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RESEARCH ARTICLE

Histopathological and Hematological Effect of Silver Nanoparticles against acute Escherichia coli Infection in Male Rats

Ghusoon A.A Alneamah[1*], Bairmani M. SH[2], Hawraa M. Murad[1]

[1]*Collage of Veterinary Medicine/Department of Pathology and Poultry Diseases AL-Qasim Green University -Babylon -Iraq.*

[2]*College of Biotechnology, Department of Genetic Engineering, AL-Qasim Green University -Babylon -Iraq.*

***Corresponding Author: Ghusoon A.A Alneamah**

Abstract

The potential treatments of silver have known long time ago. Recently many researchers have used silver nanoparticles in the area of medication because their antimicrobial, bio affinities and their easy ways to synthesis. In the present study we have estimated the antimicrobial and hematological effect of silver nanoparticles (AgNps) against acute *E.coli* infection in male rats. firstly the Ag Nps was synthetic and characterization by SEM which reviled size in about 25 nm in diameter, UV-vis have shown peak absorption in 410 nm. (40) Male rats use in the experiment was divided equally into (4) groups as following (n=10): Group I: Rats served as control and gavage 0.4 ml/ rat from distilled waters for three days. Group II: Rats served as experimental and gavage 0.4 ml/rats from AgNps for three days .Group III: Rats infected with *E. coli* (0.2)ml/rat single dose (I/P).Group IV: Rats infected with1×10^{9}CFU of *E.coli* (0.2)ml/rat single dose (I/P) + gavage 0.4 ml/rats from AgNps for three days. After three days the animals were sacrificed, the blood collected directly from the heart for hematological analysis, and the specimens from liver, and intestine were dissected out for histopathological examination. The hematological results showed significant decrease in RBCs, Hb compared with control group and increase in WBCs in infected group compared with infected group .While this value improved in group (II, IV). Regarding the histopathological results the infected group showed inflammatory cells infiltration, necrosis, and hemorrhage in both organs and micro thrombi in liver. After treatment with AgNps showed improvement in the both hematological and histological parameters.

Keywords: Silver nanoparticles (AgNps), *Escherichia coli*, Histopathology, Rats.

Introduction

Numerous of researches now days are focusing on the use of conventional and innovated nanoparticles on several biological systems because of their new properties and consequently their applications .Due to their high efficiency, biosafety, biocompatibility, easy to synthesis and cost effective scientists have synthesized various types of nanoparticles to fight pathogenic bacteria.

Among those properties is increasing surface ratio compering to the volume, nonetheless size depend property is the key issue that nanoparticles relaying on. Materials will be more efficient and reliable biocompatible tools if their size will be reduced [1]. Medically this property has been used in various fields of researches including diagnostics, drug delivery, gene delivery and antimicrobials [2]. Microorganisms have developed many strategies to overcome various types of antibiotics within the time, the matter which raised an important issue called antibacterial resistance. Nanoparticles however have been candidate as a useful implement to over whelmed this notorious phenomenon.

The safe and high efficiency of the antibacterial activity of silver nanoparticles compared with the conventional antibiotics made it powerful competitive in the race of

the new antibacterial drugs. Where it has the ability to kill about 650 types of diseases causing microorganisms [3, 4].Silver nanoparticles have shown valuable biocide beside a variety types of bacteria including *E-Coli* [5, 8]. Because of its reaction with oxygen silver ion will be directly formed in silver nanoparticle surface which is responsible for the antibacterial activity of silver nanoparticle [9].

Silver have been used in various medical applications on human because of its safety [10]. This work was carried out to synthesis silver nanoparticles and to conform its nanosize by SEM, to infect rats by E-coli, to estimate the antimicrobial activity of Ag NPs *in-vivo*.

Material and Methods

Experimental Animals

Forty male rats were used for the present study. The animals were housed in metal cages in the animal house of the Veterinary Medicine College- University of Al Qasim green and were fed on standard rat pellets, with water provided *ad libitum*, they were allowed to acclimatize for 10 days at room temperature.

Chemical

Silver nanoparticles was synthetic according to [11]

Scanning Electron Microscope (SEM)

Morphology and size of AgNps were estimated and characterization by SEM.

Experimental Protocol

Forty male rates use in the experiment aged [12] weeks and weight (70 -120) gm were divided equally into (4) groups as following (n=10): Group I: Rats served as control and gavage 0.4 ml/ rat from distilled waters for three days. Group II: Rats served as experimental and gavage 0.4 ml/rats from AgNps for three days. Group III: Rats infected with *Escherichia coli* (0.2) ml/rat single dose (I/P).Group IV: Rats infected with *E. coli* (0.2) ml/rat single dose (I/P) + gavage 0.4 ml/gm from AgNps for three days.

Induce Infection by E Coli

Infection was induced by I/p administration of 0.2 ml/rat sterile phosphate-buffered saline containing 1×10^{9}CFU of *E. coli* [12].

Hematological Analysis

Was made by mythic 18 vet device for whole blood analysis /France

Histopathological Study: According to the [13].

Statistical Analysis

Least significant difference (L.S.D.) was used to compare the significant difference between means. Data were analyzed using statistical analysis system (SAS) (2001) program

Results

Characterization of Silver Nanoparticles

Silver nanoparticles have been characterized using SEM and Uv- Vis where SEM have shown spherical shape and size in about 25 nm.(Fig. 1).

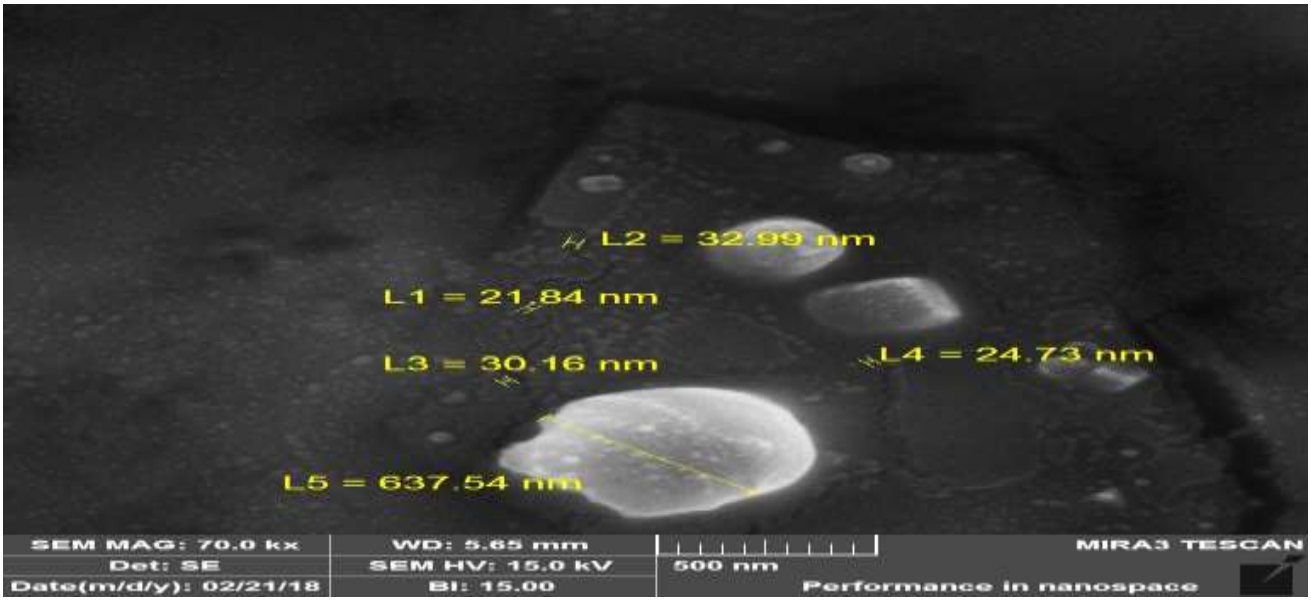


Fig1: showing the morphology and characterizations of silver nanoparticles in SEM

While UV-Vis have shown absorption peak at 410 nm (Fig 2) which was the same absorption obtained by [14]. Which indicate the total conversion of silver Ion to silver nanoparticles.

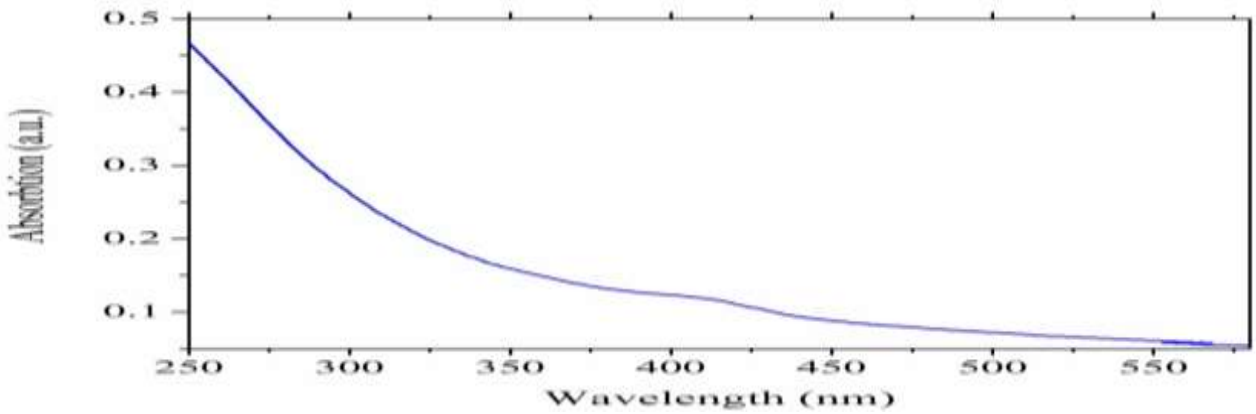


Figure 2: UV Visable Spectrophotometer of silver nanoparticles

Hematological Study

The results of blood parameters for different groups of experiment showing in Table (1).

Table 1: Showing the mean ± SE value of blood parameters for different experimental groups

Groups	RBCs x10⁶/mm³	WBCs X10³/mm³	Hb g/dL	Lymphocytes %	Neutrophils %
GI	47.73±0.12A	7.50 ±0.01C	12.49 ±0.20A	40.80±0.12D	35.80 ±0.03D
GII	39.77±0.12D	10.70±0.08A	9.62 ±0.07A	55.76±0.20B	45.81±0.01A
GIII	45.77±0.04B	7.51 ±0.14C	11.48±0.13B	50.55±0.07C	40.21±0.23C
GIV	44.68±0.92C	8.48±0.07B	12.30±0.07C	52.43 ±0.23A	43.40±0.06B

The different capital letters mean Significant differences at (P≤ 0.05)

Histopathological Study

Control groups (GI)

Showed normal histological strictures (Fig 3, 8).

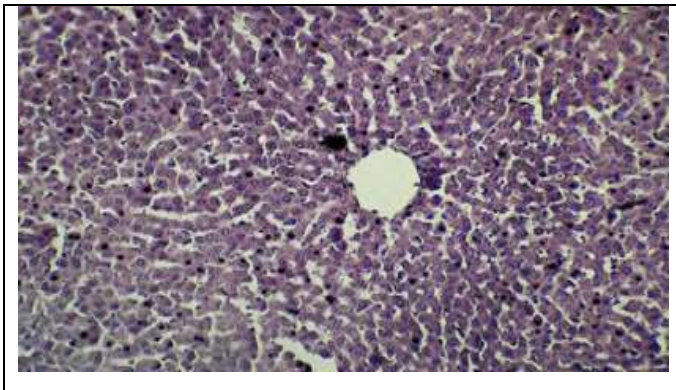
Treated Groups

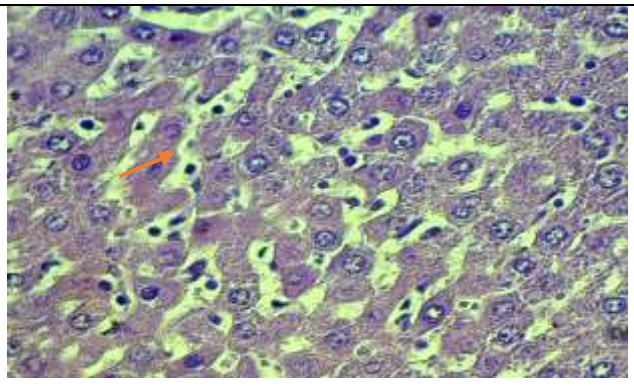
Liver

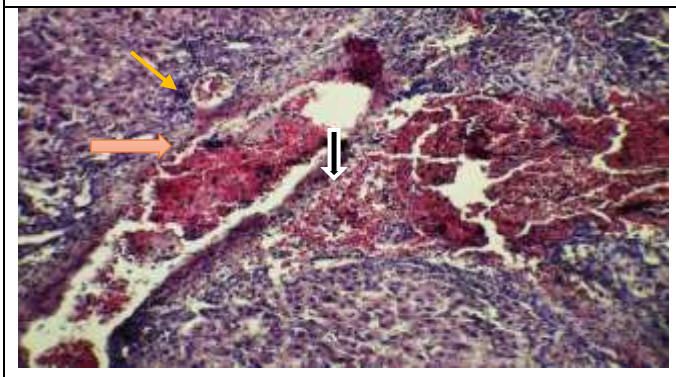
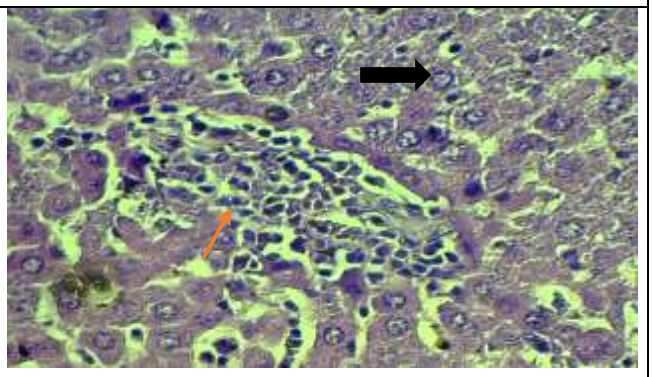
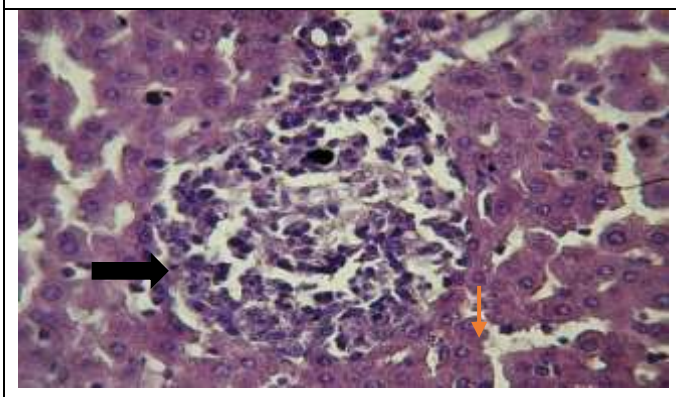
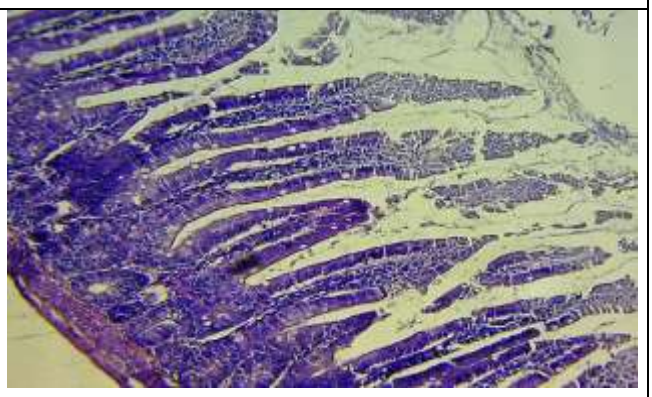
The main histopathological changes in the groups those treated with (GII) was only mild dilatation in the sinusoids with increase in number of kupffer cell (Fig4).Whereas in the infected group with E.coli (GIII) showing sever congestion and dilatation of central vein with wildly distraction in the liver tissues leading to oozing of blood and inflammatory cells in to damaged area with formation of micro thrombi (Fig5) dilation of the sinusoids with hepatocellular necrosis and inflammatory cells infiltration was also seen (Fig 6). (GIV) was showed formation of immune granuloma (Fig7).

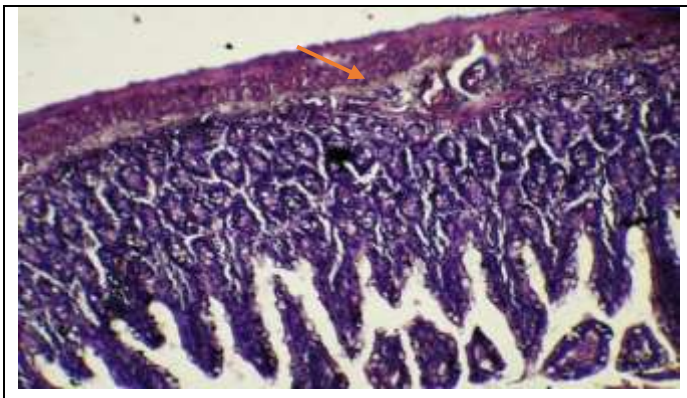
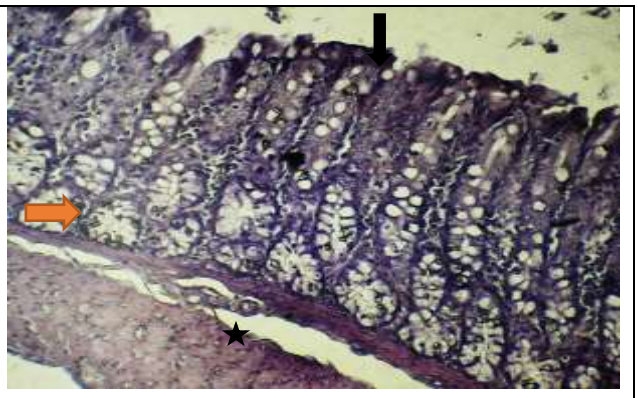
Intestine

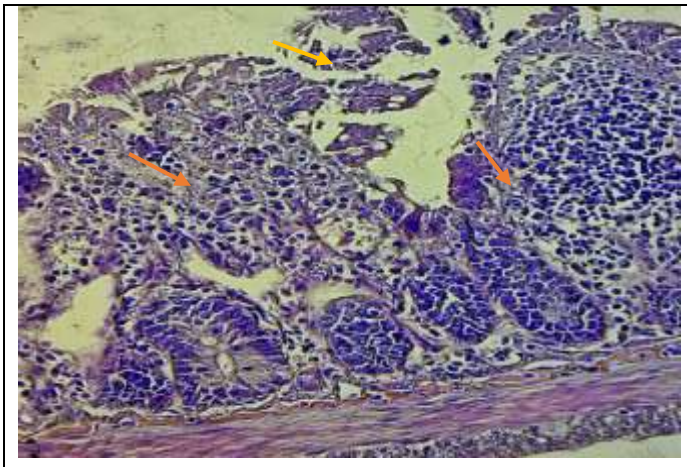
Histopathological section of (GII) treated groups showed only mild congestion in the intestinal blood vessels (Fig 9).In (GIII) showed several intestinal effects including flattened of the intestinal villi with crypts necrosis, the submucosa and separated from muscular is layers (Fig10), with necrosis of intestinal villi with inflammatory cells infiltration (Fig11).In the (GIV) showed normal intestinal glands with hyperplasia of gut lymphoid tissue (Fig12).



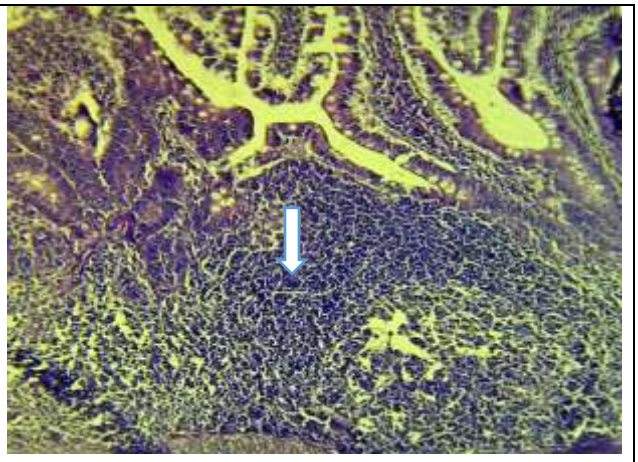


3:Histopathological section of liver of rat for control group(GI) showing normal histological stricture H&E (100X)	4: Histopathological section of liver of rat of (GII) showing slightly dilatation in the sinusoids with increase in number of kupffer cell (→) H&E (100X).
	
5: Histopathological section of liver of (GIII) showing sever congestion and dilatation of central vein (⇨) with sever distraction in liver tissues leading to oozing of blood and inflammatory cells into distracted area (⬇) micro thrombi also seen (→)H&E (100X).	6: Histopathological section of liver of rat of (GIII) showing diffuse hepatocellular necrosis (➡) with inflammatory cells infiltration (→) H&E (400X).
	
7: Histopathological section of liver of rat of (GIV) showing formation of immunological granuloma (➡) with dilation of sinusoids (→) H&E (400X).	8: Histopathological section of intestine of rat for control group (GI) showing normal histological stricture H&E (100X).

	
9: Histopathological section of intestine of rat of (GII) showing only mild congestion in the intestinal blood vessels (arrow) H&E (200X).	10: Histopathological section of intestine of rat of (GIII) showing flattened of the intestinal villi (⬇) with crypts necrosis (➡), the submucosa and separated from muscular is layers (★) H&E (200X).



11: Histopathological section of intestine of rat of (GIII) showing necrosis of intestinal villi (→) with inflammatory cells infiltration (→). H&E (400X).



12: Histopathological section of intestine of rat of (GIV) showing hyperplasia of gut lymphoid tissue (arrow). H&E (400X).

Discussion

Silver nanoparticles have been known with their wide spectrum antimicrobial activity because their strong toxicity to a broad range of microorganisms [15, 16]. In this investigation we have studied the antimicrobial activity of silver nanoparticles against E-coli infections. However the results showed that infection with E. coli caused significant reduce in a blood parameters RBC, Hb (39.77±0.12, 9.62 ±0.07) respectively this results may be occur due to break down of erythrocytes by hemolytic enzymes produced by E -coli, or maybe due to e-coli induce enteritis and lead to nutritional deficiency and this lead to decrease in erythrocytes number and hemoglobin concentration this result agreed with [17, 18, 19].

Also The infected group showed significant increase in WBCs, lymphocytes and neutrophil percentage (10.70± 0.08, 55. 76±0.20, 45.81±0.01) respectively compared with control group this results occur due to E.coli infarction cause increase in total leukocytes count or due to response to systemic infection like colibacillosis and salmonellosis this agreed with [20,21,17].

Although AgNps cause elevated level of WBCs and Hb and immune cells this was may be immunological impact or stimulation of bone marrow Productions [22]. Also AgNps activation of immune response which indicated by focal mononuclear cells infiltration, formation of immune granuloma in liver and hyperplasia of gut lymphoid tissue anal intestine [23].

The histopathological results show slightly dilutions and the sinusoidal on the Liver tissues where in the intestine was no pathological lesions only mild congestions in the blood vessels this changes may be occurring due to In the group that infected with e-coli the result was sever dilutions and congestions of blood vessels of both liver and intestine with inflammatory cells infiltration, necrosis also seen in the both organs.

In the liver necrosis of the hepatocyte leading to distractions of the wide area and oozing of the blood into the area causing hemorrhage and micro thrombi the diffuse inflammation and necrosis may be due to attachment of bacteria to the intestinal cells which caused disquamanation of microvillus and may be flattened and change in the cells morphology [24].

The necrotic changes with hemorrhage that occur in the intestine and liver may be due to alpha and beta hemolysis that cause lysis of the organs [25,18] also showed hemorrhagic foci and micro thrombi by which may cause due to intravascular hemolysis and vascular damage [26].

Intestine also showed atrophy of submucosal glands with a flattened in the intestinal villi this changes possible occur due to LPS in the bacterial cell wall it has immunological role, this pathway due to aggregation of leukocytes with distractions of the endothelial layer finally may lead to organ failure agreed with [27, 28].

E-coli have affinity to epithelial cell type [29]. Properties assess the E.coli cause damage to epithelial cells to different organ and leakage of the blood and inflammatory cells into the adjacent area.

This result agreed with [30] he deported that hemorrhage and congestion were observed in various organs indicates septicemic natures of disease [30, 31]. Also reported that equals histopathological changes in mucosal epithelia of intestine including hemorrhage, necrosis and desquamation, Inflammatory cell infiltration, also mucosa was showed vacuolations and flatting in there villi the lesion in the above organs was possibly due to response and reaction in detoxification and execution of the endotoxin release by E. coli.

After treatment with silver nanoparticles the histopathological changes showed different degree of improvement especially in the liver and intestine that leading to formation of immune granuloma, improvement in the intestinal glands with decrease in the amount of inflammation in these organs, this improvement may be due to the bactericidal activity of silver nanoparticles this results agreed with other studies applied by [32, 1].

They reported that "Antimicrobial effect of silver nanoparticles against E.coli and recommended them for new bactericidal agents."There are several mechanism used to discriped the antimicrobial activity of silver nanoparticles, many studies detected that effect may be due to interaction with bacterial cell wall and lead to formations of pores in this wall, and accumulations of the silver nanoparticles in pits caused increase the permeability of cell membrane [33, 34], there suggestions that the nanoparticles affections the bacterial cytoplasmic protein and lead to cell death [35, 36].

Studies reported that silver nanoparticle can accumulate in the bacterial cell membrane leading to increase the permeability and this leakage of cell content me be because reactive oxygen species [37, 38, 32, 40]. Other research for postulated that the silver nanoparticles can effect on DNA replication by inhibition of bacterial DNA replication damage of bacteria cytoplasmic membrane decrease in the level of the intracellular adhesions Triphosphate (ATP) and finally cause cell death [15, 35].

Conclusion

The present study showed that AgNps have strong antimicrobial effect against E.coli infection and ameliorate of hematological parameters and histopathological lesions also activation of immune response which indicated by focal mononuclear cells infiltration, formation of immune granuloma in liver and hyperplasia of gut lymphoid tissue of intestine.

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