



## HEPATOPROTECTIVE EFFECT AND ANTIOXIDANT ACTIVITY OF SILVER NANOPARTICLES BIOSYNTHESIS FROM CYMBOPOGAN CITRATUS (LEMONGRASS)

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**Abstract:** Green synthesis of silver nanoparticles (AgNPs) is non-toxic, rapid and eco-friendly than commonly used physicochemical methods. This study focuses on synthesis, characterization, hepatoprotective effect and antioxidant activity of AgNPs synthesized from *Cymbopogon Citratus* (Lemongrass). Silver nanoparticles were formed within 30 minutes by heating to 80°C magnetic stirrer using aqueous solution of AgNO<sub>3</sub> (0.1 N) with fresh leaves methanolic extract of *Cymbopogon Citratus*. The synthesized silver nanoparticles were characterized by using UV-visible spectrophotometer analysis and transmission electron microscope (TEM). The antioxidant activity of nanoparticles were determined *in vitro* by reducing power, total antioxidant capacity and free radical scavenging activity by (DPPH). and experimental study was designed to evaluate the antioxidant activity and hepatoprotective effect of silver nanoparticles, methanolic extract and essential oil comparing with vitamin E.

**Results:** (UV-Vis) spectrum of synthesized AgNPs shows a peak at 412 nm and the size of silver nanoparticles was 13.5 nm by (TEM).

Silver nanoparticles showed potent hepatoprotective effect and antioxidant activity in both *in vitro* and *in vivo* methods.

**Key words:** Silver nanoparticles (AgNPs), green synthesis, lemon grass, antioxidant activity, DPPH (2, 2-diphenyl -1- picrylhydrazyl)

### INTRODUCTION

Nano, “dwarf” in Greek, is known as 10<sup>-9</sup> m or one billionth. To explain this in a simple way; the average diameter of human hair strand is about 75,000 nm. Also, the length of ten linked H atoms is roughly 1 nm (Su and Huang, 2007) Nanotechnology is a technology that forms and utilizes particles and materials which own specific characteristics due to their small size (Power, 2011). The description of nanotechnology refers to the study and application of small things in the range of 1-100 nm. These

nanoparticles or nanomaterials can be used in different disciplines such as materials science, engineering, biology, physics and chemistry, The description of nanotechnology refers to the study and application of small things in the range of 1-100 nm (Ananda *et al.*, 2015). The green synthesis of AgNPs attracted more attention than before due to the novel advantages of this synthesis such as nonuse of hazardous chemicals, low cost, simplicity, eco-friendliness and Safety (Raja *et al.*, 2017). Plants and their parts contain carbohydrates, fats, proteins, nucleic acids, pigments and

several types of secondary metabolites which act as reducing agents to produce nanoparticles from metal salts without producing any toxic by product. Similarly, biomolecules such as enzymes, proteins and bio-surfactants present in microorganisms serve as reducing agents. For instance, in many bacterial strains, bio-surfactants are used as capping and/or stabilizing agents (Siddiqi and Husen., 2017). The genus *Cymbopogon* is of great interest due to its commercially valuable essential oils and also in native medicines, The essential oils from *Cymbopogon* have been reported to be cytotoxic to human cancer cells, possess antitumor activity in mice, hepacoprotective and has been shown to have antifungal and antimicrobial properties (Chao *et al.*, 2000) Lemongrass is an herb that has gained interest as a nutritional supplement and is widely used in human foods in tropical countries. The main constituent of lemongrass extract is citral which is essential for vitamin A synthesis. Lemongrass herb has been reported to have antibacterial, antioxidant and anti-hyper ammonia-producing ruminal bacterial activities (Wanapat *et al.*, 2008). Lemon grass extracts have protective effects against oxidative stress induced cytotoxicity, we hypothesized that lemongrass extracts would also decrease the liver damage in rats. In general, serum enzymes such as AST, ALT, ALP, and GGT are released into plasma when the liver is damaged, and lemon extracts can help for reduce levels of those parameters (Verma, *et al.*, 2015) Lemongrass leaf extract can also be used for the synthesis of nanoparticles of silver and gold and tested for bactericidal effects against pathogenic bacteria. (Shankar *et al.*, 2004). The synthesis of silver nanoparticles by reduction of aqueous metal ions during exposure of *Cymbopogon citratus* leaves extract can

be easily monitored by using UV-visible spectrophotometry. the absorbance spectra of reaction mixture containing aqueous solution of 1 mM silver nitrate and extract of *Cymbopogon citratus* leaves after microwave irradiation. Reaction mixture showed an absorbance peak around 430 nm, which is characteristic of silver nanoparticles, due to its surface plasmon resonance absorption band (Shalaka *et al.*, 2011).

## MATERIALS AND METHODS

### Materials

The mature male albino rats (150 ± 10gm) were obtained from Research Institute of "Laboratory Animals Research Center", Faculty of Veterinary Medicine, Benha University, Qalubia, Egypt. Animals were placed for 15 days as an adaptation period. Water and food were always available throughout the experiment.

Kits for enzymes activity (SOD, CAT, ALT, AST, ALP) and total protein, albumin, GSH and MDA purchased from the Biodiagnostic Company, Cairo, Egypt.

### Plant collection and identification

Lemon grass plant was collected from Agriculture Research Center in El-qnater El-khairia, Kaliobia, Egypt in September 2016. plant was Identified in Horticulture department, Faculty of Agriculture, Minufiya University.

### Methanolic extract

500 grams of plant sample powders were steeped in 5000 ml of methanol 80% and the mixture was then kept in shaker incubator for 24 hrs at room temperature then filtered through filter paper and centrifuged at 3000 rpm. The filtrate was placed in Rotary Vacuum Evaporator to evaporate alcohol from it. We used to obtain a dried powder, as described by (Mukhtar and Ghori, 2012).

### **Synthesis of silver nanoparticles (AgNPs) from methanolic extract of lemon grass**

Take 1.0 gram from dry methanolic extract prepared in the preceding step, in conical flask and dissolved in 100 ml distilled water, adjust pH at 12 using 0.01N sodium hydroxide then keep all this system under magnetic stirring, when the temperature reaches 80°C add 1ml 0.1N silver nitrate and keep under magnetic stirring for 30 min (EL-Bisi *et al.*, 2013).

### **Nanoparticle composition**

The successive formation of AgNPs was indicated by the appearance of brown color, this is because of excitation of surface Plasmon vibrations in nano-silver) It was a quick interaction as demonstrated by the immediate color change on blending the solution of silver nitrate and methanolic extract of lemon grass. This color change demonstrates performing of redox reaction, whereby ions of Ag<sup>+</sup> are reduced to Ag<sup>0</sup> by the extract components, which are oxidized to different species (EL-Bisi *et al.*, 2013)

### **Characterization technique of silver nanoparticles synthesis from lemon grass methanolic extract.**

#### **Ultraviolet-visible (UV-vis) spectra**

UV-vis spectra have been proved to be quite sensitive to the formation of silver colloids because AgNPs exhibit an intense absorption peak due to the surface Plasmon excitation which describes the collective excitation of conductive electrons in a metal.

#### **Transmission Electron Microscopy (TEM)**

Shape and size of AgNPs were practically obtained using TEM; JEOL-JEM-1200. Specimens for TEM

measurements were prepared by placing a drop of colloidal solution on 400 mesh copper grid coated by an amorphous carbon film and evaporating the solvent in air at room temperature. The average diameter of the prepared AgNPs was determined from the diameter of 100 nanoparticles found in several arbitrarily chosen areas in enlarged microphotographs.

### **Determination of free phenolic compounds, total flavonoids, and antioxidant parameters in vitro**

The concentration of free phenolic compounds in extract was determined colorimetrically by the method of Folin-Ciocalteu's as described by (Gulcin *et al.*, 2002), while the total flavonoids contents were determined using the method reported by (Dewanto *et al.*, 2002), DPPH (2, 2-diphenyl -1- picrylhydrazyl) free radical was measured to methanolic extract according to (Lee *et al.*, 1996) and total antioxidant capacity was determined according to (Prieto *et al.*, 1999).

### **Experimental animals**

- The mature albino rats were obtained from the "Laboratory Animals Research Center", Faculty of Veterinary Medicine, Benha University in Mushtaher - Tukh - Qalubia Egypt.
- The present studies were performed on 36 male albino rats having an average weight of 150 ±10 gm (1.5 – 2.0 months old). Animals were allowed to acclimatize to laboratory conditions for a minimum period of 2 weeks prior to the experiment. animals were kept on a balanced diet throughout the experimental period.

### **Experimental design.**

Thirty six rats were divided into six groups: group (A) control negative without any treatment, groups (B, C, D, E

and F) were treated with H<sub>2</sub>O<sub>2</sub> 0.5% in drinking water through the experiment period. Group (B) saved as positive control without any supplementation, and the other four groups of each experiment were allowed to treated with lemon grass methanolic extract (400 mg/kg b.wt.) as group (C), lemon grass oil (300 mg/kg b.wt.) as group (D) silvernano particles green synthesis from lemon grass (0.5 ml/kg b.wt.) as group (E) and standard vitamin E (10 mg/kg b.wt.) as group (F).

### **Blood Samples**

After 60 days of treatment period, the animals were deprived of food overnight and anesthetized and then sacrificed by cervical decapitation. Blood samples were collected from orbital sinus veins technique using heparinized capillary tubes at the end of experimental period, into clean, dry, and labeled eppendorf tubes (1.5 ml). The tubes contained heparin as anticoagulant.

Samples were centrifuged at 3600 rpm for 15 min in a refrigerated centrifuge to separate plasma. Plasma samples were kept in a deep freeze at (-20 °C), till the different assays were carried out.

### **Measurement of biochemical parameters.**

Alkaline phosphatase (ALP) was determined in serum according to Moss *et al.*, (1987), while serum aspartate transaminases (AST) and alanine aminotransferase (ALT) were measured according to Young, (1990), also total protein was determined in plasma as described by Schultze and Heremans, (1966), and albumin was measured in plasma as described by Cannon *et al.*, (1974). The lipid peroxidation end product, MDA was measured according to Ohkawa *et al.*, (1979) Also, Catalase (CAT) activity was determined as described by Aebi, (1984), Superoxide dismutase (SOD) activity was measured

using the method of Nishikimi *et al.*, (1972) and Kinetic determination of glutathione reductase (GSH) activity was done according to the method of Goldberg and Spooner, ( 1983 ).

### **Statistical analysis.**

Statistical analysis was done using analysis of variance (ANOVA), Least Significant Difference (LSD) were obtained to compare the means of treatments, using Costat version 6.311 (Copyright 1998-2005, CoHort software. Duncan's multiple range test (Duncan, 1955) was used to compare between the treatments means. The mean values within each column followed by same letters are not significantly different at 0.05 %.

## **RESULTS AND DISCUSSIONS**

Characterization technique of silver nanoparticles synthesis from lemon grass methanolic extract.

### **UV-Visible spectrum of the prepared (Ag NPs) extracts**

Fig. (1) Shows the characteristic absorption peak of AgNPs in the UV-Vis spectra which were located between 400 – 450 nm (ca.415 nm). Our result was in the same line with (Shalaka *et al.*, 2011 and Bandita *et al.*, 2019) they mentioned that lemon grass methanolic extract acts as both the capping and the reducing agent in green synthesis approach and UV-Vis spectra ranged from 400– 450.

### **Transmission electron microscopy (TEM) of (AgNps) extracts**

Data in Fig. (2), showed that the Transmission electron microscopy (TEM) was utilized to elucidate shape and size of the prepared silver nanoparticles of lemon grass investigation was made by

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using JEOL JEMIOxIO Electron microscope-Japan. It was noted That the prepared Silver nanoparticles have a spherical shape and well dispersed in the polymer matrix with average particle size of 13.5 nm.

Our results is in the same line with many author such as ( Shalaka *et al.*, (2011) and Ashish and Deepak , 2015 )

they mentioned that Silver nanoparticles were synthesized by the bio-reduction of silver nitrate solution (1 mM) using methanolic extract of lemongrass leaves,synthesized silver nanoparticles was analyzed by Dynamic Light Scattering (DLS) technique which revealed their average (nm) size 40-100 nm

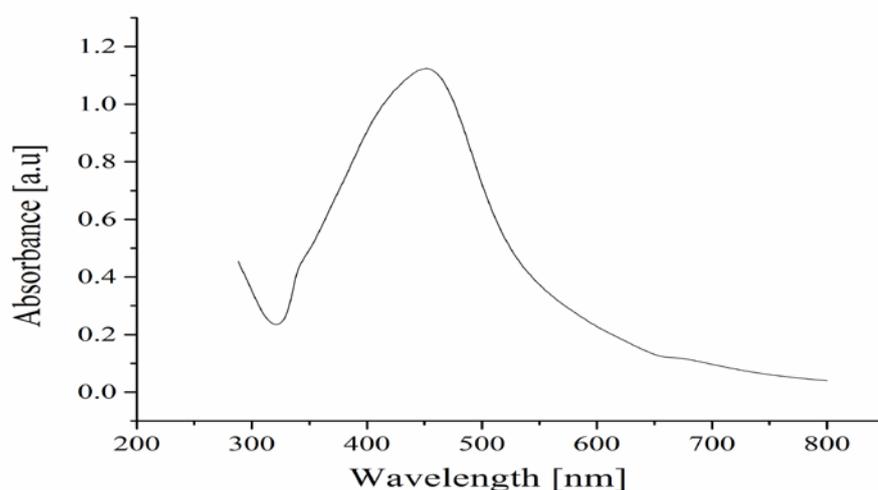


Fig (1): UV-Visible spectrum of the prepared Ag NPs of Lemon grass methanolic extract.

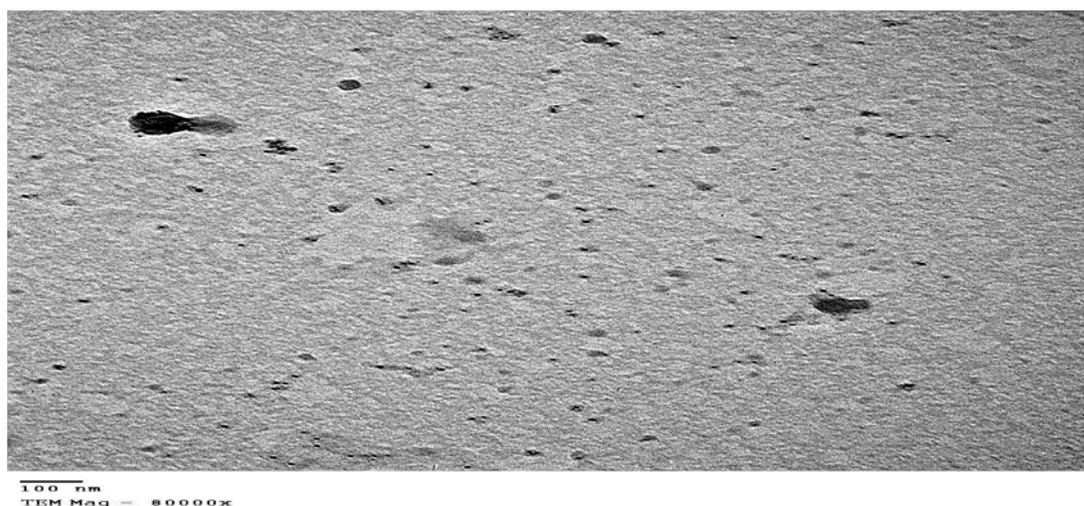


Fig. (2): TEM image of the prepared Ag nano particles from lemon grass Methanolic extract

### The Antioxidant biomarker in *vitro* of silvernanoparticles, methanolic extract and oil.

Total phenolic, total flavonoids, DPPH activity, reducing power and total antioxidant capacity

The obtained results as shown in Table (1) clarified that silvernano particeles from lemon grass methanolic extract contain high amount of total phenolics (309 mg/100g) but methanolic extract record high amount of total flavonoids ( 16.6 mg/100g), and high percent of DPPH activity (86.1%), in the same time silvernano particeles from lemon grass methanolic extract recorded high level of reducing power (512 Mol As. Equ100mg/ml ) and high level of total antioxidant capacity (403  $\mu$ mol ascorbic equ100mg/ml) .The above results are in agreements with those obtained by many author such as (Mittal *et al.*, 2012 ). They mentioned that the synthesized nanoparticles have antioxidant activity due to capped phenolic compounds and can be used against deleterious effects of free radicals.

### Effect of lemon grass extracts, oil and vitamin E on liver functions in rates plasma

The obtained results as shown in Table (2) and Revealed that hydrogen peroxide caused significant increasing in the liver enzymes levels (AST, ALT, ALP compared with control group; while non-enzymatic liver functions (total protein and albumin) significantly decreased in

hydrogen peroxide group compared with control group.

In contrast, administration of silvernano particeles from lemon grass methanolic extract, lemon grass oil and methanolic extract significantly decreased liver enzymes activities (AST, ALT, ALP); on the other hand prevented the decreasing in non enzymatic marker levels (total protein and albumin) compared with hydrogen peroxide group.

Our results were in the same (Lai *et al.*, 2016 and Chien *et al.*, 2018) they investigate the hepatoprotective effect of methanolic extracts of *Cymbopogon citratus* has hepatoprotective effect properties against oxidative stress in rats which might be ascribed to its antioxidant and free radical scavenging property and can reduce the elevation of liver functions such as Albumin, T. Protein and (ALP) also (Nakamura *et al.*, 2003) reported that treatment the effects of hepatotoxicity of the liver cells by the lemon grass extract observed a reduction in the rate of ALT compared to the positive control group. lemon extracts contain many active component which can protect liver against Oxidative stress and used as Liver support to improve liver functions such as (AST, GGT and ALP) (Verma, *et al.*, 2015). *C. citratus* has a potent protective effect against H<sub>2</sub>O<sub>2</sub> -induced liver injury. *C. citratus* treatment significantly reduced the increase in liver enzyme activities and attenuated oxidative stress-induced pathological changes Rahim *et al.*, (2014)

Table (1): Total phenolic, total flavonoids, DPPH activity, reducing power and total antioxidant capacity

	Total phenolics (mg/100g)	Total flavonoids (mg /100g)	%Inhibition forDPPH 100mg/ml	Reducing power assay (Mol As. Equ100mg/ml)	Total antioxidant capacity.µmol ascorbic equ100mg/ml
LME	144	16.6	86.1	149	383
LO	339	6.61	71.7	318	412
LMESNP	309	8.4	72.67	512	403

(LME) lemon grass methanolic extract, (LAE) lemon aqueous extract (LO) lemon grass oil, (LMESNP) lemon grass methanolic extract silver nanoparticles.

Table (2): Effect of lemon grass extracts, oil and vitamin E on liver functions in rates plasma

	AST	ALT	ALP	T.protein	Albumin
Group A	14 ±1.17 <sup>a</sup>	13 ± 1.17 <sup>a</sup>	54 ± 3.27 <sup>a</sup>	7.91 ±.23 <sup>a</sup>	4.02 ±.17 <sup>a</sup>
Group B	169 ±3.38 <sup>f</sup>	159 ±2.84 <sup>e</sup>	137± 4.37 <sup>e</sup>	6.57 ±0.20 <sup>b</sup>	2.92 ±.26 <sup>c</sup>
Group C	90±2.60 <sup>d</sup>	91 ± 4.89 <sup>c</sup>	109± 3.12 <sup>c</sup>	6.80 ±0.35 <sup>b</sup>	3.30 ±0.24 <sup>b</sup>
Group D	87± 3.61 <sup>d</sup>	89 ± 2.11 <sup>c</sup>	111± 2.25 <sup>c</sup>	6.80 ±0.26 <sup>b</sup>	3.50 ±0.18 <sup>b</sup>
Group E	86±2.43 <sup>d</sup>	78 ± 3.04 <sup>b</sup>	111± 2.25 <sup>c</sup>	6.80 ±0.22 <sup>b</sup>	3.50 ±0.17 <sup>b</sup>
Group F	70±2.56 <sup>b</sup>	71 ± 3.23 <sup>b</sup>	85 ± 4.27 <sup>b</sup>	7.30 ±0.30 <sup>a</sup>	3.70 ±0.12 <sup>b</sup>

Table (2) Values represent means ± S.D obtained from 6 rats , means in the same column followed by the same letters do not differ significantly, and when the means followed by different letters differ significantly at (p ≥ 0.05).

### Effect of lemon grass extracts, oil and vitamin E on Antioxidant parameters in rates plasma

Data in Table (3) showed that positive control (group A) showed increasing in all antioxidant parameters (SOD, MDA, CATand GST) comparing with negative control (group B) and all treated group showed significant decreasing at (p ≥ 0.05) comparing with positive control, this means that lemon grass oil, lemon grass methanolic extract and silvernano

particeles have a potent antioxidant activity and hepatoprotective effect against oxidative stress. Our results were in the accordance with ( Wang *et al.*, 2013 and Madhu *et al.*, 2017 ) they reported that Antioxidant enzymes such as catalase, (SOD), (GST), glutathione peroxidase (GPx), and glutathione reductase help to counteract the toxicity of Reactive oxygen species under normal physiological conditions and oxidative stress. Also silver nanoparticles have

antioxidant effect and that can reduce the evaluation of antioxidant parameters. antioxidant enzymes such as catalase, (SOD), (GST), (GPx) and glutathione reductase help to counteract the toxicity of ROS under normal physiological conditions. However, during condition of oxidative stress, damage to the brain, liver and reproductive tissues may occur due to the interaction of ROS and other free radicals with carbohydrates, lipids, DNA and proteins components of these tissue. Silver nanoparticles can reduce the evaluation of antioxidant parameters. (Ganjewala *et al.*, 2008 and Amos *et al.*, 2017) reported that lemon grass (*Cymbopogon flexuosus*) have been reported to be cytotoxic to human cancer cells and possess antitumor activity in mice lemon grass oil is also thought to help with stress-related disorders, and has been shown to have antifungal and antimicrobial properties. antioxidants can be categorized in multiple ways. Based on their activity, they can be categorized as enzymatic and nonenzymatic antioxidants. Enzymatic antioxidants

work by breaking down and removing free radicals. The antioxidant enzymes convert dangerous oxidative products to hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and then to water, in a multi-step process in presence of cofactors such as copper, zinc, manganese, and iron. Non-enzymatic antioxidants work by interrupting free radical chain reactions. Few examples of the non-enzymatic antioxidants are vitamin C, vitamin E, plant polyphenol, carotenoids, and glutathione. The antioxidants can also be categorized according to their size, the small-molecule antioxidants and large-molecule antioxidants. The small molecule antioxidants neutralize the ROS in a process called radical scavenging and carry them away. The main antioxidants in this category are vitamin C, vitamin E, carotenoids, and glutathione (GSH). The large-molecule antioxidants are enzymes (SOD, CAT, and GSHPx) and sacrificial proteins (albumin) that absorb ROS and prevent them from attacking other essential proteins Satish and Dilipkumar, (2015)

Table (3): Effect of lemon grass extracts, oil and vitamin E on Antioxidant parameters in rates plasma.

	SOD(U/L )	MDA (nmol/ml )	CAT ( U/L )	GST( U/ml )
Group A	53 ± 3.33 <sup>a</sup>	12 ± 1.21 <sup>a</sup>	108 ± 1.89 <sup>a</sup>	19 ± 1.21 <sup>a</sup>
Group B	177 ± 2.31 <sup>f</sup>	81 ± 3.98.0 <sup>f</sup>	318 ± 11.47 <sup>f</sup>	73 ± 12.53 <sup>b</sup>
Group C	145 ± 2.92 <sup>f</sup>	45 ± 2.38 <sup>c</sup>	229 ± 8.49 <sup>a</sup>	48 ± 4.26 <sup>c</sup>
Group D	129 ± 6.78 <sup>d</sup>	50 ± 3.55 <sup>c</sup>	183 ± 6.92 <sup>a</sup>	51 ± 5.44 <sup>c</sup>
Group E	110 ± 3.15 <sup>e</sup>	43 ± 2.89 <sup>c</sup>	213 ± 6.36 <sup>a</sup>	48 ± 7.52 <sup>c</sup>
Group F	101 ± 3.28 <sup>b</sup>	29 ± 2.32 <sup>c</sup>	180 ± 6.12 <sup>b</sup>	35 ± 3.05 <sup>b</sup>

Table (3) Values represent means ± S.D obtained from 6 rats , means in the same column followed by the same letters do not differ significantly, and when the means followed by different letters differ significantly at (p ≥ 0.05).

## Conclusion

We have found that the lemon grass oil and methanolic extract exhibit a promising potent antioxidant activity and hepatoprotective effect against oxidative stress. The green synthesized silver nanoparticles were quick, reliable, easy, one step synthesis and cost effective. Our results concluded the potential effect of silver nanoparticles as hepatoprotective effect and antioxidant activity.

Overall, this study shows green synthesis of silver nanoparticles might be a potential agent for cancer therapy. Further investigation is required to elucidate the molecular mechanism of silver nanoparticles and its application in future drug therapy.

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## التأثير الواقي للكبد والنشاط المضاد للأكسدة لنترات الفضة النانومترية المخلقة حيويًا من نبات حشيشة الليمون

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### الملخص العربي

التخليق الحيوي لجسيمات الفضة النانومترية غير سام، سريع وأكثر أمانًا من الطرق الفيزيائية والكيميائية. هذه الدراسة اهتمت بتخليق نترات الفضة النانومترية باستخدام مستخلص ميثانولي من نبات حشيشة الليمون، ثم دراسة خواص نترات الفضة النانو وعمل تجربة حيوية على فئران التجارب لدراسة دورها في حماية الكبد ضد الإجهاد التأكسدي . تحويل نترات الفضة إلى الصورة النانو باستخدام مستخلص ميثانولي من حشيشة الليمون استغرق حوالي ٣٠ دقيقة بالتسخين عند درجة حرارة ٨٠ على مقلب مغناطيسي عند درجة حموضة ١٢ . تم استخدام محلول مائي من نترات الفضة ٠,١ عيارى والمستخلص الميثانولي من حشيشة الليمون، تم دراسة خواص نترات الفضة باستخدام جهاز سبكتروفوتومتر والميكروسكوب الإلكتروني، تم دراسة النشاط المضاد للأكسدة بقياس القوة الاختزالية والنشاط المكافح للشقوق الحرة . صممنا تجربة حيوية على فئران التجارب لقياس قدرة المستخلصات على حماية الكبد من الإجهاد التأكسدي وذلك مقارنة بفيتامين E الصناعي

### النتائج

نترات الفضة أعطت طول موجي ٤١٢ على جهاز سبكتروفوتوميتر وكان حجم الجزئ المحول للنانو ( ١٣,٥ نانوميتر) باستخدام الميكروسكوب الإلكتروني، نترات الفضة النانومترية المخلقة حيويًا باستخدام المستخلص الميثانولي من حشيشة الليمون كان لها تأثير كبير في حماية الكبد من الإجهاد التأكسدي يليها الزيت الطيار لحشيشة الليمون ثم المستخلص الميثانولي مقارنة بفيتامين E الصناعي.

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