including Alzheimer disease.
- ✓ The high protection ability of our treatment not limited to biological parameters level but extended to microscopic level which shows their high protection in cells stabilization against neuro and hepato injury induced by oxidative stress. We shed the powerful effect of U_{30}ACS and purslane in our histological sections. This allows us to considered that these treatments have potential effect to limiting the neurodegeneration which associated with Alzheimer disease.

Finally, Based on all of the above, we can conclude that the new approach U_{30}ACS relying on nanotechnology has proven highly effective against Alzheimer's symptoms, in all biochemical, hematological, immunological, histological and oxidative stress aspects, which opens up new hopes for Alzheimer's patients on the possibility of treating them to reduce their suffering.

Perspective

Given the importance of these results, they open experimental perspectives and other indepth studies that should allow us to clearly identify:

- ➢ The orientation to nanotherapy in order to ameliorate the acute or another chronic disease.
- ➢ The extraction of purslane alkaloids and their application to inhibit the factors currently widely recognized to be involved in AD.
- ➢ Try to make a specific drug form fromU_{30}ACS approach.
- ➢ Improve the U_{30}ACS approach efficiency and evaluating the long-term effect of its use.
- ➢ Evaluation of the effect of U_{30}ACS approach on synthesis and secretion of tau protein, amyloid β and secretases enzymes.

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Annex

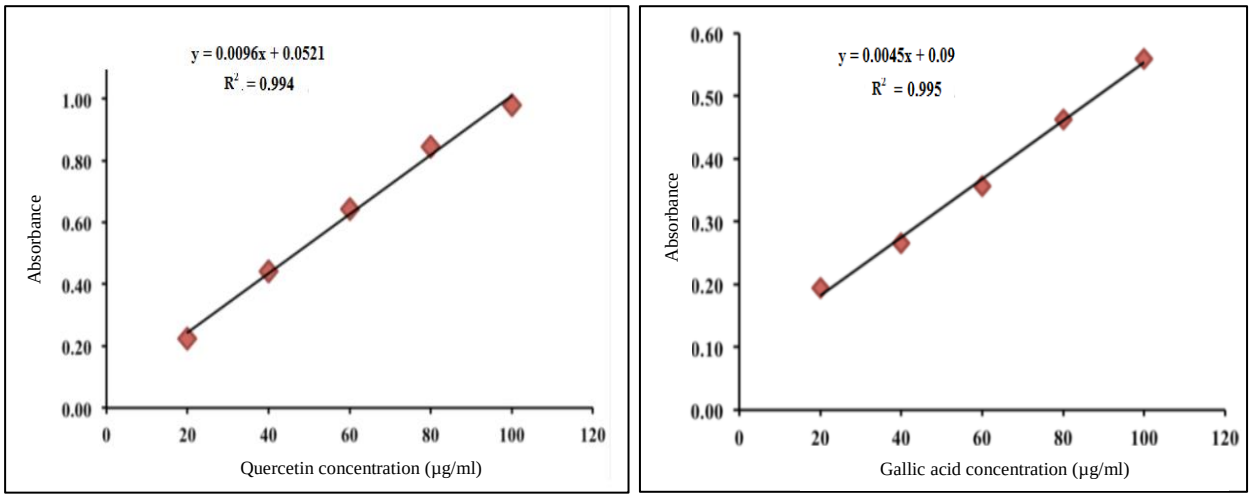

Figure: Calibration curves of Quercetin and Gallic acid

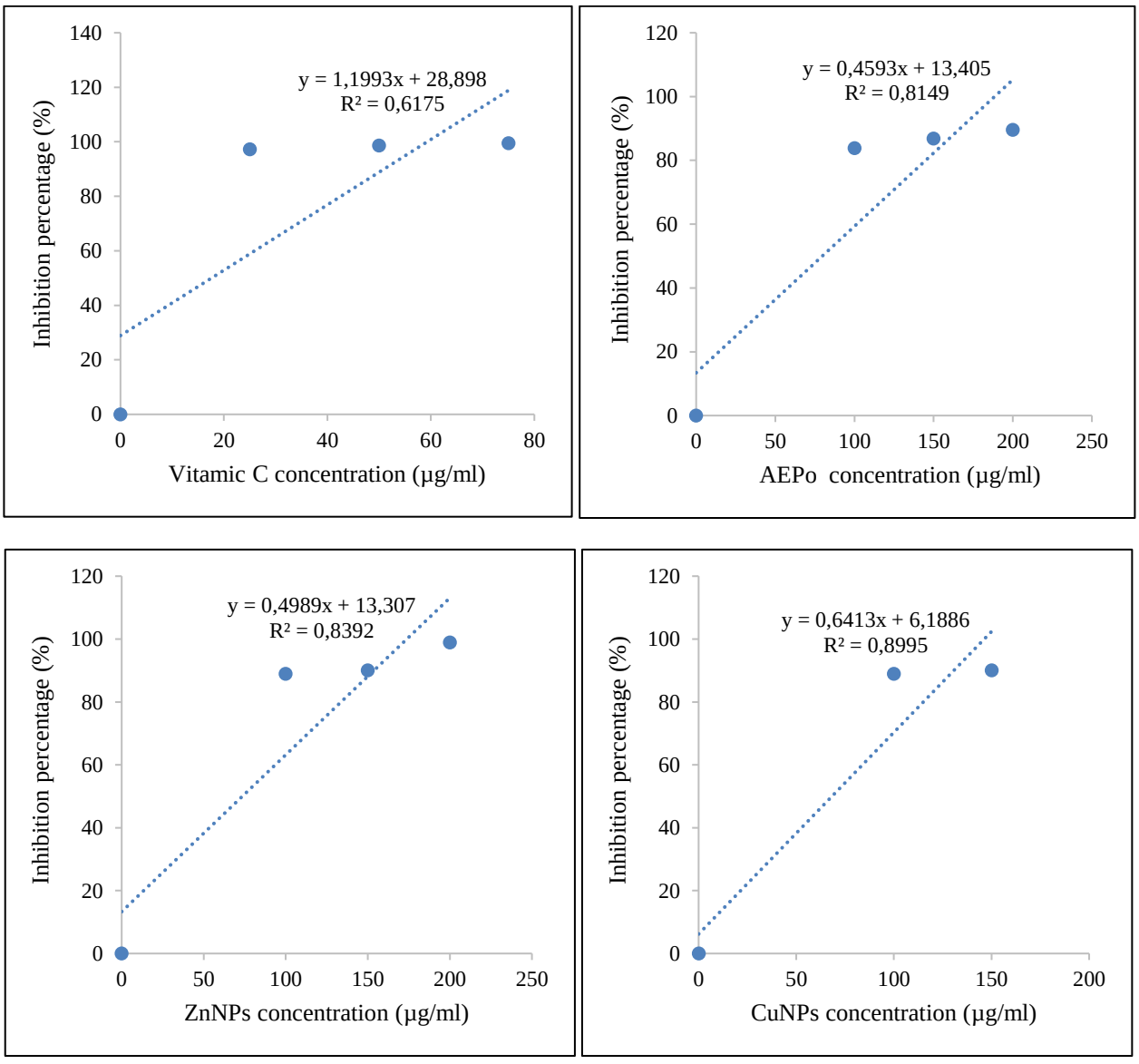

Figure: Inhibition percentage versus ascorbic acid, purslane aqueous extract, ZnNPs and CuNPs concentrations for the determination of IC50 for FRAP activity

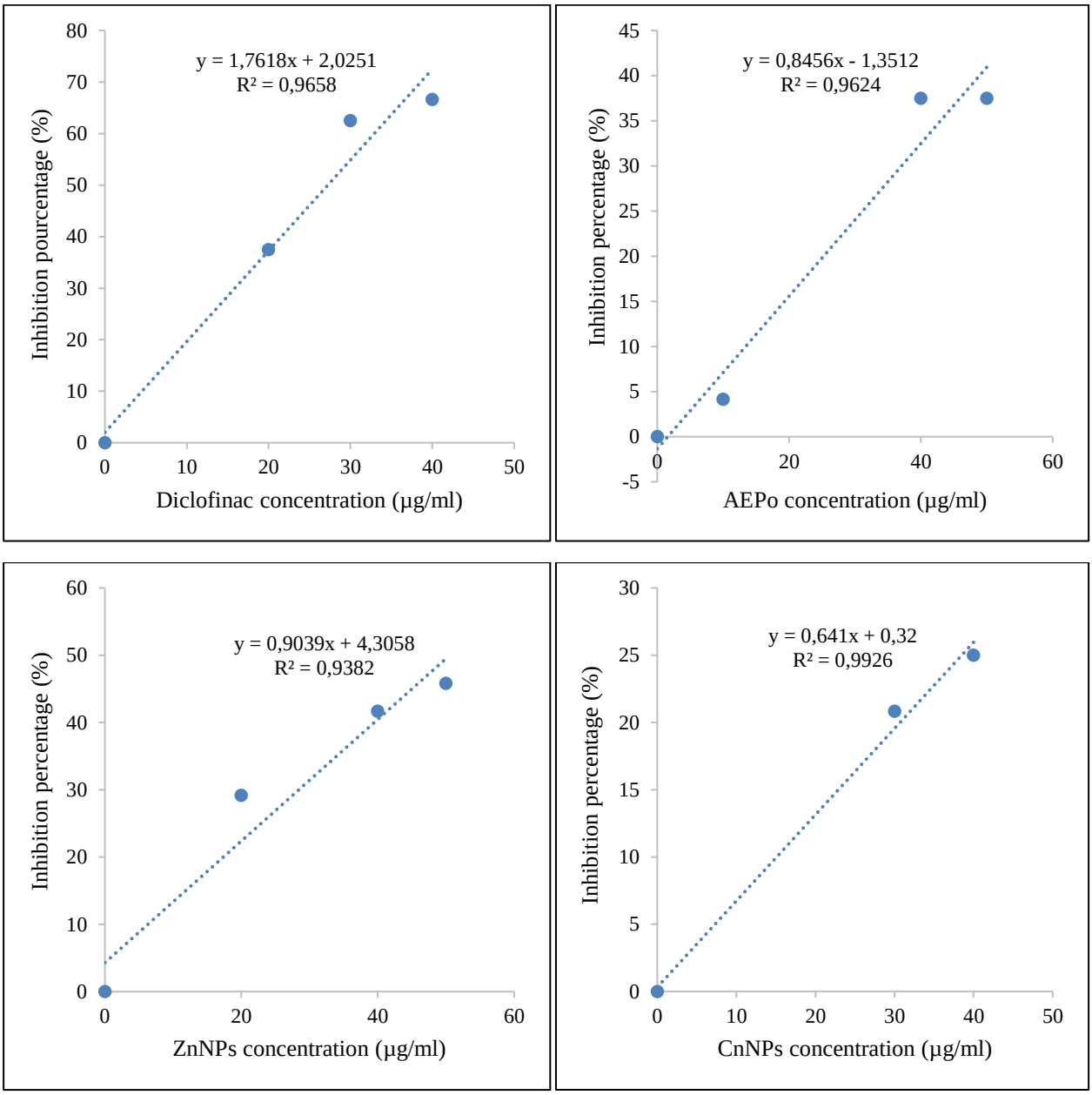

Figure: Inhibition percentage versus diclofinac, purslane aqueous extract, ZnNPs and CuNPs concentrations for the determination of IC50 for anti-inflammatory activity

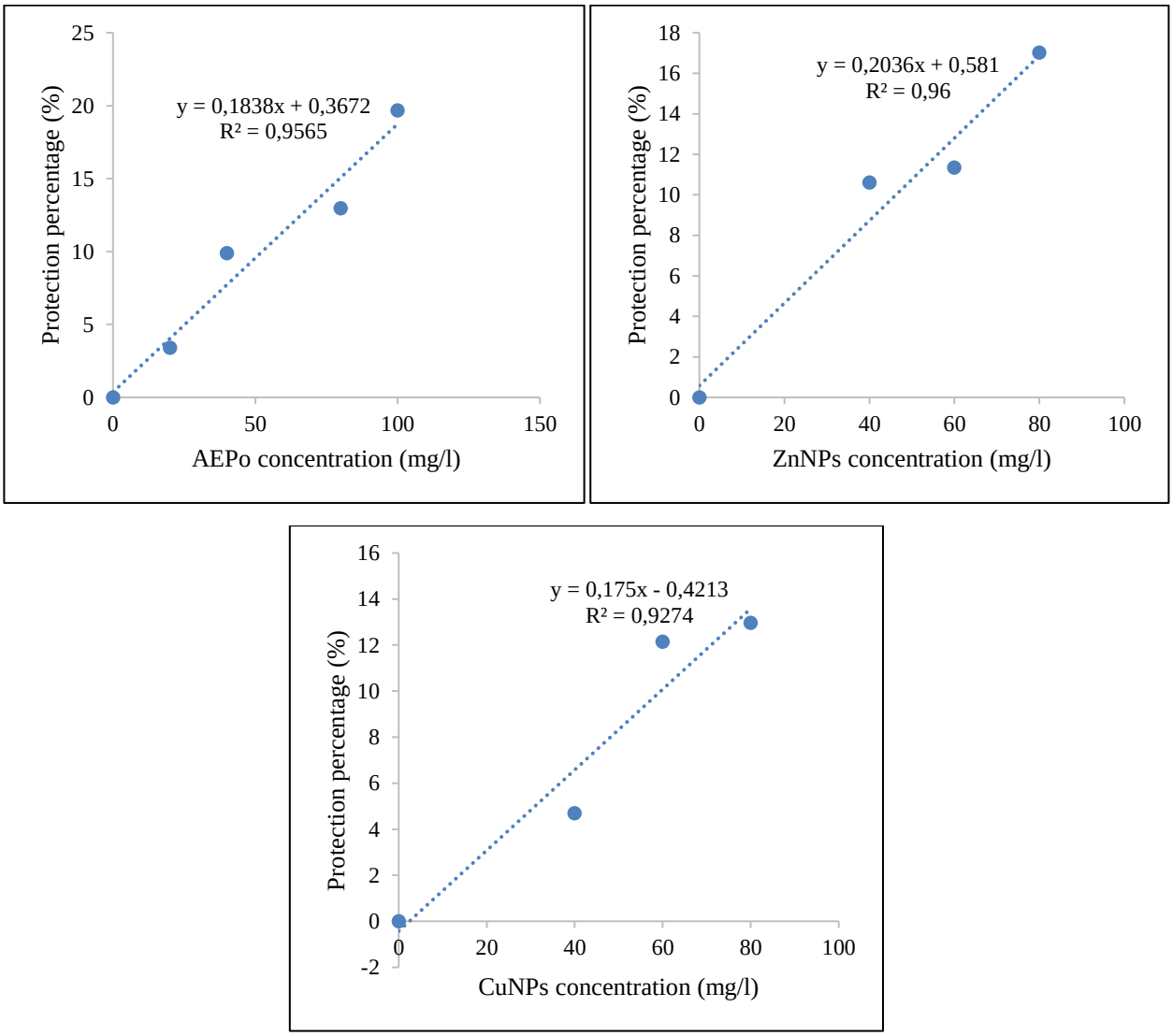

Figure: Protection percentage versus purslane aqueous extract, ZnNPs and CuNPs concentrations for the determination of IC50 for hemolysis assay

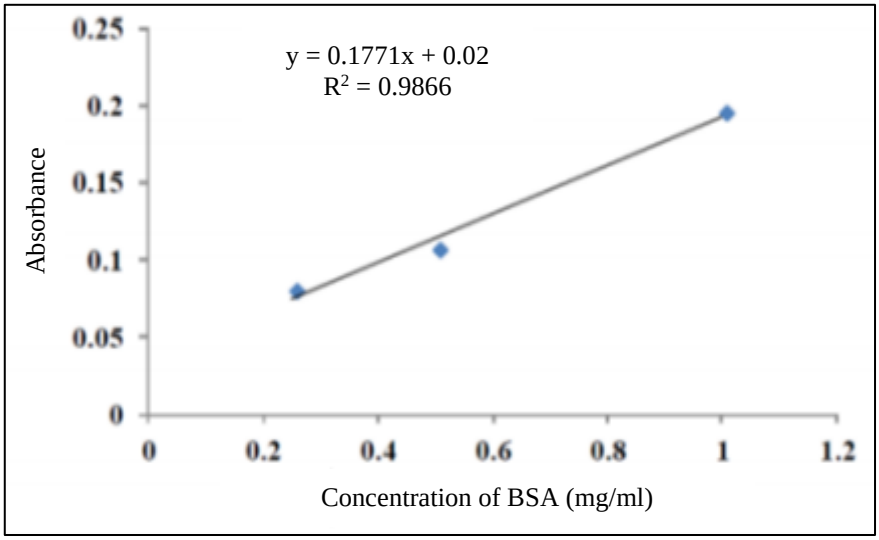

Figure: Calibration curves of bovine serum albumin for the determination of protein