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In-vitro and *in-vivo* antifungal effect of silver oxide and *Syzygium aromaticum* in *Wistars* rats

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Abstract

The objective of this work is to study the therapeutic effect of the aqueous extract of *S. aromaticum* and silver nanoparticles AgNPs on certain biochemical, hematological and oxidative stress parameters and on liver and testis histological in male wistar rats infected by *Aspergillus niger*.

The experimental study carried out in the laboratory on 25 male Wistar rats weighing 130-245 g were divided into four groups, the first group serves as a control, the second is contaminated by *Aspergillus niger* spores, and the third is contaminated by fungal spores and treated with AgNPs nanoparticles (80 µg/kg) and the fourth is contaminated with fungal spores and treated with the aqueous extract (200 mg/kg). Some biochemical, hematological and oxidative stress markers were determined. Biosynthesized AgNPs were characterized by analytical methods and the in-vitro antifungal activity was applicated using standard methods.

Results of in vitro phytochemical and HPLC analysis results revealed that *S. aromaticum* contains most of active compounds, especially flavonoids (naringin and chlorogenic acid) with high antioxidant activity and without toxicity in rats. But the AgNPs has a little toxicity in rate up a dose (800µg/ kg). Characterization of AgNPs confirmed the involvement of biological molecules in AgNPs synthesis with size ranged from 19.8 to 92.8 nm and the average diameter was 46 nm. Results of in-vivo rats study showed a change in hematological and some biochemical parameters in rats infected by fungal spores compared to control. Also, results illustrated an oxidative stress in rats infected by *Aspergillus niger* with high level of MDA and low level of GSH compared to control. Histopathology of liver and testis tissues tested confirmed the altered in these tissues with inflammation, necrosis, hemorrhagic in rats infected by fungal sport compared to control. However, the obtained Results show a partial improvement of all of previous alteration in rats treated with *S. aromaticum*plant and AgNPs compared to infected rats.

In conclusion, the present study suggests that AgNPs and *S. aromaticum* extract could be a substantially promising anti-inflammatory, antioxidant and antifungal effect which can be used in treatment of disease caused by this fungal.

Keywords: *Aspergillus niger*, *Syzygium aromaticum*, AgNPs, Caracterization, Oxidative stress, inflammation, Wistar rats.

الملخص

الهدف من هذا العمل هو دراسة التأثير العلاجي للمستخلص المائي لنبات *S. aromaticum* وجزيئات الفضة النانوية AgNPs على بعض المعايير البيوكيميائية ومعايير الدم ومعايير الاجهاد التأكسدي وعلى نسيج الكبد والخصية في ذكور فئران ويستار المصابة بعدوى الفطريات *Aspergillus niger*

الدراسة التجريبية أجريت في المختبر على 25 ذكور من جرذان ويستار وزنها 130-245 جرام حيث تم تقسيمها إلى أربع مجموعات ، المجموعة الأولى شاهدة ، والثانية مصابة بعدوى الفطريات ، والثالثة مصابة بعدوى أبواغ فطرية و معالجة بالجسيمات النانوية AgNPs 80 ميكروغرام / كغ والرابعة مصابة بعدوى الجراثيم الفطرية معالجة بالمستخلص المائي لنبات *S. aromaticum* (200 مغ / كغ). تم تحديد بعض المعايير البيوكيميائية والدم والإجهاد التأكسدي. كما تم تعيين وتحديد AgNPsذات التركيب الحيوي بالطرق التحليلية. تم تطبيق النشاط المضاد للفطريات في المختبر باستخدام الطرق القياسية.

أظهرت نتائج التحليل الكيميائي النباتي و HPLC في المختبر أن *S. aromaticum* تحتوي على معظم المركبات الفعالة ، خاصة مركبات الفلافونويد (النارينجين وحمض الكلوروجينيك) ذات الفعالية المضادة للأكسدة العالية وبدون سمية في الجرذان. لكن AgNPs لها سمية قليلة عند الجرعات التي تقارب (800 ميكروغرام / كجم). التحليل الطيفي لجزيئات النانو اظهرت الحصول على الحجم النانوي للفضة بما يقارب 42 نانومتر لحجم لهذه الجزيئات. أظهرت نتائج الدراسة التي أجريت على الفئران تغيرا في خصائص الدم وبعض المعايير البيوكيميائية في الجرذان المصابة بالجراثيم الفطرية مقارنة بالشاهدة. كما أوضحت النتائج وجود إجهاد تأكسدي في الفئران المصابة بالجراثيم الفطرية مع مستوى مرتفع من MDA ومستوى منخفض من GSH مقارنة بالشاهدة. من جهة اخرى أكد التشريح المرضي لأنسجة الكبد والخصية التي تم اختبارها حدوث تغير في هذه الأنسجة مع التهاب ونخر ونزيف عند الفئران المريضة مقارنة بالشاهدة. ومع ذلك ، فإن النتائج التي تم الحصول عليها تظهر تحسنًا جزئيًا في جميع التغييرات السابقة في الفئران التي عولجت بالمستخلص المائي لنبات *S. aromaticum* و AgNPs مقارنة بالفئران المصابة.

في الختام ، تشير الدراسة الحالية إلى أن AgNPs ومستخلص نبات *S. aromaticum* يمكن أن يكون علاجا واعدًا مضادًا للالتهابات ومضادًا للأكسدة ومضادًا للفطريات مما يمكن استخدامه في علاج الأمراض التي تسببها هذه الفطريات.

الكلمات المفتاحية: *Aspergillus niger* ، *Syzygium aromaticum* ، جزيئات الفضة النانوية ، التحليل الطيفي ، الاجهاد التاكسدي ، الالتهابات ، فئران ويستار.

Abbreviation list

A.niger: *Aspergillus niger*

Ag : Silver

AgNPs: Silver nanopartecils

AgO: Silver oxide

BHT: Butyl hydroxy toluene

Ca2+: calcium

CAT: catalase

CBC : complete blood count

CINC-2α : cytokine-induced neutrophil chemo- 2 alpha

COX-2: cyclooxygenase-2

CYA: Czapek Yeast Agar

CYP450: Cytochrome P450

DNA: deoxyribonucleic acid

DNAase: deoxyribonuclease

DPPH: 1,1-diphenyl-2-picryhydrazyl

DTNB: Acide 5,5'-dithiobis(2-nitrobenzoique) ou reactif d'Ellman

EDS: energy-dispersive spectroscopy

EDTA: ethylenediaminetetraacetate

EGCG: EGC 3-gallate

FDA: Food and Drug Administration

FRAP: Ferric Reducing Antioxidant Power

FTIR: Fourier-transform infrared spectroscopy

G25N: Glycerol Nitrate Agar

GOT: Glutamic Oxaloacetate Transaminase.

GPT: Glutamic Pyruvate Transaminase.

GSH: reduced Glutathione

HCC : hepatocellular carcinoma

HCT: haematocrit

HGB: haemoglobin

HPLC: High-performance liquid chromatography

IC50: Inhibitory Concentration of 50%

ICAM-1: Inter Cellular Adhesion Molecule -1

IFNγ : Interferon, gamma

IL-10 : interleukins 10

IL-1β : interleukins 1β

IL-6 : interleukins 6

IL-8: interleukins 8

LPO : Lipid peroxydation

MDA: Determination of malondialdehyde

MEA: Malt Extract Agar

mRNA: Messenger RNA

NADPH: Nicotinamide adenine dinucleotide phosphate

NF-κB: nuclear factor-kappa B

OTA : Ochratoxin A

PDA: Potato Dextrose Agar

RBC: red blood cell

ROS : reactive oxygen species

S.aromaticum: *Syzygium aromaticum*

SDA: Sabouraud Dextrose Agar

SEM: Scanning Electron Microscope

SOD : Superoxyde dismutase

TBA : Thiobarbituric acid: L'acide thiobarbiturique

TCA: Trichloroacetique

TNF α: tumor necrosis factor

UV-vis: UltraViolet-Visible

WBC: white blood cell

WHO: World Health Organization

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Introduction

Introduction

Food poisoning is considered as one of the most common cause of illness and death in developing countries **(Sapkota *et al*., 2012)**. In addition, pathogenic microorganisms that did not receive much resonance are fungi poisoning or fungal spore poisoning. Fungi have long been known to affect human well-being in various ways, including disease of essential crop plants, decay of stored foods with possible concomitant production of mycotoxins, superficial and systemic infection of human tissues, and disease associated with immune stimulation such as hypersensitivity pneumonitis and toxic pneumonitis. Diseases associated with inhalation of fungal spores include toxic pneumonitis, hypersensitivity pneumonitis, tremors, chronic fatigue syndrome, kidney failure, and cancer**. (Sorenson, 1999)**

Prevention of food spoilage and their etiological agentis traditionally achieved by the use of chemical preservatives **(Yamamura *et al*., 2000; Shan *et al*., 2007)**. Despite of the proven efficiency of these chemical preservative in prevention and outbreak control of food poisoning diseases, their repeated applications has resulted in the accumulation of chemical residues in food and feed chain, acquisition of microbial resistance to the applied chemicals and unpleasant side effects of these chemicals on human health **(Akinyemi *et al*., 2006; Bialonska *et al*., 2010)**.

Plants are a source of bioactive molecules and have been widely used both traditionally and commercially to increase the shelf-life and safety of foods **(Sasidharan *et al*., 2008)**.

Clove (*Syzygium aromaticum* L.), is one of the most ancient and valuable spices of the Orient. Whole and ground cloves are used to enhance flavor foods, for his are highly valued in biomedicine as a carminative and stimulant and are said to be a natural anthelmintic **(Srivastava *et al*., 2003).** In the last several years, clove extracts is used extensively as a phytotherapy. It is used in medicine for its antibacterial, antiseptic and antibiotic properties **(Kim *et al*.,1998),** anti-mutagenic, anti-inflammatory, antioxidant, antiviral, anti-thrombotic, anti-parasitic and antifungal **(Hussein *et al*., 2000)**
Furthermore, silver nanoparticles (AgNP) are prominent in applications as antimicrobial agents that can eliminate microorganisms by interacting with their cells, thus preventing the cellular respiration, and consequently its replication **(Vilas *et al*., 2014).**

The use of plant derivatives for the synthesis of nanoparticles is widely used nowadays, and their compounds often have important biological functionalities that can act synergistically with the nanoparticles for various applications in different areas from the food to the pharmaceutical and medical industry **(Hongfang *et al.,* 2017; Raja *et al.* ,2017; Keshari *et al*., 2018).**

In this context, the original idea of our work was to evaluate the phytotherapeutic effect of the aqueous extract of *Syzygium aromaticum* L and AgNPs on metabolic and physiological disturbance induced by fungal spores toxicity in rats. For this, we are going to focus on the assay of blood and hematological biochemical parameters as well as the evaluation of tissue oxidative stress parameters and to complete our study we also carried out a histological study on the testicles and the liver.

First part

Bibliographic synthesis

Chapter I

Syzygium aromaticum and Silver nanoparticles

1. Generality

The traditional medicinal system based on the use of herbal remedies still plays an important role in the health care system. In recent decades, medicinal plants have been gaining wider acceptance due to the perception that these plants being natural products have lesser side effects and improved efficacy than their synthetic counterparts **(Abushouk *et al*., 2017)** Currently, about 80% of the world's inhabitants rely on traditional medicines as a major form of their primary health care **(Ekor, 2014).** Pharmacologically, various herbal plants possess bactericidal, virucidal, fungicidal activities; they are used in embalmment, in food preservation, and have anti-inflammatory, antimicrobial, spasmolytic, sedative, analgesic, and local anesthetic activities, many plant species have been reported to have pharmacological activities attributable to their phytoconstituents such are glycosides, saponins, flavonoids, steroids, tannins, alkaloids, terpenes and accordingly (**Batiha *et al.*, 2018).** Up to date, herbal remedies have been documented as a vital source for discovering novel pharmaceuticalmolecules that have been used to treat serious diseases. These identified phytochemicals have beenconsidered a remarkableleading compound in the search for effective and new drugs **(Beshbishy *et al*., 2019).**

2. Definition of *Syzygium aromaticum*

Syzygium aromaticum, also known as clove, is a dried flower bud belonging to the Myrtaceae family that is indigenous to the Maluku islands in Indonesia but has recently been farmed in different places worldwide,the clove tree is composed of leaves and buds (the commercial part of the tree) and the flowering bud production begins four years after plantation. Afterward, they are collected either by hand or using a natural phytohormone in the pre-flowering stage **(Cortés-Rojas *et al.,* 2014)**. Interestingly, they are commercially used for many medicinal purposes and in the perfume industry, and clove is considered one of the spices that can be potentiallyused as preservatives in many foods, especially in meat processing, to replace chemical preservatives due to their antioxidant and antimicrobial properties **(Chomchalow *et al*., 1996).** Several reports have documented the antibacterial, antiviral, anticarcinogenic, and antifungal activities of some aromatic herbs including cinnamon, oregano, clove, thyme, and mint. However, clove has gained much attention among other spices due to its potent antimicrobial and antioxidant activities. the effective role of clove in the inhibition of different degenerative diseases is attributed to the presence of various chemical constituents in high concentrations with antioxidant activity **(Astuti *et al.*, 2019)**. As well, in America, clove has been traditionally used in inhibiting food-borne pathogens to treat viruses, worms, candida, and different bacterial and protozoan infections **(Bhowmik *et al.,* 2012).**

3.Systematic and taxonomy of *S. aromaticum*

The botanical description of the clove plant (*S. aromaticum*) is as follow **(Teuscher, 2006)**, up to 20 m tall, evergreen tree with pyramid-shaped top. The petiolated leaves are leathery, elliptic to lanceolate, 9 to 12 cm long, 3.5 cm to 4.5 cm wide. The terminal flowers are arranged in tripartite paniculate corymbs. Flowers 10 to 14 mm long, with 2 scale-like prophylls, tubular calyx 1 to 1.5 cm long, 4 thick sepals, 4 petals, whitish pink to carmine-red, numerous stamina, inferior ovary, which is partially enclosed by and fused with a tubular hypanthium (cup-like structure of the flower axis). The fruit is a dark red berry, 2.5 cm to 3 cm long, 1.3 to 1.5 cm wide, crowned by 4 curved sepals and containing 1,rarely 2 seeds.

The evergreen clove tree is adapted to tropical climate around the see with altitude not exceeding 300 m. In many literatures, the clove tree is described to have an average height of 8–12 m although it has a potential to grow up to 20 m high. The most precious part of the plant, the flowers **(Fig. 1),** occur after a long period of growth of around 8–10 years (some reports say after 6 years) with full flowering appear at about 20 years. The economic return of the clove crop thus comes after two decades of waiting but sustained for several decades as the plant is known to provide flowers on annual bases for over 80 years. Theunopened flower buds in clusters or inflorescences bearing the cloves are handpicked, and sun-dried **(Fig. 2)**.

Fig. 01: The clove plant during flowering buds period.

Fig. 02: Cloves dried flow

(Habtemariam, 2019)

The cloves collected with care not to damage the young branches are less than 2 cm long and the overall yield from the tree vary with age but can reach up to 7 kg per tree annually. Yield could vary, however, from far smaller in younger to some report even say up to 9 kg from mature trees. It is interesting to note the floral feature of the plant from which clove derives. The long nail feature comes from the calyx ending with four sepals and the unopened four petals or rather pieces of the chalice forming the central ball-like (nail head) feature at the centre. Upon sundrying which normally takes 3–4 days, the darker-brown clove appears. The hard-dry feature together with its preservation with its chemical components like eugenol, clove can be kept for a loner period without losing its aroma. The taxonomic hierarch of clove is shown below by **(Merr and Perry, 2019)**

Kingdom: Plantae—Plants

Subkingdom : Tracheobionta—Vascular plants

Superdivision: Spermatophyta—Seed plants

Division: Magnoliophyta—Flowering plants

Class: Magnoliopsida—Dicotyledons

Subclass: Rosidae

Order: Myrtales

Family: Myrtaceae—Myrtle family

Genus: SyzygiumP. Br. ex Gaertn.—Syzygium

Species: *Syzygium aromaticum*(L.)

4. Distribution of *Syzygium aromaticum*

The genus Syzygium gaertn comprised of around 1200 species that occur in the Old World tropical and subtropical regions of Africa, Asia, Australia, New Zealand and the Southwest Pacific regions. Some estimates even suggest that up to 1800 species may be represented by the genus making it as the largest genera of woody flowering plants in the world.The distribution of this plant around the world is further displayed in the map **(Fig. 3).**

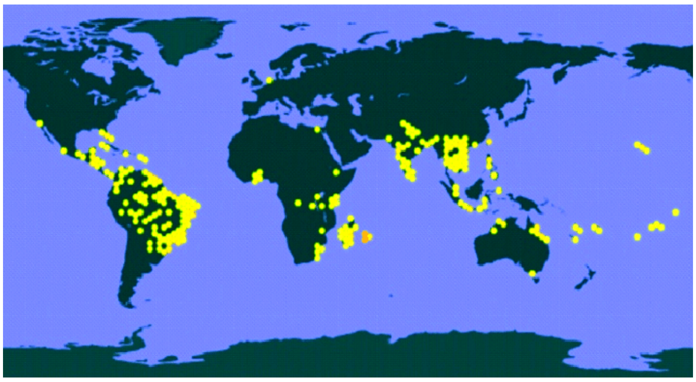
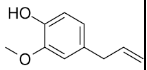
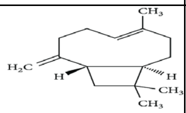
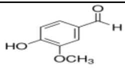
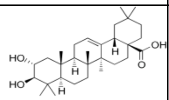
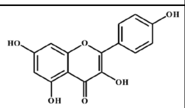
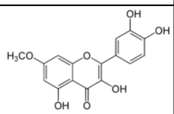
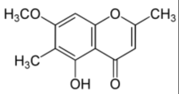
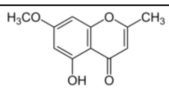
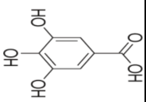
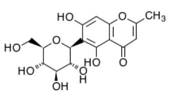
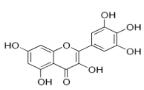


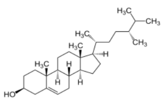
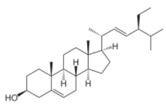
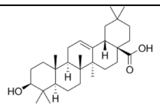
Figure 03: Distribution map of *Syzygium aromaticum* (**Shivaji *et al.,* 2020).**

5. Chemical Constituents

Pharmacologically, clove has been documented as the main source of phenolic molecules like hidroxibenzoic acids, flavonoids, hidroxiphenyl propens, hidroxicinamic acids, and eugenol ($C_{10}H_{12}O_2$)—which is the major bioactive molecule—and gallic acid derivatives like hidrolizable tannins that are found in high amounts in the fresh plant **(Neveu *et al.*, 2010)**. Moreover, clove contains flavonoids namely quercetin and kaempferol and phenolic acids like ferulic, caffeic, ellagic, and salicylic acids **(Cortés-Rojas *et al.*, 2014)**. Notably, **(Gülçin, 2011)** reported the in vitro antioxidant effectiveness of clove and discussed the relationship between compounds and activity. They showed that clove allows the donation of the hydrogen atom and subsequently fixes the phenoxil radical, which results in the formation of steady molecules that do not establish or increase oxidation. Additionally, the clove compounds have a pleasant carbon chain link with the aromatic ring which can be involved inphenoxil radical stabilization by resonance. In table below shows the most important different compounds isolated from *Syzygium aromaticum*.

Table 01: Pharmacological activities of different phytochemicals isolated from *S.aromaticum*

Name of Phytoconstituent	Structure of Phytoconstituent	Biological Activity	Reference
Eugenol		Antimicrobial, Analgesic, Antioxidant Anticancer, Anthelmintic, Antiulcer, Anti-inflammatory, Anti-depressant	**(Kamatou *et al.*,2012)**
β -caryophyllene		Antitumor, anti-apoptotic Anesthetic Anti-lishmanial Anti-inflammatory Antioxidant, antibiotic	**(Cai & Wu, 1996 ; Soares *et al.*, 2013)**
Vanillin		Antimicrobial Antioxidant Antidepressant	**(Fitzgerald *et al.*, 2004; Shoeb *et al.*, 2013)**
Crategolic acid		Antitumor	**(Tena *et al.*, 2013)**
Kaempferol		Antimicrobial, Antioxidant, Anti-inflammatory, Anticancer	**(Jnawali *et al.*, 2014)**
Rhamnetin		Anti-inflammatory, Antioxidant Cardio protective , Antifungal	**(Andrioli *et al.*, 2012)**
Eugenitin		Antifungal	**(Couto *et al.*, 2013)**
Eugenin		No activity reported	
Gallic acid		Antimicrobial, Antioxidant	**(Saavedra *et al.*, 2013)**
Biflorin		Antibacterial ,Antioxidant, Anticancer	**(Vasconellos *et al.*, 2011)**
Myricetin		Antimicrobial ,Antioxidant, Anticancer, Anti-inflammatory	**(Zheng *et al.*, 2012)**

Campesterol		Antibacterial, Antinociceptive , Anti-carcinogenic	**(Cai& Wu, 1996)**
Stigmasterol		Antimicrobial, Antitumor , Acaricidal, Block cartilage degradation	**(Choi *et al.,* 2007)**
Oleanolic		Anti-diabetic Antimicrobial , Anticancer,	**(Feng *et al.,* 2009)**

6.Crude Clove Extracts Efficacies

Several *S.aromaticum* molecules namely kaempferol, biflorin, 5, 7 dihydroxy-2-methylchromone-8-C-β-D-glucopyranoside, orsellinic acid glucoside, myricetin, rhamnocitrin, gallic acid, oleanolic acid, ellagic acid, and flavonoidstriglycosides have been documented for their effectiveness in inhibiting oral pathogens **(Koba *et al.,* 2011).**As the aqueous *S. aromaticum* extract showed high antioxidant efficacy in additionto its hepato protective activity on liver damage caused by paracetamol treatment **(Nassar *et al.,* 2007). (Essawi and Srour, 2000)** tested the antimicrobial efficacy of six medicinal herbal extracts in vitro toward four bacterial species methicillin-resistant Staphylococcus aureusand Bacillus subtilis were the most inhibited microorganisms. *Syzygium aromaticum* extract was the most active against multidrug-resistant.

7. Biological Activities

Biological and biochemical properties of *S.aromaticum* have revealed the antiviral, antimicrobial, antifungal, anticancer, antioxidant, and anti-inflammatory activities **(Han and Parker, 2017)** . Clove extract influenced on the cancer cell cycle control, the problems of pathogen resistance, as well as the toxic residues to most of the commercially available antimicrobial drugs **(Rapp, 2004).** Therefore, it is clear that the development of new and effective antimicrobial treatment options is vital for improving disease treatment and control. Clove is a well-known and significant herbal remedy because of its broad pharmacological efficacy **(Bouchentouf *et al.,* 2017).** Recent studies have examined the *in vivo* increase in the lipid peroxidation and blood sugar in diabetic rats and reestablished the levels of the antioxidant enzyme after nutrition supplement with cloves , additionally that the dietary cloves *in vivo* reduced the tissue damages in the livers, lens, and cardiac muscles in rats

(Shukri *et al.,* 2010) Pharmacologically, clove is used in a wide range as an antiseptic in oral diseases and for the treatment of toothaches, allergy disorders, asthma, acne, scars, and rheumatoid arthritis, and it showed antispasmodic and acaricidal effects toward Dermatophagoides pteronyssinusand Dermatophagoides farin **(Kim *et al.*, 2003; Wongsawan *et al.,* 2019).**

8. Other biological activities

8.1. Antifungal activity

Many studies have reported antifungal activity for clove extracts against yeasts and filamentous fungi, such as several food borne fungal species and human pathogenic fungi **(Eugenia *et al.*, 2009 ; Gayoso *et al.,* 2005).** The phenolic and flavonoids compounds of clove play a key role as an antifungal, carvacrol and eugenol are clove compounds, are known to possess fungicidal characteristics, including activity against fungi isolated from onychomycosis **(Manohar *et al.*, 2001 ; Gayoso *et al.*, 2005)**. Antifungal activity of clove against different strains and reported following scale of sensibility- *Mucor sp .> Microsporum gypseum > Fusarium monoliforme NCIM 1100> Trichophytum rubrum> Aspergillus sp.> Fusarium oxysporum* determined by **(Rana *et al.*, 2011).**

In chromatographic analysis eugenol (one of the biologically active compounds of cloves) was found to be the main compound responsible for the antifungal activity, due to lysis of the spores and micelles. A similar mechanism of action of membrane disruption and deformation of macromolecules produced by eugenol was also reported by **(Devi *et al.*, 2010)**. The large spectrum of fungicidal activity of clove oil and eugenol was reported on Candida, Aspergillus and dermatophytes and the mechanism of action was attributed to the lesions of the cytoplasmic membrane **(Eugenia *et al.*, 2009).** Aditionally **(Burt, 2003)** proposed that different modes of action can be involved in the antifungal activity of clove. The activity may in part be due to their hydrophobicity, which is responsible for their partition into the lipid bilayer of the cell membrane, leading to an alteration of permeability and a consequent leakage of cell contents.

8.2. Antioxidant/Free radical scavenging activity

Clove has the highest antioxidant capability and perhaps one of the best known herbs.The aqueous extract *S.aromaticum* showed strong radicals scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) **(Abd El Azim *et al.*, 2014).** Free and bound phenolic extracts of clove buds also exhibited high antioxidant activities, as shown by their high reducing power and 2, 2-azinobis-3- ethylbenzo-thiazoline 6-sulfonate (ABTS) radical scavenging abilities, as well as inhibition of Fe2+ -induced lipid peroxidation in rat pancreas in vitro **(Adefegha and Oboh, 2012).** Clove extract also inhibited the malonaldehyde (MDA) formation from horse blood plasma oxidized with Fenton's reagent **(Lee and Shibamoto, 2001).**

9. Efficacy in Diseases

Aqueous *S.aromaticum* extract has the potent antioxidant efficacy, may be due to the strong hydrogen donating ability, scavenging of hydrogen peroxide, free radicals and superoxide and metal chelating ability **(Gülçin *et al*., 2004).** clove extracts play a significant role in treating memory deficits resulting from oxidative stress **(Mehta *et al*., 2010).** (**Halder *et al.,* 2011**), revealed that clove extract pre-treatment reduced the oxidative stress evaluated by glutathione as well as malondialdehyde levels in mice's brains. They concluded that the ability of clove to restore memory and learning deficiencies resulted from short- and long-term scopolamine treatment is attributed to its effectiveness in reducing oxidative stress. Moreover, **(Daniel *et al*., 2009)**, reported the in vivo analgesic efficacy of eugenol using the abdominal wriggling method stimulated by acetic acid. Interestingly, the anti-carcinogenic and cytotoxic activities of the colve extract have been reported against human tumor cell lines PC-3 and Hep G2 **(Ogunwande *et al*., 2005). (Chaieb *et al.,* 2007)**, documented that eugenol and dehydrodieugenol have been shown to stimulate human cancer cell death. Clove exerted anti-inflammatory and immunomodulatory activities by suppressing the lipopolysaccharide(LPS) action as well as the nuclear factor-κB (NF-κB) pathway. (**Han and Parker, 2007)**,reported that the anti-inflammatory activity of clove may be related to the active compound, eugenol. Moreover, eugenol was found to protect against hepatotoxicity caused by CCl4 when administered with CCl4 therapy **(El-Hadary *et al*., 2016).** Interestingly, other phytochemical compounds isolated from *S.aromaticum* extracts including sang uinarine and benzophenanthridine alkaloids have been documented for protection from liver damage **(Ali *et al*., 2014). (Shyamala *et al*., 2003),** proved that clove intake tends to recover ALT, urea, AST, and lipid levels in kidneys, serum, and liver in comparison with normal values in hyper lipidemic rats. The antidiabetic efficacy of *S.aromaticum* extracts may be attributed to the existence of insulin-stimulating agents (**Kuroda *et al*., 2012**). In vivo experiments revealed that the normal blood sugar has been enhanced in *S.aromaticum* extracts-treated mice **(Sanae *et al.,* 2014)**.

The in vitro and in vivo experiments have documented the antiobesity efficacy of *S.aromaticum* extracts by reducing the serum triglycerides and cholesterol levels **(Przygodzka *et al*., 2016)**. Additionally **(Jung *et al*., 2012)**, documented that diet supplemented with *S.aromaticum* extracts decreased serum insulin, leptin, and hepatic lipid levels along with the body weight of high-fat diet mice, suggesting its prospect as a natural anti-obesity supplement and its ability to decrease the hepatic lipid accumulation. **(Several, 2015)** reports documented that clove can reduce the risk of arterial sclerosis, cardiovascular disorders, and other disease associated with oxidative stress. Eugenol also exhibits reversible, dose related vasodilator as well as negative inotropic activities in heart muscle and showed smooth muscle relaxant and hypotensive efficacy **(Pulikottil and Nath, 2015).** Clove has been documented to possess nervous stimulating as well as sexual behavior boosting effect in male mice **(Tajuddin *et al*., 2003)**, and this action may be attributed to their nervine enhancing activity. Moreover, it showed an increase in mating performance in mice compared to an increase in sexual motivation. **(Cortés-Rojas *et al.,* 2014)**, reported the ability of clove oil to inhibit prevent premature ejaculation.

The sexual behavior in humans caused of clove has been enhanced by stimulating the testosterone level. Clove extract has been documented as thromboxane synthesis and platelet aggregation inhibitors and showed an anticoagulant activity. Moreover, clove prevented the platelet aggregation caused by the platelet-activating factor, arachidonic acid or collagen, and the results revealed that clove extract ismore efficient in inhibiting platelet-activating factor- and arachidonic acid-induced aggregation than collagen **(Saeed *et al*., 1995).** The myogenic antispasmodic effect of eugenol has been documented on the airway smooth muscle of rats. It was found to act by blocking Ca2+ channels managed by voltage and receptors, enhancing the release of Ca2+ from the sarcoplasmic reticulum and decreasing the sensitivity of the contractile proteins to Ca 2+ **(Lima *et al*., 2011)**

10. Toxicity Doses

Food and Drug Administration (FDA) has confirmed the safety of clove and oleoresins as a food supplement; however, there has been considerable attention regarding its toxicity recently **(Vijayasteltar *et al*., 2016). (Prashar et *al*., 2006)** have examined the cytotoxic activities of clove in vitro against human fibroblasts and endothelial cells, and they documented they recognized them as safe. On the other hand, other reports revealed that clove has an allergic efficacy when used in dentistry **(Anuj and Sanjay, 2010)**. Moreover, eugenol, as well as clove was reported to have a spermicidal effect in vitro in six male partners of infertile couples **(Mishra and Singh, 2008).** The World Health Organization (WHO) has proven that the acceptable daily amount of clove in humans is 2.5 mg/kg body weight **(Ogunwande *et al*., 2005). (Janes *et al*., 2005)**, documented the acute side effects (e.g., disseminated intravascular coagulopathy, generalized seizures, and hepatotoxicity after clove administration). Recently, **(Johannah *et al*., 2015)**, demonstrated the remarkable detoxification and the cardiac health effects in humans by reducing lipid peroxidation and increasing the endogenous red/ox enzyme levels.

11. Generality about sliver nanoparticles

Silver nanoparticles (AgNPs) have been the focus of many studies in recent times. This is related to the fact that AgNPs have been used in many applications. They have been used as an antibacterial agent in cotton fabric and in drinking water treatment, in products of cosmetics, in medical devices and dental applications **(Wattimena *et al*., 2021)**

Nanoparticles, the rudiments for nanotechnology, are nowadays produced using noble metals like Ag, Pt, Au and Pd with the advancement of new materials with nanometer size including nanoparticles, nanotubes, nanowires, and so forth. In the recent times, silver nanoparticles (AgNPs) have attracted intensive research interest because of their advantageous applications in biomedical, drug delivery, food industries, agriculture, textile, water treatment industries, catalysis and surface enhanced Raman scattering **(Chaloupka *et al.,* 2010 ; Sharma *et al*., 2009)**

Diverse methods are used for the synthesis of silver nanoparticles. And the most commonly available known method is the chemical reduction of metal salt precursor using chemical reducing agents such as, citrate, polymer substances, borohydride,N,N-dimethyl formamide sodium hydroxide, 2-mercaptobenzimidazole, or the physical methods include, laser ablation method **(Maity *et al*., 2011 ; Gauri *et al*., 2017)**. Sono chemical deposition photochemical reduction, gamma ray and solar irradiation **(Wei *et al.,* 2012)**

Although the commercial methodologies have proven as efficient tools for synthesizing AgNPs, but their continuous use may pose a great threat to human health and the environment because of the use of toxic and hazardous reagents and generation of toxic by-products in some instances. These products tend to bind to the AgNPs surface and may adversely affect their character and performance **(Gengana *et al*., 2013).** There is a great need tofind alternative methods for AgNP synthesis, which are nontoxic and eco-friendly.

Some of the recently developed green methods utilizing biological materials show favorable routes for their synthesis. The use of plants for the synthesis of AgNPs is in the focus of intensive research because of its eco-friendly nature. The use of plants boasts of several advantages such as the elimination of elaborate processes of maintaining cell cultures, easy scale up for large-scale synthesis and cost-effectiveness. Moreover, plant extracts may act both as reducing agents and stabilizing agents in the synthesis of nanoparticles **(Kumar *et al*., 2010)**. Typically,aplant extract-mediated bio-reduction for photosynthesis of silver nanoparticles involves mixing the aqueous extract with an aqueous solution of the silver nitrate salt **(Shankar *et al.,* 2003).**

12.Green synthesis of silver nanoparticles using *Syzygium aromaticum*

The present study directs the advantageous of silver nanoparticles from silver nitrate through a simple green route utilizing the extract of cloves (*Syzygium aromaticum*) as the reducing agent. Cloves (*Syzygium aromaticum*), are the aromatic flower buds of a tree in the family Myrtaceae and numerous restorative uses have been most broadly connected to a toothache, and for mouth and throat aggravation. Cloves show antiseptic, antibacterial, antifungal and antiviral properties.Thus, the study proceeds with the synthesis of silver nanoparticles utilizing *Syzygium aromaticum* and their cytotoxicity of biosynthesized silver nanoparticles was studied against MCF-7 and A549 cancer cell lines.

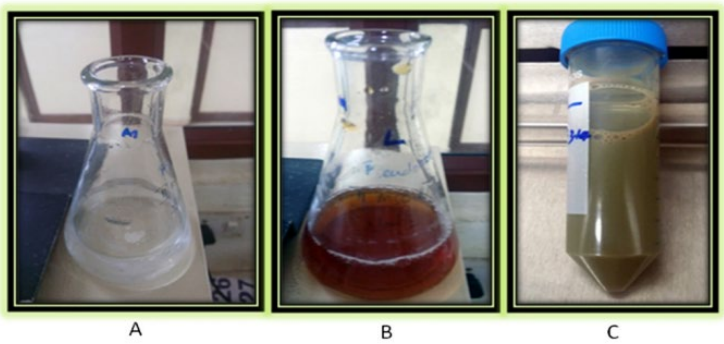


Fig. 04: Nanoparticle synthesis using *Syzygium aromaticum* plant extract; (A) silver nitrate solution, (B) *Syzygium aromaticum* extract broth, (C) silver nanoparticle solution **(Venugopal *et al*., 2017).**

Cloves show antiseptic, antibacterial, antifungal and antiviral properties. It is a popular remedy for dental disorders, respiratory disorders, headaches and sore throats in regular medicines of Australia and Asian countries. Besides its antimicrobial, antifungal and antiviral properties, clove displays cytotoxic, anesthetic and anti-inflammatory properties. As a consequence of its antiseptic and antibiotic properties, clove is habitually used to treat oothaches and as a basic constituent in prevalent toothpastes and mouth fresheners. This plant holds particular promise for preparing AgNPs owing to their phytoconstituents. Despite the advantageous features of S. aromaticum, it has received relatively little attention as a material for the preparation of AgNPs. The ethnomedical aspect of the *S.aromaticum* flower bud extract is a particular motivating factor for us to study its use for the biocompatible synthesis of AgNPs. **(Ajitha *et al*., 2018)**

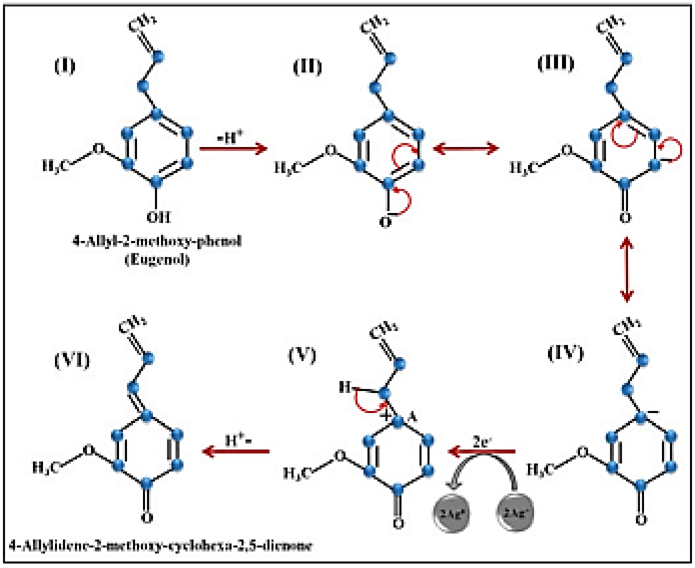


Figure 05: Schematic representation of the mechanism by whichAgNPs formed in the presence of a phytoconstituent of clove extract. **(Ajitha *et al*., 2018).**

Chapter II

Aspergillus niger

& Oxidative stress

1.General characteristics

Aspergillus niger is a versatile filamentous fungus found in soil, water, air, decaying plant material and large number of food and feeds all over the world **(Pitt and Hocking, 1997). (Raper and Fennell, 1965)** designated 15 species as comprising the *Aspergillus niger* group, which includes all of the aspergilli with black conidia, but now the concept of of the *A. niger* group based on black conidia seems dominant. The taxonomic description is as follows:

1.1. Taxonomy of *Aspergillus niger*

Domain : Eukaryota

Kingdom : Fungi

Phylum : Ascomycota

Subphylum : Pezizomycotina

Class : Eurotiomycetes

Order : Eurotiales

Family : Trichocomaceae

Genus: *Aspergillus*

Species : *Aspergillus niger*

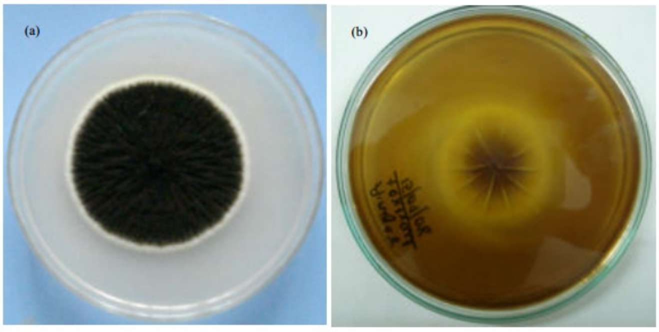


Fig 06: Characteristics of *A.niger*: (a) Morphological and (b) Reverse side of colony.

1.2. Aspergillus niger associated with human diseases

A.niger is believed to be most common storage fungi posing serious threat to contamination of stored commodities in tropical warm regions of the world. Food and herbal drug industries are very much suffering from the mycotoxin contamination of *A. niger*. It is studied that less than 10% of these strains were tested positive for ochratoxin A and fuminisins under conditions that were favorable **(Schuster *et al.*, 2002)**. Livings beings including humans, when contacted with *A. niger* and its mycotoxinsusually through consumption may cause many negative effects, immunotoxcitiy, carcinogenicity and hepatotoxicity. The effects on animals include decrease in antibody responses, size reduction in immune organs and an alteration in the production of cytokine which are proteins and peptides specifically used in signaling. Poultry feed if contaminated by *A. niger* has major effect on the poultry industry. Different animals, such as chicken, turkey and ducks, are very prone to ochratoxin**(Schuster *et al.*, 2002).**

A.niger is commonly regarded as a pathogenic allergen generassociated with lung infections in individuals with weak immune system. Because the conidi and conidiophores are small, readily air borne, can easily breathed in and cause deep or systemic mycosis **(Kierownik, 1990) (Table 02).** Ear is the location of *A. niger* infection (**Fig. 07**). Local lesions in both external and middle ear, as well as in post-operative cavities, can create favorable conditions for fungal growth and subsequent otomysis **(Kaur *et al.,* 2000).** *A. niger* can produce a secondary metabolites include oxalic acids and cyclic pentapeptides having moderate to high acute toxicity **(Ueno and Ueno, 1978).** Oxalate crystals of oxalic acids produced by *A. niger* can cause pulmonary oxalosis **(Nakagawa *et al.*, 1999)** (**Fig. 08).**

Table 02: Human diseases caused by *Aspergillus niger.*

Name of disease	Target organ	Reference
Weak immune system	Any organ of the body	**(Louthrenoo *et al*., 1990)**
Systemic mycosis	External body part	**(Louthrenoo *et al*., 1990)**
Ear infection	External auditory system	**(Padhye, 1982; Walsh and Pizzo, 1988)**
Aspergillosis	Lungs	**(Ueno and Ueno, 1978; Bennet, 1979 ; Richard *et al*., 2008)**
Asthma and allergic alveolitis	Respiratory tract	**(Edwards and AlZubaidy., 1977)**

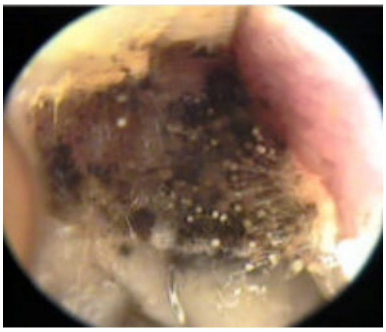


Fig .07: Ear Infection by *A.niger* external auditory. **(Rihard *et al*., 2008)**

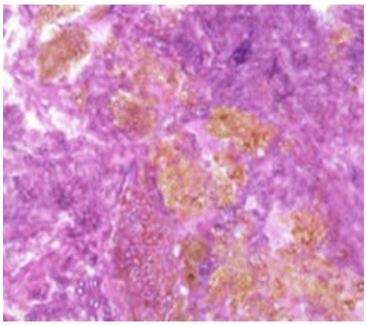


Fig 08: Pulmonary oxalosis due to fungus ball of *A.niger*. The band of necrosis isdue to the diffusion of OTA&oxalosis crystals **(Rihard *et al*., 2008)**

1.3. Generalities about Ochratoxins

Ochratoxins are fungal secondary metabolites produced during storage by fungus of the genera*Aspergillus*, mainly in tropical and warmer regions and by *Penicilliumverrucosum*, in temperate and colder areas **(Pohland *et al*., 1992**). The most commonly occurring and most toxic member is ochratoxin A (OTA **Figure 09**).

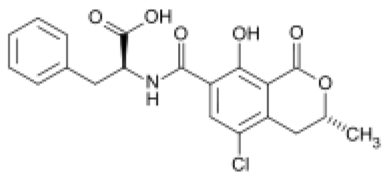


Fig 09: Chemical formula of Ochratoxin A

1.4. Ochratoxin induced inflammation

OTA induces renal, hepatic and intestinal toxicity, characterized by inflammation and cell death **(Klahr and Morrissey, 2003)**. In the acute and initial phase of the inflammatory process, the vascular permeability increase and the neutrophils (PMN) infiltrate the tissue; here they contribute to the inflammatory response by producing several mediators, such as ROS **(Hedenborg and Klockars, 1987)**.Although OTA causes intestinal inflammation*in vivo*, the mechanisms responsible for this effect are poorly understood **(Bouhet and Oswald, 2005)**. This inflammatory effect could result from a direct stimulatory effect on the production of pro-inflammatory cytokines by intestinal epithelial cells. Indeed, it has been shown that OTA can directly stimulate cytokine production by immune cells, suggesting a possible similar direct effect of this mycotoxins on cytokines production by gut epithelial cells.**(Al-Anati and Petzinger, 2006).**

2. Oxidative stress

Oxidative stress is imbalance between the levels of reactive oxygen species produced and the ability of a biological system to detoxify the reactive intermediates, creating a perilous situation by contributing to cellular damage. **(Derouiche *et al*., 2018)**. Briefly, oxidative stress is imbalance in the balance between antioxidants and pro-oxidants in favor of antioxidants. **(Derouiche *et al*., 2018).** Additionally, oxidative stress is an important factor causing metabolic and physiological alterations and various diseases in the body**. (Derouiche, 2020).**

2.1 Oxidative stress and Ochratoxin A

Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species (ROS) and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids and DNA **(Chandra *et al.*, 2015).** It was the toxin itself that was thought to induce cytotoxicity, genotoxicity and inflammation in vivo and in vitro, but now that role has been assigned to ROS and oxidative stress induced by OTA **(Abd El-Haleem *et al.,* 2016; Abdel-Wahhab *et al.,* 2017).**OTA induced the increase of NADPH and P450 enzyme, which activates the caspase signaling pathway and induces apoptosis. The increase of ROS caused mitochondria and endoplasmic reticulum oxidative stress, inducing calcium release and inhibiting the cell cycle, mRNA splicing, DNA replication, lipid and nucleotide metabolism. All of these could lead to cell apoptosis. Figure 10 shows OTA-induced apoptosis and cell signaling.

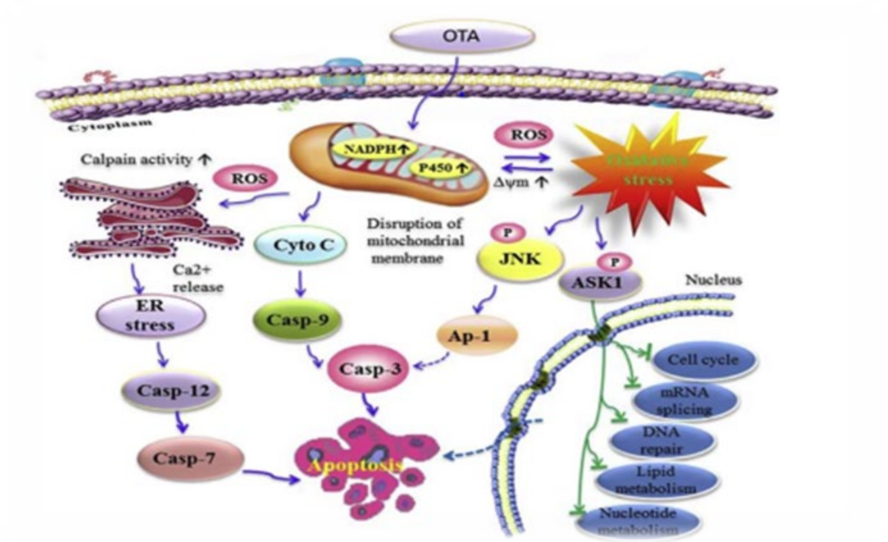


Fig. 10: OTA-induced apoptosis and cell signaling. **(Arbillaga *et al.*, 2007).**

It is important to note that the reactive nitrogen species levels may also increase in OTA-treated cells. OTA facilitates the expression of inducible nitrogen oxide synthase (iNOS) enzyme and also increases the expression and activity of dimethylarginine dimethylaminohydrolase (DDAH) with subsequent elevation of nitrogen monoxide (NO) synthesis and increased nitrite/nitrate concentrations **(Sorrenti *et al.*, 2013)**. High levels of NO may cause nitrosative stress because it can react with $O_2^{\bullet -}$ resulting in the formation of peroxynitrite ($ONOO^-$), which in turn gives nitrogen dioxide (NO_2) and $OH^\bullet$. A significant increase in the levels of protein nitrotyrosine residues was observed with OTA. In addition, OTA was found to increase the level of DNA abasic sites in both cell culture systems in one study. Treatment of cells with L-N6-(1-iminoethyl) lysine, a specific inhibitor of iNOS activity, inhibited the OTAmediated over-nitration of proteins but did not reduce the level of DNA abasic sites **(Cavin *et al.*, 2009).**

2.2. OTA-mediated oxidative damage

2.2.1 Damage to lipids

It has been demonstrated by **(Rahimtula *et al.*, 1988)** that OTA enhances lipid peroxidation. They suggested that OTA stimulates both NADPH-dependent and ascorbate-dependent lipid peroxidation in microsomes, iron (Fe^{3+}) being the co-factor. An OTA–Fe^{3+} complex would be formed, which would facilitate the reduction of iron in the presence of the

NADPH-cytochrome P450 reductase system. The resulting complex OTA–Fe2+ would then generate OH•, leading in turn to membrane lipid peroxidation. OTA-enhanced lipid peroxidation affects the permeability of the plasma membrane to Ca2+ and thus impairs calcium homeostasis by enhancing Ca2+ influx, releasing it from intracellular stores and influencing calcium-sensitive channels. In the presence of NADPH-CYP450 reductase, OTA–Fe3+ complex facilitates the reduction of Fe3+, and the OTA–Fe2+ complex formed initiates the appearance of free radicals, leading to lipid peroxidation **(Omar *et al.*, 1990; Rahimtula *et al.*, 1988).**

2.2.2. Ochratoxin A iduced damage to DNA

OTA could lead to oxidative chromatin and DNA damage in vitro, resulting in growth inhibition of canine umbilical cord matrix mesenchymal stem cells **(Rutigliano *et al.,* 2015).** Oxidative DNA damage was shown at cytotoxic concentrations when formamidopyrimidine DNA glycosylase (FPG) and endonuclease III (EndoIII) were introduced into the assay with or without metabolic activation. Furthermore, at these concentrations, elevation of ROS was measured and pre-incubation with N-acetyl-L-cysteine was able to produce a slight protective effect on OTA-induced oxidative DNA damage as well as cytotoxicity. These data suggest that OTA does not act as a direct genotoxic carcinogen and that oxidative stress is implicated in the genotoxicity and cytotoxicity observed in these human renal cells **(Arbillaga *et al.*, 2007)**.

DNAfragmentation was also found for a pig kidney cell line (LLC-PK1 cells), but was reduced by pretreatment with free radical-scavenging agents (epigallocatechin gallate, EGCG, and epicatechin gallate, ECG) **(Costa *et al.*, 2007**). In mouse lymphoma cells, OTA exposure resulted in a significant increase in both direct and oxidative DNA damage, with induction of oxidative damage being greater **(Ali *et al.*, 2014).** What's more, OTA could induce DNA strand breaks and chromosome aberrations in Het-1A cells (Liu et al., 2015). The capability of OTA to form DNA adducts is still conflicting, but the OTA–DNA damage potential is mainly related to free radical overproduction and oxidative stress (**Giromini *et al.*, 2016).** Formation of 8-oxoguanine adducts occurred in a timedependent manner, which correlates well with the OTA induced production of ROS in these cells. the formation of 8-oxoguanine in cell cultures treated with pro-oxidants. The OTA concentrations elevating the ROS levels and inducing the formation of 8- oxoguanine were of the same order of magnitude. Recently, OTA in the presence of Fe(III)- porphyrin facilitates single-strand cleavage of supercoiled plasmid DNA through the production of ROS (i.e. hydroxyl radical HOS). The author proposes that OTA is oxidized to a (hydro-) quinone. Quinones are known to redox cycle, thus creating free radicals that bind covalently to a variety of cellular macromolecules.**(Ringot *et al.,* 2006).**The formation of the (hydro-) quinone of OTA implicates the reported involvement of oxidative metabolism in the genotoxicity of OTA**(Sorrenti *et al.,* 2013).** In figure 11 shows how OTA induced damage to DNA.

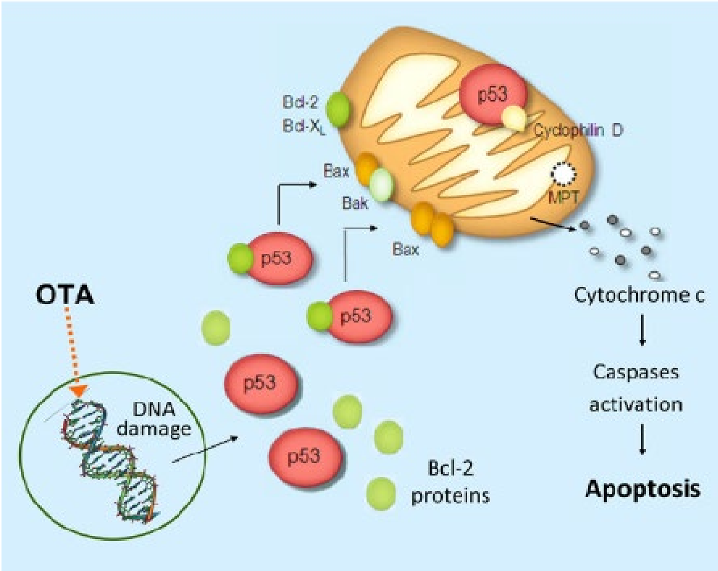


Fig 11 : Damage to DNA **(Periasamy *et al.*, 2016).**

2.2.3. Inhibition of proteins and RNA synthesis

OTA increases ROS concentrations and the expression of several metallothioneins, while reducing superoxide dismutase (SOD) activity and catalase mRNA levels **(Zheng *et al.,* 2013).** A report shows that OTA decreased the protein expression of several markers of the Nrf2-regulated gene batter.

OTA can act on all metabolic systems involving inhibition of PhetRNA synthase and inhibition of Phe in prokaryotes, mammalian cell cultures and in vivo animal studie. Irreversible hydroxylation of Phe to Tyr is the key regulatory step in the catabolism of this amino acid. A tight control of Phe hydroxylase is required because too rapid degradation of its substrate will lead to depletion of Phe. On the contrary,the accumulation of Phe will lead to impairment of the production of compounds derived from Phe as seen in phenylketonuria disease. The enzyme is activated by its substrate, Phe, by binding to a specific site, distinct from the substrate-binding site. The Phe moiety of OTA can bind to both sites and impair the hydroxylation reaction **(Richardson and Fisher, 1993)** so it is thought that the mechanism involved in inhibiting protein synthesis is the inhibition of peptide elongation through competition with Phe-tRNA synthetase **(Marin-Kuan *et al*., 2008).**

2.2.4. Alterations in antioxidant status

Taking into account that OTA enhances the production of free radicals, the activity of redox-regulated transcription factors and antioxidant enzymes including catalase (CAT), glutathione peroxidase (GPx) and SOD may be affected by OTA. It has been recently suggested that disruption of Nrf2-related signal transduction pathways is involved in OTA-induced impairment of antioxidant defense and cellular detoxification **(Marin-Kuan *et al.,* 2006).** The enzymatic antioxidant defense systems are responsible for protecting from ROS. Despite several studies carried out with antioxidants, manganese (III) porphyrins (MnPs) with high SOD mimetic activity have never been studied in the context of OTA toxicity. These compounds have the ability to mimic natural SOD enzymes and to scavenge a plethora of different ROS, modulating the cellular redox status **(Batinic-Haberle *et al.*, 2014)**. Most of the SOD mimics thus far developed belong to the classes of Mn-(MnPs) and Fe porphyrins (FePs), Mn(III) salens, Mn(II) cyclic polyamines and metal salts. Several MnPs are of potency similar to SOD enzymes. Assuming that all diseases have in common the perturbation of the cellular redox environment, developing SOD mimics still seems to be an appropriate strategy for the design of potent redox-active therapeutics**(Batinic-Haberle *et al.,* 2015).**

Second Part

Experimental Part

Chapter I

Materials and Methods

1. Materiels

1.1. Plant materials

In this study, (*Syzygium aromaticum*) were obtained from the market. These herbs were powdered by mechanical grinder until a fine powder was obtained. The powders of *Syzygium aromaticum* are stored at room temperature in airtight containers protected from bright light until the beginning of the experiment.

1.1.2. Aqueous extract preparation

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

1.1.3. HPLC fractionation and analysis

Extract of *Syzygium aromaticum* (10mg) were fractionated by CTO-20AC model HPLC with a photodiode array detector (HPLC-DAD) (pf 425–250 Interchim, C8 column Zorbax, 150 × 21.5 mm (250 bars)) at a concentration of 100 g/L. The mobile phase consisted of methanol (A) and water (B) with the following elution gradient: 0 min 0% A 100% B, 26.0 min 100% A 0% B; 26.1–29.4 0% A 100% B, 29.4–29.5 0% A 100% B. The flow rate was 20 mL/min and the column temperature was set at 25 °C. Chemical characterization of *Syzygium aromaticum* extract was carried out by comparing the detected polyphenol peaks with respect to retention times with those of standardchemicals (such as chlorogenic acid, caffiec acid, vanillic acid, quercetin, naringin, rutin, vanilin, p-coumaric acid, gallic acid) that were monitored at 250 nm using the same HPLC system. All standards were purchased from extrasynthese or Sigma-Aldrich. Identity and purity of the chemical standards were assessed by HPLC analysis.

1.2.Biosynthesis of Silver nanoparticles

For the biosynthesis of silver nanoparticles, the suitable reaction mixture was prepared by adding 1 ml of aqueous leaf extract and 9 ml of 1 mM $AgNO_3$ solution in a clean 25 ml Erlenmeyer flask. On the contrary, same experimental set up of 1 ml of aqueous leaf extracts with 9 ml distilled water was kept as control. Both flasks were incubated for 2–4 h in the rotary shaker under dark conditions at 25°C. Later, the synthesized silver nanoparticles

(AgNPs) were separated and purified by continuous centrifugation (9000 rpm; 20 min; 10°C) with distilled water. The dried AgNPs were kept at 4°C for further characterization and bioactivity study.

1.3. Characterization of silver nanoparticles

1.3.1. UV-Visible Spectroscopy

Synthesis of silver nanoparticles solution with *Syzygium aromaticum*extract may be easily observed by ultraviolet-visible (UV-Vis) spectroscopy.The bio-reduction of the Ag+ions in solutions was monitored by periodic sampling of aliquots(1 mL) of the aqueous component and measuring the UV-Vis spectra of the solution.UV-Vis spectra of these aliquotswere monitored as a function of time of reaction on a 6705 Uv-Vis spectrophotometer JENWAY in 400-600 nm range operated at aresolution of 1 nm.

1.3.2. Scanning electron microscope (SEM) and energy dispersive X-ray (EDX)

SEM is a type of electron microscope that images a sampleby scanning it with a high-energy beam of electrons in araster scan patterns. SEM and EDXanalysis was done using VERTIV-MODEL 6390 machine. Thin films of the sample were prepared on a carboncoated copper grid by just dropping a very small amount ofthe sample on the grid, extra solution was removed using a blotting paper and then the films on the SEM grid wereallowed to dry by putting it under a mercury lamp for 5 min.

1.3.3. X-ray diffraction (XRD) analysis

The particle size and nature of the silver nanoparticle were determined using XRD. This was carried out using Shimadzu XRD-6000/6100 model with 30 kv, 30 mA with Cuk a radians at 20 angle. X-ray powder diffraction is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. The analyzed material is finely ground, and average bulk composition is determined. The particle or grain size of the particles on the silver nanoparticles was determined using Debye Sherrer's equation. $D=0.94\lambda \times B \cos\theta$

1.3.4.FTIR spectroscopy

Todetermine the biomolecules present in the extract,FTIR analysis was carried out for the reduction of Agions with the spectral range of 400-4000 cm-1. Herethe sample was centrifuged at 9500 rpm for 20 min dried using hot air oven and ground with KBr to form apellet. Then, the pellet was analyzed using Cary 630 model FTIR instrument.

1.4. *Aspergillus niger* isolated from decayed Lemon fruits

Aspergillus niger was isolated from moldy Lemon fruits which were surface disinfected with 4% sodium hypochlorite for 2 min, the surface of disinfected fruits was plated on sterilized Petri dishes contained Potato Dextrose Agar (PDA) which was prepared according to (**Mackie, 1996)**. The plates were incubated at 28 ºC for seven days. The identification of *Aspergillus niger* isolates was based on cell and colony morphology characteristics according to the method described by **(Klich, 2002; Samuel *et al*., 2015)**.

1.5. Animal materials

1.5.1. Animal and husbandry condition

In this study, 25 male Wister rats aged **10 weeks** old and weighting **130-245g**, were obtained at the Animal Service of the Pasteur Institute, Algeria. The animals were carried under the same conditions, photoperiod (12h of light/12h of black) with a relative humidity of 65.3% and an ambient temperature of (25 ± 2) C° for four weeks. Animals have free access to water and food by a standard diet **(Southon *et al*., 1984)** ,The experiment was conducted over a period of 15 days.

After a period of adaptation, the animals were divided into Five experimental groups of 5 animals eachas follows:

1.5.3. Treatment animals

- **Group 1(Control groups):** were normal rats received 1mL of distilled water gavage.
- **Group 2 (FUNGAL treated groups):** were rats received 1mL of fungal solution prepared in distilled water by gavage for 10 days.
- **Group 3 (FUNGAL +AgNPs treated rats):** were rats received dose of AgNP (80 ug\kg) by Gavagefor 10 days.
- **Group 4 (FUNGAL + *S. aromaticum* treated rats):** were rats received dose of aqueous extract of *S.aromaticum* (200 mg\kg) by Gavage for 10 days.

1.5.4. Sacrifice, blood and tissues collection

After 12 hours of fasting, these animals were sacrificed under slight anesthesia by chloroform (94%) by inhalation; blood samples were collected during the slaughter of animals into EDTA tube to carried FNS and dry tubes. The serum was obtained by centrifugation for 10 min at 3000 tour/min and used for biochemical analysis assays; blood sugar level measured during rat's slaughter using glucometer.

Then the liver, Testicles, and lung were isolated from these animals and washed in normal saline. Then it was laid flat and the number and degree of erosions were counted and scored. Live and Lung stored at -20 C for oxidative stress , also A piece of the liver and one testicle of each group of these animals were takenand placed in 10% formaldehyde for histological analysis.

1.6. Reagents and products

Sodium Chloride (Nacl), Methanol, Chloroform, Comassie Blue, Phosphoric Acid (H3PO4), Bovine SerumAlbumin (BSA), Gallic Acid, Trichloroacetic Acid (TCA), Thiobarbituric Acid (TBA), ButylatedHydroxytoluene (BHT), Chloride Hydrogen HCl, Tris, Salicylicacid, DTNB (5-5'-dithiobis2-nitrobenzoic acid), hydrogenperoxide (H2O2), GSH, FeCl3, magnesium (Mg), Fehling liquor, sulfuricacid. Aluminum chloride (AlCl3), Dipotassium phosphate (K2HPO4), Monopotassium phosphate (KH2PO4), Potassium nitrite KNO2), Magnesium sulfate($MgSO_4$), Potassium chloride (KCl), Sacharosse, Glucose, Glycerol.

2. Methods

2.1. 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)**

2.1.1. Phenols

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

2.1.2. 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.

2.1.3. 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.

2.1.4. Tannins

In a test tube, introduce 5 ml of extract and add 1 ml of a 2% aqueous solution of ferric chloride (FeCl3). The presence of tannins was indicated by a greenish or bluish-blackish coloration.

2.1.5. 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.

2.1.6. 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.

2.1.7. Saponins

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

2.1.8. Steroids

For 1ml of plant extract, add 0.5ml of acetic acid solution, followed by 0.5ml of concentrated H2SO4. If the solution does not give any green color, it proves the presence of unsaturated steroids. In a second tube, the same volume of H2SO4 was added. The presence of the red color indicates the presence of steroid derivatives.

2.2. Total phenols and flavonoids compounds

2.2.1. 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 500 µl 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.

2.2.2. 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.

2.3. Antioxidant activity

2.3.1. Free radical scavenging activity, DPPH assay

The free radical scavenging activity was measured by a modified DPPH assay. Briefly, the DPPH assay was carried out as described by **(Cuendet *et* al., 1997)**, 100 µL of various concentrations of *Syzygium aromaticum*sample in methanol was added to 1.9 mL of a methanol solution of DPPH (0.004 %). The mixture was shaken and then allowed to stand at room temperature for 30 min in the dark. The absorbance was measured at 517 nm. The scavenging activity on the DPPH radical was expressed as inhibition percentage using the following equation:

$$\textbf{DPPH scavenging-radical (\%)} = [(A0 - As) / A0] \times 100$$

A0: is the absorbance of control reaction

As: is the absorbance of sample solution containing the test compound.

BHT were used as a positive control. The IC50 of extract was calculated from the graph of inhibition percentage plotted against extract concentrations.

2.3.2. Ferric Reducing Antioxidant Power Assay, FRAP assay

Take 500µl of sample and Add 1.25ml of the buffer solution (0.2 M, PH = 6.6). Add to 1.25 potassium fericianure**.**Then incubation during 20 min in a water bath at 50 ° C. After cooling, add 1.25ml of the aqueous TCA solution (10%) to stop the reaction. Centrifugation at 3000 rpm for 5 minutes. Then take1.25 ml of supernatant are then mixed with 1.25 ml distilled water and 250 µl $FeCl_3$ (0.1%). The absorbance was measured at700 nm against a blank.

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

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

2.4. Hemolysis assay

Hemolysis assay was done as described by **(Henkelman *et al.*, 2009),** 5mL of blood was collected from healthy volunteers in the tubes containing 5.4 mg of EDTA to prevent coagulation and centrifuged at 1000 rpm for 10 min at 40 ºC. Plasma was removed carefully and the white buffy layer was completely removed by aspiration with a pipette with utmost care. The erythrocytes were then washed for additional three times with 1X PBS, pH

7.4 for 5min. Washed erythrocytes were stored at 4oC and used within 6 h for the hemolysis assay. 50 µL of 10 dilutions (100 uL Erythrocytes suspension: 900 µL 1XPBS) of erythrocytes suspension was mixed with 100 µL of test samples*Syzygium aromaticum* (4-8µg/mL), 100 µL of 1XPBS was used as negative control and 100 uL of 1% SDS as positive controls. Reaction mixture was incubated at 37ºC water bath for 60 min. Volume of reaction mixture was made up to 1 mL by adding 850 µL of 1XPB. Finally, it was centrifuged at 300 rpm for 3min and the resulting hemoglobin in supernatant was measured at 540 nm by spectrophotometer to determine the concentration of hemoglobin. Percentage haemolysis was calculated as follows

$$\textbf{Hemolysis inhibition (\%)} = 100 - [\text{Abs Sample} \div \text{Abs Control}] \times 100$$

2.5. Confirmation of the strain tested

2.5.1. Gender confirmation

The identification of mold mainly appeals to cultural and morphological characters (**Botton *et al*., 1990):** Cultural characters: these are the macroscopic criteria such as the speed of growth, the texture, the color of the thallus, the color of the underside of the crop. , the odorexudate and the presence of a diffusible pigment **(Chabasse *et al*., 2002).**

2.5.1.1. Morphological characters

This is the microscopic study of the mycelium, the nature of differentiated organs and the biometric study. The study of morphological characters is carried out by the micro culture technique described by **(Haris, 1989)**.

2.5.1.2. Micro culture technique

Described by **(Haris , 1989),** this technique consists in inoculating the mold spores on slides led with small squares of solidified ANS and covering them with lamellae. The spores are seeded on the peripheral edges of the medium to provide them with a high oxygen potential so that they can germinate. The whole is packaged in a sterile and humid chamber and then incubated at 25 ± 2 °C for 3 to 5 days.

After incubation, the coverslips to which the mycelium adheres are transferred to other sterile slides containing a few drops of lactophenol for microscopic observation at magnifications 10, × 40, × 100.

- The genus Aspergillus is determined by cultural and microscopic characters by referring to the manual by **(Barnett, 1972).**

2.5.2. Confirmation of the species

The identification of Aspergillus species is carried out by the **Pitt and Hocking** method, this method is called « **Single Spore** », it consists of the inoculation of a few pores on four different media which are:

- Malt Extract Agar **(M.E.A)** at **25 °C**
- Glycerol Nitrate Agar **(G25N)** at **25 °C**
- CzapekYeast Agar **(C.Y.A)** at two different temperatures: **5 °C, 25 °C** and **37 °C**
- Potato Dextrose Agar **(P.D.A.)** at **25 ° C**

These media are inoculated as shown in the following figure. The reading is taken after 7 days by referring to the identification keys of **Pitt and hocking (2009).**

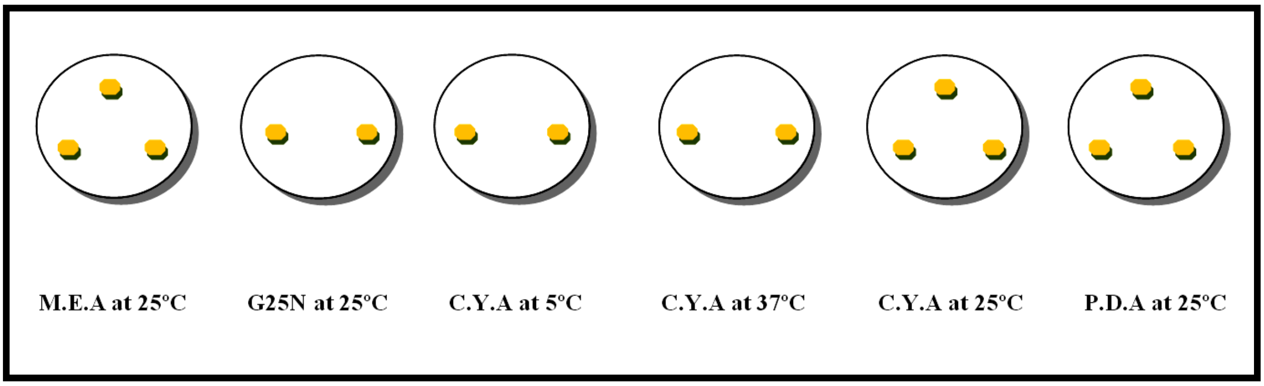


Figure 12 : Type of inoculation of the different isolates **(Pitt and hocking, 2009).**

2.6.Antifungal activity

The antifungal activity of the extracts was tested by the direct contact method in solid medium. It is used to determine the active extracts by evaluating the level of inhibition according to the method of **(Fandohan *et al*., 2004).**

Quantity of 1ml of the extract tested at different concentrations (3, 1.5, 0.75 and 0.375 mg / mL) is incorporated separately into tubes containing 19ml of the PDAa medium maintained in supercooling. Each tube is instantly homogenized by manual stirring and then its contents are poured into a petri dish. A 6mm diameter mycelial disc taken from the young culture of the fungus was inoculated.

At the same time, two petri dishes were inoculated one with 20 ml of PDAserves as a control and the other as a standard containing 19 mL of PDA and 1ml Fluconazole 50mg. The results were read after 7 days of incubation at 25 ± 2 °C by measuring the diameter of the growth zone.

The antFungal effect of our extracts on the growth strain tested is determined by measuring the rate of growth inhibition using Ebbot's formula **(Motiéjunaité and Peiculyté, 2004):**

The calculation of the antifungal index (Percentage inhibition) is determined by the formula:

Antifungal index = (1-**Da** / **Db**) x100 **(Wang *et al.*, 2005)** with:

Da: the diameter of the growth zone of the test.

Db: the diameter of the growth zone of the control.

The excerpt says:

- Very active when it has an inhibition of between 75 and 100%, the fungal strain is said to be very sensitive;
- Active when it has an inhibition of between 50 and 75%, the fungal strain is said to be sensitive.
- Moderately active when it has an inhibition of between 25 and 50%, the strain is said to be borderline.
- Little or no activity when it has an inhibition of between 0 and 25%, the strain is said to be insensitive or resistant.

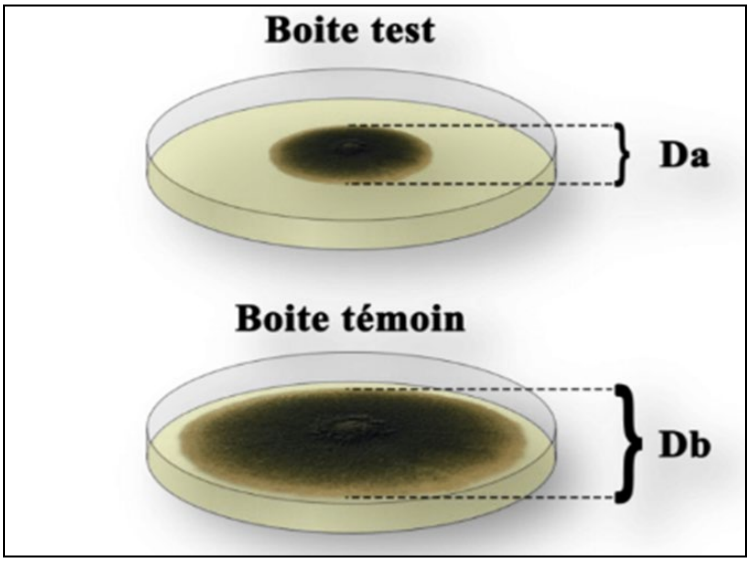


Figure 13: Expression of the results of the radial growth method.

2.7. Hematological parameters analysis

The determination of hematological parameters performed using fully Auto Blood CellCounter (ERMA) commercial reagent kits from **Biomeghreb (Tunisia)** using manual-analyzer.

2.8. Biochemical parameters analysis

Serum lipid levels were determined using the commercial kit from spinreact, Spain (ref: cholesterol-20111, triglyceride-20131). And for enzymes are also measured by the use of commercial kits (Spinreat_ ref GOT-20042, GPT-20046).

2.9. Oxidative stress parameters

2.9.1. Preparation of homogenates

About 1g of liver was homogenized in 9ml of buffer solution of Tris buffer saline (TBS, pH=7.4). Homogenates were centrifuged at 4000xg for 20 min and the obtained supernatant was used for the determination of antioxidant activity.

2.9.2. Determination of malondialdehyde (MDA) level

MDA was measured according to the method described by **SASTRE et al., (2000)**. In brief, Pipette 100 µl of sample, 400 µl of TBA reagent into the glass and screw test tubes and seal. Heat the mixture in the Marie bath at 100 °C for 15 minutes. Then cool in a coldwater bath for 30 minutes leaving the tubes open to allow evacuation of the gases formed during the reaction. Centrifuge at 3000 rpm for 5 minutes and read the absorbance of the supernatant at 532 nm using a spectrophotometer. TBARS concentration was determined using the MDA molecular extinction coefficient (å= 1,53 105 M-1 cm-1). The results were expressed in µmol / mg proteins.

2.9.3. Determination of reduced glutathione (GSH) level

GSH concentration was performed with the method described by **Ellman**. based on the development of a yellow color when DTNB is added to compounds containing sulfhydryl groups. In brief, 0.8 mL of tissue homogenate was added to 0.2 mL of 0.25% sulphosalylic acid and tubes were centrifuged at 2500 g for 15 min. Supernatant (0.5 mL) was mixed with 0.025 mL of 0.01 M DTNB and 1 mL TBS (pH 7.4). Finally, absorbance at 412 nm was recorded. Total GSH content was expressed as nmol GSH/mg proteins.

2.10. Histopathological study of liver and testicles tissues

After rats sacrificed, Liver and Testicles tissues were removed and immersed in fixative (solution 36% formaldehyde) intel the time of slices preparation. Whish dehydrated in ascending graded series of ethanol, cleaned with toluene, immersed in paraffin, and colored with hematoxylin and eosin. Histopathological evaluation was performed with light microscope.

2.11. Statistical analysis

Our statistical study is carried out by the Minitab software using (Student t test) to compare means among our different experimental group. If we compare the control group with the rest experimental groups, we put (*, ** or *** depending on significant at $p<0.05$, $p<0.01$ or $p<0.001$, successively). And if we compare the fungal group with *S.aromaticum* and AgNPs groups.we put (a,b or c depending on significant at $p<0.05$, $p<0.01$ or $p<0.001$, successively).

Chapter II

Results & Discussion

1. Results

1.1. Yield for aqueous extract of *S.aromaticum*

The extract obtained after a decoction of 120 minutes and maceration for 24 hours at room temperature, is subjected to filtration, then dried and weighed to determine its yield and properties (color and aspect); it is represented in the table below.

Table 03: Yield and characteristics of aqueous extract of *S.aromaticum* studied

Characteristics	Aspect	Color	Yield
Plant extract	**Powder**	**Brown**	**12.24 ± 0.693**

1.2. In vitro essays of *Syzygium aromaticum*

1.2.1. Phytochemical study for aqueous extract of *S.aromaticum*

1.2.1.1. Qualitative phytochemical analysis

Results of phytochemical essays shows that aqueous extract of *S.aromaticum* rich on different important chemical compounds such as flavonoïds, phenols, carbohydrates, ,saponins, tannins and terpenoïds but our extract plant is poured from alkaloids, in table below:

Table 04: Phytochemical essays for aqueous extract of *S.aromaticum*

Compounds	Alkaloids	Flavonoïds	Terpenoids	Phenols	Tannins	Reducing compound	Saponins
Aqueous extract Of *S.a*	-	+	+	+	+	+	+

- **(+): Present , (-): Absent**

1.2.1.2. Qualitative phytochemical analysis

1.2.1.2.1 Dosage of polyphenols

The quantitative study of the extract carried out by spectrophotometric assay using the method of Folin Ciocalteu aimed to determine the total content of polyphenols present in aqueous extract of *S.aromaticum*. The content of these compounds was calculated from of the calibration curve for Gallic acid, expressed in mg of Gallic acid / g of dry extract.

1.2.1.2.2. Determination of flavonoids

From the Quercitin calibration curve, the flavonoid content was determined in the extract, expressed as mg of Quercetin / g of dry extract. The results of this assays (polyphenols and flavonoids are shown in **Table 05 .**

Table 05: Total Phenols and Flavonoïds concentration in aqueous extract of *S.aromaticum*.

Compounds	Polyphenols (mg of GAEq/g of extract)	Flavonoïds (mg QEq/g of extract)
Aqueous Extract of *S.aromaticum*	**77 ± 1,66**	**34,666 ± 1,527**

1.2.2. Antioxidant activity

1.2.2.1. DPPH radicals scavenging activity and IC50 value

In figure 14 below **,** shows the scavenging activity of aqueous extract of *S.aromaticum* and BHT on DPPH radicals at various concentrations. The greatest inhibitory activity observed was in the case of Aqueous extract of *S.aromaticum*, reaching as high as 86.96% at 0.5 mg/mL, while for BHT a concentration at 0.5 mg/mL was needed to achieve the inhibition of DPPH radicals of 77.63%.The concentration of aqueous extract of *S.aromaticum* resulting in a 50% inhibition of the free radical, IC_{50}, was 0.233 mg/mL. IC_{50} values with high regression coefficient (R^2 =0.9264) (for aqueous extract of *S.aromaticum*). The standard BHT had IC_{50} values of 0.257 mg/mL with (R^2 =0.9246).

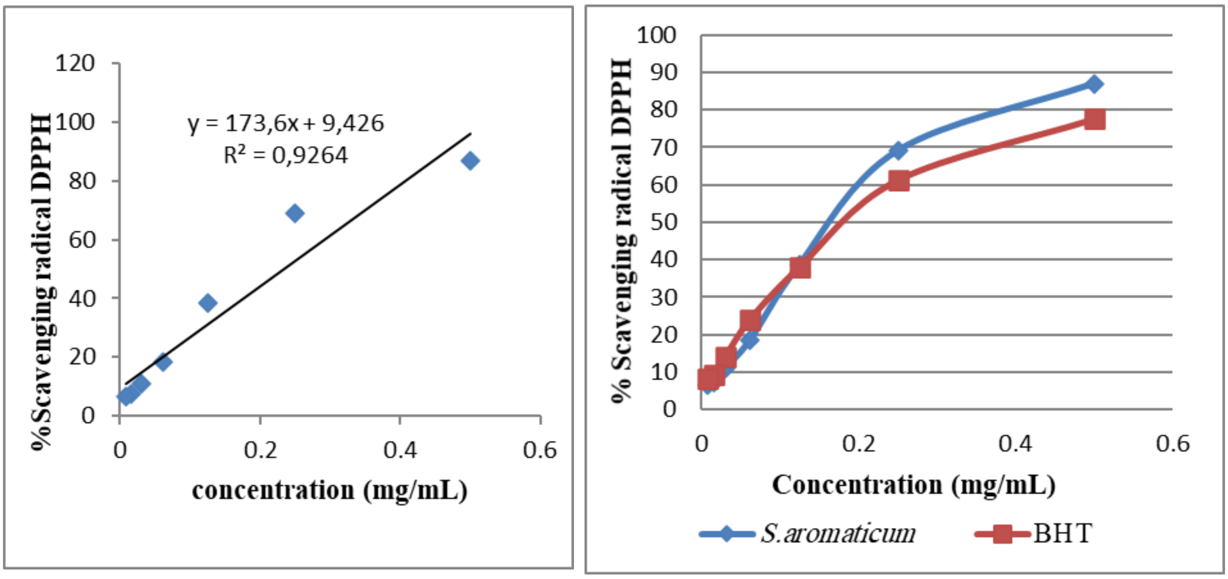


Figure 14: Scavenging effects on the DPPH radical of S.aromaticum and BHT.

1.2.2.2. FRAP assay

The reducing power property of the aqueous and ascorbic acidextracts is illustrated in **Fig.15.** In this study, the reductive capabilities of the *S.aromaticum* extract was increased with increase in their concentration. The aqueous extract of *S.aromaticum* exhibited relatively good reducing activity with IC_{50}17.32µg/mL reaching an inhibition 92.63%at 0.5 mg/mL,however the ascorbic acid showed the highest activity (IC_{50} 4.12 µg/mL) with percentage inhibition of 96.14% at 250 µg/mL. IC_{50} values with regression coefficients (R^2=0.7967 and $y = 18.14\ Ln(x) - 1.737$), for aqueous extract of *S.aromaticum*. The standard Vitamin C had IC50 values of 4.12 µg/mL with highregression coefficients(R^2 =0.9423 and $y = 12.59\ Ln(x) + 32.16$)

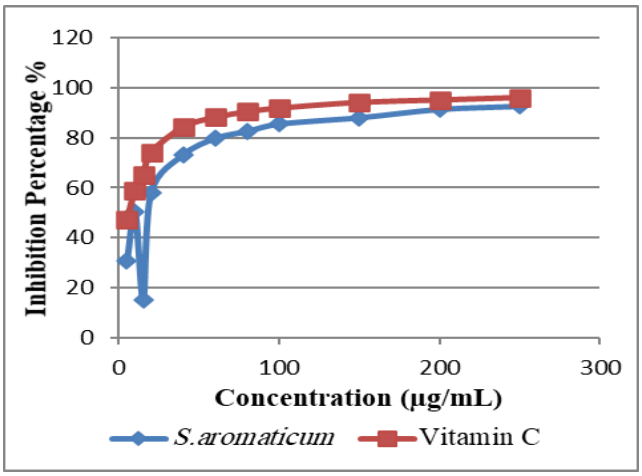


Figure 15: FRAP assay for aqueous extract of *S.aromaticum* and Vitamin C.

1.2.3. Hemolysis assay

Dehydration and delayed proton equilibria of human blood erythrocyte membrane mediated by phosphate buffer (XPBS, pH= 7.4) induces membrane damage and subsequently hemolysis. The antihemolytic activity of the various Concentration(4_8µg/mL) of aqueous extract of *S.aromaticum* and AgNPs on human blood erythrocytes are presented in Fig 16.
At the concentration 8µg/mL of aqueous extract of *S.aromaticum* showed maximal antihemolysis activity (50.95%) in other the maximal antihemolysis activity of AgNPs showed (41.86%) at cocentration 4µg/mL.

Interestingly, at variousconcentrations of AgNPs , the lower the concentration, the greater of antihemolysis activity. On the other hand, we notice the exact opposite, at various concentrations of aqueous extract of *S.aromaticum*, the higher the concentration, the higher of antihemolysis activity.

antihemolysis activity with high regression coefficient (R^2 =0.9283) (for aqueous extract of *S.aromaticum*) and in AgNPs with regression (R^2 =0.8619).

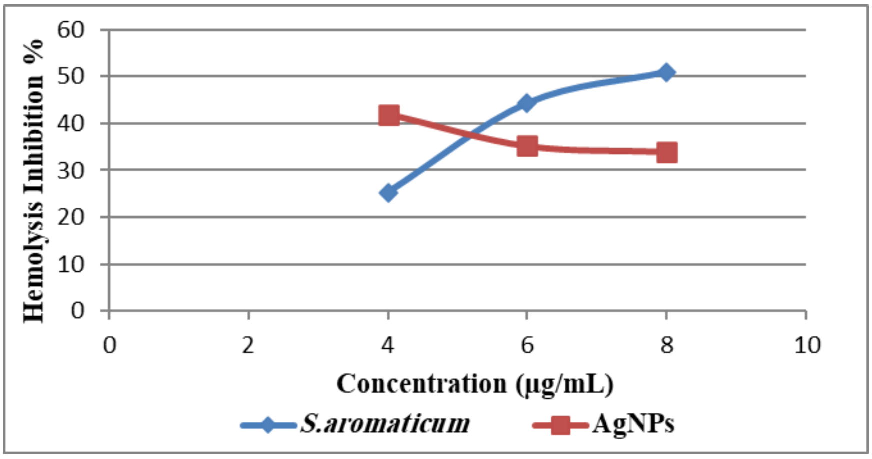


Figure 16 : Antihemolytic activity of aqueous *S.aromaticum* extract and AgNPs.

1.2.4. HPLC analysis of aqueous extract of *S.aromaticum*

HPLC chromatogram results of *S.aromaticum* showing in Figure17 show that S.aromaticum had a different number of phenolic compounds (chlorogenic acid, caffiec acid, vanillic acid, quercetin, Naringin and Rutin) and the predominance of chlorogenic acid with concentration (27.95µg/mL), then Naringin with concentration (15.12 µg/mL).

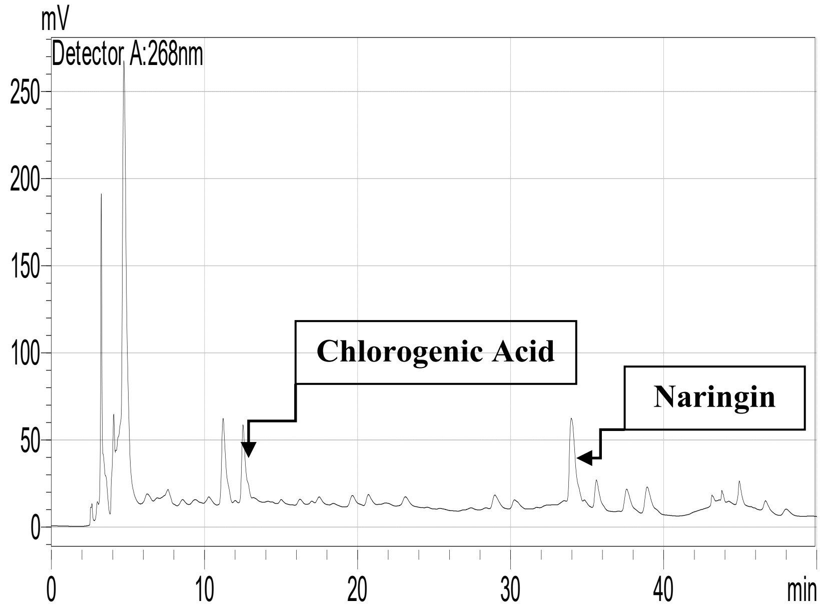


Figure 17: HPLC chromatogram of the aqueous extract of *S.aromaticum.*

1.3. Characterization of Silver nanoparticles

Formation of Silver nanoparticles (AgNPs)

Silver nitrate solution is colorless and extract of cloves is dark red in color (Fig. 18.A). After adding *Syzygium aromaticum* extract to Silver nitrate solution, the solution became grayish red in color (Fig.18.B). The color change confirms that the silver nitrate was reduced and transformed into silver nanoparticles.

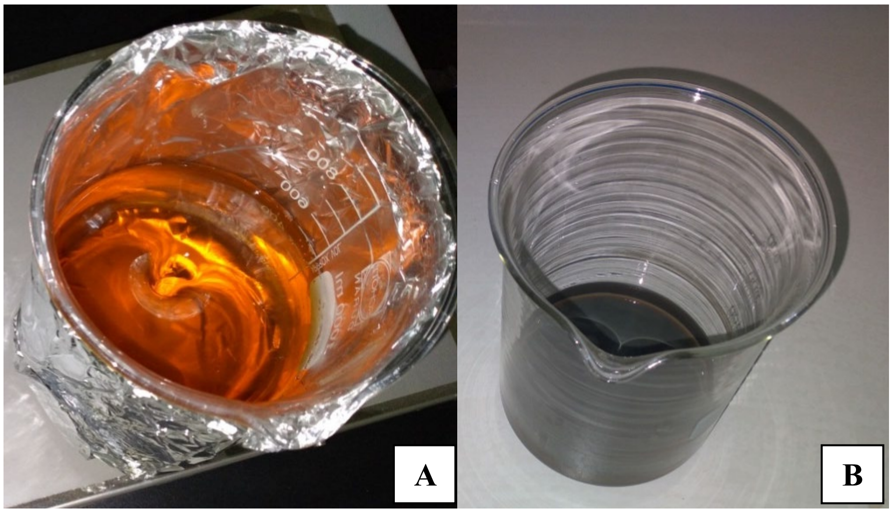


Figure 18: Biosynthesis of Silver nanoparticles (AgNPs) using aqueous extract of *S.aromaticum*

1.3.1. UV-Vis spectral studies

The presence of nanoparticles was confirmed by obtaining a spectrum in the visible range of 350 nm -510 nm using UV-visible spectrophotometer (Fig. 19). From this analysis, absorbance peak was found at around 435 nm, which was specific for Ag nanoparticles. Based on the UV-ViS spectra, the sharpness of the absorption peak was found to be dependent on the concentration ratio of *Syzygium aromaticum*extract, thus, it was sharper with a higher concentration ratio.

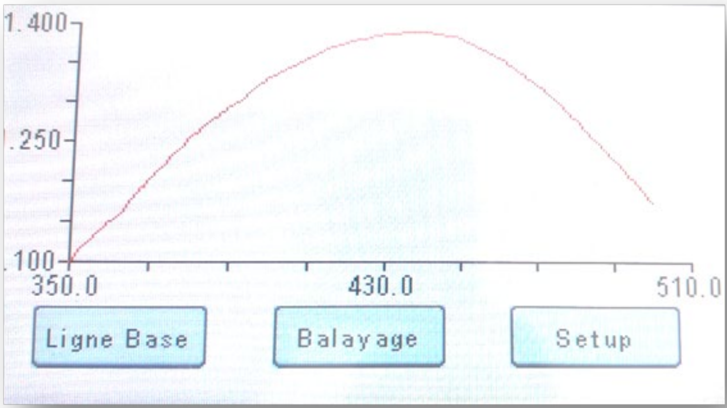
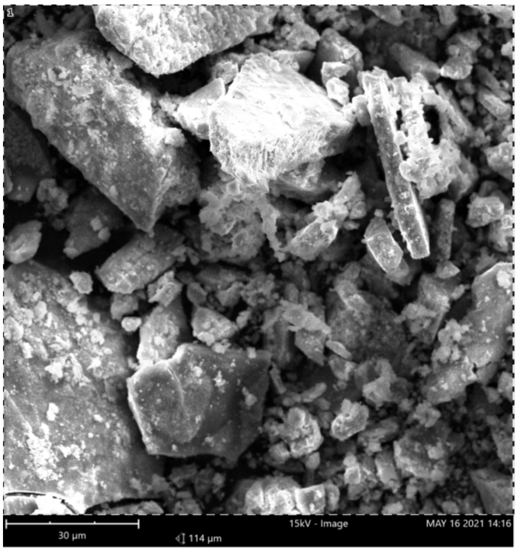


Fig 19: UV-Vis spectra of silver nanoparticles using aqueous extract of *Syzygium aromaticum.*

1.3.2. SEM and EDX studies

SEM technique was employed to visualize the size and shape of silver nanoparticles. In figure.20, SEM images were obtained with 10% of *S.aromaticum.* The SEM (JEOL MODEL 6390) used SEM grids which were prepared by placing a small amount of sample powder on a copper coated grid and drying under lamp. The formation of silver nanoparticles as well as their morphological dimensions in the SEM study demonstrated that the average size was 46 nm with inter-particle distance. The shapes of the silver nanoparticles proved to be Multifaceted. EDX spectra recorded from the silver nanoparticles were shown in Figure.21. From EDX spectra, it is clear that silver nanoparticles reduced by *S.aromaticum* have the weight percentage of silver as atomic concentration 16.77% and weight concentration 57.60%.



Element Symbol	Atomic Conc.	Weight Conc.	Oxide Symbol	Stoich. wtConc
Ag	16.77	57.60	Ag_2O	100.00
O	83.23	42.40		

Figure 20: SEM image of silver nanoparticles formed by *Syzygium aromaticum.*

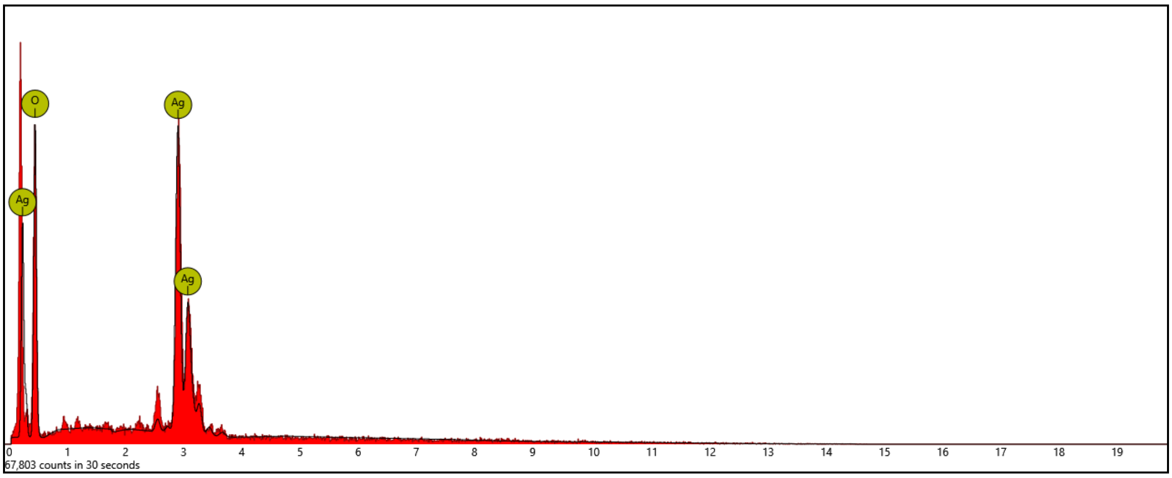


Figure 21: EDX spectra recorded from a film, after formation of silver nanoparticles with different X-ray emission peaks labeled

1.3.3. XRD studies

Figure.22 showed the XRD confirming the existence of silver colloids in the sample.The braggs reflections were observed in the XRD pattern at 2θ= 32.8, 38.1, 54.6, 64.2 and 67.5 . These braggs reflections clearly indicated the presence of (111), (200), (202), (222), (311) and (400) sets of lattice planes and further on the basis that they can be indexed as face-centered-cubic (FCC) structure of silver. Hence XRD pattern thus clearly illustrated that the silver nanoparticles formed in this present synthesis are crystalline in nature. In addition to the bragg peaks representative of FCC silver nanoparticles, additional as yet unassigned peaks were also observed suggesting that the crystallization of bioorganic phase occurred on the surface of the nanoparticles.

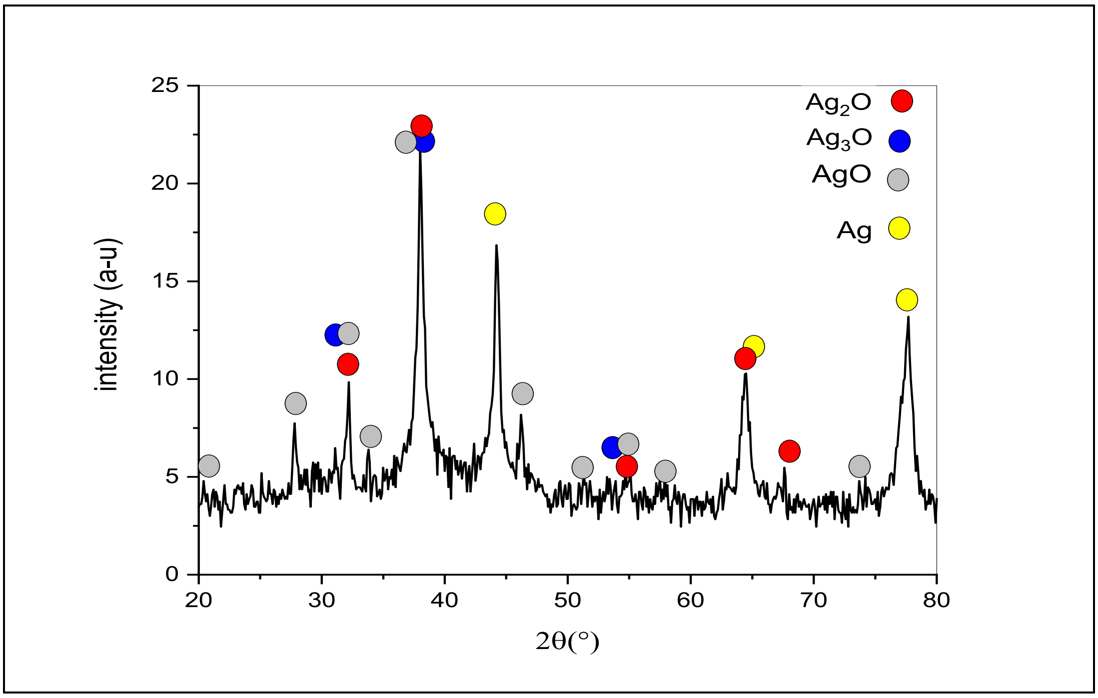


Figure 22: XRD pattern of the synthesized AgNPs using *Syzygium aromaticum* extract.

1.3.4. FTIR spectroscopy

It has been shown that phytochemical analysis of *S.aromaticum* extract reveals whether the aqueous extract contains carbohydrates, glycosides and flavonoids. The presence of carbohydrates, glycosides and flavonoïds in *S.aromaticum* extract may play an important role in Ag reduction reaction. FTIR spectroscopy was used to characterize and identify the chemical composition of the AgNPs surface. As can be seen in Fig. 23, the peak at 3400 cm-1 revealed that water and OeH absorption frequency .

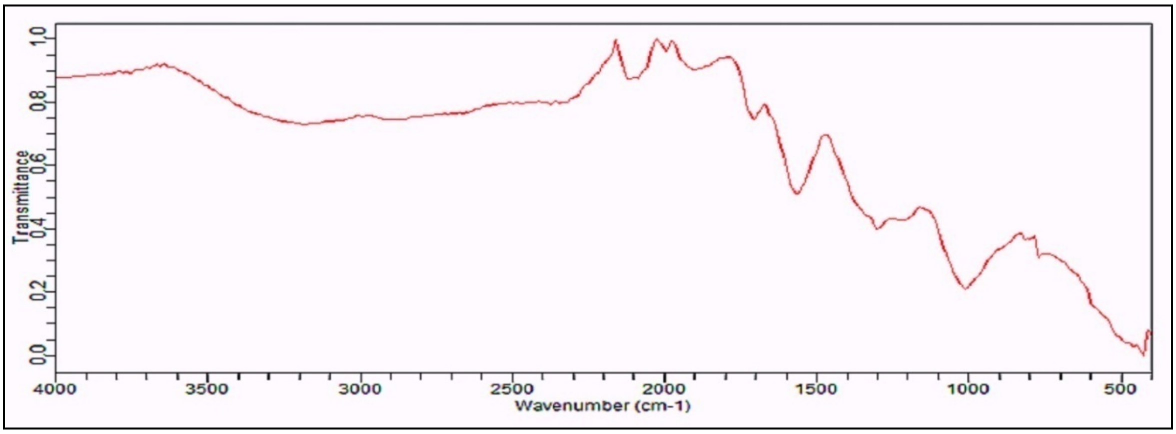


Figure 23: FTIR spectra of silver nanoparticles using *Syzygium aromaticum* extract

1.4. Isolation,Confirmation and Antifungal activity of the fungal strain tested

1.4.1. Isolation of the strain tested

After the isolated from moldy Lemon fruits which were surface disinfected with 4% sodium hypochlorite and cultured moldy lemon in SDA on Petri dishes. After incubating on Petri dishes for 7 days at a temperature 25° C, we obtained black fungal colonies, shown in the Fig.24 below:

Figure 24 : Isolation Fungal colonies **(original photo)**

1.4.2. Confirmation of the fungal strain tested

The microscopic study carried out previously is supplemented by a macroscopic study in order to determine the specie of the selected strain *Aspergillus*. This study is essential for the identification of mold (appearance of the colony color and reverse and growth rate, ...). In this case *Aspergillus* is incubated at 5 ° C, 25 ° C and 37 ° C for one week on fournutrient agar media.

The identification of specie of the gener Aspergillus was carried out after cultivation on different media and at different temperatures. To do this, we referred to the works of **(Pitt and Hocking, 2009)**, while using a taxonomic scheme based on morphological characters (reading diameters, color of mycelia) after 7 days of incubation. The morphological characters obtained are summarized in the table below illustrate the fungal stain Aspergillus on the different culture media as well as their microscopic appearance.

The macroscopic study is carried out by observations with the naked eye and diameter measurements. The results obtained are gathered in **table 06.**

Table 06: Macroscopic identification of *Aspergillus niger***.**

		Reading	
Genus and species	**Middle**	**Color**	**Diameter (mm)**
Aspergillus niger	P.D.A 25°C	Black	**45.33 ± 10.71**
	G25N 25°C	White	**50.42 ± 13.20**
	M.E.A25°C	Black	**45.17 ± 9.73**
	C.Y.A 25°C	Black	**25.56 ± 6.12**
	C.Y.A 37°C	white	**15.33 ± 2.06**
	C.Y.A 5°C	ND	ND

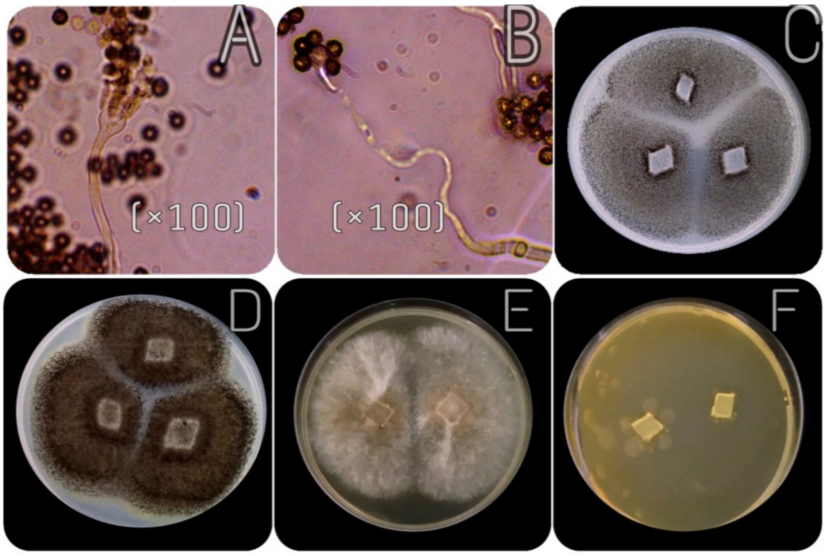


Fig 25: *Aspergillus niger*: (A) and (B) under microscope (×100), (C) on P.D.A at 25 °C, (D): on M.E.A at 25 °C, (E): on G25N at 25 °C, (F): on C.Y.A at 37 °C.

1.4.3. Antifungal activity

The direct contact technique, which involves both contacting between extract and microorganism and observing the growth of the fungal strain tested, suggested that the extract under study of *S.aromaticum* and their AgNPs exerted an inhibitory activity on mycelium growth of *A. niger*

The AgNPs extract was the most potent against fungal strain tested with the high percentage of inhibition of 53.07% at the concentration 0.75 mg/mL. While, the aqueous extract was less effective against *A.niger* with inhibition equal to 13.84% at the same. However, the AgNPs and the aqueous extract provided an effect with 20% and 12.30% respectively at the same concentration (0.375 mg/mL). Our results were compared to the standard used Fluconazol, which has a good effect against *A.niger* with percentage of inhibition 55.38% at the concentration 3 mg/mL. The figure 26 showing the antifungal activity of AgNPs and aqueous extract of *S.aroamticum* against *A.niger*.

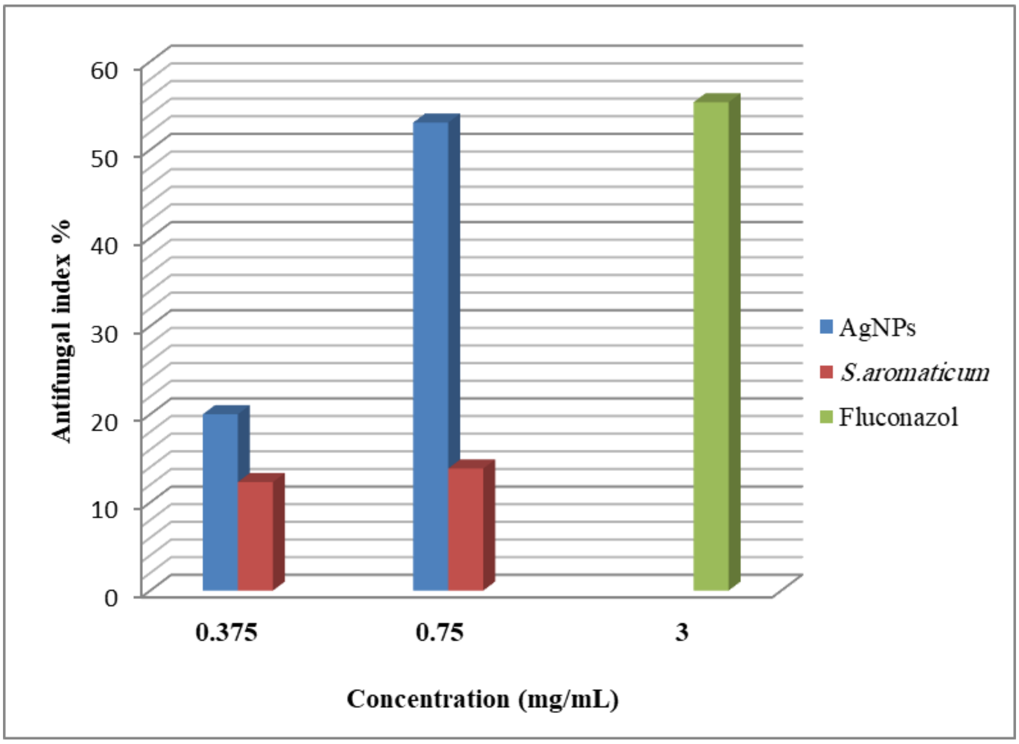


Figure 26: Antifungal activity of AgNPs and aqueous extract of *S.aroamticum.*

1.5. *In vivo* essays of AgNPs and *S.aromaticum*

1.5.1. Hematological parameters

Hematological parameters illustrated in Figure 27 show that, there is no effect of FUNGAL or different treatment on white blood cell, lymphocytes , granulocytes and mean corpuscular volume levels in hole the experimental rats but Hemoglobin , platelets and show a high signification ($p<0.01$) in AgNPs and *S.aromaticum* groups compared to FUNGAL .

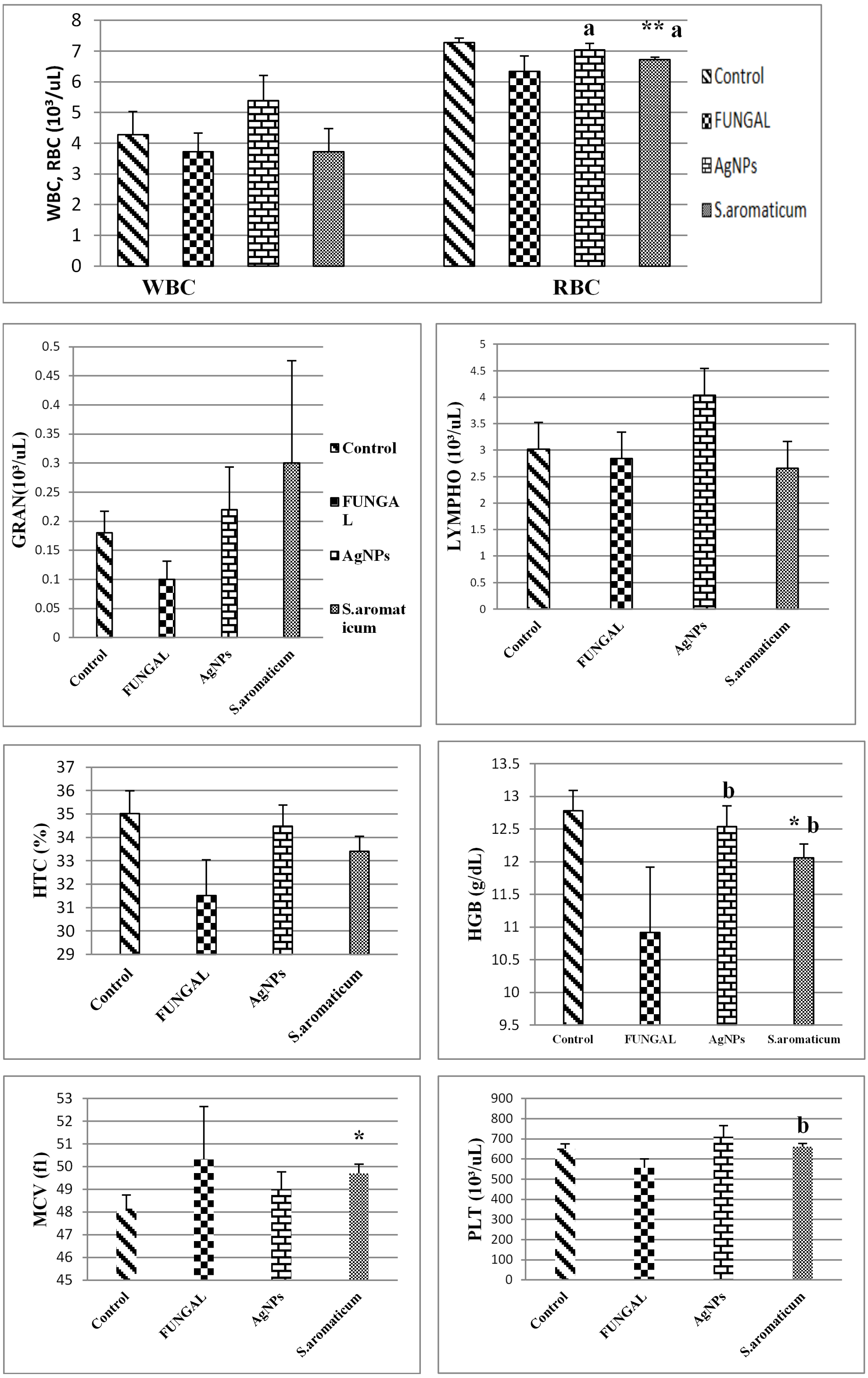


Figure 27: WBC, RBC, GRA, MCV, HGB, HTC, PLT and LYMPHO levels of control and experimental groups.

Result show there is no effect of FUNGAL on RBC levels compared to the control, also RBC levels show a high signification (P<0.01) in *S.aromaticum* and AgNPs groups with increase compared to FUNGAL.

1.5.2. Biochemical parameters

As for which level illustrates in Figure 28,Below, the results of blood glucose parameters show a decrease in FUNGAL group compared to the control. In the other side fig.28 shown that blood glucose was significant decrease in FUNGAL rats treated with *S.aromaticum* compared to the control group.

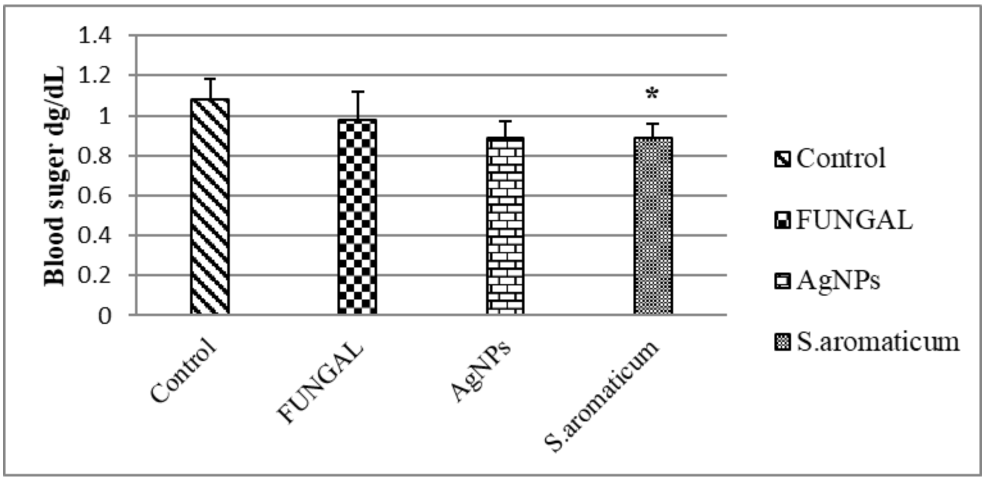


Figure 28: Blood glucose level in the control and experimental groups

As shown in Figure 29 the results of transaminases enzymes activities appeared significant increase of GPT (p<0.01) and GOT activities (p<0.05) in FUNGAL group compared to the control. GOT and GPT levels in *S.aromaticum* group show a high significant (P<0.001) and (P<0.01), respectively compared to FUNGAL group.

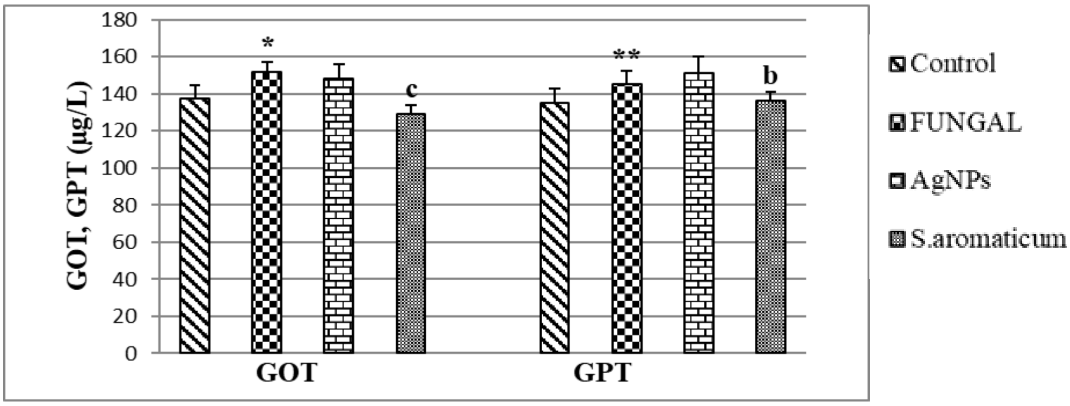


Figure 29: GOT, GPT activities in the control and experimental groups.

In Figure 30 show that, there is no effect of FUNGAL or different treatment on Triglyceride levels in hole the experimental rats, but Cholesterol level show a high signification (p<0.01) in FUNGAL group compared to the control, also treatment with AgNPs shows a signification compared to control group .

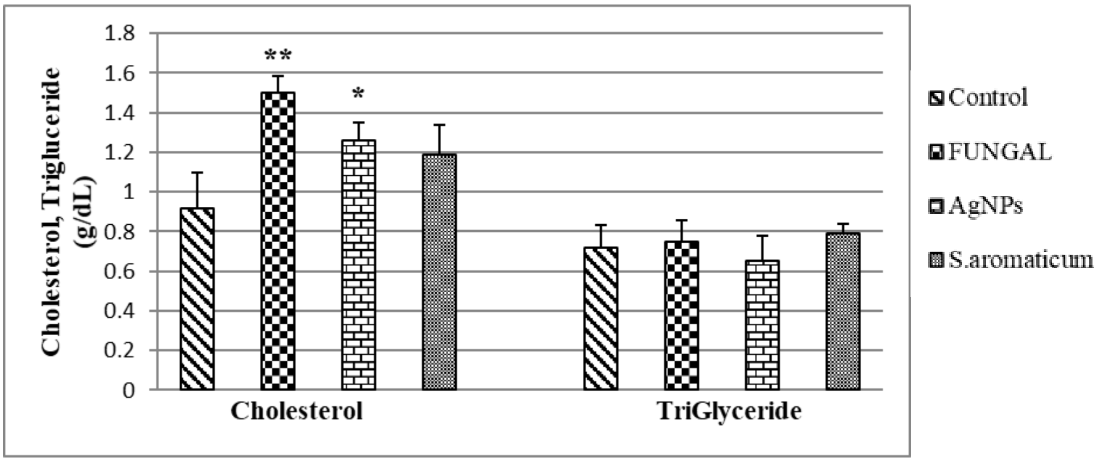


Figure 30: Cholesterol and triglyceride levels in the control and experimental groups

1.5.3. Oxidative stress parameters

1.5.3.1. Malondialdehyde (MDA) levels

MDA levels of liver and lung shows in Figure 31. MDA level in liver and lung shows a signification (p<0.05) increases in FUNGAL group compared to the control. Also MDA level shows decrease significantly (p<0.001) in AgNPS and *S.aromaticum* groups compared to the FUNGAL group. AgNPS and *S.aroamticum* treatment groups showed efficacy in decreasing MDA level.

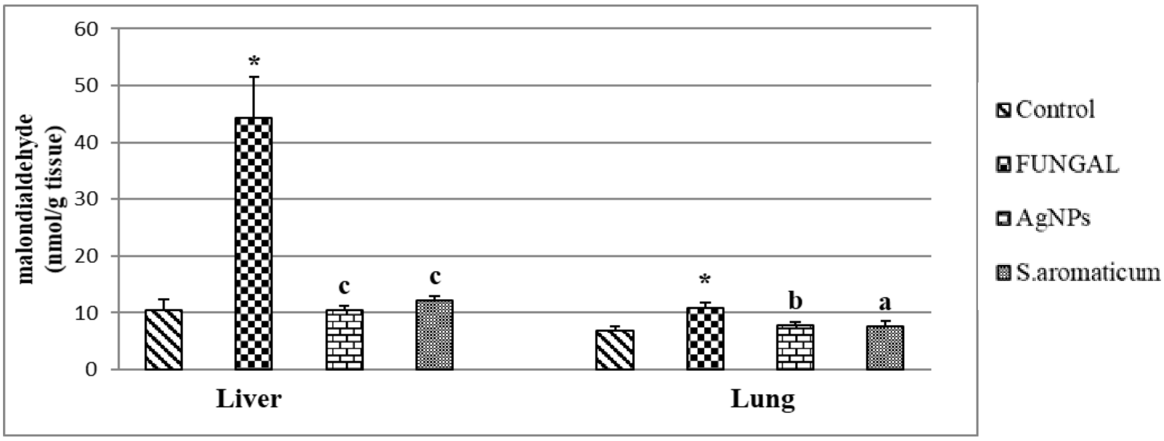


Figure 31: MDA levels in Liver and Lung in control and experimental groups.

1.5.3.2. Reduced glutathione (GSH) level

Liver and Lung GSH levels shows in Figure 32. For Liver and Lung GSH levels, there is decreasing level of GSH in FUNGAL group compared to the control, AgNPS and *S.aroamticum* groups, decrease of GSH level in FUNGAL group compared to the AgNPS and *S.aroamticum* treatment groups with (p<0.001) (p<0.05) in Liver and Lung respectively. AgNPS and *S.aroamticum* treatment groups showed efficacy showed efficacy increasing GSH level.

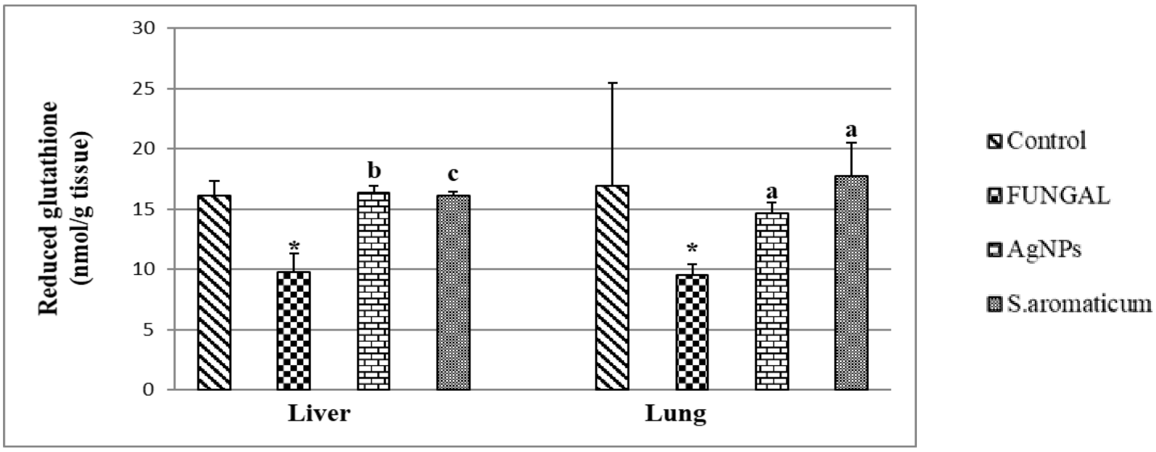


Figure 32: GSH levels in Liver and Lung in control and experimental groups.

1.6. Acute toxicity study

There is no mortality during the 24 hours, but at the two acute doses of AgNPs related to behavioral profiles, slow physical movement was observed, and diarrhea was also observed at 800µg/mL acute dose observed during the first 24 hours and during follow-up for 14 days Table 07.

Table 07: Effect of AgNPs with 2 various concentrations on physiological parameters of Wister albino rats.

	Parameter	Death rate	EyesRedness	Sleep	Diarrhea
0 h	Control	0	N	N	N
	Test (400 µg/mL)	0	N	N	N
	Test 800 µg/mL	0	N	N	N
2 h	Control	0	N	N	N
	Test400 µg/mL	0	N	N	N
	Test800 µg/mL	0	N	N	N
6 h	Control	0	N	N	N
	Test400	0	N	N	N

	µg/mLTest800 µg/mL	0	+	+	+
24 h	Control Test 400 µg/mL Test 800 µg/mL	0 0 0	N N +	N N +	N N +
Day- 14	Control Test400 µg/mL Test 800 µg/mL	0 0 0	N N +	N N +	N N +

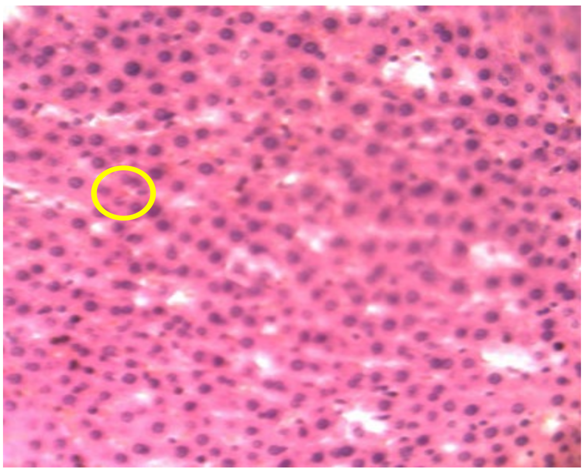
1.7. Histological results

Liver and testis histological results

For liver as shown in (Figure 33-a1, ×40), results indicated normal epithelium cells layer structure with almost non-existent around hepatic pits appearedin control group, conversely in FUNGAL group the histological results show a deep lesion in hole epithelium layer with hemorragic necrosis and a huge destruction in Hepatic pits in hole epithelium cells laye (figure33-b1) . Histological observations of the liver morphology of the rats treated with *S.aroamticum*extract show tolal correction in morphological better than a Control group (Figure 33-c1**).** Finely in AgNPs using *S.aroamticum* extract group the histological results show a partial correction in morphological a deep lesion in Hepatic necrosis compared to FUNGAL group (Fig33-d1).

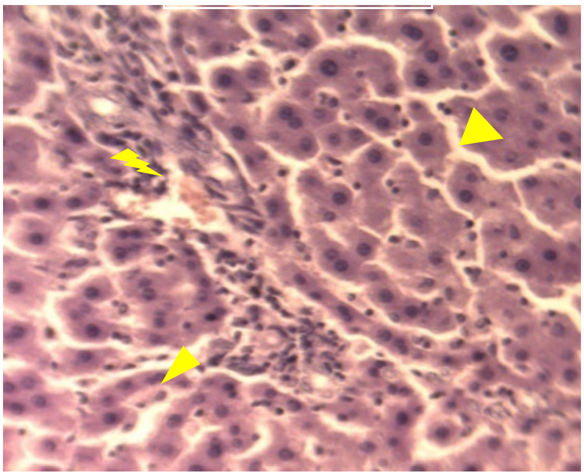
- **Liver (× 40)**

CONTROL group



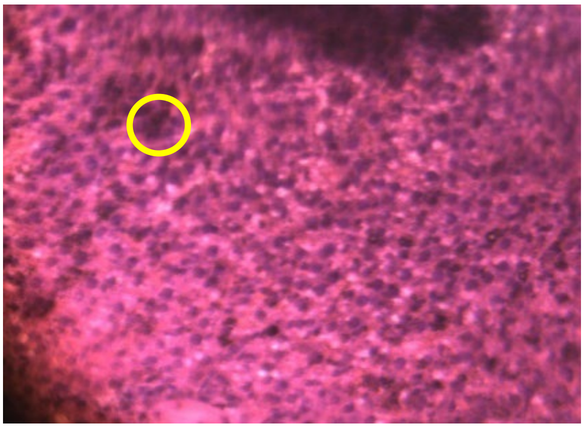
a1

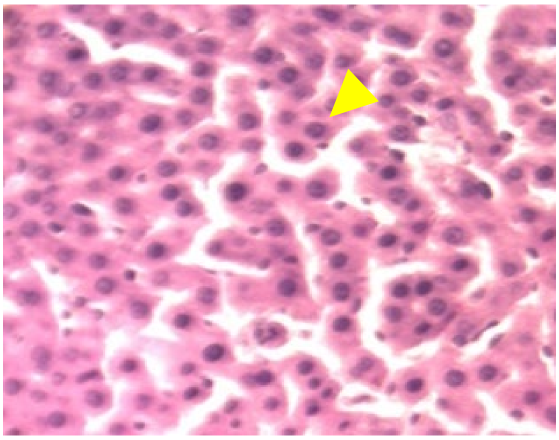
FUNGAL group



b

***S.aroamticum* group** **AgNPs group**



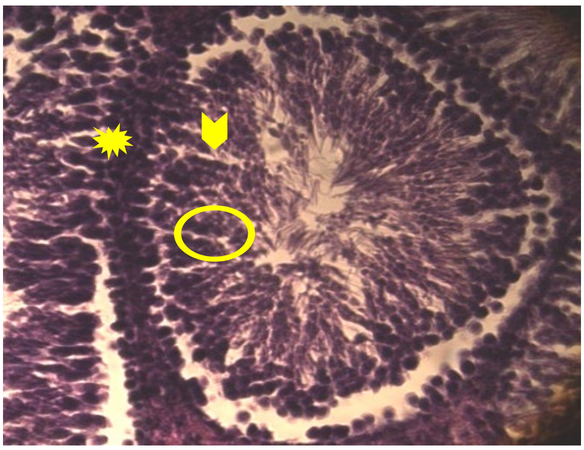


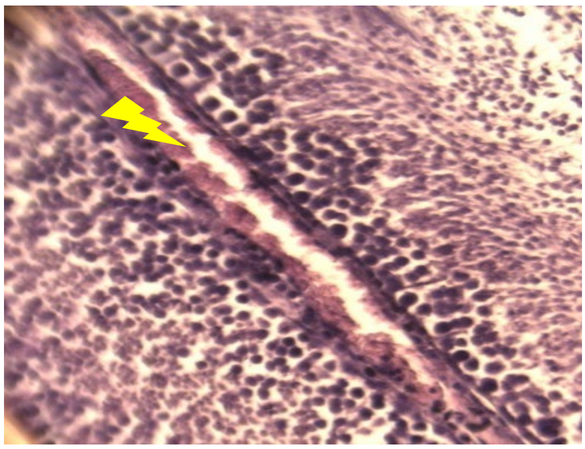
c1 **d1**

Figure 33: Histological examination of the liver. **(× 40)**

- **The Testis (× 40)**

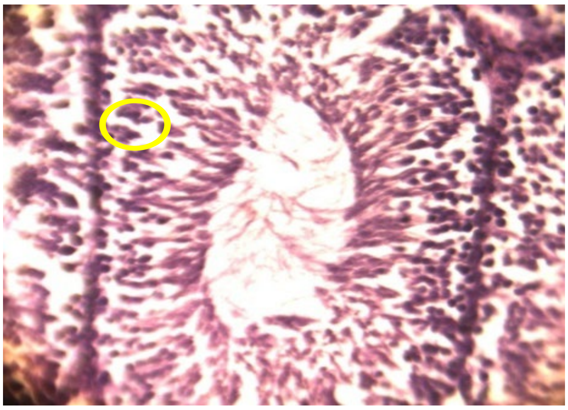
CONTROL group **FUNGAL group**



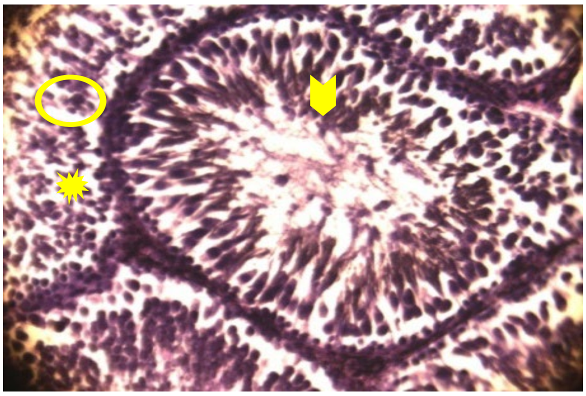


a2 **b2**

***S.aroamticum* group** **AgNPs group**



c2



d2

Figure 34: Histological examination of the testis. (× 40)

For testis as shown in (Figure 34-a2, ×40),in Control group results indicated normal morphologly structure of :tubes seminiferes, Interstitial cells of Lyding, sertoli cells. Compared to FUNGAL group(Figure 34-b2, ×40),there is a total damage the structure of tubes seminiferes with a presence of acute hemorragic due of the infection by Fungal Spores, resulting to extension and malformation of tubes seminiferes and ejection of the sperm.(decrease sperm level in FUNGAL group). In grouptreated with *S.aroamticum* extract (Figure 34-c2, ×40), the histological results show almost total correction in morphological , and there are also a few parts that are almost destroyed by FUNGAL spores in tubes seminiferes compared to FUNGAL group**.** Finely in AgNPs group (Figure 34-a2, ×40),the histological results show a partial correction in morphological , and there are also a some parts that are almost destroyed by FUNGAL spores and few parts had hemorragic in tubes seminiferes compared to FUNGAL group.

FUNGAL treated rats showing some structural alteration **(Figure 33- b1 and figure 34-b2 , ×40).** Rats group of aqueous *S.aroamticum* extract at dose (200 mg/Kg) showing normal appearance of liver and testis cells (**Figure 33 c1 and figure 34- c2**). Liver and testis sections of the rats administered AgNPs using *S.aroamticum* dose (80µg/Kg) showed moderate degree of liver and testicules damage (**Figure 33 d1 and figure 34- d2**).

✸ = epithelium cells layer / seminal epithelium , ⛉ = tubes seminiferes , ⚡ = deep lesionsin (epithelium cells/ seminal epithelium) , ◎ = normal hepatic/testicules pit,

▽ = Hapatic/testicules pits destruction).

2. Discussion

The objective of our study is green preparation of AgNPs using S.aromaticumand evaluation of their (AgNPs and *S.aromaticum)*t herapeutical property against acute inflammation induced by fungal spores in Wistar rats.

2.1. In vitro assay of *S.aromaticum*

The results of phytochemical essays shows that aqueous extract of *S.aromaticum*rich with different important second metabolic (flavonoids, phenols, carbohydrates, saponoside, tannins and terpenoids) but absence alkaloids. According to **(Milind *et al*., 2011)** phytochemical result same of our result. secondary plant metabolites and have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism (**Mamta *et al*., 2012)**. according to **(Wink, 2015)**Terpenes show cytotoxic activities against a wide range of organisms, ranging from bacteria and fungi to insects and vertebrates and have been widely used in herbal medicine against infections, in some cases, steroids, triterpenes and saponins structurally resemble endogenous anti-inflammatory hormones, e.g., glucocorticoids. The total phenolic compounds content in the aqueous S.aromaticum extract was 34.66 GAE mg /g , which is higher than values reported by **(Radünz *et al*., 2018),**(18.59 mg GAE/g), which suggests that the concentration is dependent on the aqueous extraction method and characteristics of the sample.

HPLC result showed the presence of flavonoids such as naringin , and this is similar to the results of **(Wojdyło *et al*., 2007)** (in herbs, C.zeylanicum,Trigonellafoenum-graecum, Myristicafragrans, *Syzygium aromaticum* and *C. longa*, no caffeic acid was detected the HPLC analysis showed that no flavonoids were present in 13 of the plants under investigation .However, quercetin (155 mg/100 g dw) was found in one spice, i.e. *S. aromaticum* **(Wojdyło *et al*., 2007)**

The anti-oxidant activity results obtained from *S.aromaticum,* aqueous *S. aromaticum* extract was found to act as strong free radical scavengers in comparison with commercial antioxidants BHT as indicated by DPPH assays. It is well known that free radicals play an important role in autoxidation of unsaturated lipids in food stuffs .These results demon started that the aqueous *S.aromaticum* extract has effective activity as hydrogen donors and as primary antioxidants by reacting with lipid radicals and scavenges oxygen-free radicals, and inhibits the enzyme xanthine oxidase. **(Halliwell *et al*., 1995).** This is because our plant contains naringin. Naringin is one of such plant-based citrus bioflavonoid well known for its various therapeutics benefits mediated majorly through its antioxidant mechanism. **(Singh *et al.,* 2004).**Its seems to be from richness of our plant from polyphenols and flavonoids, accprding to **(Gülçin *et al*., 2004)** that the potent antioxidant efficacy of aqueous *S.aromaticum* extract may be due to the strong hydrogen donating ability, scavenging of hydrogen peroxide, free radicals and superoxide and metal chelating ability. Additionally **(Mehta et*al*.,2010)** Revealed that antioxidant agents like clove extracts play a significant role in Treating acute infections resulting from oxidative stress. In **(Mittal *et al*., 2014)** study show that aqueous *S.aromaticum* extract has the highest antioxidant capability. **(Gulcin *et al*., 2012)** studies showed the aqueous *S.aromaticum* extract demonstrated scavenging activity against the 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical at concentrations lower than the concentrations of butylatedhydroxytoluene (BHT**)** and this resultit similar of our study.

The ability of the extract to reduce Fe^{3+} may be attributed to the hydrogen donating effect of phenolic compounds **(Shahidi *et al*., 1992)**. It has been reported that phenolic compounds were the main antioxidant components, and their total contents were directly proportional to their antioxidant activity **(Angkawijaya *et al*., 2014)** Therefore, in this study, the presence of the flavonoids and phenols in the tested extract of *S.aromaticum* might have contributed to the antioxidant activity.

2.2. Biosynthesis and characterization of silver nanoparticles using *S.aromaticum*

Mechanism of bioreduction AgNPs were synthesised using *S.aromaticum* extract, which naringin is the main component responsible for bioreduction. At the ortho and para positions of –OH group of naringin, electron withdrawing methoxy and allyl groups are present, the inductive effect of which facilitates the release of a proton from –OH group thereby rendering a negative charge on narngin. This is followed by the formation of resonating structures of the anionic form of naringinand the two electrons released in the process are responsible for the reduction of 2 Ag+ions to 2AgO. The appearance of brown coloration up on addition of extract to AgNO3 solution suggested the formation of AgNPs. **(Kaur *et al.,* 2013)**. Our results is similar of depending of **(Huang *et al*., 2007; Mallikarjuna *et al.,* 2011)** study, compares the absorption spectra of *S. aromaticum* extract with AgNPs, clearly showing the formation of AgNPs having a λ max of 440 nm. The spectra obtained are in agreement with other researchers, which have synthesized AgNPs from *S.aromaticum*

2.2.1. UV–Vis SpectraAnalysis

UV–Vis spectroscopy is one of the essential systems used to determine the primary existence of metal nanoparticles in a liquid medium. The color change demonstrating the presence of Ag nanoparticles was further characterized by UV–Vis spectrophotometer and observed by taking readings at a distinctive temperature. The absorption peaks show the UV–Vis spectra of silver nanoparticle formation at temperature room using *Syzygiumaromaticum* nanoparticle extract. The peaks shows due to surface plasma resonance of silver colloid for diverse temperature were seen in the range of 420 to 470 nm spectral lines were obseved and the UV visible spectra. The intense SPR bands were observed around ~435 nm. Our results confirm the observations of (**Shalini *et al.,* 2012; Venugopal *et al.,* 2017)**

2.2.2. FTIR spectroscopy

FTIR spectra of AgNPs demonstrated the peaks at 3400, 1750, 1600, 1300 and 1050 cm−1. According to ; Theband at 3444 cm−1 corresponds to“polymeric”OH stretching mode. The 1722 cm−1 peak corresponds to normal aldehyde group. The absorption at 1621 cm −1 represents amide and Open chain imino (\\C_N\\). 1388 cm−1 relates methyl C\\H says./sym bend. The 1388 cm−1 peak relates to trimethylor“tert butyl”(multiplet). The 1055 cm−1 peak corresponds to the C\\C stretch and the aliphatic fluoro compounds C\\F stretch. The peaks at 587 cm−1 correspond the aliphatic do compounds, C\\I stretch, alcohol and OH out-of-plane bending. This confirmation proposes that the protein particles could perform the capacity of the arrangement and adjustment of AgNPs in the aqueous medium **(John Coates, 2000; Shah *et al*., 2013).**

2.2.3. XRD analysis

The diffraction peaks correspond to (100), (111) and (200) planes of face centred cubic crystals and hexagonal phases in the spectrum **(Philip, 2009; Sathishkumar *et al*., 2009; Mallikarjuna *et al*., 2011; Narasimha *et al*., 2011; Sulaiman *et al*., 2013).** The average size from the XRD analysis is in agreement with the findings from DLS and TEM.

2.2.4. SEM and EDX studies

The SEM image showed relatively spherical shape nanoparticle formed with diameter in the range of 35-55 nm. Similar phenomenon was reported by (**Chandran *et al.,* 2006)** and **(Udayasoorian *et al.,* 2011*)***.Energy dispersive spectrometry (EDS) micro-analysis is performed by measuring the energy and intensity distribution of X ray signals generated by a focused electron beam on a specimen. EDX spectra were recorded from the silver nanoparticles. From EDX spectra, it is clear that silver nanoparticles reduced by *S.aromaticum* have the weight percentage of silver as 20.16% and 16.41%.

2.3. Antifungal activity

Plants have become a very interesting, safer and more effective source of substances than synthetic antimicrobial agents **(Mahmoud, 1994).** These plants produce constitutively active antimicrobial compounds. The first observation drawn up by the results shows that our extracts (aqueous *S.aromaticum* and AgNPs) both had an effective effect on *A. niger*.These results corroborate with the works of **(Kushwaha *et al*., 2021 ; Karm, 2019; Pinto *et al*., 2009)**.In addition, **(Karm, 2019)** report that Analysis of the antifungal activity shows that the aqueous extract had a powerful inhibitory effect on all concentrations tested with a maximum inhibition of 53.07%, this effect could be explained by its richness in bioactive chemicals (flavonoids, phenols, tannins, terpenes **(Karm, 2019)**. **(Kushwaha *et al*., 2021)** have reported that *S. aromaticum* oil as well as their extract has been showed antimicrobial activities. Additionally, **(Pinto *et al*., 2009)** explained that the clove extract exhibited wide-spectrum antifungal activity against *Aspergillus* and *Candida* strains. **(Nostro *et al.*, 2004)**, has reported a low MIC values ranging from 0.08 to 0.64 µl ml^{-1}, these studies confirmed the importance of the phenolic hydroxyl groups of eugenol for inhibiting the growth of microbial species. Another study realized by **(Ahmad *et al.*, 2017)**

Was found that clove oil possesses strong antifungal activity against opportunistic fungal pathogens such as *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus.*

The synthesized AgNPs using *S. aromaticum* have strong antimicrobial activities against *Candida albicans*, *Candida parapsilosis* and *Aspergillusniger* according to(Devi et *al*.2014). Likewise, **(Maciel *et al*., 2019)** reported thatthe clove *S. aromaticum* contains in total 23 components. Among them, the most dominant is eugenol (76.8%), β-caryophyllene (17.4%), α-humulene (2.1%) and eugenol acetate (1.2%) **(Jirovetz *et al.*, 2006)**. As well**, (Kim *et al*., 2010)** reported that spherical AgNPs showed potent activity against *Candida albicans*compared with that of commercially available antifungal agents. Treating infection caused by fungi becomes a hectic problem due to serious side effects like renal and liver dysfunction associated with amphotericin B and nystatin **(Mashwani *et al.,* 2016).** The explanation of these potent activity maybe due to the Ag^{+} which forms complexes with bases of DNA and giving a potent inhibitor of fungal DNAases **(NCCLS *et al*., 2012).** Also the nanoparticles is likely to be well correlated with its decreased size and shape owing to increased surface area with enhanced antimicrobial effect**. (Devi *et al.,* 2014)**.

2.4. Hematological parameters

The results of hematological parameters in AgNPs, showed that no effects in RBC levels in all the experimental group compared to Control, but it showed a very high signification decrease level in WBC and LYM in AgNPs group. Most of the hematologic effects of AgNPs are thought to result from an immune reaction and are, there for Toxin specific **(BARBARA *et al*., 1991).** Several studies confirmed that AgNPs induced agranulocytosis such as **(JAMA *et al*., 1986 ; Kamakshi, 2014**). The cause of metal induced agranulocytosis is not fully understood, but mechanisms direct toxicity been proposed. Direct toxicity to myeloid cells, particularly neutrophils, has been shown with medications such as AgNPs **(Tesfa *et al*., 2010).** The toxicity may be due to either the parent metal or a toxic metabolite or by product. The severity of neutropenia associated with these toxins is often dose dependent, but the occurrence of reactions is still idiosyncratic. Agranulocytosis associated with direct toxicity is usually associated with a slower decline in neutrophils. Although that the mechanism is not quite clear but we can say that the toxicity of AgNPs probably caused agranulocytosis or (decrease in WBC and LYM levels). **(Pontikoglou *et al*., 2008).**However, HCT, HGB and palettes results shows decrease and high significant decrease of RBC in AgNPs group compared to control. Decreases of these parameters may be due to increase RBC hemolysis resulting from increase erythrocyte fragility and impairment of the antioxidant defense mechanisms.

2.5. Blood glucose and serum biochemical parameters

According to **(Heydrnejad *et al*., 2015)** were found Hepatic function is evaluated by measuring AST and ALT. In the other words, liver damage induced by nanosilver particles of the present investigation physiologically affected AST (in particular) and also ALT in male and female. Increase serum AST and ALT levels indicated that liver tissues were damaged. Liver enzymes like aspartate amino transferase (AST), alanine amino transferase (ALT) These enzymes showed a increase in rats AgNPs group. In addition, these were confirmed by histological microscopy and by some other studies. For instance, in a histological analysis reported by (**Gatti *et al*., 2004)**, inorganic particles, heterogeneous in nature but homogeneous in size, were identified in the liver. The results of investigation are consistent with other studies **(Cheraghi *et al*., 2013)** with nano silver on these enzymes showing elevation of hepatic enzymes so that AST level in serum was elevated in male and female rats as compared to the control. On the other hand, the observed increase in ALT in the current study may be due to the free radicals released from the nano silver particles when attacking hepatocytes and releasing ALT stored in them and entering into the blood serum **(Cheraghi *et al*., 2013; Griffitt *et al*., 2009)** Similarly, the increased level of WBC in the present study may follow phagocytosis of silver nano silver **(Takenaka *et al*., 2001; Cheraghi *et al*., 2013; Griffitt *et al*., 2009).**The immune response of rats to an external factor has been the deacrese of the number of white blood cells for phagocytosis of nano silver particles**(Cheraghi *et al*., 2013),** where as rats effects of nano silver particles have been evaluated at different doses on serum. In this study, the level of ratio of WBC components changed which is in accordance with other studies. In fact, silver nanoparticles can lead changes in lymphocytes granulocytes ratios so that the lymphocyte/granulocyte ratio may change sharply **(Tang *et al*., 2009; Aniya *et al.,* 2005)** This study achieved a dose-dependent effect of the silver nanoparticles, the gender-related difference between the male and female rats livers has not been previously reported. Likewise**, (Kim *et al*., 2009)** found the gender-related distribution of silver nanoparticles in the rat kidneys (but not in the liver.Also **(Ibrahim, 2020)** mentioned thatcarbohydrates (glucose) and lipids (cholesterol and Triglyceride) are the main sources of animals energy and indicators of stress resulting from toxins, lipids also have a fast metabolic transformation **(Harabawy and Ibrahim, 2015)**. The present results showed a significant increase in serum glucose level (hyperglycemia), Triglyceride and cholesterol of rats AgNPs group. (**Harabawy and Ibrahim, 2014)** noted that hyperglycemia caused by liver glycogenolysis due to plasma catechol amines and corticosteroid hormones elevation in addition to amino acids through the activation of gluconeogenesis process. Also, reported hyperglycemia in rats of AgNPs group, maybe due to its release to blood from the glycogen of the liver. Cholesterol and triglyceride increase may be due to the disturbance of lipid metabolism as a result of AgNPs group **(Shalaby, 2007).**

2.6. Oxidatif stress

Acute exposure to Fungal spores of Aspergillus sp is believed to be an important factor for acute inflammatory resulting from A.niger. Ochratoxin A is one of secondary metabolites of A.niger, wich is considered a causative agent of many inflammatory diseases , including hepatomegaly, damage to the testicles and Pulmonary dysfunction **(Janette *et al*., 2012)**.

Therefore, many intervention strategies are set to prevent or alleviate FUNGAL spores-induced (OTA) disorders; aqueous *S.aromaticum* extract and AgNPs. In current study, the role of aqueous *S.aromaticum*extract and AgNPs on the FUNGAL spores-toxicity was investigated in male rate. The results of our experimental showed that FUNGAL treatment group elevated MDA concentration in liver and lung tissues, which indicated to the increase of lipid peroxidation. MDA is an end product of lipid peroxidation compared to control group, and this may be considered a late biomarker of oxidative stress and cellular damage (**Chico *et al*., 1998).** The results of this study confirm and extend previous data which have demonstrated that OTA induces a significant increase in LPO in liver and lung induced by OTA as increasing in malondialdehyde (MDA) production **(Petrik *et al*., 2003).** Previously**, (Cui *et al*., 2020)** stated that OTA did evoke oxidative stress, which might contribute, at least in part, to OTA hepatic and Pulmonary toxicity and carcinogenicity in rats during long-term exposure. To assess the balance of reactive oxygen species (ROS) production in liver and lung , levels of non-enzymatic antioxidants GSH was measured. Current results showed that OTA caused significant decrease in the levels of GSH, this decrease indicated the cell damage of liver and lung tissues **(Doorten *et al*., 2004)**. **(Özçelik *et al*., 2004)** stated that intracellular GSH status appears to be a sensitive indicator of cell's overall health and its ability to toxic challenges, whereas, the decrease in GSH activity will increase the level of superoxide radicals, leading to an increase in oxidative stress enhancing early cell death, probably by apoptotic mechanisms. Our finding of decrease in the activity of GSH corroborates with that of previous studies **(Ozcelik *et al*., 2004; Abdel-Wahhab *et al*., 2008).** According to **(Katary *et al.,* 2017),** increased lipid peroxidation expresses the of oxidative stress induced by OTA by several mechanisms, on the one hand, OTA inhibited the mitochondrial oxidative phosphorylation leading to the release cytochrome c from mitochondrial inter membranous space into cytosol and to the release of ROS such as superoxide anion and H2O2. These free radicals declined the intracellular ATP concentration, leakage of Ca2+ out of mitochondria, cellular osmotic imbalance and lipid peroxidation, resulting in increased permeability and subsequent hpatic and pulmonary toxicity . Oxidative stress induced generation of ROS and its over production is one of the prime an etiologic factor that cause acute inflammatory **(Derouiche *et al*., 2019).** As a result, some relevant biochemical marker status gets affected as peroxidation of lipids, proteins and nucleic acids, which may lead to cellular damage and cell death **(kumar, 2017).** On the other hand, MDA, is more cytotoxic to cells affects the membrane structure and causes further destruction in the cells, accompanied by hemorrhagic necrosis **(Mehmet *et al*., 2013).**

Interestingly, When the endogenous antioxidant systems is exhausted to combat the oxidative stress-induced cellular damage, we may have to depend on the exogenous antioxidants. In this context, the plant-based secondary metabolites such as flavonoids, phenolic compounds and tannins are well known for their antioxidant potency, and naringin is one of such plant-based citrus bioflavonoid well known for its various therapeutics benefits mediated majorly through its antioxidant mechanism. **(Singh *et al.*, 2004).** The richness of our plant like Naringin make it one the most potent antioxidants plants. According to **(Viswanatha *et al.*, 2017),** that naringin (100 mg/kg) supplementation for 7 days could alleviate the anti-oxydantssystam through increasing (SOD, GSH..) levels,and reducing the levels of MDA, TNF-a, IL-1b, and IL-6. In additionally, decreasing the gene expression and concentration of pro inflammatory cytokines (TNF-a, IL-6, NF-kB p65 Unit, iNOS, IL-1b, COX-2), decreasing the MMPs (MMP-2, MMP-9), decreasing the expression of apoptotic factors (Cascapase-3, Caspase-9). **(Liu *et al.*, 2016).**

In result of (**Liu *et al.*, 2016)** that naringin (100 and 200 mg/kg) administration has showed a significant improvement in diabetes-associated cognitive impairment. Via combination of multiple mechanisms by activation of peroxisome proliferation activated receptor-gamma (PPAR-g), reducing the proinflammatory cytokines (TNF-a, interleukin-6 (IL-6), interleukin-1 b (IL-1 b)), enhancing the oxidative defense and reducing the expression of caspase-3 and caspase-9 (**Zhanghua *et al.*, 2015).**Further, a similar study conducted by (**Liu *et al.*, 2016) ,** reported that naringin (100 mg/kg) supplementation for 7 days could alleviate the OTA-induced cognitive impairment through increasing SOD levels, reducing the levels of MDA, TNF-a, IL-1b, and IL-6 in the hippocampus, also showed significant increase in PPAR-g expression in hippocampus of naringin-treated group compared to OTA-control group. (**Liu *et al.*, 2016).** Also **(Golechha *et al.*, 2014)** evaluated naringin against PTZ-induced seizures and oxidative stress, and reported that naringin (80 mg/kgi.p.) has attenuated the PTZ induced convulsions and cognitive deficits through GABA Areceptor mediated mechanism along its potent antioxidant (reduced MDA, increased GSH) and antiinflammatory (decreased TNF-a) mechanisms **(Golechha *et al.*, 2014).**

The results of the experimental rate treatd with aqueous *S.aromaticum* extract and AgNPs showed that treatment with aqueous *S.aromaticum*extract and AgNPs after FUNGAL spores gavage ameliorated antioxydants system, where MDA level decreased and GSH activity increased.

The plant extract can decrease the MDA by polyphenols and flavonoids contained a hydroxyl group in their structure wish make them have a very important antioxidant activity, many flavonoids chelate free Fe and Cu that could otherwise increase ROS generation, and also reduce ROS such as O2·−, and HO· **(Bhattacharyya *et al*., 2014).** Hydroxyl groups on this nucleus donate hydrogen and an electron to hydroxyl, peroxyl, and peroxynitrite radicals, stabilizing them and giving rise to a relatively stable flavonoid radical. **(Sofna *et al*., 2014).**

In our study FUNGAL decrease the reduced glutathione (GSH) level. GSH is probably the most important antioxidant present in the cells. Therefore, other enzymes that help to generate GSH are critical to the body’s ability to protect itself against oxidative stress. The functions of GSH are removal of oxygen free radical species and maintenance of membrane protein thiols **(Gopinathan *et al*., 2015).** In addition, GSH has been shown to scavenge a wide range of reactive species (RS) including reactive oxygen species (ROS) and reactive nitrogen species (RNS) **(Derouiche *et al.,* 2020).** In this way, GSH can scavenge superoxide anion (O2), hydroxyl radical, and singlet oxygen directly, by donating electrons and becoming oxidized to their radical **(FRANCO *et al*., 2007**). In **(Hussain *et al*., 2016)** study, tripeptide glutathione (GSH) is an intracellular thiol antioxidant; lower level of this GSH causes higher ROS production, which results in imbalanced immune response, inflammation, and susceptibility to infection **(Ghezzi *et al*., 2011)**,study was conducted on the role of GSH and its oxidized form and their regulatory function and gene expressions beyond free radical scavenging activities linked with GSH. GSH takes part in the redox regulation of immunity **(Ghezzi *et al.,* 2013),** through mixed disulfides between protein cysteines and glutathiones; it is known as glutathionylation which operates signaling proteins and transcription factors **(Fratelli *et al*., 2002)**. In addition, *S.aromaticum* treatment group show signification effect of GSH activity compared to control, which this is refer to the high protection of aqueous *S.aromaticum* extract to cells damage probably due to their second metabolic compounds. The results obtained in our study shown also that AgNPs treatment ameliorate the stat of oxidative stress decreasing MDA level in liver and lung tissues. AgNPs could inhibit oxidative stress via protection and stabilizer of lipids and proteins.

In our work, we assessed the *in vitro* effects of AgNPs and *S.aromaticum* with various concentrations on on human erythrocytes, and showed that AgNPs can induce hemolysis. we can explain this a follow as: oxidative stress and increase cytosolic Ca2+, annex in V binding and calpain activity **(Ferdous *et al*., 2018**). Additionally **(Kim *et al*., 2014; Choi *et al*., 2011)** working on incubated the erythrocytes with vehicle *S.aromaticum* and AgNPs for 4 h; a time point similar to previous in vitro studies. the various concentrations of AgNPs and *S.aromaticum* (4, 6 and 8 µg/ml) used in our study are also similar to previous studies evaluating nanotoxicity and blood compatibility of erythrocytes incubated with AgNPs and *S.aromaticum***(Chen *et al*., 2015; Huang *et al*., 2016).** Our concentrations are comparable with previous studies using AgNPs on human, and rats a erythrocytes and the highest dose of 8µg/mL , is much very lower than the optimal dose of AgNPs (200 µg/ml) that has been reported to induce hemolysis in human erythrocytes **(Chen *et al*., 2015; Kim *et al*., 2014; Huang *et al*., 2016; Krajewski *et al*., 2013 ; Kwon *et al*., 2012).**

2.7. Histological analysis

The analysis of our study about liver and testis histology concluded that less necrosis and damage were generated in the aqueous *S.aromaticum* extract and AgNPs treated groups, compared with the FUNGAL group. These results confirmed the above findings related to MDA and GSH levels. The results of studies on the mechanism of hepatic necrosis suggest that lesion genesis proceeds in a sequence of events **(Mclean *et al*., 1978 ;Yayla *et al*., 2014).** TheRelationship between oxidative stress and inflammation has been documented by many authors, evidences indicated that oxidative stress plays a pathogenic role in inflammatory diseases **(Derouiche, 2020).** Additionally **(Popa *et al.,* 2016)** reported that damage by oxidative stress such as oxidized proteins, glycated products, and lipid peroxidation results necrosis tissus. Referring to **(Clarkand *et al.,* 2006 ; Varga *et al.,* 1996; Al-Anati *et al*., 2006)** OTA is among the toxic secondary metabolites of *Aspergillus niger*, which causes severe inflammation in several organs, sometimes damaging them. OTA is known to be immune toxic in animal studies**.** The immune suppressant activity of OTA in animals has been characterized by size reduction of vital immune organs like the thymus, spleen and lymph nodes, depression of antibody responses, alterations in the number and functions of immune cells, and modulation of cytokine production **(Al-Anati *et al.,* 2006).** There are also complex relationships between Aspergillus, T regulatory lymphocytes and candidiasis **(Montagnoli *et al*., 2006),** which can be clinically relevant in humans.

(Hope *et al*., 2012; Klahr and Morrissey, 2003) clearer that OTA induces renal, hepatic and intestinal toxicity, characterized by inflammation and cell death. In the acute and initial phase of the inflammatory process, the vascular permeability increase and the neutrophils (PMN) infiltrate the tissue; here they contribute to the inflammatory response by producing several mediators, such as ROS (**Hedenborg and Klockars, 1987). (Ferrante *et al*., 2006)** studyin rats, a single oral OTA administration (10 mg/kg) induced oxidative damage and PMN infiltration in parenchymal organs .(**Marin *et al*., 2018)** studied, there are less evidence concerning the ability of OTA to induce liver cancer. For example, **(Rossiello *et al*., 1993)** has shown that single intraperitoneal injection of AA (10 mg/kg b.w.) increased the incidence of liver neoplastic nodules in rats when coupled with the liver tumor promoter orotic acid. The liver is one of the major organs for toxin biotransformation **(Gu and Manautou, 2012; Qi *et al*., 2014)**. The development of hepatocellular carcinoma (HCC) is one of the most extensively investigated inflammation-based carcinogenic processes because more than 90% of HCCs develop in the context of chronic liver damage and inflammation **(Nakagawa and Maeda, 2012).** The toxins accumulate in the liver in concentrations similar to those found in the kidney **(Vettorazzi *et al*., 2011)**, so there are high possibilities that inflammation could have an important role in the hepato-carcinogenic potential of OTA. Indeed, OTA-DNA adducts were detected in the liver **(Schmeiser *et al*., 1988)** and OTA like mutational patterns were identified in hepato-cellular carcinoma **(Poon *et al.*, 2013).** On the other side, the liver is the target of xenobiotics because of its central role in xenobiotic metabolism, its portal location within the circulation, and its anatomic and physiologic structure **(Sturgill and Lambert, 1997).** Abnormal ROS generation results in structural and functional abnormalities in the liver through the interaction with the cellular structures of hepatocytes **(Cichoz-Lach and Michalak, 2014).** In general, an increased level of oxidative cell damage may be the result of an impairment of the antioxidant system, and this process could lead to carcinogenesis **(Halliwell, 2007).**

Oxidative stress is a disturbance in the balance between the production of reactive oxygen species (ROS) and the antioxidant defense **(Derouiche *et al*., 2018),** and is often associated with toxin exposure **(Nita and Grzybowski, 2016)**. ROS can stimulate proliferation, invasiveness, angiogenesis, and metastasis, and inhibit apoptosis being considered as a pro-neoplastic factor **(Halliwell, 2007).** Cells of eukaryote organisms have created defense mechanisms as antioxidant enzymes in the purpose to limit the damage caused by the action of free radicals **(Lobo *et al.,* 2010).** When the system fails and the antioxidant control mechanisms are exhausted or overrun, the cellular redox potential shifts toward an oxidative and nitrosative stress, which leads to DNA mutations and genomic instability **(Federico *et al.*, 2007).** Studies of **(Cosyns *et al*., 1999; Cui *et al*., 2005; Hadjiolov *et al*., 1993)** have shown that OTA has a high inflammatory effect in the liver as shown by the increase of TNF alpha, IFN gamma, IL-1 beta, IL-8, and IL6, and a decrease of IL-4 in the liver. OTA was classified by IARC as a potent carcinogen and oral administration of OTA to rats resulted in neoplastic lesions of liver, for stomach, renal pelvis, urinary bladder, ear duct, thymus, small intestine, and pancreas.We can explain this ahigh inflammatory effect as follows: Cytokines are proteins that play an important role in the inflammatory response. The majority of the cytokines are produced by activated endothelial and epithelial cells are able to produce these proteins, too. Cytokine expression is regulated by NF-κB and AP-1 and may be triggered by LPS, ROS and microbial species, among others. Interleukin (IL)-1B and tumor necrosis factor (TNF)-α are two of the main cytokines involved in the inflammatory response **(Nayely *et al.*, 2016**). Accordin to **(Katary *et al.*, 2017**) results study, lesions of liver are associated with inflammation, OTA activates NF-κB subsequently NF-κB translocates into the nucleus to up regulate the expression of pro-inflammatory cytokines genes such as TNF-α and cytokine-induced neutrophil chemo attractant (CINC-2α). These data were confirmed by induction of hepatic tissue levels of pro inflammatory cytokines such TNF-α, IL-1β and IL-6 as well as decreasing hepatic level of anti inflammatory cytokine IL- 10 by OTA, such as TNF-α, IL-1β and IL-8, are considered key inducers of hepatic injury induced by FUNGAL spores like OTA. Through the induction of adhesion molecules on both leukocytes and vascular endothelial cells. It was reported that IL-1β and IL-8, as well as TNF α, can promote inflammatory response and are involved in OTA-induced hepatic necrosis in humans and animals **(Zhang *et al.,* 2008).**TNF-α, the major pro-inflammatory cytokine released from the migrated macrophages, TNF-α, produced by macrophages, results in injury to multiple organs and causes inflammation, edema, ischemia, hemorrhage, and neutrophilic leucocytes accumulation wich plays an important role in the pathogenesis of necrosis and damage hepatic through stimulation of ICAM-1 expression on vascular endothelial cells **(Singh *et al.*, 2015).** TNF-a activates the cytotoxic and phagocytic activities of neutrophils, which, if directed towards endothelial cells, could destroy them **(John *et al.*, 1987).**

Histopathological analyses of testis showed that OTA-exposure could exert pathological impact on the testes tissue in detail. The first remarkable finding showed that OTA induces degeneration in seminiferous tubules (STs). Further steps of **(Malekinejad *et al.*, 2011)** study showed that the toxic effects of OTA are not limited to simple degenerative damage in the testes tissue. Histomorphometric analyses along with histopathological examinations showed more severe damages in OTA-exposed animals, such as lowering in germinal cells layers (TDI), sperm number, and a severe infiltration of immune cells in the interstitial connective tissues indicating a negative impact of OTA on testes. Previous data have shown the pathological impact of OTA on other organs including the kidneys.**(Kuiper *et al.*, 1989 ; Kumar *et al.,* 2007).**Also **(Malekinejad *et al.*, 2011)** noted important histopathological findings of thier study are the vasodilatation, thrombosis and prevascular and subcapsular edema, which are accompanied with the huge infiltration of inflammatory cells. All mentioned pathological signs represent an inflammation as a result from OTA exposure. The OTAinduced inflammatory reactions in gastrointestinal tract and renal systems in rats have previously been reported**. (Kanisawa *et al.,* 1990; Sauvant *et al.,* 2005).** The histochemical technique of Sudan Black B **(Kerr *et al*., 1984)** was conducted to evaluate the lipid accumulation in the cytoplasm of the germinal epithelium of the STs and interstitial connective tissue of the testes. Comparing the reaction density between control and OTA-treated groups indicates that OTA causes a significant increase of spermatogonia cells. By contrast, in control group, spermatids showed relatively high density. According to these histochemical results, OTA exposed rats showed a significantly increased lipid content in the germinal epithelium of the STs and in the cells of the interstitial connective tissue. To explain how OTA causes enhancement of the lipid accumulation in mentioned cells, it should be noted that lipid content in the sertoli cells differs depending on various conditions. For instance, when these cells phagocyte residual bodies or degenerated cells, the lipids increase in the cytoplasm of sertoli cells. **(Laughlin *et al*., 2004)**. Therefore, it would be more logic to hypothesize that due to OTA exposure, the rate of degenerated cells is increased as we showed. In turn, this results in more active phagocytosis by sertoli cells, which reacted by intensive Sudan Black B staining. Another reason for the lipid accumulation and consequently dominant Sudan Black B staining in OTA-exposed animals may be an interruption in glucose metabolism or transport as the main source of the energy for protein synthesis in the germinal cells**. (Farooqui *et al*., 1997)** reported, since the glucose transporter is the main uptake of glucose to the STs and spermatogonia, any degenerative event caused by OTA, could result in a reduced glucose metabolism and ultimately lipid increase in the cytoplasm of the cells specially those close to lumen of the STs. PAS staining technique, which is used to demonstrate the carbohydrate level in examined tissues, confirmed the previous findings as in OTA-exposed animals a very tiny PAS reaction was observed. According to **(Malekinejad *et al*., 2011)** studied,since the degeneration, inflammatory cells infiltration, lipid accumulation, and carbohydrate levels in the other groups were less than in the OTA-exposed group, the protective property of aqeuos*S.aromaticum*extact and AgNPs is postulated to OTA exposure, the role of oxidative stress in generating of OTA-induced detrimental impact on the testis may be considered, as well. There is accumulating data indicating the major role of oxidative stress in the pathogenesis of several diseases **(Derouiche *et al*., 2020).** Additionally, previous studies demonstrated that inflamation increased the ROS in freshly isolated kidney cells, which in turn could cause pathological damages in various cells. In the other side histological results of liver and testicles of aqeuos*S.aromaticum*extact and AgNPsshows a high protection against tissues lysis induced by OTA. our in-vitro result appeared that *S.aromaticum*containing different second metabolic compound which they have anti inflammatory property such as flavonoïds (naringin) , naringin is important modulators of pro-inflammatory cytokines, such as IL-1β, IL-6 and TNF-α. However, the effect of naringin on intracellular signalling pathways and on other inflammatory mediators still remains to be investigated, since it would depend on the type of cells, the studied disease and the applied stimulus **(Zhanghua *et al*., 2015).** In addition, flavonoids such as quercetin and luteolin, showed higher inhibitory effect on TNF-α release than those with only one hydroxyl group in B ring **(Comalada *et al*., 2006),** the high protection property of our plant *S.aromaticum*is due of inhibition of pro inflammatory cytokines that refer to their second metabolic.

Conclusion

Conclusion

Infections of the body's organs are among the dangerous factors that cause total or partial damage. Any defect in the organs leads to the failure to perform their vital functions.
One of these major problems is destruction of liver tissue and testicles. So, the aim of this study is to evaluate the biological protection (hepatoprotective effect) effect of aqueous extract of *S.aromaticum*and their silver nanoparticalesAgNPs against inflammation induced by fungal spores of *A.niger*.
The phytochemical screening showed the richness of aqueous extract with phytocompounds like: polyphenols, flavonoïds, tannins,terpenoïds, saponins, steroïds, carbohydrate and alkaloïds.
The *in vitro* study of aqueous extract and AgNPs appeared an important activity against inflammation induced by oxidative stress.In the same time,The *in vivo* study of aqueous extract show up their important therapeutic activity, and revealed that this extract had a good effect high than AgNPs.

Thehematological analysis concluded that the rats treated by aqueous extract of*S.aromaticum* may have a positive impact in the hematopoietic system.Interestingly, there is a very clear effect treated by AgNPs may have a negative impact in the hematopoietic system.As for biochemical parameters, AgNPs showed protection against lipogenesis such as: (triglycerides and cholesterol), in contrast, it has a negative effect on liver enzymes: GOT GPT.

In addition,theaqueous extract gave remarkable results of erythrocyte protection against hemolysis.The high protection ability of this extract it's not limited *in vitro*, but extended to microscopic level which shows their high therapy to return cells normal against inflammatory injury by fungal spores which induced oxidative stress. However contradictory action of the aqueous extract and theirAgNPswas observed in the decrease of MDA and increase of GSH level.

For future studies, we hope that studies will focus more on detect all compounds of *S.aromaticum*, especially the effective compound like eugenol, naringin and essential oil that can be more potent while testing them in inflammation, and why not work on the extraction and the separation these compound.

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Annexes

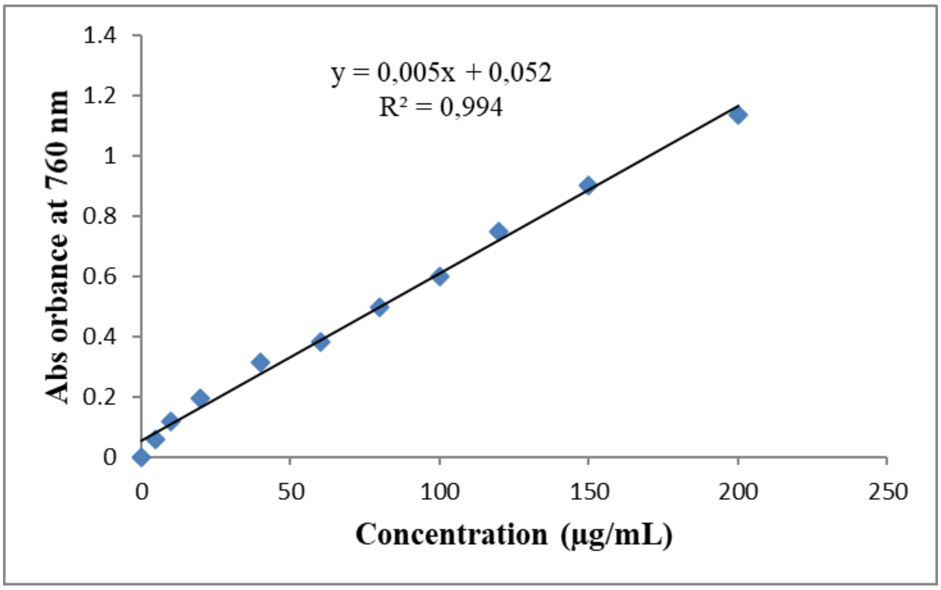

Figure: Calibration curve for Gallic acid determination of polyphenols.

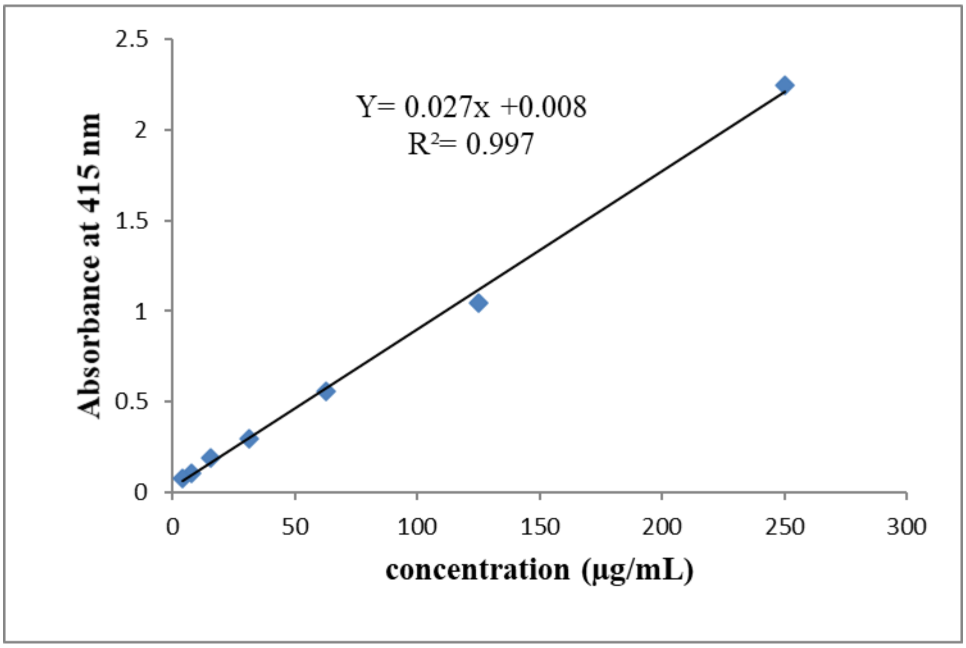

Figure: Calibration curve for Quercetin for determination of total flavonoids

المركب	Tr	المعادلة	R^2
Gallic Acid	5,29	$y = 54681x$	$R^2 = 0.9956$
Chlorogenic Acid	13,392	$y = 21665x$	R^2 =0.9853
Vanilic Acid	15,531	$y = 65077x$	R^2 =0.9921
Caffiec Acid	16,277	$y = 84066x$	R^2 =0.9974
Vanilin	21,46	$y = 58930x$	R^2 =0.9966
p-Coumaric Acid	23,817	$y = 49495x$	R^2 =0.9961
Rutin	28,37	$y = 28144x$	R^2 =0.9869
Naringin	34,788	$y = 19379x$	R^2 =0.9968
Quercetin	45,047	$y = 45378x$	R^2 =0.9962

Lamda= 268 nm

Figure : HPLC chromatogram of flavonoids standard.

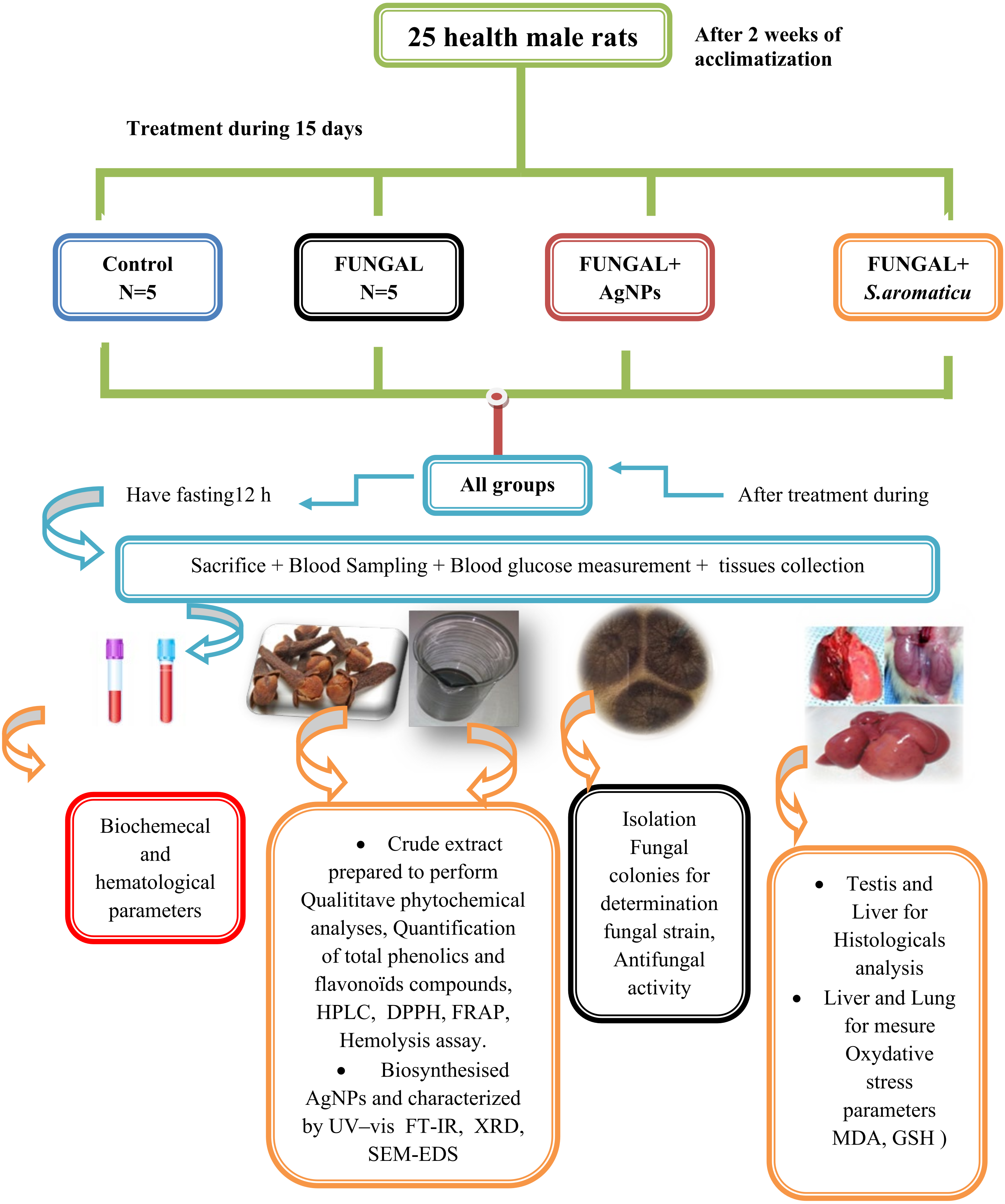

Figure : Experimental design of study