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Effect of Silver Nanoparticles Synthesis from Sphingomonas paucimobilis in Leishmania donovaniin vivo and in vitro

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Abstract : In this study, synthesis of silver nanoparticles (AgNPs) was carried out by using *Sphingomonas paucimobilis* then theefficiency on *Leishmania donovani* was studied on its *in vivo* and in vitro. No cases of death have been recorded in the mice which indicate that there was no-toxic effect of silver nanoparticles, also the results showed that nanoparticles have a significant impact in reducing the percentage of a vital promasitgote in concentration (0.88 mg /ml) which led the vital to drop to 0% after (5 min) only while in concentrations (0.22 and 0.44mg /ml) gradually effected and reached zero after (15 and 30 min)respectively, the pentostam drug reached to zero after(30 min) compared with the control group where the number of living cells reached to 90%after 60 minutes , With a high significant difference ($p< 0.05$) between different concentrations, also nanoparticles and pentostam lead to significantly decrease in the numbers of parasites of spleen tissue compared with control. The histopathological study liver tissues appeared that naonparticles causes hydropic degeneration, infiltration of lymphocyte in liver tissue. So the results showed that the silver naonparticles extract from *Sphingomonas paucimobilis* has a good antiparasitic activity against visceral leishmaniasis.

Keywords : *Sphingomonas paucimobilis,* pentostam, naonparticles, *leishmania donovani*.

Introduction

Visceral leishmanias is a zoonotic disease transmitted by bite of sand fly [1]. It is characterized by neutropenia, fever, hepatosplenomegaly and anemia [2], the global incidence of visceral leishmaniasis is evaluated by the world health organization to be approximately 500,000 new cases per year [3].According to the modes of transmission there are two causative parasite species responsible for visceral leishmaniasis in Europe and North Africa *leishmania infantum* and *L.donovani* and *L. chagasi* in Latin America [4]. The ready treatment options for visceral leishmaniasis have problems relating to cost, adverse effects and efficacy. The main drug used for treatment of visceral leishmaniasis are the systemic like amphotericin, paromycin and antimony and now oral drug miltefosine [5].*Sphingomonas paucimobilis* non-fermentative gram-negative bacillus non-spore forming, strictly aerobic and is a yellow pigmented[6,7]. It is rarely causes infections in humans and its consider as opportunistic pathogen[8], it is present widely both in natural and nosocomial environments, like laboratory equipment and hospital water system[9,10]. Nanotechnology is the use of a single atom for molecular manufacturing in material science and technology [11].It has been used in cancer treatment bio molecular optical tracking applications and new materials. The antimicrobial activity of MnONps synthesis from green leaf of *A. malabarica* shown agood sensitivity towards *Staphylococcus aureus, Proteus vulgaris* and *E.coli* the22 µl[12].Silver nanoparticles (AgNPS) are the most engineered nanomaterials used in catalytic performance and antibacterial effects.[13-16],Polyester fabrics were treated with ZnO nanoparticles to improve its light fastness,

self-cleaning, UV-protective, antibacterial activities[17].AgNPS, now consider as a part of daily life and widely found in consumer products as well as in medical devices,recently, the use of several microorganisms in the synthesis of AgNPS has been reported, such as *Sphingomonas*, *Escherichia coli*, *Bacillus licheniformis* and *Brevibacterium casi*[18-20]. Considering this background, the first aim of this study was to synthesis of AgNPS using *Sphingomonas paucimobilis*, the secondary aim was to evaluate the efficiency of these biologically prepared AgNPS on *L.donovaniin vitro* and *in vivo*.

2 Materials and Methods:

2.1 Bacterial strain:

Sphingomonas paucimobilis obtained from Lab of AL-Kindy Hospital in Baghdad. Brain hart infusion broth was prepared, sterilized and inoculated with fresh growth of test strain *S. paucimobilis*. The culture flasks were incubated at 37c° for 72 hrs. in an orbital shaker at 150 rpm. After the incubation period, the culture was centrifuged at 10,000 rpm for 5min , the supernatant was used for the synthesis of silver nanoparticles (AgNPS). The supernatant of *S. paucimobilis* culture was separately added to the reaction vessels containing silver nitrate at a concentration of 0.1gm /L. The reaction between the supernatant and silver ions was carried out in good conditions for 72 hrs.[21]

2.2 Leishmania strain:

It was obtained from the department of biology science AL-Nasirya University. *L.donovani* strain was maintained in vitro by serial passage in semisolid medium, then washed three times in phosphate buffered saline(PBS) pH7.2 and adjusted to concentration 1×10^7 promastigote/ml.

2.3 Experimental Steps

2.3.1 Characterization of silver nanoparticles by atomic force microscopy

Atomic force microscopy image was taken using park system AFMXE100.A thin film of the sample was prepared on a glass slide by dropping 100 ml of the sample on the slide and was allowed to dry for 5min. The slides were then scanned with the AFM [22].

2.3.2 Animals

Twenty-Four male albino mice aged 12-13 weeks, weighing 15-17gm were obtained from the animal house in college of Medicine Baghdad University were housed under standard condition. Then, 18 mice were infected by injected with 1×10^7 promastigote/ml intraperitonial.After one day , the infected mice were divided into 4 groups each group contain 6 mice, the last 6 non infected mice remain as negative control. Then each group inoculated as a follow:

1. **Group one**: inoculated orally by stomach tube (0. 1ml/day) from AgNa silver every day.
2. **Group two**: injected with (0. 1ml/day) from pentostam by intraperitonial each day.
3. **Group three**: inoculated orally by stomach tube (0. 1ml/day) normal saline consider as positive control.
4. **Group four** (none infected): inoculated orally by stomach tube (0. 1ml/day) normal saline consider as negative control.

After 21days post inoculation all the mice were scarified, liver and spleen were removed.

2.3.3 Splenic parasite burden

Impression smears from spleens were stained with Giemsa to evaluate parasite burden. The number of amastigote per host cell nucleus was determined by counting 1000 host cells as described by Bardiy *et al*[23]. The relative total numbers of parasites per organ named Leishman- Donovan units (LDU) and total Leishman-Donovan units (total LDU), were calculated according to the formula:

$$\text{LDU} = \frac{\text{number of parasites}}{\text{1000 host cell nucleus}} \qquad \ldots(1)$$

Total LDU = LDU x organ weight (mg) x 2 x 10^5

2.3.4 Histopathological changes

Liver was removed after 21days and fixed in 10% formalin then processed ,staining with hematoxylin and eosin then examined under the light microscope to study histopathological changes caused by inoculated of nanoparticles.

2.4 Prepare concentration of sphingonanoparticles (AgNPS);

Two different concentrations were prepared concentrations (0.22 and 0.44mg /ml) from the original stock of nanoparticles (AgNPS) was (0.88 mg /ml).

2.4.1The effect of sphingonanoparticles on vital Leishmania donovani in vitro

Parasites were collected on the fifth day after cultured and distributed solution containing the parasite on 24 tubes at (1ml) per tube divided for four groups each group contain 6 tubethen addition nano concentration as follow:

1.**First group**: add (1ml) of solution for each lock tube as control group.

2.**Second group**: add (1ml) ofnanoparticles with concentration(0.88gm/ml)each tube.

3.**Third group**: add (1ml) of nanoparticles with concentration (0.44gm/ml).

4.**Fourth group**: add (1ml) of nanoparticles with concentration (o.22gm/ml).

It was measured viability of the parasite after almost (5, 10, 15, 30, 60) min. of incubated degree26c°.According to the method of Hodgkinson *et al*. [24] by using Erythrocin-B stain 0.4%, then examined under the microscope was estimated percentage of vital cells according to the following formula:

$$\text{Percentage of vital} = \frac{\text{number of living cells}}{\text{the total number of cells}} \times 100\% \qquad \ldots(2)$$

2.5 Statistical Analysis

The data for various parameters were subjected to statistical analysis SPSS,software program using analysis of variance (ANOVA).

3 Results

3.1 Characterization of silver nanoparticles

In this study, silver nanoparticles were successfully synthesized by extracellular in the culture supernatant of *Sphingomonas paucimobilis*, the first indication of silver nanoparticles synthesis when addition of 1 mM silver nitrate into the tube containing culture supernatant of the bacteria, the medium gradually upset brown, while no color change was observed in the control culture without bacteria . The results showed that the examination diameter rate of these particles producer from bacteria *Sphingomonas paucimobilis* totaled 91.74 nm nanometrucma in Figure(1) also the double and triple morphologically can be described of particles silver nanoparticles produced by these bacteria Figure (2: A and B).

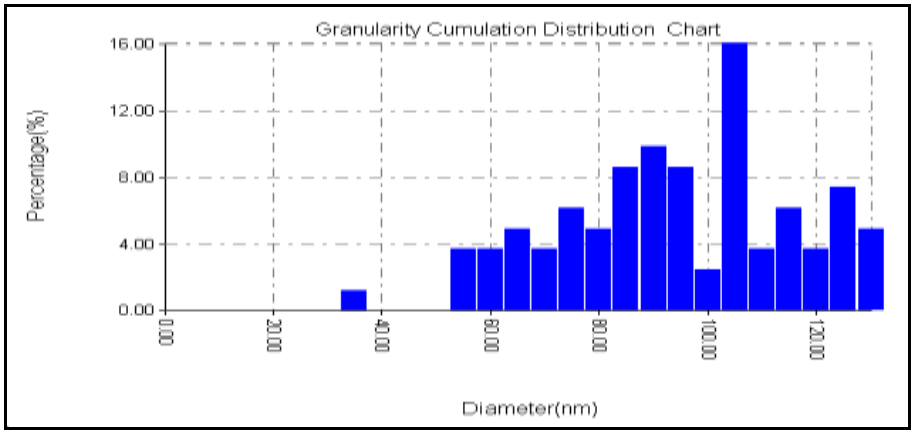
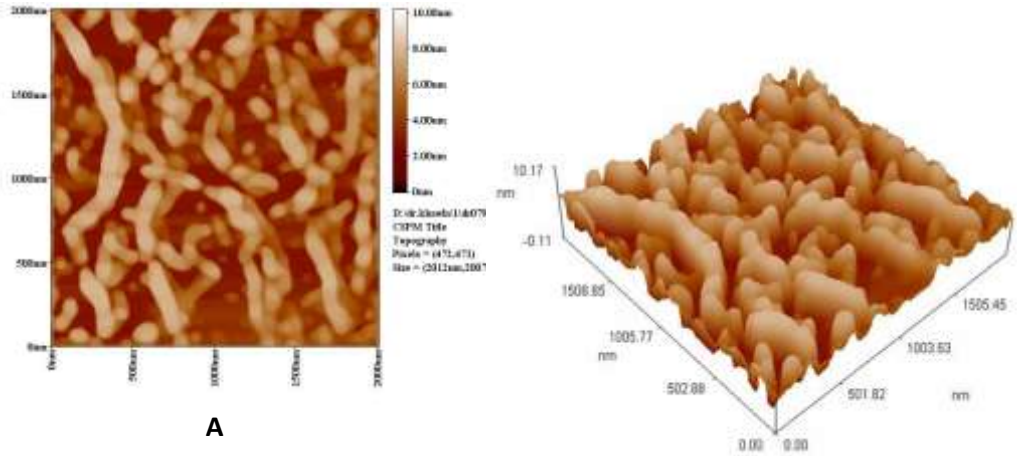


Figure 1: Particles size distribution of anisotropic silver nanoparticlesaccording volume.



A B

Figure 2:Shows double(A) and triple (B) morphological for silver nanoparticles produced by *Sphingomonas paucimobilis* bacteria.

Antiparasitic activity of silver nanoparticles against *Leishmania donovani in vitro*

The results showed that nanoparticles have a significant impact in reducing the percentage of a vital promasitgote stage for *L .donovani* directly proportional to the concentration of the nanoparticles and for all time periods as follows: the nanoparticles with concentration (0.88 mg\ml) As shown in Table(1), led to the vital drop to 0% after only (5 min) treatment, the other concentrations (0.22and 0.44 mg\ml) gradually affected in the vitality of the parasite until reached zero after (15 and 30 min) , while the pentostam drug also effects on vitality of parasite and reached to zero after(30 min),compared with the control group where the number of living cells after 60 minutes reached to 90%, with a high significant difference's ($p<0.05$) between different concentrations and the control group.

3.2 Enumeration the numbers of parasite in spleen and determination of LDU

The enumeraouse number of parasites in spleen showed that both the nanoparticles and petnostam lead to decrease the numbers of parasites in spleen tissue after 21days as shows in table (2),the numbers of parasites in nanoparticles and petnostam group were [800 and 1000]parasites /1,000 host cell respectively compared with control groups was [16000] parasites /1,000 host cell and there was significant difference between control group and two treated groups, while there was no significant difference($p>0.05$) between nanoparticles group and petnostam group.

3.3 Histological study

Study the effects of nanoparticles on the intestine and liver tissue was carried out, after 21days removed the tissues from the mice and staining by hematoxylin and eosin then compared with the control group . The results showed that the naonparticles causes in the liver tissue inoculation with nanoparticles causes hydropic degeneration and infiltration of lymphocyte cells fig (5),compared with negative control shows normal tissuefig .(3), positive control the parasite causes aggregation of lymphocyte ,hyperplasia in kupffer cells and necrosis fig.(4), and with petnostam group shows infiltration in lymphocytes, hyperplasia in kupffer cells and necrosis fig.(6).

Table (1): Shows the percentages of vitality (%) for promastigote of L. donovani after treatment by nanoparticles extracted from *S. paucimobilis* bacteria.

Time in minutes / Nano concentration mg\ml	percentages of vitality (%) for promastigote					
	0	**5**	**10**	**15**	**30**	**60**
0.88*	**23±1.2**	**4.8±0.8**	**0**	**0**	**0**	**0**
0.44*	**43±2.16**	**16.5±1.29**	**6±1.82**	**0**	**0**	**0**
0.22 *	**79.75±*5.73**	**55±3.5**	**24.25±3.86**	**6.7±0.95**	**0**	**0**
Control*	**96.5±1.29**	**95±1**	**95±0**	**91.75±0.95**	**90.75±0.95**	**90±0**
pentostam*	**37±0.81**	**19±2.58**	**6±0.81**	**1±0.81**	**0**	**0**
*** (P<0.05)**						

Table (2): Shows mean number of parasites, LDU (Leishman- Donovan units) and totalLUD in spleen of mice treatment with nanoparticles and pentostam compared with positive control.

Groups	**No of parasites** /1000 host cell	**LDU**	**Total LDU× 10^5**
Positive control	16000 ±1000*****	16	9.6
Nanoparticles	800 ±150[a]	0.8	0.4
Pentostam	1000 ±200[a]	1	0.6
*****significant difference P<0.05between tow groups and control group. a- no significant difference(P>0.05) between two groups.			

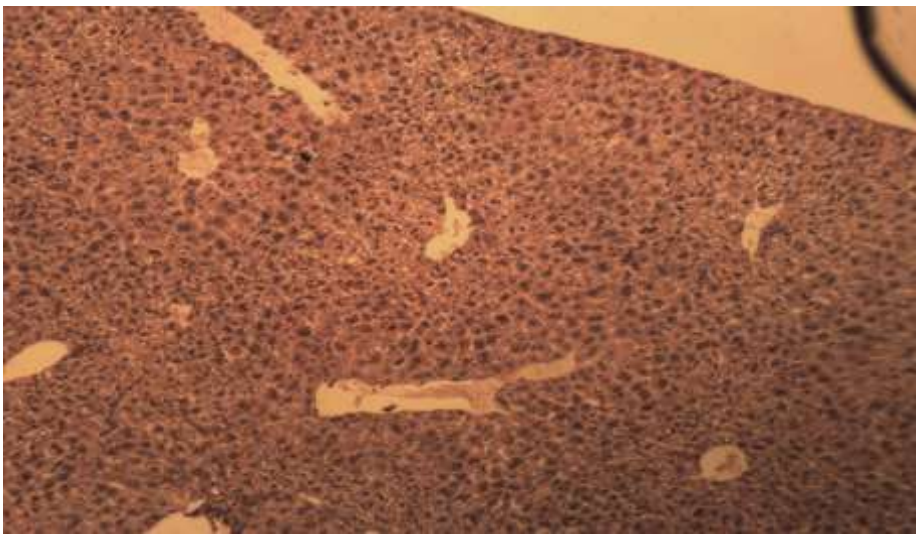


Figure 3: Shows normal liver tissue of mice in negative control group. H&E (20X).

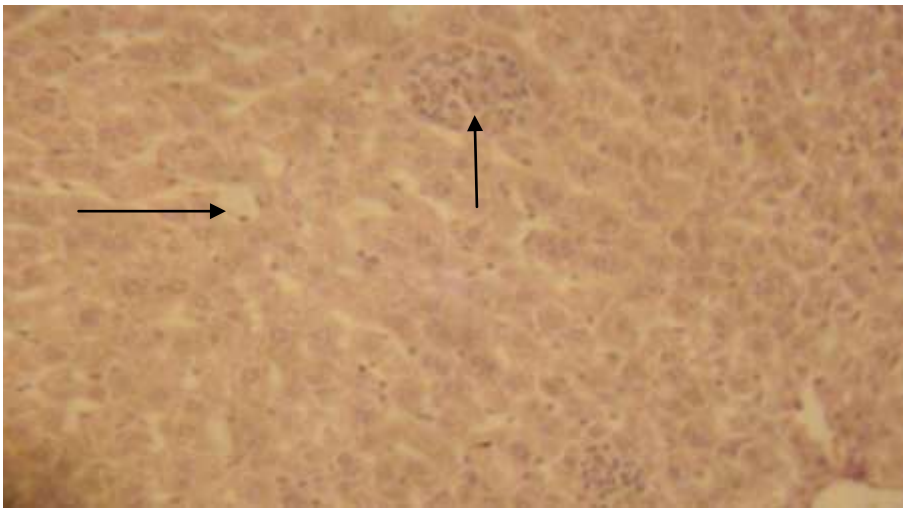


Figure4: Section in the liver tissue positive control group shows aggregation of lymphocyte (↑) hyperplasia in kupffer cells and necrosis (→) H&E(20X).

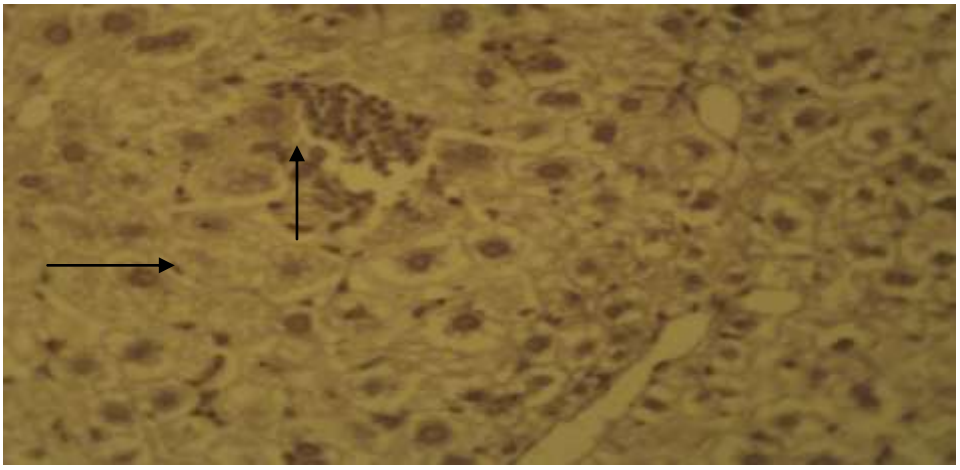


Figure 5:Shows liver of mice infected with *L. donovani* and treated with silver nanoparticles, hydropic degeneration(→) and infiltration of lymphocytes(↑)H&E (40X).

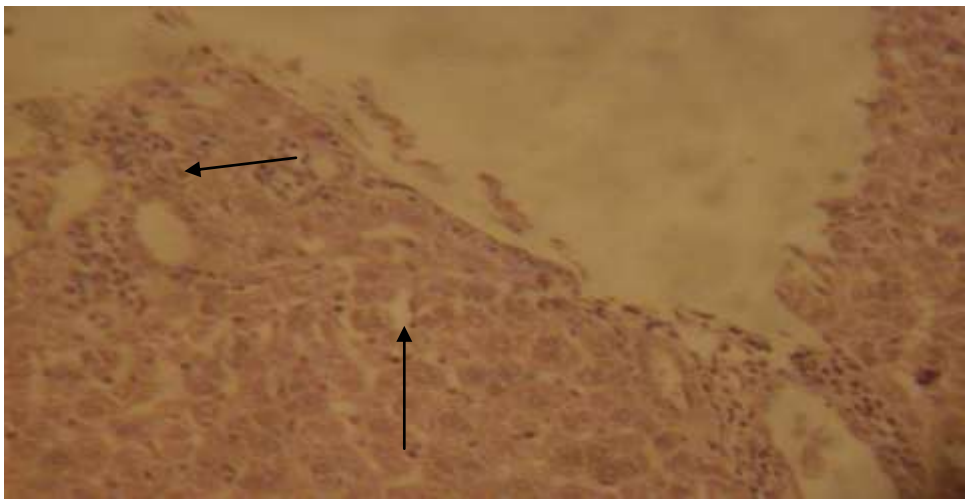


Figure 6: Section in the liver mice infected with *L. donovani* and treated with petnostam shows necrosis, hyperplasia of kupffer cells (↑) and infiltration of lymphocytes (←) H&E (40X).

Discussion

4.1 Characterization of silver nanoparticles

In this study, silver nanoparticles were synthesized from *Sphingomonas paucimobilis* bacteria , the first indication that the silver nanoparticles was synthesis in culture of bacteria when addition silver nitrate the medium color changes to brown.The brown color could be due to the stimulation of surface plasmon vibrations, which would arise from the formation of silver nanoparticles in the reaction media [25]. This reaction occurs with presence of light does not occur in the dark, Ranganath *etal*. [26]reported that the reduction of silver ion Ag + the Ag0 happens existence enzyme nitrate reductase This enzyme releases to the culture media by the bacteria, which could have reduced silver nitrate, when particles silver nanoparticles atomic force microscope (AFM) were used to characterize the size and shape of the silver nanoparticles,when examine the diameter rate of these particles was 91.74 nm. Gou *et al*.[27] found that the biosynthesis of silver nanoparticles (AgNPs) by using *Sphingomonas paucimobilis*sp BDS1 under ambient conditions were characterized with powder ultraviolet-visible spectroscopy, X-ray diffraction, Fourier transforms infrared spectroscopy (FTIR), field emission scanning electron microscopy and energy dispersive X-ray spectroscopy, the results revealed that well-scattered face centred cubic spherical AgNPs in the range of 50-80 nm were produced on the surface of *Sphingomonas paucimobilis* sp.

3.4 Antiparasitic activity of silver nanoparticles against *Leishmania donovaniin vitro*and *in vivo*

For acknowledgment, this is the first report of silver nanoparticles efficiency synthesized by *Sphingomonas paucimobilis* bacteria on *Leishmania donovani*. Many researches propose that the biosynthesized silver nanoparticles of various shapes can be separated by centrifugation, and sedimentation behavior show dependent on shape nanoparticles [28]. *Sphingomonas* is incoming and numerous microbial purse for biodegradation of aromatic compounds. It has great potential in environment protection and industrial production. The use of microorganisms for the synthesis of nanoparticles is in the limelight of modern nanotechnology since it is non-toxic, friendly to the ever-overwhelmed environment also cost effective[27].Ghosh *et al*. [29] reported that inhibitory effect of minutes silver nanoparticles against bacteria by affecting the molecule of DNA and lose the ability to replicate, as well as the presence of silver ion Ag+ become ineffective cell proteins as well as possible crashing of these proteins through the silver ion functional aggregates of the protein link. It has long been known that silver ions are considered strong and disincentives killer of bacteria and the effect on a wide range [30.]Klueh *et al*.[31]recorded that the effectiveness of silver nanoparticles minutes through a silver can link with sulfur groups (-SH) with enzymes and as a result become in effective. The nanomaterial's can occurring irregularly holes in the outer membrane of the bacterial cell and the changing of the impermeability ,that leading to the loss of important molecules of the cell and kill it [32],also silver ion enters the cell and interferes with the purine bases and Albraimaidin in a molecule of DNA, leading to breakup[33].Davies and Etris [34]suggested that the silver ion involved in stimulating the oxidation processes of the cell, leading to be bi-sulfur bond (R-S-S-R) by stimulating the interaction between oxygen and hydrogen molecule in the cell and thus liberated the water, leading to the rupture of the cell and analyzes .and the nanoparticles release silver ion which provide additional bactericidal effect[35].The efficacy of antibacterial antibiotics was found to be increased in the combination of silver nanoparticles[36].To prepare silver nanoparticles using aqueous solution of silver nitrate, dextrose, PVP and sodium hydroxide[37] because there is no similar study found ,therefore is able to provide comprehensive information about the total efficacy of the nanoparticle on parasite in the liver, and effects it on liver tissue. Therefore,we have adopted in this study repairs caused by the nanoparticles on the liver tissues and reducing the number of parasites in the spleen compared with drug petnostam and the damage caused by the parasite in the liver. Squires *et al*. [38] reported that *Leishmania donovani* causes granuloma, hemorrhage infiltration of lymphocyte and hypertrophy of liver cells. Al-Zugaibi [39] notedthatspread large number of Kupffr cells and macrophages in the liver of the infected dogs with *Leishmania donovani*.

5 Conclusion:

Silver naonparticles synthesis from *Sphingomonas paucimobilis* issafety, nontoxic and has a good antiparasitic activity, it can be used as antileshmanial drug or can be used as supportive treatment of visceral leshmaniasis.

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