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Antiparasitic Effect Of The Silver Nanoparticles Capparis Spinosa On Immune Response In Infected Mice With Visceral Leishmaniasis

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Abstract: To evaluate the efficacy of the Capparis silver nanoparticles synthesis from the leaves of the Capparis spinosa plant on the levels of immune drivers IL-12 and IFN-γ in the serum of infected laboratory mice with visceral Leishmaniasis, they were dosed with (0.1 ml/day) and pentostam drug (0.01 ml/day) for 21 days. The results showed that the level of both IFN- γ and IL-12 in mice dosed with nanoparticles was significantly decreased for three weeks compared to the positive control group.

Keywords: leishmania donovani, silver nanoparticles (AgNPs), Capparies spinosa, IFN- γ, IL-12.

Introduction: Leishmania parasiteis can be transmitted anthropologically or zoonotically by the bites of approximately 30 different species of Phlebotomine sand flies [1]. Found in reticuloendothelial cells like macrophages, neutrophils, endothelialcells of liver, spleen, bone marrow. The most three clinical syndromes of leishmaniasis include Visceral leishmaniasis (VL), Cutaneous leishmaniasis (CL) and Mucocutaneous leishmaniasis (MCL) [2]. The possible symptoms of VL leishmaniasis are fever, anaemia, wasting, hepatosplenomegaly and immune suppression [3]. Meglumine antimoniate and sodium stibogluconate were some of the primary anti-leishmanial drugs, however, these drugs showed a number of side effects related to their ingestion [4]. Upon the development in research and technology, presently, a number of therapeutic drugs are accessible for the treatment of leishmaniasis such as pentamidine, paromomycin, miltefosine and amphotericin B [5]. Both innate and adaptive immunity plays a role in defence against the Leishmania parasite. 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 more than 90% of all inoculated parasites within a few minutes[6]. Cytokines and cells of the innate immune system strongly participate to early protection against Visceral leishmaniasis. Interleukin IL-12, produced by dendritic cells for triggers natural killer (NK) cell activation, also NK cells are the initial source of interferon gamma (IFN- γ) production and so they are able to limit parasite spread until a specific T-cell response has been mounted [7]. In fact, the role of cytokines such as IFN-γ is to trigger macrophages and increase the microbicidal activity of these cells to destroy intracellular

pathogens through the generation of reactive oxygen species and reactive nitrogen species. Furthermore, IFN-γ acts on B cells to inhibit switching to stimulate the differentiation of CD4+ T cells to the T- helper 1 (Th1) subset [8]. NPs now commonly used as medicines for treating different diseases and improving human health because the display antibacterial, antiviral and anti-parasitic efficacy [9]. Nanomedicine is known to be one of the encouraging areas in this field which has been uninterruptedly developing. It is well-known that the standard drugs for Leishmania treatments possess some drawbacks such as controlling difficulty, long-term treatment and low tolerability[10].

Materials and Methods:

Preparation of aqueous extract of C.spinosa: Fresh leaves of C.spinosa were taken, At first, it is washed 2-3 times with tap water, and then with distilled water, 20 gm is added to 100 ml of distilled water. The mixture was heated for 10 min at 60 °C with occasional stirring and then allowed to cool at room temperature, the mixture was filtered using Whatman No. 1 filter paper and then centrifuged for 10 min, the extract was stored in the refrigerator at room temperature 4 °C [11].

Preparation of silver nanoparticles (AgNPs): AgNO3 powder (0.22gm) was dissolved in 200 mL of deionized water. AgNO3 solutions were mixed with 30 ml of aqueous extract of C. spinosa leaves in a beaker, and then heated at 60 °C for half an hour, the transformation of the solution was observed to a dark brown color, this indicates 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 into plates and placed in an incubator at 38 °C for drying. Then it was collected in powder form and part of it was used for the purpose of nanoparticle characterization.

Optical and structural method to characterize AgNPs: Absorption spectra of suspensions of AgNPs at different concentrations are: (100%, 75%, 50% and 25%) and were measured with a UV-visible-T60 spectrophotometer 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 [12].

$D = 0.9\lambda\ \beta\ Cos\theta$ ----(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.

The X-ray diffractometer instrument (XRD-6000) operates at 40 kV with an interval of 2 seconds at room temperature (27) °C. Data was taken from the 2θ range from the 2° range from 30° to 80° with a step of 0.02°. The morphology and average particle size of Ag were determined by Atomic Absorption Spectroscopy Flame (AAS) and samples were prepared for AAS. AAS was generated with phenix -986, the sample was examined at directorate of materials research, Ministry of Science and Technology, 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. Xe-Ray diffractometer (XRD-6000) instrument operating at 40 kV with 2 s

time interval at the room temperature (27) C°. Data were taken from the 2θ range of 30 to 80 degrees with a step of 0.02 degrees. The sample was examined in the directorate of materials research, Ministry of Science and Technology [13].

Parasite strain and culture: The parasite was obtained from University of Baghdad Department of Biological Sciences, then cultured and maintained by serial passage in NNN media every 8thday and incubated at 26 °C.

Animal grouping: Sixty four mice were infected with 1 × 10^7 parasites/mL of Leishmania donovani promastigotes by injection intraperitoneal [14].

•Group1: ingested orally with normal saline (infected).

•Group2: ingested orally with AgNPs and considered as an AgNPs- treated group.

•Group3: injected with 0.01mL/day from Pentostam drug by intramuscular each day considers therapeutic control group.

•Group4: ingested orally with normal saline considers a negative control.

Blood collection: After the 7th, 14th, 21th days, from the facial vein collected 2 ml of blood in a sterile plain tube, then left at room temperature for 35 minutes, centrifuged the clotting blood, and obtained clear serum and the level of cytokines were measured.

Determine the level of cytokines in mouse serum: The practical work used ELISA Kit (MBS2510359) and (MBS2500105) for estimating levels of both cytokines IL-12, IFN-γ respectively, was done according to the instructions of manufacture by My biosource **/** USA.

Statistical analysis: the statistical analysis was carried out by SAS (2012) program. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significantly compare between means in this study.

The 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 AgNO3 to get solution in different concentration of AgNPs are: (100%, 75%, 50% and 25%). The absorbance spectra of all the samples have been measured by UV-visible in the range (300-750 nm), as shown in the figure(1).

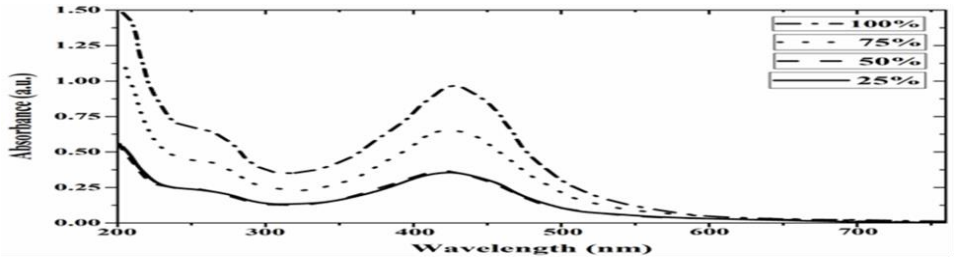

Figure (1): UV-Vis absorption spectra of colloidal solution of AgNPs with different concentrations of C. spinosa fresh leaves extract (25%-100%) at room temperature.

The synthesis of the C. spinosa leaves extract-stabilised Ag NPs was also definitely achieved by UV-V is spectroscopy analysis. The localized surface plasmon resonance band centred that observed in the Ag NPs is around 425, an absorption wavelength does not affect by the change of Ag NPs concentrations. Although the changing in the colours of AgN Ps is a result of the use of different concentrations (100%, 75%, 50% and 25%), no gave shift in the wavelength maximum values. This indicates the formation of Ag NPs close in the size to each other. The high absorption value refers to the high concentration of silver particles, while the lowest absorption values are for the low concentrations of silver particles. It can be indicated that the decrease in the agglomeration levels of the solution is explained by the formation of mostly uniform Ag NPs and the symmetry of the plasmon resonance absorption bands[15]. The powder extracted from Ag NPs was investigated by X-ray Diffraction analysis by using CuKα radiation (λ = 1.5418 Å), under 40 kV/30Ma-X-ray, 2θ/θ Scanning mode. The step of degrees was taken 0.02 data in the range of about zero to100 degrees of 2 thetas. The plane of Ag NPs was observed (111),(200),(220) and (311) corresponding at 2 theta values of 38.08, 44.03, 64.25, and 77.33 degree, respectively as shown in figure (2).

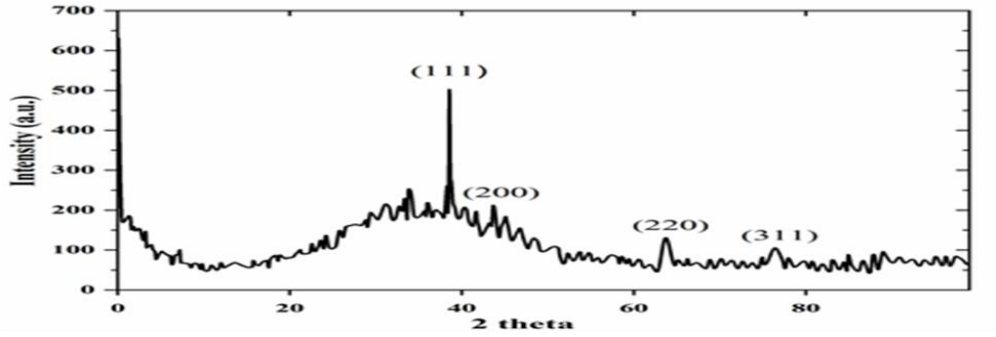

Figure (2): XRD pattern of AgNPs thin film synthesised using *C. spinosa* leaves extracted.

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

Determine the level of interleukins in mice: The role of C.spinosa Ag NPs inregulating the immune response in an experimental mice model of Leishmania was determined by

measuring the serum level of interleukins IL-12 and IFN-γ in all experimental groups (control ,silver nanoparticles and pentostam)after 7,14 and 21 days.

Interferon-gamma(IFN-γ) level: The serum level of IFN-γ among groups of the present study were high level in C.spinosa Ag NPs group was (255.81pg/mL) after 7 days while the level of IFN-γ in pentostam and control +ve groups were with lower(136.52,130.37) pg/mL respectively. There was significantly different ($p \leq 0.01$) among different groups.While after 21 days the level of IFN-γ decreased and became lower in pentostam group was lower level compared with other groups (91.39pg/mL) and in C.spinosa Ag NPs was (150.54pg/mL), while in control +ve groups increased and reached to (237.04pg/mL) .There was significantly different ($p \leq 0.01$) among different groups as table (1).

Table(1) The serum levels of INF-γ(pg/mL)in the study groups.

Groups	Mean ± SE of IFN-γ (pg/mL)		
	7 days	14 days	21 days
Control – ve	136.52 ±11.32 b	136.52 ±11.32 b	136.52 ±11.32 bc
Control +ve	130.37 ±22.576 b	172.29 ±12.78 ab	237.04 ±22.74 a
C.spinosaAgNps	255.81 ±36.39 a	186.97 ±14.34 a	150.54 ±12.86 b
Pentostam	168.73 ±24.72 b	131.02 ±24.61 b	91.39 ±12.36 c
LSD value	74.828 **	49.068 **	45.802 **
Means having with the different letters in same column differed significantly. ** (P≤0.01).			

Interlukine-12 (IL-12) level: The serum levels of IL-12 among groups of the present current study were high level in C.spinosaAgNPs then in was(255.81pg/ml) , while in the pentostam group the level of its rise was less than control +ve group (197.41,212.68pg/mL) respectively after 7 days , while the results after 21 days there was a clear decrease in the level of IL-12 level in the C.spinosa Ag NPs and pentostam groups (142.16,157.68) pg/mL respectively, compared with the control +ve group, which continued to rise untile reached to (326.04 pg/mL).There was a significantly differenc (P≤0.01)among different groups, as table(2).

Table :(2)The serum levels of IL-12 (pg/mL)in the study groups.

Groups	Mean ± SE of IL-12 (pg/ml)		
	7 days	14 days	21 days
Control – ve	181.61 ±18.06	181.61 ±18.06 b	181.61 ±18.06 b
Control +ve	212.68 ±30.63	279.39 ±20.55 a	326.04 ±20.44 a
C.spinosa AgNps	255.81 ±36.39	186.97 ±14.34 b	142.16 ±19.85 b
Pentostam	197.41 ±21.77	250.72 ±26.05 a	157.68 ±13.88 b
LSD value	81.644 NS	59.609 **	53.811 **
Means having with the different letters in same column differed significantly. ** (P≤0.01).			

Discussion: Cytokines are important for immunotherapy against experimental and human VL so it is quite essential to explore their role in detail, also the expression profile of various cytokines during disease progression as well as in host protection could give a clue to the development of new diagnostic tools and therapeutic measures against VL [16]. IL-12 resembles as an immune bridge between innate and adaptive immunities Likewise, IL-12 drives the activation and differentiation of naïve T cells into mature cells able to IFN-γ production , although the IL-12 act in promoting cytokine which supports a wide range of variety inflammatory cytokines[17]. Under VL conditions, the immunity response depended on the development of T helper type I immune responses (cell mediated immunity) were initiated IL-12 production by antigen presenting cells (APCs) that induce Interferon-γ (IFN- γ) secreting Th-1 cells[18]. In VL, the CD4+ T cells secrete pro-inflammatory cytokines such as TNF-α and IL-12, providing immunity against the parasite [19]. The high abundance of IL-12 increased the ability of CD4+ T cells to produce IFN- γ. In other words, macrophage cells as yet active able to express more IL-12 cytokines for inducing naïve T-cells after cell-cell conjugation occurred between CD40 costimulater and CD40 ligand for limited infection, a lot of naïve macrophage cells where be induced to mature as a response to T-cells differentiation. IFN-γ plays an important role in macrophage mediated anti-leishmanial activity, participate in parasite removal and the following resolution of infection [20]. NK cell-derived IFN-γ play a more prominent role in host defense by activating macrophageto kill the intracellular parasite through the generation of reactive oxygen intermediated (ROI) or reactive nitrogen in the control of intracellular parasite, by inducing IFN-γ production [21]. Mutiso et al [2013] [22] reported that the depletion of NK cell within the first 7 days of Leishmania infection in mice leads to higher parasite burden due to not enough IFN-γ production. IFN-γ signalling in infected macrophages promotes expression of inducible nitric oxide (NO) synthase (iNOS, NOS2) and NO production that, together with reactive oxygen species (ROS) generated during phagocytosis, are essential to kill intracellular parasites [23]. Kip et al [2015] [24] mentioned in his research that IL-12 and IFN-γ levels were found to be significantly elevated in patients with active visceral leishmaniais, also confirmed by Khoshdel et al [2009] [25] reported that the serum cytokines levels of IL-12, IL10, and IFN-γ were higher in patients with active visceral leishmaniasis than in family members and control persons. Kumar et al [2014] [26] demonstrated that the elevated levels of IFN-γ in patients with active VL serve to limit parasite replication and mentioned that the therapeutic administration of IFNγ may still hold potential. Neutralization of IL-12 leads to disease exacerbation in L. major and L. donovani infections[27]. It is well known during host defense in VL the efficacy of anti-leishmanial therapy is systematically associated with restored expression of IL-12 and INF-γ consequence leishmania-specific T-cell response[28]. Mondal et al. [2011][29] reported that the onset of chemotherapy and treatment with sodium antimony gluconate (antileishmanial drugs) leads to upregulation of IFN-γ and IL-12 synchronized with a decline in IL-10 in addition to the antileishmanial activity and immunomodulatory activities on peripheral blood mononuclear cells (PMNCs) resulting in decline of IL-10. Moutia et al [2016][30] reported that the C. spinosa leaf extract was shown to exhibit anti-inflammatory activity in vitro in human peripheral blood mononuclear cells (PBMC) obtained from healthy subjects also lead to significant increase in interleukin IL-4 gene expression and a significant decrease in IL-17 gene expression. El Azhary et al

[2017][31] suggested that the C. spinosa leaf extracts exhibit anti-inflammatory activity by inhibiting the pro-inflammatory cytokine expression and immune cell infiltration. AgNPs undergo oxidation and release free Ag + ions, which target intracellular amastigotes to death. after their phagocytosis by macrophages increases the release of Ag + ions by oxidation.

Conclusion: The use of C. spinosa Ag NPs enhance the production of IFN-γ and IL-12 are indicative of the its effect on immune response..

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