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Effects of the silver nanoparticle synthesis from the leaves of the *Capparis spinosa* plant on the liver of mice infected with visceral leishmaniasis

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ABSTRACT

To know the effect of the *Capparies* silver nanoparticles (AgNPs) synthesis from the leaves of the *Capparis spinosa* plant on the liver enzymes GOT, GPT and the histology of the liver in infected mice with visceral leishmaniasis (VL), they were dosed with 0.1 mL day^{-1} and pentostam drug (0.01 mL day^{-1}) for 21 days. The results showed that the silver nanoparticles of the *Capparies spinosa*, led to a significant decrease in liver enzymes, i.e., GOT and GPT after three weeks, compared to the positive control group. Also, it was noted that the liver tissue was less affected with a slight expansion of the hepatic sinusoids and a decrease in lymphocyte infiltration.

Keywords: *Leishmania donovani*, Silver nanoparticles (AgNPs), *Capparies spinosa*, Liver enzymes, GOT, GPT.
Article type: Research Article.

INTRODUCTION

Leishmania parasite is a phagocytic host cell-invading obligate intracellular parasite (Cabezas *et al.* 2015). It can be transmitted anthropologically or zoonotically by the bites of *Phlebotomine* sand flies (de Freitas *et al.* 2016). Visceral leishmaniasis (VL), also known as kala–azar disease, is caused by the *Leishmania donovani* (WHO 2010). Both innate and adaptive immunities play a role in defence against the *Leishmania*. Among protective innate mechanisms, the complement system is very rapidly activated once promastigotes penetrate the dermis and react with serum, resulting in efficient killing of over 90% of all inoculated parasites within a few minutes (Maurer *et al.*2009). Nanobiotechnology is a branch of nanotechnology that allows to build nano particles (NPs) from biological materials for different applications with less dangerous impacts (Johari *et al.* 2016; Bagherzadeh Lakani *et al.* 2016; Ghazanfari *et al.* 2020). There are various biological materials in nature, such as plants, algae, fungi, yeast, bacteria, and viruses, all of them could be used for NP synthesis (Ahmad *et al.* 2003). Nanomedicine is known to be one of the encouraging areas in this field which has been uninterruptedly developing (Saleem *et al.* 2019). *Capparis* species belonging to family Capparaceae are common plants with medicinal attributes. *C. spinosa* which grows wild in dry regions around the Mediterranean basin and have been reported for their traditional uses due to its therapeutic characteristics (Hamed *et al.* 2007; Dameh *et al.* 2022).

MATERIALS AND METHODS

Preparation of aqueous extract of *C. spinosa*

Fresh leaves of *C. spinosa* were collected, then washed thoroughly 2-3 times with tap water and once with sterile water. Thereafter, 20 g were weighed and placed into a beaker with 100 mL distilled water. The mixture was heated for 10 min at 60 °C while stirring occasionally and then allowed to cool at room temperature. The mixture was filtered using the Whatman No. 1 filter paper and then centrifuged for 10 min. The extract was stored in the refrigerator at 4 °C (Benakashani *et al.* 2016).

Preparation of silver nanoparticles (AgNPs)

$AgNO_3$ powder (0.22 g) was dissolved in 200 mL deionized water. Then $AgNO_3$ solutions were mixed with 30 mL aqueous extract of *C. spinosa* fresh leaves in a flask, followed by heating at 60 °C for half an hour. After 30

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min, the solution was turned to dark brown, indicating the formation of silver nanoparticles. About 1 mL was taken for the purpose of performing UV-Vis spectrophotometer. As for the rest of the solution, it was distributed in plates and placed in an incubator at 38 °C for drying. Then it was collected in the form of powder and part of it was used for the purpose of characterization of nanoparticles.

Optical and structural method to characterize AgNPs

The absorption spectra of AgNPs suspension with different concentrations were 100%, 75%, 50% and 25% measured by UV-Visible spectrophotometer-T60 within the wavelength range between 300-750 nm by localized surface plasmon resonance (LSPR) of AgNPs. The crystallite size of the AgNPs was determined by X-Ray diffraction analysis using Debye-Scherrer formula (Thirumagal & Jeyakumari 2020):

$$D = 0.9\lambda\ \beta\ \mathrm{Cos}\theta \quad \text{----(1)}$$

where D is crystallite size, β is the FWHM (full width at half maximum), the wave length of X-ray is: λ = 0.1541 nm and θ is the diffraction angle.

Xe-Ray diffractometer (XRD-6000) instrument operates at 40 kV with 2 s time interval at room temperature (27 °C). Data was taken from the 2θ range of 30 to 80 degrees with a step of 0.02 degrees. The morphology and the mean particle size of the Ag were determined by Atomic Absorption Spectroscopy Flame (AAS). The samples were prepared for AAS. The AAS was established using phenix -986. The sample was examined in Directorate of Materials Research, Ministry of Science and Technology, Iraq (Fayaz *et al.* 2010), where D is crystallite size, β is the FWHM (full width at half maximum), the wavelength X-ray is: λ = 0.1541 nm and θ is the diffraction angle.

Parasite strain and culture

The parasite was obtained from Department of Biological Sciences, University of Baghdad, then cultured and maintained by serial passage in NNN media every 8^{th} day and incubated at 26 °C.

Animal grouping

Sixty-four mice were infected with 1×10^7 parasites mL^{-1} of *Leishmania donovani* promastigotes by injection intraperitoneal (Morimoto *et al.* 2016).

•Group 1: ingested orally with normal saline (infected).

•Group 2: ingested orally with AgNPs and considered as an AgNPs- treated group.

•Group 3: injected with 0.01 mL day^{-1} from Pentostam drug by intramuscular each day considers therapeutic control group.

•Group 4: ingested orally with normal saline considered as a negative control.

Blood collection

After the 7^{th}, 14^{th}, 21^{th} days, 2 mL blood were collected from the facial vein in a sterile plain tube, then left at room temperature for 35 min, centrifuged the clotting blood, and obtained clear serum, then the level of each GOT and GPT were measured.

Measurement of liver enzymes

Serum levels of GOT and GPT were determined using a commercially available kit purchased from Sigma-Aldrich glutamic oxaloacetic transaminase (GPT) assay kit method (My Biosource, USA), and GOT assay kit method (Sigma-Aldrich, USA) depending on the colorimetric method.

Histological study

Liver samples were fixed in 10% formaldehyde, dehydrated in graded alcohol, clarified in xylene and embedded in paraffin, histological sections were cut at a thickness of 4.0 µm and stained with haematoxylin-eosin (H&E) for analysis under conventional light microscopy.

Statistical analysis

The statistical analyses were carried out by SAS (2012) program. Least significant difference (LSD) test (Analysis of Variance: ANOVA) was used to significantly compare between means in this study.

RESULTS

Optical and structural measurements of AgNPs

Colloidal of the AgNPs have been synthesised using an aqueous solution of *C. spinosa* fresh leaves powder mixed with $AgNO_3$ to get solution in different concentrations of AgNPs were 100%, 75%, 50% and 25%. The absorbance spectra of all the samples have been measured by UV-visible in the range of 300-750 nm, as shown in Fig. 1.

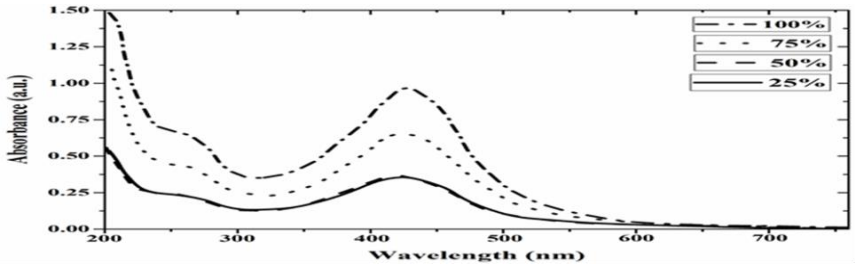


Fig. 1. UV-Vis absorption spectra of colloidal solution of AgNPs with different concentrations of *C. spinose* fresh leaves (25%-100%) at room temperature.

The synthesis of the *C. spinosa* leaves extract-stabilised AgNPs also definitely achieved by UV-V is spectroscopy analysis. The localized surface plasmon resonance band centred that observed in the AgNPs is around 425, an absorption wavelength does not affect by the alterations in the AgNP concentrations (Fig. a). Although the alterations in the AgNPs colour is a result of using different concentrations (100%, 75%, 50% and 25%), however, no shift was found in the maximum wavelength values. This indicates the formation of AgNPs close in the size to each other. The high absorption value refers to the high concentrations of silver particles, while the lowest absorption values are related to the low concentrations. It can be indicated that the decrease in the agglomeration levels of the solution is explained by the formation of mostly uniform AgNPs and the symmetry of the plasmon resonance absorption bands (Stamplecoskie & Scaiano 2010). The powder extracted from AgNPs was investigated by X-ray diffraction analysis using CuKα radiation (λ = 1.5418 Å), under 40 kV/30 Ma-X-ray, 2θ/θ scanning mode. The step of degrees was taken as 0.02 in the range of about zero to100 degrees of 2 thetas. The plane of Ag NPs was observed as 111, 200, 220 and 311 corresponding to 2 theta values of 38.08, 44.03, 64.25, and 77.33 degree, respectively (Fig. 2).

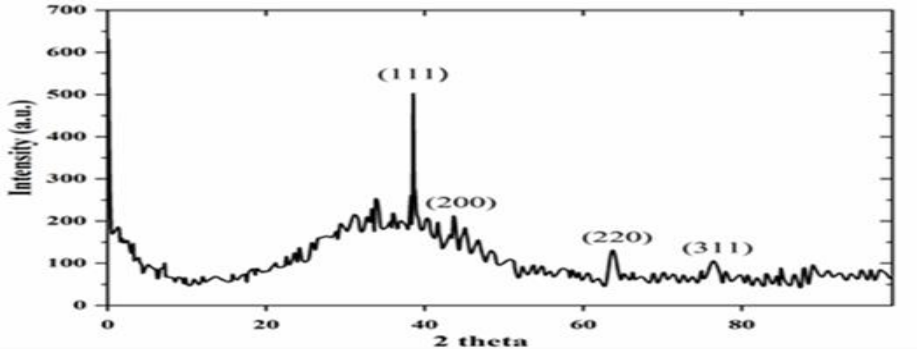


Fig. 2. XRD pattern of AgNPs thin synthesized using of *C. spinosa* leaves extract

All of peaks were compared with standard powder diffraction card of Joint Committee on Powder Standards, silver file No. 04-0783. By XRD investigations of AgNPs the crystalline nature of the dominate plane (111) was confirmed and the crystallite size was calculated by Debye-Scherrer formula about 16.6 nm.

Measurement of liver enzymes

The results in this study are shown in Tables 1 and 2 demonstrating that there were significant alterations in the level as follows:

The serum GOT increased in test groups compared to control -ve group in uninfected mice (20.81 ± 0.47 U L^{-1}) after 7 days and reached 42.54 U L^{-1} in *C. spinosa* AgNPs group and also 35.27 U L^{-1} in pentostam group, while in control +ve group was 48.70 U L^{-1} ($p < 0.0$). Then the level became deceased gradually and reached 25.73 U L^{-1} in *C. spinosa* AgNPs and 23.03 U L^{-1} in pentostam groups, after 21 days, while in the control +ve group continued to elevation and reached 72.05 U L^{-1}. Statistically significant differences in serum GOT were observed among groups. The serum GPT increased in test groups compared to control -ve group in uninfected mice (21.94 ± 0.18 U L^{-1}) after 7 days and reached to 46.37 U L^{-1} in *C. spinosa* AgNPs group and 38.01 U L^{-1} in pentostam group, while serum GPT in control +ve group was 48.50 U L^{-1} ($p < 0.01$). Then the level became deceased gradually and reached 28.47 U L^{-1} in *C. spinosa* AgNPs group and 24.40 U L^{-1} in pentostam group after 21 days, while the control +ve group continued to rise until reaching 75.2 U L^{-1}. There were statistically significant differences in serum GPT across the groups.

Table 1. The serum level of GOT (U L^{-1}) in the study groups.

Group	Mean ± SE of GOT (U L^{-1})		
	7 days	**14 days**	**21 days**
Control -ve	20.81 ±0.47^{c}	20.81 0.47^{c}	20.81 ± 0.47^{b}
Control +ve	48.70 ±5.97^{a}	62.33 ±2.89^{a}	72.05 ± 7.10^{a}
C. spinosa AgNps	42.54 ±4.04^{ab}	34.14 ±6.77^{b}	25.73 ± 2.54^{b}
Pentostam	35.27 ±5.02^{b}	30.41 ±3.30^{bc}	23.03 ± 0.72^{b}
LSD value	12.989**	11.939 **	11.202 **

Means having with the different letters in same column differed significantly ** ($P < 0.01$).

Table 2. The serum level of GPT (U L^{-1}) in the study groups.

Group	Mean ± SE of GPT (U L^{-1})		
	7 days	**14 days**	**21 days**
Control – ve	21.94 ± 0.18^{b}	21.94 ± 0.18^{c}	21.94 ± 0.18^{b}
Control +ve	48.50 ± 5.90^{a}	62.07 ± 2.70^{a}	75.29 ± 6.26^{a}
C.spinosa AgNps	46.37 ± 3.61^{a}	40.64 ± 7.06^{b}	28.47 ± 2.91^{b}
Pentostam	38.01 ± 4.82^{a}	33.06 ± 3.40^{bc}	24.40 ± 1.29^{b}
LSD value	12.441 **	12.242 **	10.37 **

Means having with the different letters in same column differed significantly. ** ($P < 0.01$).

Histological study of the mice liver

After infecting the mice by the *L. donovani* promastigotes, they were treated with pentostam and dosed with *C. spinose* AgNP, then sacrificed after 7th and 21th day, taken the liver and made slide sections stained with haematoxylin and eosin for finding the histological alteration. After 7th day the alterations in the liver of infected mice control +ve were appeared clearly compared to uninfected mice control -ve. The latter displayed normal-looking hepatic cells arranged around the central vein, presence of sinusoid and kupffer cells (Fig. 3), while the liver section for control +ve after 7th days exhibited necrosis of hepatic cells with inflammatory cell infiltrations (Fig. 4). The liver section of mice treated by *C. spinosa* AgNPs illustrated slight sinusoidal dilatation with mild depletion of glycoprotein inside the hepatocyte cells and congestion, infiltration of lymphocytes (Fig. 5). The liver section of mice treated with pentostam drug revealed large area of necrosis and inflammatory cell infiltrations along with slight depletion of glycoprotein inside the hepatocyte cells and congestion (Fig. 6). After 21th day, the damage of liver tissue in control +ve was becoming more severe, exhibiting a large area of hepatocyte necrosis with sinusoidal dilatation (Fig. 7), while the changes noticed in parenchymal liver tissue for mice dosed with *C. spinosa* AgNPs including the reduced infiltration of inflammatory cells and slight sinusoidal dilatation compared with control positive (Fig. 8). The liver tissue taken from a group of mice treated with pentostam indicated that the damage was less and the tissue looks return to natural with slight dilatation of sinusoid and focal dispersed necrosis with a few inflammatory cell infiltrations (Fig. 9).

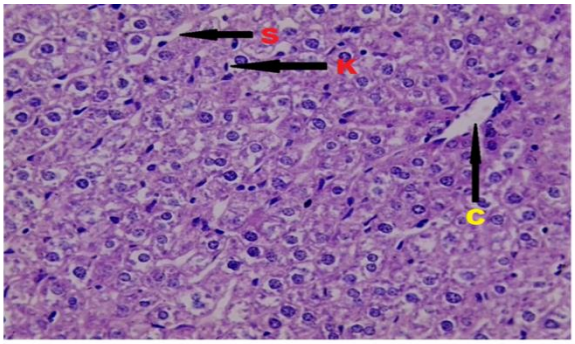
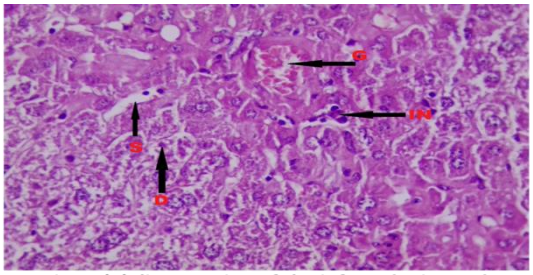


Figure (A) : The liver section of uninfected mice

Fig. 3. Liver section of uninfected mice (Control –ve) after 7th days shows normal looking liver cells arranged around the central vein (C), sinusoid (S) and the Kupffer cells (K; H&E; 400X).



Figure(C): liver section of the infected mice and

Fig. 5. Liver section of infected mice and dosed orally with *C. spinosa* AgNP after 7th days shows depletion glycoprotein (D) inside hepatocytes, slight sinusoidal dilatation (S), congestion (G), infiltration of lymphocytes (IN; H&E; 400X).

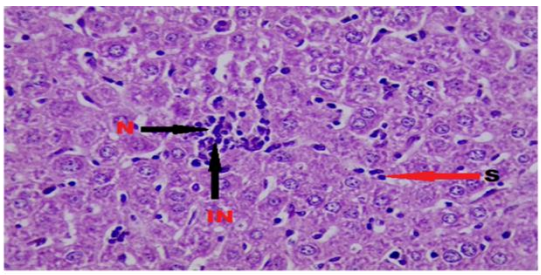
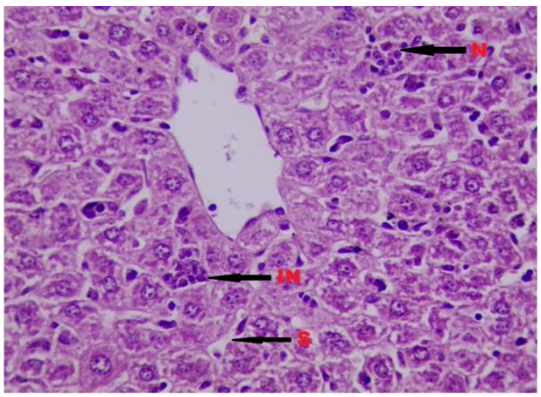


Fig. 7. Liver section of infected mice (Control + ve) with *L. donovani* promastigote after 21th day shows focal area of necrosis (N), infiltration of inflammatory cells (IN) and sinusoidal dilatation (S; H&E; 400X).



Figure(G): Liver section of mice treated with

Fig. 9. Liver section of infected mice treated with pentostam after 21th day shows slight sinusoidal dilatation (S), focal dispersed necrosis (N) and infiltration of inflammatory cells (IN; H&E; 400X).

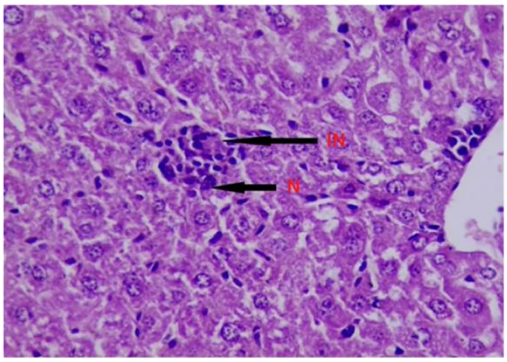
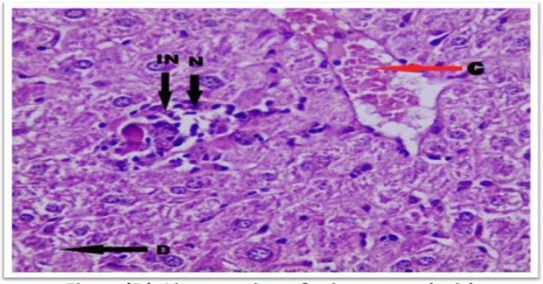


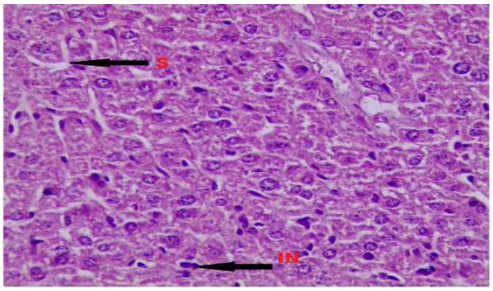
Figure (B) : The liver section of infected micecontrol

Fig. 4. Liver section of infected mice (Control + ve) with *L. donovani* promastigote after 7th days shows necrosis (N) with infiltration of mononuclear inflammatory cells (IN; H&E; 400X).



Figure(D): Liver section of mice treated with

Fig. 6. Liver section of infected mice treated with pentostam after 7th days shows focal area of necrosis (N), infiltration of inflammatory cells (IN) with slight depletion of glycoprotein (D) inside hepatocytes and congestion (G; H&E; 400X).



Figure(F):liver section of the infected mice and dosed

Fig. 8. Liver section of infected mice and dosed orally with *C. spinosa* AgNP after 21th days shows slight sinusoidal dilatation (S), congestion (G), and a few infiltration of inflammatory cells (IN; H&E; 400X).

DISCUSSION

Visceral leishmaniasis (VL) causes alterations in liver function, hence patients develop severe, life-threatening hepatitis (Mathur *et al.* 2008). VL causes hepatomegaly in affected patients and shows a value higher than GOT and GPT levels (Naseralla *et al.* 2015). Mathur *et al*. (2008) reported that there is an increase in the level of GOT and GPT in confirmed cases of VL. Likewise, Ferreira *et al*. (2020) mentioned that the disturbance of host metabolic pathways by *Leishmania donovani* exhibits crucial consequences for the activation status of immune cells and the outcome of infection, so, GOT level rises. A study conducted by Parang & Moghadamnia (2018) showed that silver nanoparticles stimulated the elevated serum levels of hepatic enzymes and other liver-related biochemical factors through induction of oxidative stress and elevation in reactive oxygen species, ultimately leading to liver injury. The rate of GPT was increased once infecting by *L. donovani*, since it attacks the visceral organs, especially the liver. So, the high level of GPT is a sign of liver damage, and progressive anaemia that occurs as a result of the presence of the parasite. Hence, it causes the blood marrow not to produce enough blood. This gives destructions to the liver to produce red blood cells. Since there is an abnormal increase in production, it gradually damages the liver due to the presence of a parasite feeding on it. It is observed that the level of GPT continues to rise (Prajapati *et al.* 2016). Kaye *et al*. (2004) demonstrated that the parasite was eventually eliminated from the liver, and that hepatic resistance to infection is the result of a coordinated host response that includes a wide range of effector and regulatory pathways targeted within specific tissue structures called granulomas. Parasite persistence is accompanied by granuloma failure and a variety of pathologies. VL causes disorder in liver functions (Tesfanchal *et al*, 2020). AgNPs presented major anti-leishmanial effects by preventing the promastigotes proliferation and metabolic activity (Allahverdiyev *et al.* 2011) . According to Mohamed *et al.* (2019), after treating infected mice with *Fusarium* AgNPs, repair occurred in the parenchymal liver after one week, with lymphocyte infiltration and hydropic degeneration, and after three weeks, only a few lymphocyte infiltrations occurred.

CONCLUSION

The use of *C. spinosa* AgNPs reduced liver damage by a reduction in the rate of liver enzymes (GOT and GPT). Also, the histological study of liver sections showed less histopathological effects in the AgNP group of treated mice compared to the pentostam group.

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