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HISTOPATHOLOGICAL CHANGES IN INTERNAL ORGANS OF ALBINO MICE TREATED WITH SILVER NANOPARTICLE AFTER INFECTED BY *B. MELITENSIS*

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ABSTRACT
The objective of the present study was to evaluate the effect of silver nanopartical on the histological state of internal organ. Forty adult *Swiss Albino* mice at the age of two months were divided into four groups. The first group (G1) was considered as control negative. Second group (G2) was injected with *B. melitensis* antigen. Third group (G3) was injected with *B. melitensis* antigen and administrated orally with (60mg /kg b.w) of silver nanopartical daily for 4 weeks. Fourth group (G4) was administrated orally with (0.3mg/kg b.w) of silver nanopartical daily for 4 weeks. At end of the experiments (4 weeks), all animals were sacrificed and Specimens were taken from internal organs like liver, kidney, spleen, heart and brain. The tissues were kept in 10% formaldehyde solution, for fixation, and then processed routinely by using the histokinete. Tissue sections were embedded in paraffin blocks, and sectioned by microtome and stained with hematoxylin and eosin stain, then examined by using light microscope. The pathological lesions showed that the animals exposed to injected with *B. melitensis* antigen was characterized by inflammatory reaction, hemorrhage, congested blood vessels, necrosis, fibrosis and multiple granuloma lesions in internal organs congested blood vessels, necrosis, fibrosis and multiple granuloma lesions in internal organs while less lesions were recorded in the groups treated with silver nanopartical showed improvement against effect of *B. melitensis*, while less lesions were recorded in the groups treated with silver nanopartical and injected with *B. melitensis* showed improvement against effect of *B. melitensis* and fifth group (G5) treated by *B. melitensis* and antibiotic choses by sensitivity test but there are deferent antibiotic uses for treated *brucella*, this antibiotics used combination between two drugs like Doxycycline plus either rifampin, an aminoglycoside (streptomycin or gentamicin), or penicillin G., ciprofloxacin and tetracycline that caused perfect action to treated brucellosis. The hormonal assay, progesterone, FSH, LH and estrogen presented a significant decrease in the serum levels in the animals injected with *B. melitensis* antigen and the animals infected by *B. melitensis* treated by silver nanoparticles and antibiotics choses by sensitivity test the hormonal were equal or increased and compared to negative groups.

KEYWORDS: silver nanopartical, Histopathological changes, estrogen, Internal organs, *Albino mice.*

INTRODUCTION

Brucellosis is a common infection produced by *Brucella* species and can infect both people and animals. It is blowout by eating infected food products and through direct contact with infected animals. The bacterial infection can affect different tissues or organs and was treatment by using antibiotics. Current recommended treatment procedures contain the used of two or more antibiotics in order to duck relapses happening and to prevent prolonged use of these drugs. Antibiotics such as tetracyclines, rifampin, and the amino glycosides streptomycin and gentamicin are effective against *Brucella* bacteria. However, the use of more than one antibiotic is needed for several weeks, because the bacteria incubate within cells[1]. Silver nanoparticles are now considered a viable alternative to antibiotics and seem to have a high potential to solve the problem of the emergence of bacterial multidrug resistance [2]. In particular, silver nanoparticles (AgNPs) have attracted much attention in the scientific field. [3]Silver has always been used against various diseases; in the past it found use as an antiseptic and antimicrobial against Gram-positive and Gram-negative bacteria [4]. AgNP which preparation as antibacterial against different microorganisms likes: fungi,viruses, bacteria and parasite[5]. Silver nanoparticales synthesized other source of metallic nanoparticales was plant extract **[6]**.And synthesized by fungus**[7]** like: *F. acuminatum* expressed effectively to an antimicrobial reaction against to gram negative and positive bacteria.The plasmatic nature of nanosilver can also be used to destroy unwanted cells. The cells can be conjugated to the target cells and then be used to absorb light and convert it to thermal energy; the thermal energy can lead to thermal ablation of the target cells[8]. Animal and human studies have indicated that nano-silver can be excreted through the hair, urine, and faces majorly[9]. The mechanisms of action and binding of silver nanoparticles to microbes remain unclear but it is known that silver binds to the bacterial cell wall and cell membrane and inhibits the respiration process[10] by which the chemical energy of molecules is released and partially captured in the form of ATP. Silver nanoparticles interact with sulfur-containing proteins of the bacterial membrane as well as with the phosphorus containing compounds like DNA to inhibit replication[11] .Bactericidal effect of silver has also been attributed to inactivation of the enzyme phosphomannose

isomerase [12]that catalyzes the conversion of mannose-6-phosphate to fructose-6phosphate which is an important intermediate of glycolysis, the most common pathway in bacteria for sugar catabolism. The antimicrobial activity of silver nanoparticles has been investigated against yeast, gram negative and positive bacteria **[13].** The Ag+ cation produced interacted with the negative charge on the cell wall and affected the membrane permeability. The nano-silver cation which had greater affinity towards sulphur and phosphorus containing compounds present in the outer membrane, respiratory enzymes, proteins and DNA, penetrate through the cell wall and plasma membrane by destabilizing them and caused protein denaturation by dissipating proton motive force, intracellular ATP depletion, respiratory inhibition[12].

MATERIALS & METHODS

Experimental Design

Forty adult *Swiss Albino* mice at the age of two months were divided into four groups. The1st group (G1) was considered as control negative.. 2nd group (G2) Second group was injected with *B. melitensis* antigen. (G3) was injected with *B. melitensis* antigen and administrated orally with (60 mg / kg b. w) of silver nanopartical daily for 4 weeks as 2nd group. 4rd group (G4) was administrated orally with (0.3mg/kg b.w) of silver nanopartical daily for 4 weeks.At end of the experiments (4 weeks)and G5 treated by antibiotics choses by sensitivity test.

Treatment

Silver nanopartical is present in the form of powder.0.3 mlgiven to one mice by fine plastic stomach tube given to G3, G4 and G5 treated by streptomycin and tetracycline choses by sensitivity test.

Preparation of silver Nanoparticles:

Step of conclusion plant material:

Firstly collection of freshly leaves of olive from olive trees. Then clean leaves olive by water and leave it for dry in room temperature about 50 days, after that collection the dry leaves and grinding by electric mill well be reach fin powder. Olive powder wishing by sterile distal water then centrifuge and testing in Baghdad with analysis report NO.1077 in project of liquidation of Muthanna stores (8\5\2017) chromatogram mar q C:\GCMS solution\Data \project1\marw.QGD

Preparation of the extract

Then added the olive powder and sterile distal water each 1gm of olive powder added 10ml of distal water mixed then heated about 10 minutes till to change the color to yellowish. That mixture finally takes in the lab for cooled at room temperature then filtration by filter paper and use it[15]

Sensitivity test and antibiotic

12 to 18-hour cultures of *Brucella melitensis* (all grown in Tryptase Soy Broth). Each person will choose one culture from this list sterile cotton swab

2 plates of Trypton soya Aga

3-antibiotic disc dispensers, each dispensing three antibiotic discs

a. Dip the sterile cotton swab into the culture to be tested. With the swab, cover the entire surface of each of the Trypton Soya Agar plates such that a confluent lawn of growth would result if nothing more were to be done on the plates. Let the plates dry at room temperature for several minutes.

b. With the disc dispensers, apply three antibiotic discs to each of the plates, making sure that all eight antibiotic are represented on your plates. The instructor will demonstrate the use of the dispenser.

c. Put disc of antibiotic on the plate and use differ disc antibiotic that treated Brucella like doxycycline, gentamicin, tetracycline, streptomycin, rifampin, pancillin G.

4. Incubation 2-3 days then measuring the reaction between discs of antibiotic and growing of bacteria resistance or sensitive Incubate the plates at 37°C.

After that determination of the zoon accrued around the disk of antibiotic and chose perfect drug for treated brucellosis those choses in this table (1):

Antimicrobial agent	Disc contents	Zone inhibition primate (diameter) m.m in m.m		
		Resistant	Intermediate	Sensitive
Streptomycin	10 m.g	11 or less	12-14	22 m.m
Tetracycline	30 m.g	14 or less	15-18	19 m.m
Gantamycine	10 m.g	12 or less	13-14	17 m.m
rephampcine	30 m.g	17 or less	13-21	10 m.m
Penicillin G	10 m.g	20 or less	21-23	15 m.m
Oxycicline	10 m.g	0	0	0

Brucella in preparation

This antigen was prepared according to[16] The microorganism was cultured on (TSA) and incubated at 37č for 1-3days, then we harvested the bacterial colonies by adding 4ml of PBS, centrifuged at 3000rpm for 30 minutes at 4˚C then the precipitate was washed three times with PBS, and the precipitate was re-suspended with PBS and put in the universal tube. The universal tube that contained *B. melitensis* suspension was sonicated for 50 minutes at intervals in ice in a water-cooled sonicator oscillator at 40 MHZ/second and the homogenate was centrifuged twice by using cooling centrifuge at 8000 rpm for 30 minutes each time, to remove cellular debris. The supernatants passed through a (0.22 µm) Millipore filter, the filtered fluid was examined by gram stain and cultured on blood agar to confirm sterility of this antigen and stored at (-20 ˚C) until used.

Hormonal analysis

Blood sample should be collected from heart in mice group in each group treated but when die animal that not collected because blood clotting and sera transferred in to epindrof tube after that kept in refrigerator in a stand

position then centrifuge at 1500 rpm for 3 minute and kept in the freezer at -20 until used.
Hormonal analysis was done according to[17] this method is called radio immune assay .

Parameter

A-Clinical signs and symptoms: Clinical signs were closely observed and continuously recorded along the period of experiment which is in 4 weeks.

B-hormonal changes: The results of the present study revealed that the serum level of progesterone in animal injected with *B. melitensis* (0.236 ± 0.04) and *B. Abortus* (0.212 ±0.04) was significantly decreased as compared with the levels in the control negative group (1.740 ± 0.17) as well as significant reduction in the serum levels of FSH (12.24 ±1.23) (14.78 ±0.42) and LH (10.10 ± 0.36) (18.76 ±0.89) in animal injected with *B. melitensis* and *B. Abortus* respectively as compared with the levels in the control negative group (36.26 ±0.44),(23.16 ±0.36). but a significantly high in the serum levels of estrogen (33.02 ± 0.53), (29.96 ±0.25). Respectively in animal injected with *B. melitensis* and treatments compared with the levels in the control negative group (27.82 ±1.04). The result showed that there was a highly significant by treated antibiotics that estrogen(50.00 ± 0.14)is high than another groups and progesterone (2.320 ±0.09) and FSH, LH (37.80 ±0.51), (29.44 ±0.43).($p<0.05$) decrease in the Estrogen, FSH, LH & Progestrone concentration (ng/ml) of mice of And Control group respectively.

TABLE 1: The effect of silver Nanoparticales on serum Estrogen, FSH, LH & Progestrone concentration (ng/ml) in adult infected mice infected mice for 4 weeks

The Group	Mean ± SE (ng/)			
	Estrogen	progestogen	FSH	LH
Control	27.82 ± 1.04 D	1.740 ± 0.17 B	36.26 ± 0.44 B	23.16 ± 0.36 B
B. melitensis	30.56 ± 1.22 C	0.236 ± 0.04 D	27.76 ± 0.89 D	10.10 ± 0.36 D
B. melitensis +AgNp	33.02 ± 0.53 B	1.160 ± 0.11 C	33.60 ± 0.11 BC	21.50 ± 2.04 C
B. melitensis + tetracycline streptomycin	50.00 ± 0.14 A	2.320 ± 0.09 A	37.80 ± 0.51 A	29.44 ± 0.43 A

C-Macroscopic and Microscopic Examination

At the end of the experiments (6 weeks), all animals were sacrificed and Specimens were taken from internal organs like liver, kidney, spleen, and brain. The tissues were kept in 10% formaldehyde solution, for fixation, and then processed routinely by using the histokinete. Tissue sections were embedded in paraffin blocks, and sectioned by microtome and stained with hematoxylin and eosin stain, then examined by using light microscope[18].

Gross pathological examination

At 9th days post infection: The no infected animals showed noticeable gross lesions characterized by splenomegaly and hepatomegaly in animals which infected by *B. melitensis* in comparison with non infected animals at 9th day post infection. The liver of animals which were infected with *B. melitensis* revealed congested and dark color on the liver parenchyma (Figure, 1), and spleen surface which atrophy (Figure, 5) but in kidney seen congestion and dark color (Figure, 3), and no significant gross lesions were reported in the other examined organs. The gross lesions that were at30th day treated by silver nanoparticles: The infected animals by *B. melitensis* treated by silver nanoparticles about 21 days and seen the gross lesion liver (Figure, 2) and kidney (Figure, 4) normal size and color. Spleen (Figure, 6) was congested and enlargement size and dark red colors firstly but when treated Agnp that disappearance and the organs recover to normal color but size still elongated but in antibiotic treated that change to perfected same AgNp but size less and color little dark that seen into liver (Figure, 9), kidney (Figure,7)and spleen (Figure, 8).

Histopathological Examinations

Histopathological changes of injected with *B. melitensis* antigen after 4 weeks the main lesions in the liver were characterized by granulomatous lesion consisting of aggregation of active macrophages and lymphocytes in the liver parenchyma and necrosis of hepatocytes (Figure, 10) and mononuclear cells aggregation in portal area around proliferation of bile duct and portal blood vessels, in other animals, it was recorded pyogranulomatous lesion consisting of aggregation of neutrophils, active macrophages and lymphocytes surrounded by fibrous connective tissue in the liver parenchyma (Figure, 11) in addition to vacuolar degeneration and necrosis of hepatocytes. In other animals coagulative necrosis in the liver was appeared parenchyma surrounded by macrophages, lymphocytes as well as dilation of sinusoid and loss of hepatic cord pattern.

Spleen:The main lesion in the spleen showed proliferation of lymphocytes in the white pulp and proliferation of mononuclear cells around sinus in red pulp form cord-like appearance. In other sections myeloid like substance deposition Kidney: expressed mononuclear cells aggregation in the interstitial tissue and atrophy of glomerular tufts with dilated of boman space and fibrosis of their walls (Figure, 14), in other animal, the kidney revealed mononuclear cells infiltration with proliferation of fibroblast between renal tubules with acute cellular degeneration of epithelial cells of renal tubules around congestion red pulp was seen.

Histopathological examination of group was injected with *B. melitensis* antigen and administrated orally with (0.3 mg / kg b.w) of silver nanopartical daily for 4 weeks liver :The histopathological examination showed granulomatous lesion in the liver parenchyma with congested sinusoids and vacuolar degeneration of hepatocytes, in addition to neutrophils and mononuclear cells aggregation in one side of central vein. In other

animals, it was reported mononuclear cells aggregation around blood vessels with apoptosis of hepatocytes (Figure, 15) and coagulative necrosis of hepatocytes characterized by pyknotic and disappearance of nuclei in addition to marked vacuolar degeneration of hepatocytes and focal mitotic division of the nuclei (Figure, 16). In other section, mononuclear cells infiltration in the liver parenchyma with mitotic figure in the hepatocytes form pseudolobules.

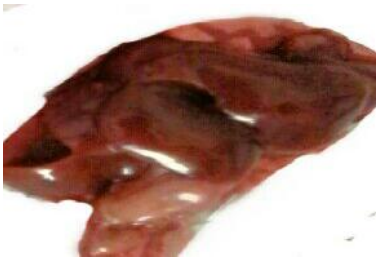
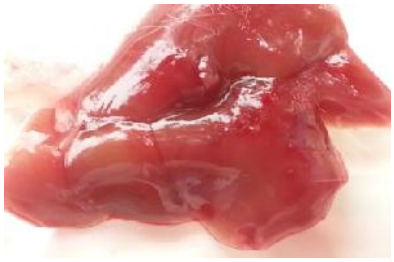
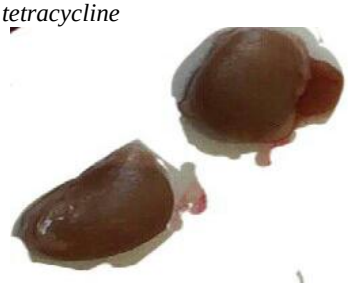
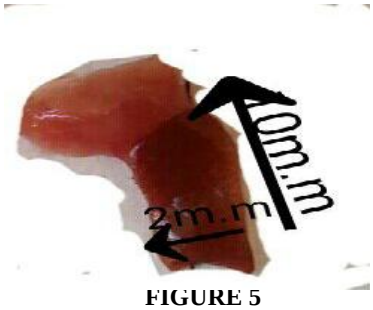
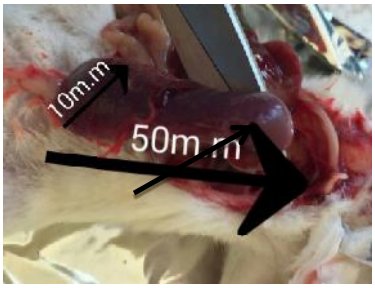
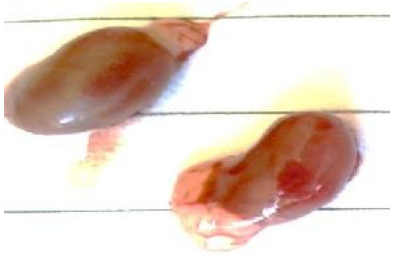
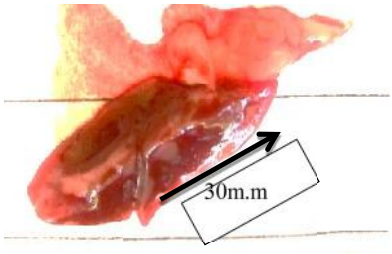
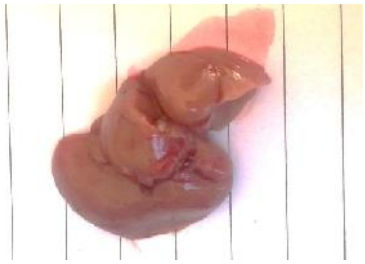
Spleen: It was noticed inflammatory cells particularly neutrophils infiltration in the congested red pulp with depletion of white pulp (Figure, 17).

Histopathological changes of groups was administrated orally with (0.3mg / kg b.w) of silver nanopartical daily for 4 weeks

Liver: (Figure, 18) There are multiple foci of mononuclear cells aggregation mainly lymphocytes around blood vessels and bile ducts (Figure, 19).

Spleen: Showed increase thickness of capsular region due to proliferation of fibrous connective tissue and lymphocyte infiltration in addition to hyperplasia lymphocytes in the periarteriolar sheath. The main lesions in the examined organs of this group characterized by mononuclear cells aggregation around blood vessels and central veins in the liver, hyperplasia of white pulp of the spleen (Fig:17) and blood vessels of other examined organs in addition to the kidney showed MNCS in the interstitial tissue and between renal tubules (Figure, 20).

Histopathological changes of groups was administrated streptomycin (I\M) and tetracycline orally daily for 4 weeks -liver multiple focal aggregation of lymphocytes and macrophage around blood vessels and bile duct and cogulative necrosis and vacuolation of hepatocyte and proliferations of coffer cell spleen decrease thickness of capsular due to high proliferation of cell in red and white pulp with proliferation of lymphocyte and present protein fiber that accumulation in white pulp defuse amyloidosis and dilation of lymphoid follicles, kidney that present less congested and same natural but well be recover organs during time .

NO.	*Brucella. melitensis*	*B. melitensis+ Agnps*	*B. melitensis+ streptomycin+ tetracycline*
Liver			
	FIGURE 1	**FIGURE 2**	**FIGURE 3**
Spleen			
	FIGURE 4	**FIGURE 5**	**FIGURE 6**
Kidney			
	FIGURE 7	**FIGURE 8**	**FIGURE 9**

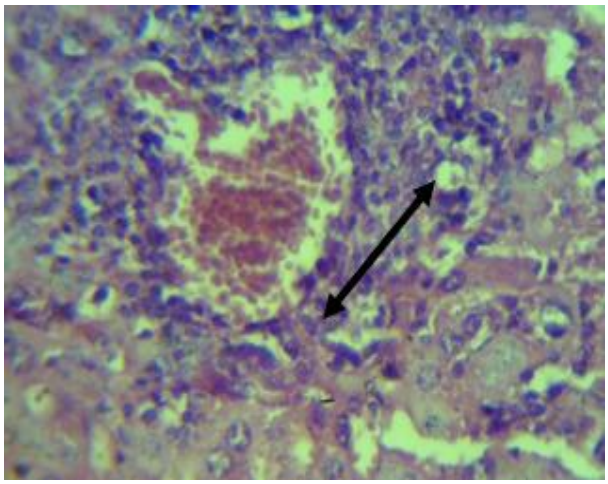


FIGURE 10:-Histopathological section in the liver of animal post-infection *B. melitensis* with shows inflammatory cells particularly neutrophils in the lumen of congested central veins and congested sinusoids (H&E stain 40X).

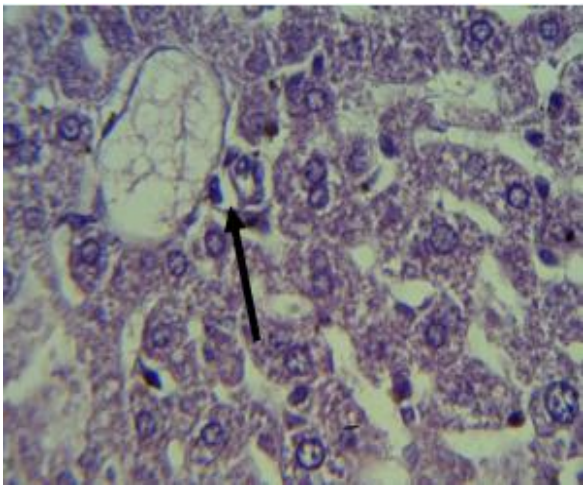


FIGURE 11:-Histopathological section in the liver of infected animal showed severe balloon vaculation of hepatocytes and proliferation of kupffers cells (H&E stain 40X)

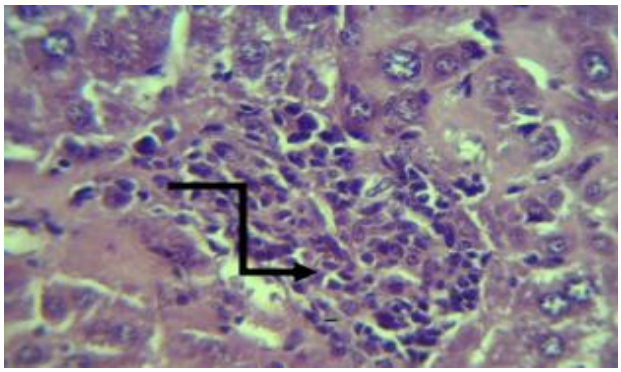


FIGURE 12:-Histopathological section in the liver of post-infection shows pyo granulomatous lesion consisting from active macrophages and lymphocytes in the liver parenchyma (H& E stain 40X).

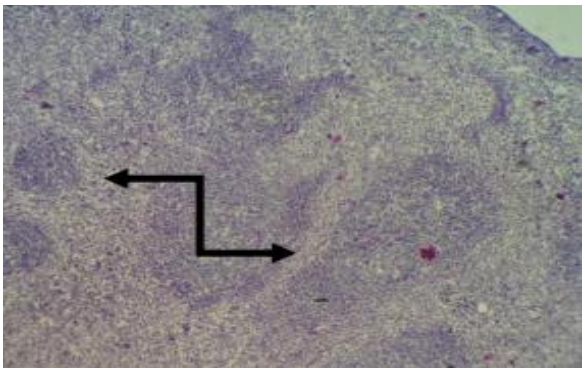


FIGURE 13:-Histopathological section in the spleen of animal at day 1 months post-infection showed hyperplasia of white pulp, and neutrophils mononuclear cells infiltration in the capsular region and extended to spleen parenchyma (H & Estain 10X)

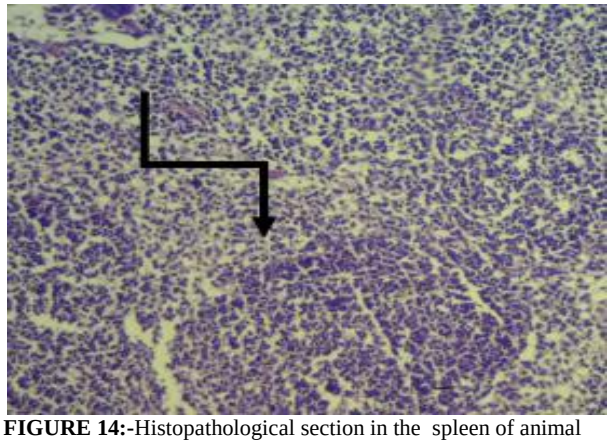


FIGURE 14:-Histopathological section in the spleen of animal post-infection showed hyperplasia of white pulp, and neutrophils mononuclear cells infiltration in the capsular region and extended to spleen parenchyma (H & Estain 10X)

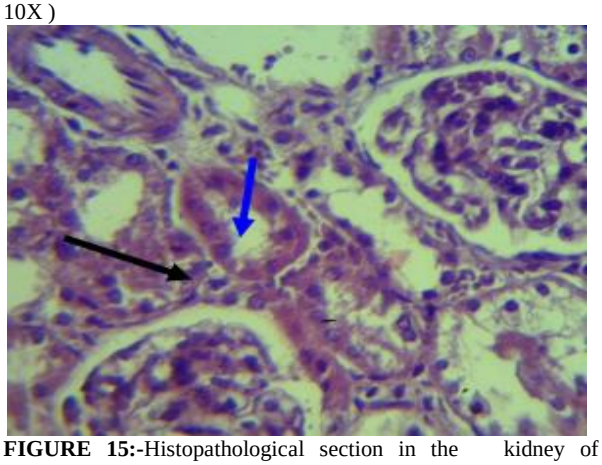


FIGURE 15:-Histopathological section in the kidney of infected animal showed marked mononuclear cells aggregation in the interstitial tissue with acute cellular degeneration of epithelial cells of renal tubules (H &E stain 40X)

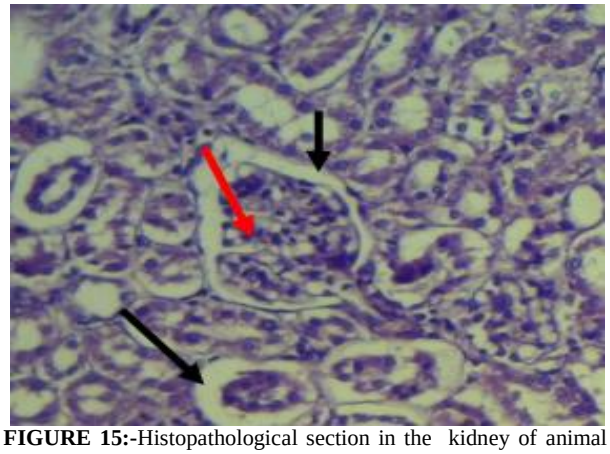


FIGURE 15:-Histopathological section in the kidney of animal post-infection showed vacuolation of glumerular tufts and macrophages aggregation in necrotic area around glumerula (H&Estain 40X) . ➡

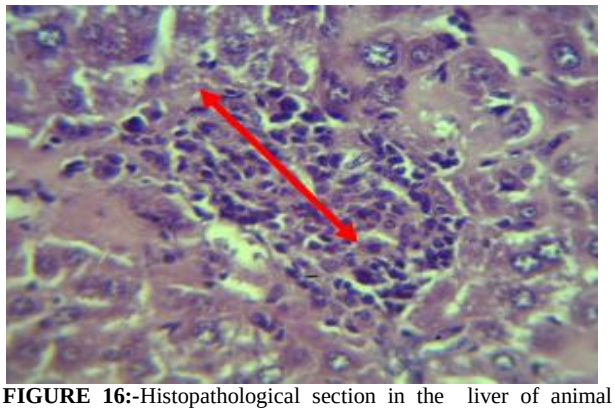


FIGURE 16:-Histopathological section in the liver of animal post-infection *B. melitensis* and administrated orally with (60 mg / kg b.w) of silver nanopartical showed multiple granulomatous lesions consisting from aggregation of mononuclear, macrophage cells, scatter in liver parenchyma and inflammatory cells in dilated sinusoids ↔ (H&E stain 40X).

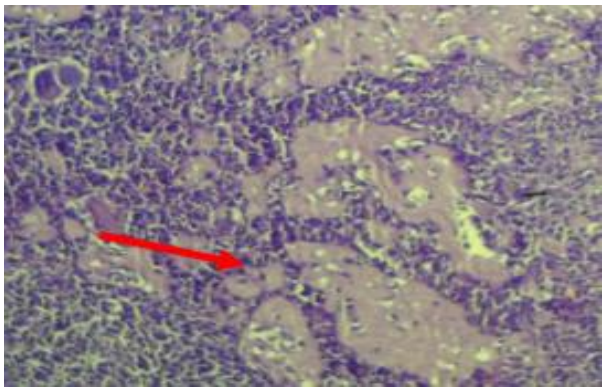


FIGURE 17:- Histopathological section in the spleen of animal post-infection with *B. melitensis* and administrated orally of silver nanopartical showed amyloid and inflammatory cells infiltration of congested red pulp with proliferation of megakarocytes ↔ (H&E stain 10X)

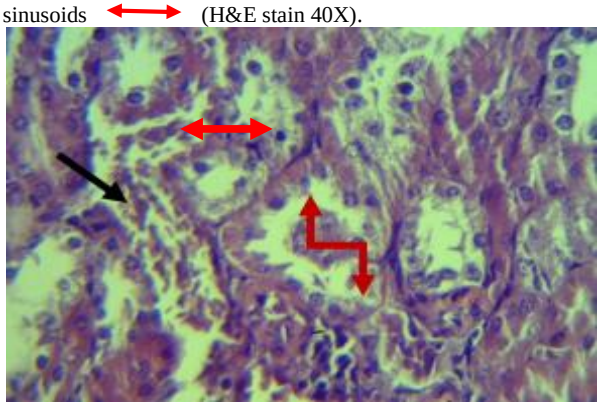


FIGURE 18:-Histopathological section in the kidneyr of animal post-infection *B. melitensis* and administrated orally of silver nanopartical showed neutrophils and mononuclear cells aggregation around and in the lumen of congested blood with acute cellular degeneration in the epithelial lining cells of renal tubules (H&E stain 40X)

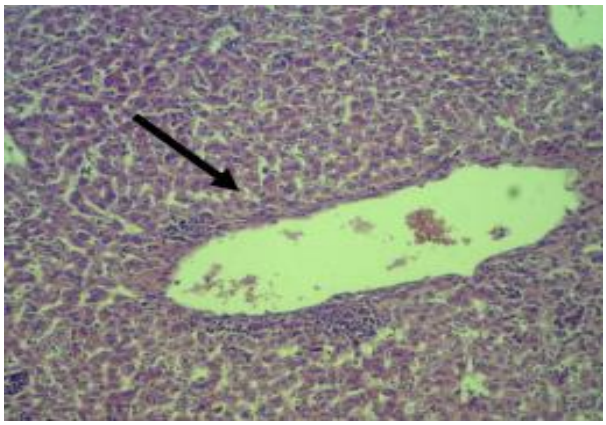


FIGURE 19:-Histopathological section in the liver of post-infection showed multiple foci of mononuclear cells aggregation mainly lymphocytes around blood vessels (H& E stain 10X).

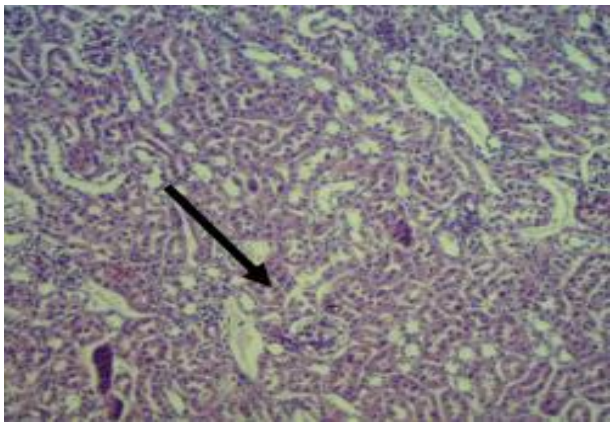


FIGURE 20:- Histopathological section in the kidney of animals administrated silver nanopartical showed neutrophils and mononuclear cells aggregation bettwen renal tubules (H&E stain 40X)

DISCUSSION

Very severe lesions were recorded in examined organs of pregnant mice with *Brucella*, as compared with those lesion were recorded in other groups this result may indicate that indicate that DTH reaction against *Brucella* [19]reported epithelioid granuloma the liver and spleen of patients during *Brucella* infection, neutrophils infiltration in the spleen and liver may indicate active chronic infection induced by *Brucella* in animals with impair immune response. This result is consistent with [20] who recorded abscess in the spleen and liver of patients during acute phase of brucellosis infection and the body

attempts to localize and eradicate the infection, this result is consistent with[21] who recorded hepatic granuloma consisting from aggregation of macrophages and DCs after one weeks post-infection of mice with *Brucella* and these organisms were localized inside macrophages within micro granuloma.

Brucella engulfed by macrophages were rapidly killed post-phagocytosis and activated macrophages were efficient killed intracellular *Brucella* but disseminated the Brucella infection in all examined organs, this may indicated that these pathogens depressed immune system of the host[22].

The presence of pathological lesions in examined organs at one month of infection may be indicated that the mice expressed chronic form of the brucellosis, this idea is consistent with [23]who demonstrated that *Brucella* can modif the immune responses that elicited during infection and lead to development of chronic infection in mouse model. We noticed moderate lesions in the internal organs of infected animals and adminstration with silver nanoparticles and animals adminstration with silver nanoparticles as compared to those lesions in control positive and negtive groups, these observations may be nanoparticles are now considered considered a viable alternative to antibiotics and seem to have a high potential to solve the problem of the emergence of bacterial multidrug resistance[24] . In particular, silver nanoparticles (AgNPs) have attracted much attention in the scientific field [25]. Silver has always been used against various diseases; in the past it found use as an antiseptic and antimicrobial against Gram-positive and Gram-negative bacteria [26] due to its low cytotoxicity. AgNPs were considered, in recent years, particularly attractive for the production of a new class of antimicrobials [27] opening up a completely new way to combat a wide range of bacterial pathogens.

The antimicrobial action of AgNPs can be classing in two types: the inhibitory and bactericidal actions. In the Ancient theory bacterial cells aren't killed but their compartment is prevented whereas in the later bacterial cells will dead due to the reaction of AgNP [28]

Silver nanoparticles effect on organs by reduction in the levels of pro-inflammatory cytokines was also demonstrated in a mouse model with burn injury when silver nanoparticles were introduced [29]. It was also found that silver nanoparticles can inhibit the activities of interferon gamma and tumor necrosis factor alpha, which are involved in inflammation [30].

In addition the plasmatic nature of nanosilver can also be used to destroy unwanted cells. The cells can be conjugated to the target cells and then be used to absorb light and convert it to thermal energy; the thermal energy can lead to thermal ablation of the target cells[31].

The present moderate lesions in organ in these groups may be due to AgNPs causes damage of the plasma membrane and apoptosis/necrosis assays detects changes in cell membrane permeability of infected cells[32].

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