Egypt. J. Chem. **Vol. 65,** No. 10 pp. 303 - 313 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

In Vitro and in Vivo Assessment of Silver Nanoparticles and Sesame Oil combination as in Treatment of Sodium Nitrate-Induced Hepatotoxicity

Seham A.A. Orabi [a*], Said E. Desouky[b], Carmen M. Sharaby [c], Mohamed M. Roushdy [b], Hanaa H. Elsayed [a], Moustafa M.G. Fouda [d*]

[a] *Department of nutritional chemistry and metabolism, National Nutrition Institute, 11562Cairo, Egypt,*
[b] *Botany and Microbiology Department, Faculty of Science, Al-Azhar University, 11884, Cairo, Egypt,*
[c] *Chemistry Department, Faculty of Science (Girls), Al Azhar University, Cairo, Egypt,*
[d] *Pretreatment and Finishing of Cellulose Based Textiles, Textile Research and Technology Institute (TRT), National Research Center, 33- El- Buhouth St, Dokki, Cairo, 12622- Egypt.*

Abstract

The liver, which carries a variety of important functions, removes toxins and toxic chemicals from the body. "Hepatotoxic substances" can interact with basic cellular organelles, resulting in a wide range of liver disorders. The main goal of this study was to investigate if silver nanoparticles (Ag NPs) which prepared using Tri sodium citrate (TSC) and Ascorbic acid (AA) as reducing agents in oleic acid with different concentrations, sesame oil (SO) and their combination could protect albino rats from sodium nitrate ($NaNO_3$) induced hepatotoxicity. UV-visible spectroscopy (UV-VIS) and transmission electron microscopy (TEM) were used to investigate the characteristics of the obtained Ag NPs. Changing the concentration of the reducing agent had no discernible impact. Ag NPs produced by TSC and AA were 28 nm and 60 nm in size on average, respectively. Following an antibacterial experiment against *Escherichia coli* and *Pseudomonas aeruginosa*. Sesame oil was combined with each concentration of Ag NPs. The antibacterial efficacy of a mixture of (SO) and Ag NPs 50 ppm versus *Pseudomonas aeruginosa* was higher than that of *Escherichia coli*. To evaluate the effect of sodium nitrate $NaNO_3$ on the liver, 70 healthy adult male albino rats" were divided into seven groups, each with ten rats. G1 received a normal diet as a negative control, G2 received $NaNO_3$ orally as a positive control to cause hepatotoxicity, G3 received (SO) only, G4 received Ag NPs 10 ppm only, Corn oil was replaced for six weeks with SO, Ag NPs10ppm, and a mixture of SO and Ag NPs10 ppm (1:1) + $NaNO_3$ at a rate of 15 mg/kg. The results showed that combining sesame oil and Ag NPs (G7) enhanced liver (AST, ALT, GGT, ALK, albumin, total protein, and globuin) and kidney (urea and creatinine) functions, as well as oxidative stress biomarkers (MDA and GSH), The relative weight of the liver in G5 and G6 was restored to that of the normal control group. Furthermore, the histological structure of the liver was normal in comparison to the other treatment groups, with normal hepatocyte and hepatic vascular structure.

Keywords: Chemical Synthesis; Silver Nanoparticles; Sesame Oil; Sodium Nitrate

Introduction

Food preservatives can help to improve and maintain safety of food, smoothness, flavour, and nutritional value. Synthetic preservatives, while effective, may have some negative side effects[1]. Sodium nitrate $NaNO_3$ a synthetic food preservative, is employed as a preservative in a variety of foods (vegetables, cured meat, and fish) [2]. When $NaNO_3$ reacts with amines found in food, it can produce nitrosamines or a significant amount of radical. These by-products may enhance lipid peroxidation, which can harm a variety of human organs [3]. Because of the increased oxidative stress, nitrate and nitrite have been linked to negative health consequences, including liver damage [4] . The liver is an essential organ for the detoxification of harmful substances such as xenobiotics. It is the major goal of toxins and xenobiotics since it plays numerous functions in metabolism, waste disposal, and the exclusion of unnecessary elements from the body [5]. Because of their safe use, anti-oxidant properties, and potential roles in intra and extracellular protection competing oxygen radicals and lipid peroxides in response to "oxidative stress, there is a growing interest in discovering defending biological function based natural composites containing nutritional flora [6] [7]. Sesame oil (SO) is the most common natural source of sesamol. It comprises a high rating of the food component, which would provide fantastic health benefits [8] [9]. Sesame oil contains 80% of “unsaturated fatty acids” and equal percent of oleic acid and linoleic acid. It also encompasses anticancer

*Corresponding author e-mail: *drseham49@gmail.com*.; (**Seham A.A. Orabi**); drmmfouda@gmail.com,
Receive Date: 18 January 2022, Revise Date: 08 February 2022, Accept Date: 17 February 2022
DOI: 10.21608/EJCHEM.2022.117010.5282

and antioxidant ingredients such as "sesame and sesamolin", which make up about 0.5-1% and 0.3-0.5% of sesame oil, respectively [10] [11].

In Nano-medicine, silver nanoparticles (Ag NPs) play an important role. Many noble metals have already been utilized for a variety of applications [12]. Ag NPs have been designated for use in a variety of applications, including cancer diagnosis and treatment. Ag NPs have been dedicated in possible presentations such as cancer diagnosis and treatment [13] [8]. Ag NPs have been discovered to have an effective antibacterial impact, and as a result, they have been widely employed in medicine for purposes such as cancer diagnosis and treatment. [14]. Ag NPs can continuously release silver ions, which could be a reflection of the microorganism-killing mechanism. "Silver ions" may observe the cell wall and cytoplasmic membrane due to electrostatic attraction and connection with sulphur proteins [4]. AgNPs can be made in a variety of ways, including physical, biological, and chemical processes, with the latter requiring two primary components: metal precursors and reducing agent. Because of advancements in Ag NPs synthesis with various characteristics, as well as physico-chemical characterisation and impacts on microbes, [6]. Description techniques like as "visible ultraviolet spectroscopy (UV-Vis) and transmission electron microscopy (TEM) have been industrialized. Using these techniques, scientists can examine Ag NPs morphology, behaviour and configuration [15].

As a result, the primary goal of this study is to examine the manufacturing of Ag NPs (10, 50 ppm) utilising tri-sodium citrate (TSC) and ascorbic acid (AA). To investigate the effect of adjusting the reducing agent on the size of the generated Ag NPs multiple technologies such as visible UV-Vis and TEM" were used to characterize the optimized Ag NPs. As a result, we chose the best reducing agent and investigated its antibacterial effectiveness in combination with sesame oil on bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Finally, using histological and biochemical features, evaluate in *Vivo* the putative involvement of sesame oil and Ag NPs against Na NO_3 produced hepatotoxicity in rat liver

2. Materials and Method

2.1. Chemicals

All reagents were of analytical grade; all solutions were prepared with deionized water. All glassware were thoroughly cleaned and washed with deionized water. Silver nitrate ($AgNO_3$) and oleic acid were provided from Sigma Aldrich, while "El Nasr Company" contributed the tri-sodium citrate (TSC) $C_6H_5O_7Na_3$ and ascorbic acid $C_6H_8O_6$. Sesame oil and wheat germ oil were obtained from the Agricultural Research Centre, Cairo, Egypt.

2.2. Silver nanoparticles preparation

Silver nitrate ($AgNO_3$) was employed as a precursor for the manufacture of silver nanoparticles Ag NPs, with ($C_6H_5O_7Na_3$) and ascorbic acid ($C_6H_8O_6$) acting as both reducing and stabilising agents to reduce Ag+ ions to Ag^0 [16], [17]. with some medication by Al-Sherbini et al. [18]. To determine the influence of these parameters, the concentrations of (TSC) and (AA) were changed. This study used the following way:

100ml oleic acid was poured into a 500 ml beaker, and the temperature was raised between 120 and 150°C using a hot plate with a magnetic stirrer. Once the solution began to boil, drop wise additions of $AgNO_3$ (1ml and 5ml) respectively from stock solution (1000 ppm) were made to prepare Ag NPs (10 and 50 ppm), (about 1 drop per second with vigorous stirring), followed by additions (1ml) of (TSC) and (AA) of concentrations 0.25%, 0.5%, 1%, 5% and 10% also about 1 drop per second with vigorous stirring).

The solution turns yellow after the addition of silver nitrate ($AgNO_3$) and (TSC) or (AA). After that, the mixture was swirled for twenty minutes. The heating was then turned off, and the solution was allowed to cool at room temperature while being constantly stirred.

2.3. Characterization of formed Ag NPs

To evaluate the functional features of the produced particles, characterization of Ag NPs is required. This characterization is accomplished utilizing analytical techniques such as UV-vis spectroscopy and transmission electron microscopy (TEM). Nanoparticle behaviour, bio distribution, efficacy, and safety are all influenced by their physicochemical qualities.

2.3.1. Ultraviolet-visible spectroscopy

Colloidal Ag NPs solutions were transported to cuvette prepared from quartz with optical path length 1 cm. (UV-Unicom UV spectrophotometer) was used. The absorbance of Ag NPs solution was then measured and definite at a wavelength of 300–700 nm.

2.3.2. Transmission electron microscope (TEM)

The size and shape of the generated Ag NPs were determined using a transmission electron microscope (JEOL-JEM 1200). The TEM was operating at a voltage of 90 kV. A little amount of produced Ag NPs was placed on the carbon coated copper grids in the TEM technique. The extra solution was removed using a drying paper after allowing the film to stand

for 2 minutes, and the grid was allowed to dry before the investigation. A beam of photons travels through an ultra-thin specimen, interacting with it as it passes through. After the electrons have passed through the specimen, the interaction produces a picture. The image was magnified and focused onto imaging equipment. [19].

2.4. Evaluation of antibacterial activity

Antimicrobial activities of six mixtures of Ag NPs (10 and 50 ppm) and sesame oil (SO) and their mixture with different concentrations of TSC (5 and 10%) on *Staphylococcus aureus*; ATCC6538 which represent positive Gram and *Pseudomonas aeruginosa*; ATCC9027 which represent Gram negative bacteria were evaluated using Micro-dilution technique in micro titer plates "MTP" to define the minimum inhibitory concentration (MIC) versus human pathogenic bacterial strains. Compounds were examined against the pathogenic strains. Briefly, 1: 100 (v/v) of overnight cultures of the test strains were added to 200 µl of Muller Hinton broth media dispersed in the wells of MTP with and without Compounds. The plates were then incubated with shaking (120 rpm) for 24 h at 37 °C. Once the incubation period end, the cell growth was read by "ELIZA reader (Tecan Elx800, USA)" at 620 nm. In the last wells with no turbidity, the MIC was determined as the lowest concentration of compounds that inhibited 100% of pathogenic microorganisms. [20].

2.5. In *Vivo* study

A total of seventy normal male adult albino rats weighing around 120 g were used in the study. At the Animal House of the Nutritional chemistry and metabolism department, National Nutrition Institute (NNI), Cairo, Egypt, all animals were housed individually in cages in a well-ventilated room. The animals were housed at temperatures below the minimum requirements (12 light: 12h dark and 22 ± 2 °C). They were given a conventional diet as well as fresh water. The investigational animals were kept and cared for in accordance with the International Guiding Principles for Animal Research.

2.5.1. Experimental design

For adaption, rats were given a standard diet for a week before being separated into seven groups. There are ten rats in each group. According to Reeves et al., the first group (G1) was given merely a standard diet [21] for 6 weeks, as a negative control group. Standard diet was prepared according to the following recipe per 100 g diet: (65.5 g corn starch, 14.0 g protein, 10.0 g corn oil, 4.0 g salt mixture, 1.0 g vitamin mixture, 5.0 g cellulose, 0.2 g choline chloride, 0.3 g methionine).

The second group (G2) was given a conventional diet + 15 mg/kg sodium nitrate ($NaNO_3$) through gavage needle, and was termed the positive control group [22]. The third group (G3) received Standard diet, with slight modification (Corn oil is replaced by SO). The fourth group (G4) received standard diet, with slight alteration also: (Corn oil is replaced by AgNPs10ppm / prepared in oleic acid). The fifth group (G5) received Standard diet, with slight alteration corn oil is replaced by (SO) + $NaNO_3$; 15 mg/kg. The sixth group (G6), received Standard diet, with slight modification (Corn oil is replaced by Ag NPs 10ppm / prepared in oleic acid) + sodium nitrate ($NaNO_3$); 15 mg/kg. The seventh group (G7), received Standard diet, (Corn oil is replaced by mixture of (SO and AgNPs10 ppm / prepared in oleic acid; 1:1 respectively) + $NaNO_3$; 15 mg/kg.

2.5.2. Biochemical assays

Blood samples were placed in vacationer containers, and all samples were centrifuged at 4000 rpm for 10 minutes at 37 °C. The serum was then transferred and stored at -20 °C until examination.

2.5.2.1. Parameters of liver functions

The method of (Young and Friedman) was used to de termine Alanine aminotransfera(ALT) and also Aspartate aminotransferase (AST) using kits provide d by Spin react[23]. According to the report of the method of for DGKC and SCE method Enzymatic-Kinetic, Alkaline Phosphatase (ALP) was evaluating using Elitech kits. [24]. Gamma – GT was determined estimated using kits supplied from BioSystems [25]. Albumin was limited according to the method as claimed by Young [26]. Total protein set according to as maintained by colorimetric method. Globulins was calculated by "Total proteins (g/dl) – albumin (g/dl) = globulins (g/dl) according to Lv et al. [27]

2.5.2.2. Kidney Function parameters

Kinetic method of creatinine using spinreact kits as supposed by (Young and Friedman) [23]. Quantitative Enzymatic colorimetric determination of Blood Urea in serum using Stanbio laboratory kits as assumed by Tabacco et al. [28] .

2.5.2.3. Oxidative stress biomarker

Lipids in liver tissue According to Uchiyama and Mihara, measured calorimetrically a pink-colored TBA-complex which formed by interaction between malondialdehyde (MDA) with thiobarbituric acid (TBA) in an acidic media [29] , Liver mass glutathione (GSH) was appreciate in the view of Beutler et al [30].

2.5.3. Histopathological examination

Animals were dissected immediately to extract the liver, which was washed in a saline isotonic solution (0.9 percent NaCl) to remove any excess blood, sanitized, fixation in 10% formalin for 1 day, dried, clarified, and then embedded in paraffin wax. Hematoxylin and eosin were used to stain paraffin

blocks into four-micron thick sections for routine histological examination (H & E)[31].

2.6. Analytical Statistics

The data were presented as mean ± standard deviation (SD). To evaluate the research hypothesis, a variance analysis (ANOVA) was used, followed by a post-hoc least significant difference test (LSD). The Statistical Package for Social Sciences was used to analyse the data (SPSS). A statistically significant P value of less than 0.05 was used.

3. Results and discussion

The target of this current work was designed through the following steps, first one: preparation of two different concentrations of Ag NPs (10 and 50 ppm) by chemical reduction way using two reducing agents (TSC and AA) with different concentrations. Secondly, antibacterial evaluation of the obtained AgNPs; 10, 50 ppm, (SO) and their mixture against pseudomonas aeruginosa and Staphylococcus aureus. Finally, in *Vivo* assessment of one selected concentration of Ag NPs; 10 ppm, (SO) and its mixture, as hepatoprotective agent in comparison with the commonly hazards food preservative material; $NaNO_3$. The coming sections are presenting our findings together with the supporting argument.

3.1. Ag NPs Synthesis and Characterization

The creation of Ag NPs was indicated by the emergence of a yellow-brown colour solution in the reaction mixes due to a surface Plasmon resonance (SPR) of Ag^0. For the primary characterization of produced nanoparticles, UV-vis spectroscopy is a very useful and reliable approach. The optical characteristics of Ag NPs are unique. Colloidal silver absorbs light in the 400-420nm wavelength range, UV-Vis results demonstrated UV-Spectrum of Ag NPs 50 ppm with reducing agent, a) tri-sodium citrate "TSC," b) ascorbic acid "AA" in Fig. 2 a &b. UV-Spectrum of Ag NPs 10 ppm with reducing agent, a) "TSC", b) AA, Figure 1 and 2 exhibited an absorption band from 350 nm to 450 nm, which is typical for silver nanoparticles [32]. The prior findings were consistent with that of the Mohamed et al. Spectral examination of silver nanoparticles Ag NPs revealed absorbance at 400 nm, which is utilised as a monitor for the generation of Ag NPs. The production of spherical Ag NPs with sizes ranging from 6 to 36 nm was revealed by TEM examination[33]. When the concentration of the reducing agent is increased, no change in absorbance is observed in the produced samples. Brooding in the absorption peak was seen with altering reducing agent concentrations while employing a mild reducing agent such ascorbic acid to generate Ag NPs, as showed in Fig.2 (b). This result in agreement with Junwei et al. [34] who studied the preparation of Ag NPs by using chemical synthesis by sodium borohydride, TSC, and ascorbic acid, which is considered as mild reducing agent, and found the absorbance of freshly prepared Ag NPs at 388 nm.

The size of Ag NPs generated using (TSC and AA) as reducing agents was clearly detected in Figure 3 using a transmission electron microscope (TEM) (a, b). This result was consistent with previous research. This finding was in line with Amany et al. [35] who showed the particle size of Ag NPs, prepared using ascorbic acid, was in ranging from 55 nm to 68 nm.

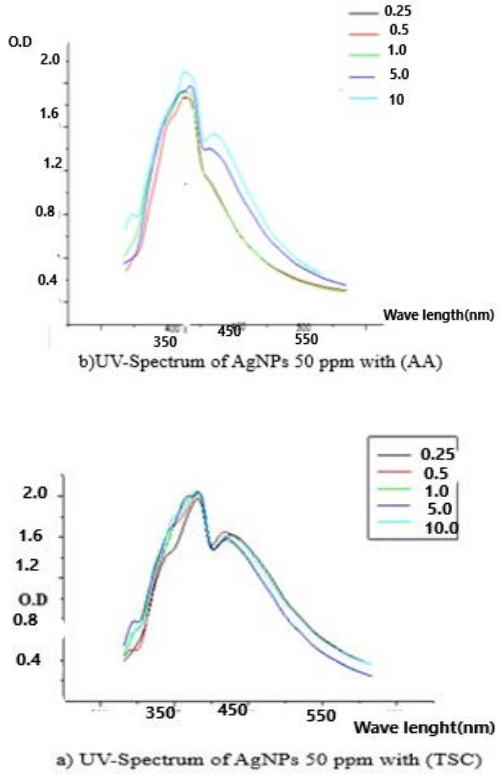
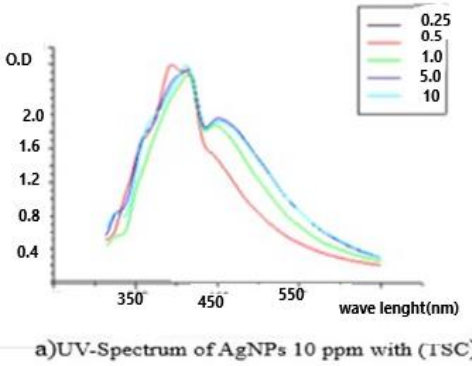
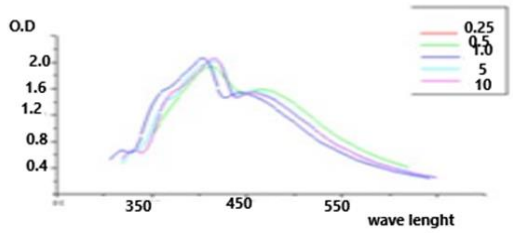


Figure 1: UV-Spectrum of Ag NPs 50 ppm with reducing agent, a) TSC, b) AA





b)UV-Spectrum of AgNPs 10 ppm with (AA)

Figure 2:UV-Spectrum of Ag NPs 10 ppm with reducing agent ,a) "TSC" , b)"AA"

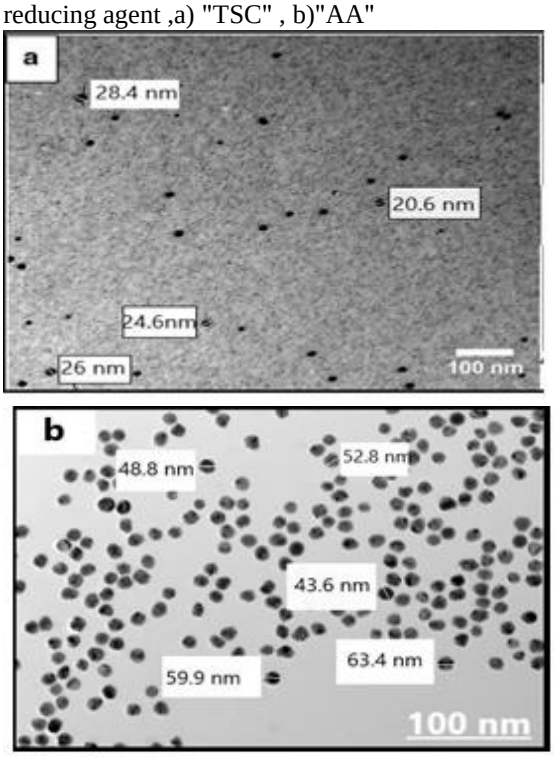


Figure 3: TEM of Ag NPs with reducing agent , a) TSC, b) AA

3.2. Antibacterial assay

The antimicrobial effect based on the results obtained by the minimum inhibitory concentration "MIC" of sesame oil and Ag NPs on *pseudomonas aeruginosa* as gram-negative and *Staphylococcus aureus* as positive Gram showed in Figure 4 which revealed that all mixtures had an antimicrobial effect. Because of their small size, Ag NPs increase the antibacterial impact of sesame oil by providing a larger surface area, which promotes reactivity and so increases their antibacterial power. In reality, Ag NPs interact with pathogenic bacteria in a size-dependent manner [36]. The present results are also in agreement with those reported by Li et al. [16] who investigated the effectiveness of dressing endotracheal tubes coated with Ag NPs versus non-coated endotracheal tubes in preventing ventilator-associated pneumonia in adults and discovered that dressing endotracheal tubes coated with Ag NPs is highly effective in preventing ventilator-associated pneumonia in adults. Beyond bulk silver, Ag NPs offer further impressive physicochemical and biological features. Ag NPs have been shown to be able to bind to the bacterial cell wall and hence enter it. This activity will produce physical changes in the bacterial membrane, such as membrane damage, which can result in the release of cellular contents and bacterial death [37].

AgNPs may interact with cellular structures and biomolecules such as proteins, lipids, and DNA when they penetrate into the microbial cell. Interaction with ribosomes, in particular, causes denaturation, which inhibits translation and protein production. Ag NPs may potentially impede intracellular biological processes by interacting effectively with the carboxyl and thiol groups of -galactosidase [38].

According to the findings of this investigation, the antibacterial impact of the mixture (Ag NPs and SO) on *Pseudomonas aeruginosa* was stronger than that on *Staphylococcus aureus*. This results in harmonious with Mandal et al. [39] who discovered that Ag NPs had a higher antibacterial effect on gram-negative bacteria than on Gram positive bacteria. The difference in cell wall thickness between positive Gram bacteria (30 nm) and Gram-negative bacteria (3–4 nm), which are primarily composed of peptidoglycan, can explain this result. Furthermore, due to the presence of carboxyl, phosphate, and amino groups, the cellular membrane of bacteria has been shown to have a negative charge. The positive charge attracts Ag NPs electrostatically and negatively charges the cell membrane of microorganisms, promoting Ag NPs attachment to cell membranes. As a result, changing the surface charge of Ag NPs to achieve a larger attractive force can boost antibacterial properties. The previous findings, as well as those of Mostafa et al. who researched the same item, show a similar pattern. The antibacterial activity of biosynthesized silver nanoparticles against *Staphylococcus aureus* and *Escherichia coli* was investigated, and Gram negative bacteria demonstrated a synergism between propolis and Ag NPs, as expected [40].

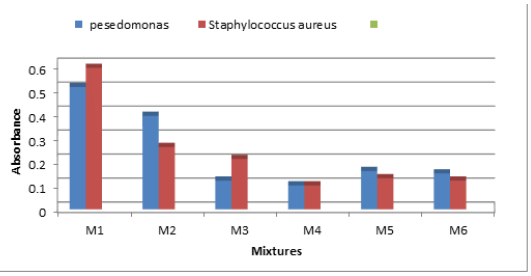


Figure 4 : MIC of different mixtures of Ag NPs and (SO) with different concentrations of (TSC) on *pesedomonasaeruginosa* and *Staphylococcus aureus*

Where, M1= Positive control (bacteria only), M2= M1+ SO, M3= (M1+ SO + Ag NPs 50ppm + TSC (10 %), M4= (M1+ SO +Ag

NPs 50ppm + TSC (5 %), M5= (M1+ SO + AgNPs 10 ppm + TSC (10 %), M6= (M1+ SO +Ag NPs (10 ppm) + TSC (5 %

3.3. In *Vivo* study

3.3.1. Effect of administration of sesame oil and Ag NPs with sodium nitrate on:

a) liver function

In this study, there was a rise in serum enzyme levels such as ALT, AST, ALP, and GGT. Albumin and total proteins (TP) were assessed and then Globulin (G) was calculated in the sodium nitrate group (G2) in comparison to the control group (G1). As shown in Fig.5 (a, b, c, d, e, f, and g). Treatment groups (G5, G6, and G7) revealed a highly decreasing significantly in ALT and ALK ($P < 0.0001$) and enzyme activities AST and GGT revealed a significant ($P < 0.001$). In the same figure, the results clarified that increasing levels of AST, ALT, GGT, and ALK in positive control, the leakage of these enzymes from the liver cytosol into the blood explains this rise. This could be related to hepatic necrosis, altered membrane permeability, and the generation of the free radical peroxynitrite (ONOO) from nitric oxide. Oxygen and NO radicals may react further to generate nitro compounds and other oxidants like peroxynitrite, which may cause liver injury and contribute to liver cell death. [37].

Figure 5(e and f) includes the decrease in total protein and albumin in the $NaNO_3$-treated group (G2); highly significant reduction ($P < 0.0001$) and ($P < 0.01$). This drop in total protein levels could lead to nitrate toxicity, which is caused by the oxidation of proteins and lipoproteins by nitric oxide or peroxynitrite. In another scenario, a decrease in protein content in response to nitrate exposure could lead to nitrite toxicity (the active metabolite). Previous research has supported the idea that the nitrite effect is mirrored in protein production [41]. AST, ALT, GGT, and ALK in sesame oil (SO) (G5), (10 ppm) Ag NPs (G6) group demonstrated a good protective impact by restoring biochemical profiles in the hepatic functioning. The effects of combining Ag NPs with sesame oil (G7) were reduced, and statistically significant differences ($p <0.05$) were restored in favour of the healthy group (G1). The preceding findings were consistent with earlier research achieved by Azab [42] who investigated serum administration of hepatic functional markers. This was also linked to the liver's proper histological structure, This is also in contrast with Uthandi and Ramasamy [43], who discovered that sesame meal treatment resulted in a significant drop in ALT, AST, and ALP activities, as well as a reduction in total proteins levels, in high fat-fed waster rats. This result was found to be consistent with Rouag et al. [45]. They tested this theory by observing the effects of nitrate causing hepatomegaly on rats and then treating them with Pumpkin Seed Oil. These findings are also consistent with the findings of Ogur et al. [46], who found that nitrate consumption promotes hepatomegaly.

The relative liver weight (RW) of rats treated with nitrate increased dramatically, as seen in Fig.5 (h). This rise could be related to the toxic effects of nitrate or its metabolites, which can cause liver damage and necrosis in hepatocytes, where; Relative Weight (RW) of liver Calculated by the following equation Aniagu et al. [44].

"RW = (organ weight / final body weight) × 100"

This result was correlated with Rouag et al. [45] They proved this notion by studying the effect of nitrate on rats and treating them with Pumpkin Seed Oil. These results also agree well with those of Ogur et al. [46] who demonstrated that nitrate consumption causes hepatomegaly.

G5,G6 and G7 which received sesame oil ,Ag NPs and mixing of Ag NPs with sesame oil showed decreasing in liver (RW)with statistically highly significant differences ($p<0.001$) restored towards negative control group, the previous results was harmony with data obtained by Hijazi et al. [47] who investigated the effect of sesame oil on the relative weight of the liver in rats fed "Monosodium glutamate (MSG)". The results reported that treatment oral intake of monosodium glutamate generated a significant rise in liver RW, while oral intake of (SO) induced a significant decrease in liver RW in the treatment groups. These findings could be attributed to the presence of antioxidants, which may act as radical scavengers by scavenging the radicals that contribute to oxidative stress.

b) Kidney function parameters

Serum blood urea nitrogen (BUN) and creatinine were assessed to see if (SO) and Ag NPs could protect against disparity medium-induced renal damage. Urea is a waste product that results from the breakdown of proteins. In most cases, urea is excreted in the urine. A high urea level ('uraemia') suggests that the kidneys aren't working properly. The term "creatinine" refers to a waste product produced by the muscles. Creatinine is a substance that enters the bloodstream and is excreted in the urine. A high creatinine level shows that the kidneys are not functioning properly. In the positive group, there was a highly significant increase in (BUN) and creatinine ($p <0.0001$), as shown in Fig.6 (a, b). While AgNPs (G6) fell significantly in the Sesame oil-treated groups (G5) compared to the positive control group (G2), renal function was recovered to almost that of the control group (G7). Some previous studies also indicated the same result with Piacenza et

al. [48] who discovered that in an "acute kidney injury (AKI)" animal model, sesame oil-treated rats provide renal protection by inhibiting renal oxidative stress. Sesame oil may also reduce oxidative stress by reducing the formation of the hydroxyl radical, which is one of the most dangerous free radicals. Renal superoxide anion production and inducible nitric oxide synthase (iNOS) expression were both lowered by sesame oil. This finding is consistent with prior research, which found that (SO) inhibits renal inducible nitric oxide synthase (iNOS) in a variety of oxidative stress conditions [49].

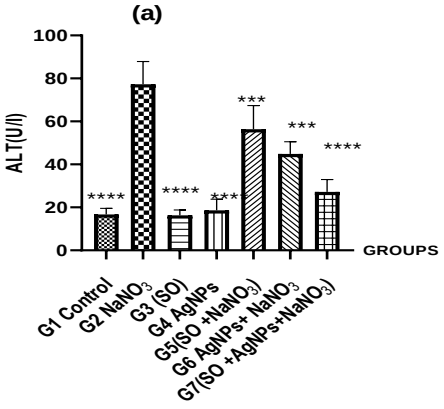
c) Oxidative stress biomarker

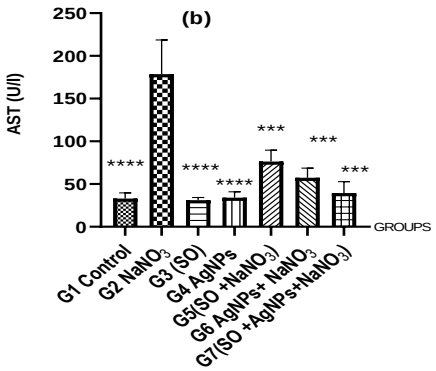
Glutathione (GSH) existing in mammalian tissues is the non-protein thiol. GSH is a powerful antioxidant found within cells. It protects cells from harm caused by lipid peroxides, reactive nitrogen, and oxygen species by acting as a cellular redox state regulator. In healthy cells, GSH serves a protective role in the detoxification of carcinogens. [50].

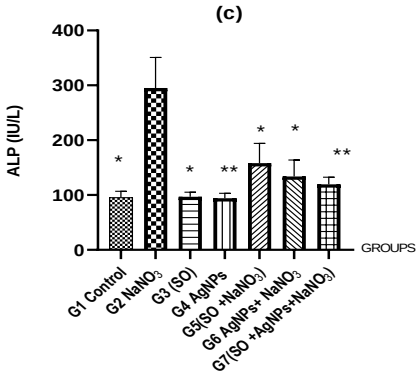
In the nitrate group (G2), the results of oxidative stress markers showed a considerable increase in MDA levels and a significant drop in GSH, as shown in Figure (7) a, b in parallel. Bouaziz-ketata et al. [51] showed that the management of $NaNO_3$ Malondialdehyde (MDA) levels in rat livers increased dramatically. The deadly action of $NaNO_3$, preventing its thiol function with direct conjugation of nitrate and/or its metabolite, or inhibiting glutamyl-cysteine-

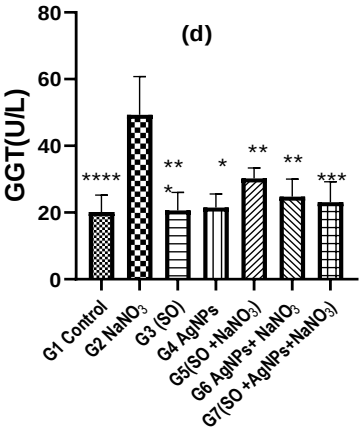
Synthesize activity. Furthermore, after rats were given sodium nitrate, the amount of hepatic GSH was shown to be lower. Furthermore, after rats were given sodium nitrate, the amount of hepatic GSH was shown to be decrease. [52].

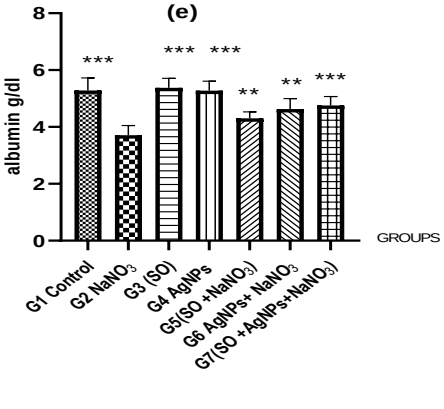
The results revealed a statistically significant difference between the sesame oil and Nano-silver treatment groups G5 and G6, where the sesame group improved first, followed by the Ag NPs group, and the best improvement in the mixture group (G7) was a decrease in MDA when compared to the positive control group.











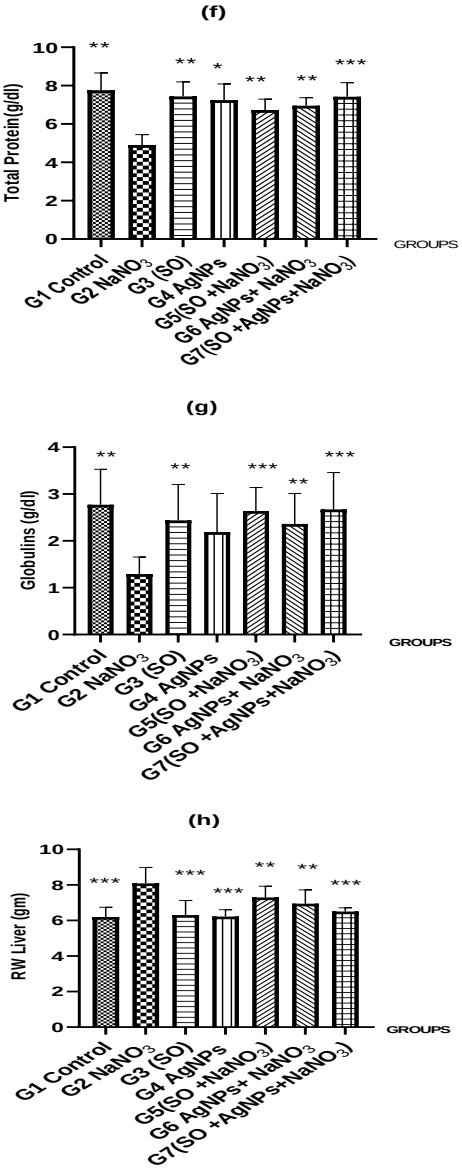
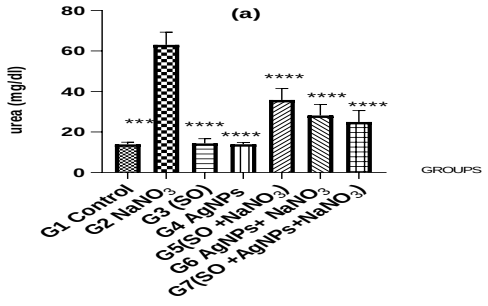


Figure 5: Effect sesame oil (SO), Ag NPs and its mixture on a) ALT, b) AST, c) ALP, d) GGT,e) albumin , f) total protein ,g) Globulin , h) relative weigh "RW"of liver Represents the mean value ± S.D. (n=10 rats / group), significantly different using One-way ANOVA.* mean (P < 0.05) ,** (P <0.01),***(p<0.001), ****(p<0.0001)



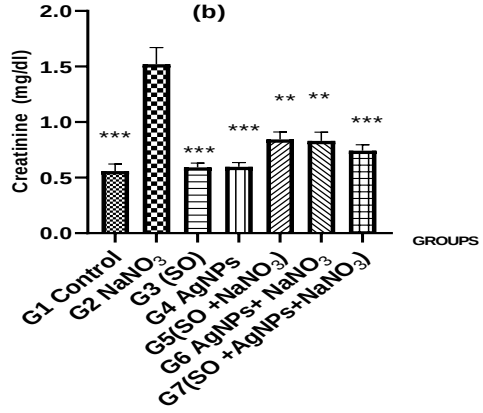


Figure 6: Effect sesame oil (SO) , Ag NPs and its mixture on a) urea, b) creatinin Represents the mean value ± S.D. (n=10 rats / group), significantly different using One-way ANOVA.* mean (P < 0.05) ,** (P <0.01),***(p<0. 001), ****(p<0.0001**)**

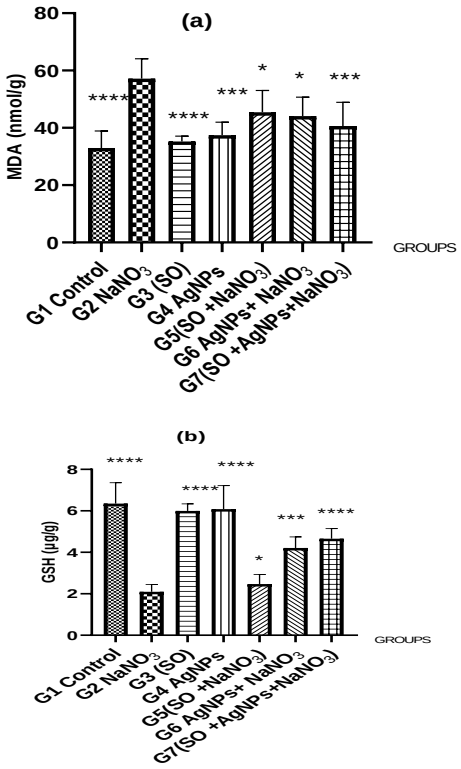


Figure 7: Effect sesame oil (SO), AgNPs and its mixture on a) MDA, b) GSH Represents the mean value ± S.E. (n=10 rats / group), significantly different using One-way ANOVA.* mean (P < 0.05) ,** (P <0.01),***(p<0. 001), ****(p<0.0001)

3.3.4. Histopathological profiles

The histopathological examination of liver showed severe congestion and thrombosis of central and portal veins with periportal fibrosis and severe congestion and thrombosis of central vein and

presence of apoptotic hepatocyte with pyknotic nucleus in Figure 8(b), which represent $NaNO_3$ group This results explained by Manal Said et al. [53] The liver contains a lot of polyunsaturated fatty acids, which are vulnerable to free radical damage due to oxidative stress. and also explained by Rouag et al. [54] who supposed that this damage in tissue due to the formation of free radicals, protein carboxylation and lipids peroxidation that caused plasma membrane destruction. Therefore, the histopathological study of liver tissues confirms the changes of the previously studied parameters in nitrate group. G1, G2 and G4 in Figure 8 (a, c, and d) showed normal structure of hepatocytes and hepatic blood vessels (HE, x100). Figure 8 (e) clarified that scattered solitary macrovesicular steatosis in the hepatocytes (HE, x100) which represent treatment with (SO) which consider relative amelioration this results explained by Hijazi et al. [55] who demonstrated that Monounsaturated oleic acid and polyunsaturated linoleic acid are the principal fatty acids found in sesame oil. Sesame oil also contains a large amount of polyunsaturated fatty acids (PUFAS), which are important targets for free radical attack. Free radicals can target PUFAS and cause it to oxidise into lipid peroxides., as showed in this study AgNPs improved sesame oil activities, G6 in the same Fig. (f). Microphotograph of rat liver clarified that mild congestion of central and portal veins (HE, x100), Figure 8(g) represent (G7)(SO + Ag- revealed normal structure of hepatocytes and hepatic vessels (HE, x100), [47].

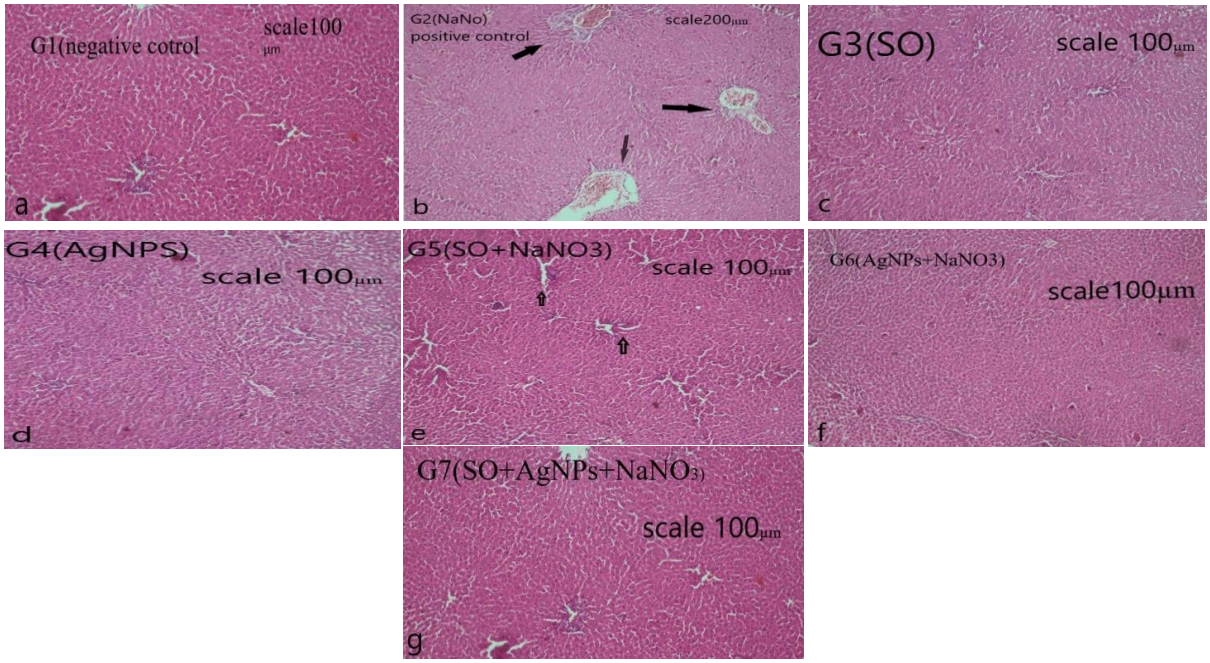


Figure 8: (a, b, c, d, e, f and g). Histological liver sections of groups, where a) negative control (G1) showing normal structure (HE, x100), b) positive control (G2) severe congestion and thrombosis of central and portal veins with periportal fibrosis (HE, x100), c) (G3) receive sesame oil only showing normal structure (HE, x100), d) (G4) receive sesame oil only showing normal structure (HE, x100), e) represent ($SO+NaNO_3$) (G5) showing scattered solitary macrovesicular steatosis in the hepatocytes (HE, x100), f) ($AgNPs+ NaNO_3$)(G6) Microphotograph of rat liver clarified that mild congestion of central and portal veins (HE, x100),g) (Mixture of SO +Ag $NPs+NaNO_3$)(G7) showing normal structure of hepatocytes and hepatic vessels (HE, x100).

Abbreviations

AgNPs	silver nanoparticles	**AA**	Ascorbic acid
TSC	tri-sodium citrate	**TEM**	Transmission electron microscopy
SO	Sesame Oil	**AKI**	acute kidney injury
H&E	Hematoxylin and Eosin	**(iNOS)**	inducible nitric oxide synthase
SPR	surface plasmon resonance	**PUFAS**	polyunsaturated fatty acids

ALT	Alanine aminotransferase	**AST**	Aspartate aminotransferase
ALK	Alkaline Phosphatase	**GGT**	Gamma-glutamyl Transferase
BUN	Blood urea nitrogen	**RW**	Relative weight
MTP	micro titer plates	**MIC**	minimum inhibitory concentration

4. Conclusion

In this current study the chemical reduction method was used perfectly in the production of silver nanoparticles (Ag NPs). Tri-sodium citrate (TSC) has been successfully used as a reducing agent. The obtained Ag NPs, were used as anti-bacterial agent against *Pseudomonas aeruginosa* and *Escherichia coli*. Also, mixing of AgNPs 10ppm with sesame oil (SO) as polyunsaturated oil increased the antimicrobial activity especially against *Pseudomonas aeruginosa*. Furthermore, the impact of this mixture against sodium nitrate-induced hepatotoxicity in albino rats has been satisfactorily assessed using histological and biochemical characteristics. To do this, seventy healthy adult male albino rats were divided into seven groups. G1 fed on the stranded diet as (negative control), G2 received $NaNO_3$ orally as (positive control) to induce hepatotoxicity, G3 received SO only, G4 received Ag NPs 10ppm only, the treated groups (G5, G6, G7) fed on standard diet with slight modification "Corn oil was replaced by SO, Ag NPs10ppm and mixture of (SO with Ag NPs10ppm (1:1) respectively + $NaNO_3$ in a dose of 15 mg/kg for six weeks. Results showed improved kidney functions as well as positive effect on oxidative stress biomarkers which were restored towards a healthy group. The possibility of using Ag NPs and its combination with (SO) in food industry application instead of using $NaNO_3$ that are commonly used daily as food preservative, irrespective, its harmful effects, opened the way for a new source of an alternative, safe, and cost effective nano-medicine. Moreover, the present study elucidated the mechanism through which, mixture of Ag NPs with (SO) could contribute as therapeutic agents in addition to their anti-bacterial action.

Conflict of interest

The authors declare that they have no conflict of interest regarding the publication of this paper

References

1 A. N. Badr, M. Youssef, A. G. Abdel-Razek, M. G. Shehata, M. M. Hassanien, and H. Amra, Egyptian Journal of Chemistry **64** (1), 285 (2021).

2 A. Z. Mahmoud and M. N. Habza, Cytotechnology **67**, 437 (2015).

3 P. Song, L. Wu, and W. Guan, Nutrients **7** (12), 9872 (2015).

4 R. A. Bapat, T. V. Chaubal, C. P. Joshi, P. R. Bapat, H. Choudhury, M. Pandey, B. Gorain, and P. Kesharwani, Materials Science and Engineering: C **91**, 881 (2018).

5 O. H. Abdelhafez, M. A. Fawzy, J. R. Fahim, S. Y. Desoukey, M. Krischke, M. J. Mueller, and U. R. Abdelmohsen, Plos one **13** (8), e0202362 (2018).

6 T. I. Shaheen, M. E. El-Naggar, J. S. Hussein, M. El-Bana, E. Emara, Z. El-Khayat, M. M. Fouda, H. Ebaid, A. J. B. Hebeish, and Pharmacotherapy, **83**, 865 (2016).

7 E. A. Marzook, A. E. Abd El Moneim, and A. A. Elhadary, Journal of Radiation Research and Applied Sciences **7** (1), 1 (2014).

8 J. Hussein, M. E. El-Naggar, M. M. Fouda, S. I. Othman, A. A. Allam, E. H. Nadwa, E. K. Rashwan, and O. J. J. o. C. S. Hendawy, **32** (2), 423 (2021).

9 P. Jayaraj, C. A. Narasimhulu, S. Rajagopalan, S. Parthasarathy, and R. Desikan, Food & function **11** (2), 1198 (2020).

10 Y. Wan, H. Li, G. Fu, X. Chen, F. Chen, and M. Xie, Journal of the Science of Food and Agriculture **95** (13), 2571 (2015).

11 A. Sedik, M. M. Mosaad, H. Othman, and A. Mohamed, Egyptian Journal of Chemistry (2021).

12 M. M. Fouda, J. S. Ajarem, S. N. Maodaa, A. A. Allam, M. M. Taher, and M. J. S. M. Ahmed, **269**, 116553 (2020); M. E. El-Naggar, T. I. Shaheen, M. M. Fouda, and A. A. J. C. p. Hebeish, **136**, 1128 (2016).

13 A. Abou-Okeil, H. Fahmy, M. M. Fouda, A. Aly, and H. J. B. Ibrahim, **11** (3), 687 (2021); X.-F. Zhang, Z.-G. Liu, W. Shen, and S. Gurunathan, International journal of molecular sciences **17** (9), 1534 (2016).

14 Y. Weng, J. Li, X. Ding, B. Wang, S. Dai, Y. Zhou, R. Pang, Y. Zhao, H. Xu, B. Tian, and Y. Hua, Int J Nanomedicine **15**, 1823 (2020).

15 C. A. Dos Santos, M. M. Seckler, A. P. Ingle, I. Gupta, S. Galdiero, M. Galdiero, A. Gade, and M. Rai, Journal of pharmaceutical sciences **103** (7), 1931 (2014).
16 J. Liu and Y. J. N. p. Lu, **1** (1), 246 (2006).
17 M. Elnaggar, H. Emam, M. Fathalla, M. Abdel-Aziz, and M. Zahran, Egyptian Journal of Chemistry **64** (5), 2697 (2021).
18 A. S. Al-Sherbini, M. M. Khalil, H. H. El-Sayed, and A. F. Soliman, Journal of Food Processing and Preservation **42** (1), e13358 (2018).
19 P. Krishnamoorthy and T. Jayalakshmi, J. Chem. Pharm. Res **4** (11), 4783 (2012).
20 S. E. Desouky, M. S. El-Gamal, A. F. Mohammed, and M. A. Abu-Elghait, World Appl Sci J **32**, 731 (2014).
21 P. G. Reeves, F. H. Nielsen, and G. C. Fahey Jr, (Oxford University Press, 1993).
22 I. U. Yarube, M. Okasha, J. O. Ayo, and K. V. Olorunshola, European Journal of Scientific Research **25** (1), 35 (2009).
23 D. S. a. F. Young, R.B. , (2001).
24 R. Keiding, M. Hörder, W. G. Denmark, E. Pitkänen, R. Tenhunen, J. Strömme, L. Theodorsen, J. Waldenström, N. Tryding, and L. Westlund, Scandinavian journal of clinical and laboratory investigation **33** (4), 291 (1974).
25 C. A. Burtis, E. R. Ashwood, and D. E. Bruns, *Tietz textbook of clinical chemistry and molecular diagnostics-e-book*. (Elsevier Health Sciences, 2012).
26 D. S. Young, *Effects of drugs on clinical laboratory tests*. (AACC press, 2000).
27 G.-y. Lv, L. An, X.-d. Sun, Y.-l. Hu, and D.-w. Sun, Clinica chimica acta **476**, 81 (2018).
28 A. Tabacco, F. Meiattini, E. Moda, and P. Tarli, Clinical chemistry **25** (2), 336 (1979).
29 M. Uchiyama and M. Mihara, Analytical biochemistry **86** (1), 271 (1978).
30 E. Beutler, O. Duron, and B. Kelly, J Lab Clin Med **61**, 882 (1963).
31 J. Banchroft, A. Stevens, and D. Turner, (Churchil Livingstone, New York, London, San Francisco, Tokyo, 1996).
32 A. Šileikaitė, I. Prosyčevas, J. Puišo, A. Juraitis, and A. Guobienė, Mater. Sci **12** (4), 1392 (2006).
33 A. A Mohamed, A. Fouda, M. S Elgamal, S. EL-Din Hassan, T. I Shaheen, and S. S Salem, Egyptian journal of chemistry **60** (Conference Issue (The 8th International Conference of The Textile Research Division (ICTRD 2017), National Research Centre, Cairo 12622, Egypt.)), 63 (2017).
34 J. Zheng, X. Li, R. Gu, and T. Lu, The Journal of Physical Chemistry B **106** (5), 1019 (2002).
35 A. Amany, S. F. G. El-Rab, and F. Gad, Der Pharma Chemica **4** (1), 53 (2012).
36 N. H. Rao, N. Lakshmidevi, S. Pammi, P. Kollu, S. Ganapaty, and P. Lakshmi, Materials science and engineering: C **62**, 553 (2016).
37 M. Seong and D. G. Lee, Current microbiology **74** (6), 661 (2017).
38 C. You, C. Han, X. Wang, Y. Zheng, Q. Li, X. Hu, and H. Sun, Molecular biology reports **39** (9), 9193 (2012).
39 D. Mandal, S. K. Dash, B. Das, S. Chattopadhyay, T. Ghosh, D. Das, and S. Roy, Biomedicine & Pharmacotherapy **83**, 548 (2016).
40 M. M. Khalil, S. O. Mohamed, and K. El-Naggar, Egyptian Journal of Chemistry (2021).
41 E. G. Helal, A. Almutairi, M. A. Abdelaziz, and A. A. Mohamed, The Egyptian Journal of Hospital Medicine **80** (3), 964 (2020).
42 E. J. A. J. B. Azab, **2** (6-2), 1 (2014).
43 A. Uthandi and K. Ramasamy, International journal of Science and Research **2** (12), 205 (2011).
44 S. O. Aniagu, F. C. Nwinyi, D. D. Akumka, G. A. Ajoku, S. Dzarma, K. S. Izebe, M. Ditse, P. E. Nwaneri, C. Wambebe, and K. Gamaniel, African Journal of Biotechnology **4** (1), 72 (2005).
45 M. Rouag, S. Berrouague, N. Djaber, T. Khaldi, M. Boumendjel, F. Taibi, C. Abdennour, A. Boumendjel, and M. Messarah, African Health Sciences **20** (1), 413 (2020).
46 R. Ogur, O. Coskun, A. Korkmaz, S. Oter, H. Yaren, and M. Hasde, Environmental toxicology and Pharmacology **20** (1), 161 (2005).
47 M. A. Hijazi, A. A. Alrasheedi, and L. M. Saeed, Middle East J Appl Sci **6** (4), 824 (2016).
48 F. Piacenza, M. Malavolta, C. Cipriano, L. Costarelli, R. Giacconi, E. Muti, S. Tesei, S. Pierpaoli, A. Basso, and M. Bracci, Toxicology letters **189** (3), 200 (2009).
49 D.-Z. Hsu, C.-T. Liu, Y.-H. Li, P.-Y. Chu, and M.-Y. Liu, Shock **33** (1), 88 (2010).
50 L. Kennedy, J. K. Sandhu, M.-E. Harper, and M. Cuperlovic-Culf, Biomolecules **10** (10), 1429 (2020).
51 H. Bouaziz-Ketata, G. B. Salah, H. B. Salah, R. Marrekchi, K. Jamoussi, T. Boudawara, F. Fakhfekh, and N. Zeghal, Biomedical and Environmental Sciences **27** (9), 695 (2014).
52 T. Manal Said and A.-B. Nawal, Food and Nutrition Sciences **2012** (2012).
53 T. Manal Said, A.-B. J. F. Nawal, and N. Sciences, **2012** (2012).
54 M. Rouag, S. Berrouague, N. Djaber, T. Khaldi, M. Boumendjel, F. Taibi, C. Abdennour, A. Boumendjel, and M. J. A. H. S. Messarah, **20** (1), 413 (2020).
55 M. A. Hijazi, A. A. Alrasheedi, and L. M. J. M. E. J. A. S. Saeed, **6** (4), 824 (2016).