

The Prophylactic Effect of Ficus carica Nanoparticles on C57BL/6 Female Mice Infected with Schistosoma mansoni

Naira Adel El-Attar

nairaadel65@gmail.com

Mansoura University

Mamdouh Rashad El-Sawi

Mansoura University

Eman Ahmed El-Shabasy

Mansoura University

Research Article

Keywords: Schistosoma mansoni, Praziquantel, Prophylaxis, Ficus carica, Ag-NPCs

Posted Date: January 6th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-5735794/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Schistosomiasis has re-spread again lately in Africa including Egypt according to World Health Organization in 2023. Bilharziasis is an endemic disease that causes damage to the lungs, gastrointestinal, and liver. Praziquantel (PZQ) is the most effective commercial treatment for all *Schistosoma* species, although it cannot cause total death after infection besides being ineffective against larvae and eggs. The thesis aimed to study the prophylactic effect of *Ficus carica* leaves extract nanoparticles (*F. carica*-NPCs), silver nanoparticles (Ag-NPCs), and in combination (*F. carica*-Ag NPCs) against *Schistosoma mansoni* infected mice. Schistosomiasis could cause hepatic injury which showed decrease in GSH, SOD and CAT (antioxidants) and an increase in oxidative stress marker MDA in comparison with the negative control group. It caused increase in inflammatory markers such as CRP, IL-6, VCAM-1 and ICAM-1 in comparison to healthy control group. It elevated P53, Bax, cytochrome C, caspase 9, 3 otherwise Bcl-2 as apoptotic & anti-apoptotic markers in comparison with negative control group. Comet tail length and tail DNA showed significant DNA damage in infected mice compared to healthy control. Finally, hepatic cell membrane leak out occurred leading to increase in serum ALT, AST, ALP, γ -GT, and bilirubin unlike albumin as liver function parameters compared with the negative control group. The

F. carica-NPCs, Ag-NPCs, and *F. carica*-Ag NPCs recorded significant amelioration in previous markers compared with the infected-untreated group. The *F. carica*-NPCs, Ag-NPCs, and *F. carica*-Ag NPCs had anti-schistosomal and hepatoprotective effects in comparison with the positive control group. The *F. carica*-Ag NPCs observed a better prophylactic effect than other groups.

1. Introduction

Schistosomiasis is a disease in which people are infected by cercariae that are carried *via* contaminated water. Female *S. mansoni* lays eggs in the venules of the liver and large intestine which can cause clinical sickness as they remain in these tissues and can cause granulomatous reactions, fibrosis, and cirrhosis (Saad El-Din et al., 2023).

Until now, the main treatments for *Schistosoma* infections are PZQ, oxaminoquine, and metrifonate (El-Shabasy et al., 2022). PZQ is the most effective against all *Schistosoma* species with a cure rate of 100% in the past and 65–90% after a single oral dose (Saad El-Din et al., 2023). Now, because of worm resistance and host tolerance, the curable rate is not recorded as 100%. In addition, it is inefficient against schistosomules (the parasite juveniles) and ova, making it inappropriate for mass therapy in high endemic regions so the reinfection still occurs (Doenhoff et al., 2008).

One of the oldest trees, *Ficus carica* L., is a member of the Moraceae family, which is mostly planted in the Mediterranean region. Its fruit is a great source of organic acids, sugar, vitamins, and minerals (El-Attar et al., 2024). *F. carica* phytochemicals are phytosterols, anthocyanins, amino acids, organic acids, fatty acids, phenolic compounds, hydrocarbons, alkaloids, aliphatic alcohols, and volatile compounds isolated from various parts of the plant (Renda et al., 2023). The majority of phytochemicals are

flavonoid substances that have hepatoprotective, renoprotective, anti-viral, anti-microbial, anti-schistosomal, anti-cancer, anti-cholinesterase, antioxidant, anti-inflammatory, and anti-DNA damage properties (Jeong and Lachance, 2001; Veberic et al., 2008).

Nanotechnology has completely changed drug delivery, according to the tiny particle size, large exposed surface area, increased physical and chemical stability, and high biocompatibility of its constituents. In addition, nanotechnology can improve the solubility, permeability, and bioavailability of a drug regardless of its physical properties (Abd El Hady et al., 2023). Rather than being physical or chemical, the green biosynthesis approach of nanoparticles has received a lot of attention because it is an efficient, cost-effective, lower systemic toxicity and environmentally friendly way of manufacturing, the silver nano metal among others have demonstrated potent schistosomicidal effects when compared with gold nanoparticles (Moustafa et al., 2018; Abd El Wahab et al., 2021).

The present study aimed to evaluate the role of *F. carica* nanoparticles and Ag nanoparticles against schistosomiasis mansoni to replace the use of PZQ as a treatment.

2. Materials and Methods

2.1. Materials

2.1.1. Chemicals

The Egyptian International Pharmaceutical Industries Company (EPICIO), located in Mansoura, Egypt, was the supplier of the PZQ tablets.

2.1.2. Experimental animal groups

Thirty black female mice (C57BL/6) were used in the presented study. They were divided randomly into 6 groups: G1: Negative control; healthy group. G2: Positive control group; infected with cercariae and non-treated. G3: PZQ; in the 7th week after *S. mansoni* cercariae infection, 200 mg/kg was given orally by oral tube for two consecutive days.

G4: Administered by *F. carica*-NPCs; 400 mg/kg b. w. *via* oral gavage day after day; 3 times (during the 1st week) before infection (El-Morsy et al., 2022; El-Attar et al., 2024).

G5: Administered by Ag-NPCs; 400 mg/kg b. w. *via* oral gavage day after day; 3 times (during the 1st week) before infection. G6: Administered by *F. carica*-Ag NPCs; 400 mg/kg b. w. *via* oral gavage day after day; 3 times (during the 1st week) before infection (El-Attar et al., 2024).

Ethical approval: The experimental protocol was carried out by the guide of the National Institute of Health for the care and use of laboratory animals (NIH publication No. 8523, revised 1996) and was conformed to the local experimental animal ethics committee of the Faculty of Medicine, Mansoura University with approval number **Sc. Ms. 22.12.12.** **The date of approval: is 16/12/2022.** It was obtained from MU-ACUC for 30 black female C57BL/6 mice in the current experiment.

2.2. Methods

2.2.1. Infection of mice with *Schistosoma mansoni* cercariae

Freshly shedding cercariae were injected subcutaneously into each infected C57BL/6 black female mouse (G2-G6) *via* a stock solution containing approximately 70 cercariae per 0.5 ml of distilled water.

2.2.2. Praziquantel solution freshly prepared

Praziquantel (200 mg/kg) was mixed with purified olive oil to paste texture, and 5.2 ml of distilled water was added with stirring (El-Attar et al., 2024).

2.2.3. Preparation of *Ficus carica* nanoparticles

Hydrothermal squeeze methods (a specialized technique for high vapor pressure, and high-temperature crystallization of compounds at Nanoscale from aqueous solutions) were adopted for the preparation process as shown in Figure 1 (Salehi et al., 2020).

Figure 1: The experimental preparation of *Ficus carica* nanoparticles

2.2.4. Silver nanoparticles preparation

Silver nanoparticles was prepared by the Green Synthesis Technique as illustrated in Figure 2 (Al-Rajhi et al., 2022).

Figure 2: The experimental preparation of silver nanoparticles

2.2.5. Preparation of *Ficus carica* extract nanoparticles loaded on silver nanoparticles

It was done by adding 10 ml of ethanolic *F. carica* extract to 10 ml of silver nitrate, with continuous stirring for 1 hr at pH=7, leaving for 24 hours in the dark, with stirring and then left until yellowish-green or violet color appears (El-Attar et al., 2024).

After administration with *F. carica*-NPCs, Ag-NPCs and *F. carica*-Ag NPCs in the 1st week, mice were injected subcutaneously with cercariae according to Liang et al. (1987). Mice were cared for 6 weeks (Peters and Warren, 1969). PZQ was administered orally in week 8 before scarification for G3.

2.2.6. Characterization of nano samples

The *F. carica*-NPCs, Ag-NPC, and *F. carica*-Ag NPCs were characterized by measuring Zeta-potential and hydrodynamic size (DLS) using the Malvern Zetasizer instrument, Malvern Panalytical Company, Worcestershire, United Kingdom.

2.2.7. Blood sampling

After 7 weeks of infection, mice were anesthetized with Ketamine (43.5 mg/ kg) and Xylazine (6.5 mg/ kg) for 35 minutes (**El-Sherif, 2019**). Mice were sacrificed by sharp sterilized blades and blood samples were collected in clean centrifuge glass tubes, left for complete clotting, and then centrifuged at 1350 xg for 15 minutes. The clear supernatants were rapidly collected. The sera were kept in labeled Eppendorf tubes and frozen at -20 °C for later experimental biochemical analyses.

2.2.8. Liver homogenate preparation

Mice livers were collected and washed. The samples from each liver were homogenized by distilled water (10% w/v), and supernatants were collected, and labeled stored as aliquots at -20 °C in Eppendorf tubes for subsequent biochemical analyses.

2.2.9. Worm recovery

Males, females, and copulated worms were collected from infected groups by the porto-mesenteric perfusion technique described by **Duvall and DeWitt (1967)**. The worms were perfused with citrate saline, and cleaned with 70% ethanol. The liver, kidney, and intestines turned pale; therefore, the perfusion process was stopped. Worms were rinsed three times in phosphate buffer saline (PBS) at pH = 7.4. They were then counted as described by **Kamel et al. (1977)**. By stereomicroscope, the % reduction of worm burden by the administration model was obtained by the equation:

Reduction % = $(1 - \text{Mean worms (test gp.)} / \text{Mean worms (+ve control gp.)}) \times 100$
(**Melman et al., 2009; Muema et al., 2015**).

2.2.10. Egg count

Three samples (1g) were obtained from the liver and intestine of mice after scarification for egg count by using a scanning power (4x) light microscope ocular lens (**Cheever and Anderson, 1971**). Deposited eggs' counting was obtained after digestion with potassium hydroxide solution (4%) of -20 °C stored slices of the liver and intestine (**Kamel et al., 1977**).

2.2.11. Oogram pattern

The pharmacological activity that affected oviposition and maturation was reflected by the pattern of degree of ova maturity and viability. Following perfusion, three segments (1 cm long) of the small intestine were divided longitudinally, cleaned in saline, and partially dried on filter paper before being squashed between two glass slides. Typically, 100 eggs were counted in each piece, and this process was repeated with further fragments until a total of 300 eggs were gathered and divided into three types: immature, mature, and dead eggs (**Pellegrino et al., 1962**).

2.2.12. Scanning electron microscopy for topographical study of *Schistosomamansonii* adult worms

Adult worms were perfused from hepatic and porto-mesenteric veins, washed by PBS many times to get rid of any debris or mucous, dipped in buffered glutaraldehyde solution (2%) left overnight at 4°C as

primary fixation, washed by PBS for 15 minutes for three times at room temperature to get rid of extra fixative solution, after that, dipped in post-fixative osmium tetroxide (1%) at 37°C for 2 hours. The samples were washed residues of solutions by PBS by using ascending serial concentrations of ethanol for the dehydration step (30, 40, and 50%) for 15 minutes each, followed by 70% for 30 minutes, and twice for 30 minutes in 90%. They were dried on stubs' holders for 30 minutes, and coated with gold under vacuum. The worms were examined by Joel JEM-1200 SEM at the Electron Microscope Unit, Faculty of Agriculture, Mansoura University, Mansoura, Egypt (**El-Sayad et al., 2017**).

2.2.13. Biochemical analyses

2.2.13.1. Antioxidant and oxidative stress biomarkers

Antioxidants such as glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT), and the oxidative stress marker as malondialdehyde (MDA) were assayed in liver tissue according to the methods described by **Beutler et al. (1963)**; **Nishikimi et al. (1872)**; **Aebi (1984)**; **Ohkawa et al. (1978)**, respectively.

2.2.13.2. Inflammatory markers

C-reactive protein (CRP), interleukin 6 (IL-6), vascular cell adhesive molecule-1 (VCAM-1), and intercellular adhesive molecule-1 (ICAM-1) were estimated according to the method described by **Banerjee et al. (2003)**; **Feng et al. (2023)**; **Carlos and Harlan (1994)**, respectively.

2.2.13.3. Apoptotic and anti-apoptotic markers

Hepatic p53, Bax, cytochrome C and caspase 9 were estimated by ELISA Kits, from CUBIO Innovation Center Houston, TX, USA. Hepatic Bcl-2 was assayed by an ELISA Kit, from Creative Biolabs Ramsey Road, Shirley, USA. and hepatic caspase-3 content was estimated by using an ELISA kit from Biovision, Grove Street, Waltham, Massachusetts.

2.2.13.4. DNA damage by comet assay

The comet assay was run according to **Singh et al. (1988)**. Comet tail length and DNA percent in the tail and tail moment were detected according to **Tice et al. (2000)**.

2.2.13.5. Liver function tests

Activities of liver function tests as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were estimated in serum according to the methods described by **Reitman and Frankel (1957)**; **Belfield and Goldberg (1971)**, respectively. Serum gamma-glutamyl transferase (γ -GT) activity was evaluated according to the method of **Theodorsen and Strømme (1976)**.

Serum albumin and bilirubin were estimated according to the methods described by **Young (2001); Martinek (1966)**, respectively.

2.2.14. Statistical analyses

The GraphPad Prism 8.0 software (Graphpad Software Inc., San Diego, California, USA) was used for preparing all statistical data. The results are shown as the mean $\pm$ standard error of the mean (SEM) (n=5). A one-way analysis of variance (ANOVA) was used to make the different statistical comparisons, followed by the Neuman-Keuls post-hoc test (**Armitage et al., 2008**). The data was considered significant when $P \leq 0.05$.

3. Results And Discussion

3.1. Characterization of nanoparticles

3.1.1. Zeta potential and size of *F. carica*-NPCs

The charge of *F. carica*-NPCs recorded a mean volt of -0.948 mV as observed in Figure 3A. Their diameter average recorded 163.2 nm as recorded in Figure 3B.

Figure 3: Characterization of *F. carica*-NPCs as A: Zeta potential and B: Size of nanoparticles

3.1.2. Zeta potential and size of silver nanoparticles

The charge of Ag-NPCs had a mean volt of -18.7 mV as observed in Figure 4A. Their diameter average recorded at 51.75 nm as shown in Figure 4B.

Figure 4: Characterization of Ag-NPCs as A: Zeta potential and B: Size of nanoparticles

3.1.3. Zeta potential and size of *Ficus carica* loaded on silver nanoparticles

The charge of *F. carica*-Ag NPCs with a mean volt of -6.46 mV is shown in Figure 5A. Their diameter average recorded at 121.9 nm as in Figure 5B.

Figure 5: Characterization of *F. carica*-Ag NPCs as A: Zeta potential and B: Size of nanoparticles

3.2. Total worm burden

Figure 6 proved that the PZQ-treated group had a significant reduction in the total worm reduction. The protected group with *F. carica*-Ag NPCs had a significant reduction in worm burden as PZQ treatment compared with the positive control group and Ag-NPCs, and *F. carica*-NPCs recorded a significant reduction in total worm burden as compared with the positive control group.

Figure 6: The recovered worms burden in control and different mice groups

The PZQ had the same results that obtained by Mokbel et al. (2020); they confirmed that the PZQ treatment didn't show a complete eradication of worms. *F. carica*-NPCs had a similar pattern of results obtained by Seif el-Din et al. (2013) who demonstrated that the active phytochemicals like tannins, flavonoids, and terpenes that were in *Calotropis procera*, *Ficus elastica*, and *Zingiber officinale* also found in *Ficus carica* have anti-helminthic properties. Ag-NPCs results also go concomitant with that of Khan and Javed (2021); Al-Rajhi et al. (2022), and Detoni et al. (2023).

According to Detoni et al. (2023), they assigned that the Bio-Ag NPCs could alter the viability and membrane integrity of juvenile forms, as well as promote oxidative stress and mitochondrial membrane depolarization in adult worms, triggering the formation of lipid droplets and autophagic vacuoles. Oral administration of Bio-Ag NPCS to *S. mansoni*-infected mice is also able to reduce parasite burden and associated pathology. *F. carica*-Ag NPCs recorded the same reduction percentage of the PZQ that was discussed according to *F. carica* and Ag may have a synergistic effect and elevate the effectiveness against schistosomiasis.

3.3. Egg count

The number of ova in gram of each hepatic and intestinal tissues of control and different mice groups was indicated in Figure 7 as the group of *F. carica*-NPCs showed lower reduction levels than were recorded in Ag-NPCs. Finally, the lowest reduction level was *F. carica*-Ag NPCs. The PZQ group recorded the highest reduction level of *Schistosoma* egg in liver and intestine.

Figure 7: Ova count of control and different mice groups

3.4. Oogram pattern

The reduction percentages of intestinal egg developmental stages in Figure 8 recorded that the *F. carica*-NPCs group had an insignificant effect on the dead ova; also, it had an insignificant decrease in mature ova and a significant increase in immature ova in comparison to the positive control group.

The Ag-NPCs group had an insignificant decrease in immature ova and a decrease in mature ova with an insignificant effect on dead ova compared with positive control. In the *F. carica*-Ag NPCs group, there was an insignificant effect on dead ova, with an insignificant decrease in immature ova and an increase in mature ova. Otherwise, the treated group recorded significant reduction in mature ova and significant reduction in immature and dead ova as compared with positive control.

Figure 8: Egg developmental stages% of control and different mice groups

Administration of PZQ showed a significant reduction in ova numbers within both liver and intestine tissues, a significant diminishing of immature and mature ova, and in contrast a significant elevation in dead ova was achieved when compared with the infected mice. These data go parallel with that of Frezza et al. (2013); El-Morsy et al. (2022) and Mohamed et al. (2023). The authors concluded that PZQ

can assault vitelline cells and ovaries, affecting the development of eggs besides altering the worm's tegument and its muscle structure.

The *F. carica*-NPCs group recorded a significant reduction in ova count within the liver and intestine tissues. It showed a significant change in immature ova percentage, and an insignificant change in dead ova and mature ova percentages compared with the positive control group. These results were agreed with Shaaban et al. (2019); Mokbel et al. (2020) and El-Morsy et al. (2022) who discussed their results that the short duration of therapy or the low chemical dose utilized may be the cause of the low effectiveness.

The Ag-NPCs and *F. carica*-Ag NPCs groups showed a significant reduction in the ova count of the liver and intestine tissue in comparison with the infected control group. The immature, mature, and dead ova change percentages showed insignificant reduction compared with the positive control group which was the same as Dkhil et al. (2020) obtained; Ag-NPCs showed a curable effect on jejunal epithelia and could recover the intestinal epithelia. Abououf et al. (2018) and El-Menawy et al. (2021) proved that the treatment had higher effectiveness than protection which appeared in different ova patterns.

3.5. Scanning electron micrographs

3.5.1. Ultra-micrographs of adult *Schistosoma mansoni* worms were obtained from positive control group

The adult male body (M) (Mean length was 6.2 mm) recovered was thicker, cylindrical, rough and shorter than the adult female body (F). The male carried the female inside the gynaecophoric canal in copulation (Figure 9A). The oral sucker (OS) and ventral sucker (VS) of the male had a normal appearance (Figure 9B). The tegument of the anterior third appeared normal with tubercles (Tu) bearing spines (S) and dome, fungiform, and cratered papillae (DP, FP, & CrP) (Figure 9C).

The female body (F) (Mean length was 7.9 mm) appeared normal (Figure 9D). The tegument of the anterior and posterior thirds had a normal architecture with spines. The tegument beard maculated and cratered papillae (MP & CrP) (Figure 9E & 9F).

Figure 9 (A-F) Scanning electron micrographs of *Schistosoma mansoni* couple recovered from positive control group

Fig. 9A: Whole mount of male (M) and female (F) couple.

Fig. 9B: Magnified ventral view of the anterior third in Fig. 9A showed normal oral sucker (OS) and ventral sucker (VS).

Fig. 9C: High magnified view in the anterior third of female in Fig. 9A showed normal tubercles (Tu) with spines (S). Appearance of dome (DP), hemispherical (HP), fungiform (FP) and cratered (CrP) papillae.

Fig. 9D: The whole mounts of the female (F) appeared normal.

Fig. 9E & 9F: High magnification of the anterior third and posterior third in the female body beard normal tegmental spines (S). Note: Maculated papillae (MP) and cratered papillae (CrP) appeared on tegument.

3.5.2. Ultra-micrographs of adult *Schistosoma mansoni* worms were obtained from mice treated with praziquantel group

Adult worms were recovered after being treated with 200 mg/kg for two consecutive days in the 7th week from infection.

The protruded female (F) (Mean length was 8.5 mm) from the male (M) body (Figure 10A). The male worm (M) (Mean length was 7.1 mm) had a coiled body (Figure 10B). It had a triangular-shaped oral sucker (OS) and pulled shrinkage ventral sucker (VS) with the eroded tegument-like embedded folds (red arrows) (Figure 10C). Its tegument had tuberclesless that appeared like small stones, the dorsal surface was deformed as appearance of peeling (P) and some eroded area (E) (Figure 10D) also, in ventral view of the end region (pre-excretory pore) showed extremely corrosive tegument (Figure 10E). In another specimen, the male (M) tegument had peeling and erosion; their tubercles were naked as blebs (Tu) (Figure 10F & 10G). The protruded female (F) from the male (M) had body tegument peeled (P), and the tegument had erosion (E) (Figure 10H).

Figure 10 (A-H) Scanning electron micrographs of *Schistosoma mansoni* male recovered from mice treated with praziquantel group

Fig. 10A: Male (M) and female (F) worms in copulation.

Fig. 10B: Whole mount of coiled male (M) worm.

Fig. 10C: Magnified view of Fig. 10B; in the anterior third showed highly flattened triangular-shaped oral sucker (OS) and pulled shrinkage ventral sucker (VS).

Fig. 10D: High magnification of dorsal tegument highly corrugated showed peeling (P) and erosion (E).

Fig. 10E: Highly magnified (end third) of Fig. (10C) as red arrows showed deformed tegument with erosion (E) as continuous lines that resulted from peeling (P).

Fig. 10F: The male worm (M) appeared coiled.

Fig. 10G: Dorso-lateral surface of Fig. 10F; it observed naked tubercles (Tu) which appeared like blebs.

Fig. 10H: High magnification of Fig. 10A showed a peeled ventral surface (P) of the female tegument that had eroded areas (E).

These results were parallel to that of **Amara et al. (2018)**; **Abd El Wahab et al. (2021)** and **El-Derbawy et al. (2022)**. In fact, PZQ caused sucker deformity which reduced the fluke's ability to adhere to blood vessels, and this render the ingestion of nutrients (more difficult). Tegmental damage along the worm

body was impaired the functioning of the tegument and destroyed the worm defense system, facilitating an attack by the host's immune system that led to worm death according to **Amara et al. (2018)**.

3.5.3. Ultra-micrographs of adult *Schistosoma mansoni* recovered worms were obtained from mice protected by *Ficus carica* leaves extract nanoparticles group

The adult recovered worms were obtained from protected mice by 3 doses of 400 mg/kg *F. carica*-NPCs day after day in the 1st week before cercariae infection.

The couples had elongated bodies; the male (M) (Mean length was 6.3 mm) carried the female (F) (Mean length was 7.7 mm) inside a remarkably opened gynaecophoric canal (GC) (**Figure 11A & 11C**). The female body tegument had a deformed flattened shrunk architecture (**Figure 11B**); its surface was peeled (P) and lost its spines. The internal tegument of the gynaecophoric canal showed its spines (S). The couple of **Figure 11C** had elongated body showing deformed suckers, shrunk oral sucker (OS) and flabby ventral sucker (VS) (**Figure 11D**). The male tegument had damaged tubercles (Tu) like blebs. The presence of cratered papillae (CrP) on tegument (**Figure 11E**), its tubercles (Tu) were flattened with a total absence of spines recorded (**Figure 11F**).

Figure 11 (A-F) Scanning electron micrographs of *Schistosoma mansoni* couples recovered from mice protected by *Ficus carica* leaves extract nanoparticles group

Fig. 11A: The whole mount of the male (M) carrying the female (F) inside a completely opened gynaecophoric canal (GC). Note: The couples had elongated bodies.

Fig. 11B: The female (F) appeared peeled and shrinkaged inside opened the gynaecophoric canal (GC). Note: Appearance gynaecophoric canal spines (S).

Fig. 11C: The appearance of highly elongated couples' bodies illustrated twisting in the middle third. Note: An opened male (M) carrying the female (F) in gynaecophoric canal (GC).

Fig. 11D: Dorsal view of male showed deformed suckers; shrunk oral sucker (OS) and flabby ventral sucker (VS).

Fig. 11E: A magnified view of Fig. 11C showed deformed peeled (P) tegument with damaged tubercles (Tu) like-blebs. Note: Presence of cratered papillae (CrP) between tubercles.

Fig. 11F: High magnification of Fig. 11E showed flattened appearance of tubercles that appeared naked (Tu) on peeled (P) tegument.

These data were similar to that observed by **El-Derbawy et al. (2022)** who used ginger plant that had similar phytochemicals as fig plant such as polyphenols, tannins, and flavonoids. Then, they interpreted their results in that; ginger cannot get rid of *S. mansoni* larval stages but it could cause a deleterious effect on suckers, so worm adherence capability to blood vessels is altered. Its effect on the teguments

along the worm's body would impair the tegumental function, devastate the worm's defense system and it could easily be attacked by the host immunity.

3.5.4. The ultra-micrographs of adult *Schistosoma mansoni* worms recovered from mice protected by silver nanoparticles group

The adult recovered worms were obtained from protected mice by 3 doses of 400 mg/kg Ag-NPCs day after day in the 1st week before cercariae infection.

The couple (M & F) had elongated partially coiled bodies (**Figure 12A**); it had an opened gynaecophoric canal (GC). In the anterior third, the worms had deformed shrinkage peeled (P) tegument with naked tubercles (Tu), it had shrinkage oral sucker (OS) and ventral sucker (VS) (**Figure 12B & 12C**). The eroded (E) tegument had deformed naked tubercles (Tu) with loss of spines (**Figure 12D**). Another conjugated male (M) (Mean length was 5.61 mm) and protruded female (F) from the gynaecophoric canal (GC) (**Figure 12E**). The anterior third of the male body had a corrugated pulled shrinkaged oral sucker (OS) and swollen ventral sucker (VS). The tegumental tubercles (Tu) appeared as spots (**Figure 12F**).

The female (F) (Mean length was 7.30 mm) protruded from the middle third of the couple's body; it had a deformed swollen peeled (P) tegument with the absence of spines (**Figure 12G**). The male body had eroded (E) tegument with deformed tubercles (Tu) and dome papillae (DP) (**Figure 12H**).

In last specimen of the male (M) and female (F) couple showed an extremely elongated body with a semi-opened gynaecophoric canal (GC) (**Figure 12I**). The male laterodorsal surface had deformed eroded tegument (E), mostly naked tubercles (Tu). The appearance of hemispherical papillae (HP) (**Figure 12J**). Ventrally, it showed a deformed tegument with a corrugated inner surface (IS) of opened gynaecophoric canal (GC) illustrated inner spine (S) of the canal (**Figure 12K**).

Figure 12 (A-K) Scanning electron micrographs of *Schistosoma mansoni* couple adult worms recovered from mice protected by silver nanoparticles group

Fig. 12A: The couple showed elongated partially coiled male (M) body. The gynaecophoric canal (GC) had a highly open appearance carrying female (F) inside.

Fig. 12B: A magnified view of Fig. 12A in the anterior third showed tegument bearing tubercles (Tu), oral sucker (OS) and ventral sucker (VS) appeared shrinkage. Note: The female (F) body exited from opened male's gynaecophoric canal (GC).

Fig. 12C: A magnified view of Fig. 12A showed shrinkage of body tegument with peeling appearance in both; female (F) and male (M). Note: Oral sucker (OS) and ventral sucker (VS) had shrinkage indulged architecture.

Fig. 12D: High magnification showed alteration in tegmental layer; deformed naked tubercles with loss of spines (Tu), peeling (P) and erosion (E).

Fig. 12E: Another male (M) and female (F) in the copulation position had twisting that showed protrusion of the female body from the gynaecophoric canal (GC). Note: Swollen of the male body was remarkable.

Fig. 12F: Magnified view of Fig. 12E showed a ventrolateral side (anterior third) in the male body with a deformed pulled shrinkage oral sucker (OS) and swollen ventral sucker (VS). Note: Tubercles (Tu) appeared like spots.

Fig. 12G: Magnified view through the second third in male (M) body showed protrusion of female (F) from the copulatory canal. Tubercles (Tu) appeared as blebs. Note: Deformed peeled (P) tegument of a female with absence of spines.

Fig. 12H: High magnification of male body showed erosion (E) of tegument; deformed tubercles (Tu) without spines and appearance of dome papillae (DP) in between tubercles.

Fig. 12I: The male (M) and female (F) couple had an extremely elongated body with semi-opened gynaecophoric canal (GC).

Fig. 12J: High magnification of dorsolateral surface showed eroded (E) tegument; mostly naked tubercles (Tu). Note: Appearance of hemispherical papillae (HP).

Fig. 12K: High magnified view of the inner surface (IS) of the gynaecophoric canal (GC) showed a deformed corrugated surface with minute spines (S).

These results matched with **Moustafa et al. (2018)** and **El-Derbawy et al. (2022)**; who returned the obtained results to the effect of Ag-NPCs that could cause complete blockage to cercarial infectivity after 30 min of exposure which reduces the host susceptibility to infection. The side effects of Ag-NPCs on cercariae were attributed to Ag ions, which can bind to the papillary sites on the cercarial surface to disrupt its function.

3.5.5. The ultra-micrographs of adult *Schistosoma mansoni* recovered worms were obtained from mice protected by *Ficus carica* loaded on silver nanoparticles group

The adult worms were recovered from protected mice by 3 doses of 400 mg/kg *F. carica*-Ag NPCs day after day in the 1st week before cercariae infection.

The first recovered specimen was elongated partially coiled conjugated male (M) (Mean length was 7 mm) and female (F) (Mean length was 8.1 mm). The gynaecophoric canal (GC) was opened till the end of the body (**Figure 13A & 13E**). Its anterior third appeared coiled and emerged female (F) from the copulatory canal was seen (**Figure 13B**). The protruded female (F) body from the gynaecophoric canal had a shrunk eroded (E) tegument. (**Figure 13C**). Dorsally, the dorsal side of the body had eroded (E) and peeled (P) tegument with damaged tubercles (Tu) (**Figure 13D**).

Another recovered specimen had a highly elongated twisted male body (M) with a semi-opened gynaecophoric canal with a protruding female body (F) (**Figure 13F**). In the middle third of the couple's

body; protruded female (F) appeared with peeled (P) tegument and erosion (E) (**Figure 13G**). In the posterior third of the male body; a closed gynaecophoric canal (GC) surrounding the female was seen (**Figure 13H**).

Figure 13 (A-H) Scanning electron micrographs of *Schistosoma mansoni* couple recovered from mice protected by *Ficus carica* loaded on silver nanoparticles group

Fig. 13A: Whole mount of elongated, partially coiled conjugated male (M) and female (F).

Fig. 13B: The magnified view in the anterior third of Fig. 13A showed a coiled male body (M) and emerged female (F).

Fig. 13C: The magnified view of Fig. 13B showed a protruded shrunk female body (F) from the gynaecophoric canal (GC), and in the tegument had erosion (E).

Fig. 13D: High magnification of tegument in anterior third showing highly deformed tegument with erosion (E), peeling (P), and damaged tubercles (Tu) without of spines that appeared naked. Note: Appearance of hemispherical papillae (HP).

Fig. 13E: Dorsal magnified view at last third showing that the tegumental surface of the male appeared a leaf-like shape with naked tubercles (Tu). Note: The gynaecophoric canal (GC) was opened till the end of the body.

Fig. 13F: Whole mount of highly elongated twisted male body (M) bearing female body (F). Note: The gynaecophoric canal had partially opened appearance.

Fig. 13G: Magnified view of Fig. 13F in the middle third of the couple's body showing protruded swollen female body (F) with peeled (P) tegument. Note: Presence of erosion (E) in some areas of tegument.

Fig. 13H: Magnification of ventral side of posterior third of couple body of Fig. 13F showing closed gynaecophoric canal (GC). Note: Erosion (E) of tegument with naked tubercles (Tu) caused by peeling (P).

These results go parallel to the results of **El-Derbawy et al. (2022)**, who observed that in combined oral administration, the obtained results showed high efficacy against schistosomes that illustrated the synergistic effect on each other rather than single form.

3.6. Antioxidant and oxidative stress biomarkers

Figure 14 (A, B, C, and D) showed **GSH** level and **SOD** and **CAT** activities **MDA** level in control and different mice groups. Hepatic GSH level, SOD and CAT activities showed significant elevation in PZQ, *F. carica*-NPCs, Ag-NPCs, and *F. carica*-Ag NPCs compared positive control group. Hepatic MDA level showed significant decrease in

PZQ, *F. carica*-NPCs, Ag-NPCs, and *F. carica*-Ag NPCs compared positive control group. GSH and SOD reached a normal status in *F. carica*-Ag NPCs protected group in comparison to positive control group.

Figure 14: Antioxidant and oxidative stress biomarkers in control and all other tested mice groups; (A) GSH (Reduced glutathione), (B) SOD (Superoxide dismutase), (C) CAT (Catalase) and (D) MDA (Malondialdehyde)

3.7. Inflammatory markers

Figure 15 (A, B, C, and D) showed CRP, IL-6, VCAM-1 and ICAM-1 contents of control and different mice groups. Serum CRP, hepatic IL-6, VCAM-1 and ICAM-1 contents showed significant decrease in PZQ, *F. carica*-NPCs, Ag-NPCs, and *F. carica*-Ag NPCs compared with positive control group.

Figure 15: Inflammatory markers in control and all other tested mice groups; (A) CRP (C-reactive protein), (B) IL-6 (Interleukin-6), (C) VCAM-1 (Vascular cellular adhesive molecule-1) and (D) ICAM-1 (intercellular adhesion molecule-1)

3.8. Apoptotic markers

Figure 16 (A, B, C, D, E, and F) showed p53, Bax, Bcl-2, cytochrome C, caspase-9 and caspase-3 levels in hepatic tissues of control and different mice groups. p53, Bax, cytochrome C, caspase 9 and caspase-3 levels showed significant decrease in PZQ, *F. carica*-NPCs, Ag-NPCs, and *F. carica*-Ag NPCs compared to the positive control group. The anti-apoptotic protein Bcl-2 showed significant increase in *F. carica*-NPCs, Ag-NPCs and *F. carica*-Ag NPCs comparison to the infected control group.

Figure 16: Hepatic apoptotic and anti-apoptotic markers in control and all other assessed mice groups; (A) P53, (B) Bax, (C) Bcl2, (D) cytochrome C, (E) caspase 9 and (F) caspase 3

3.9. DNA damage by comet assay

Figure 17 (A, B, C, D & E) and Figure 18 (A, B & C) observed data of DNA tail length (TL), %DNA, and tail moment (TM) in different mice groups; negative control showed normal damaged spots and untailed DNA. In addition, the positive control group showed increase in abnormal spots also, the length and diameter of the nuclear DNA (Tailed). The PZQ treated group showed abnormal damaged spots of DNA. While, protected mice groups showed significant reduction in *F. carica*-NPCs, Ag-NPCs, and *F. carica*-Ag NPCs compared with infected control mice.

Figure 17: Photomicrographs of DNA damage of all studied groups (Scale bar = 100 µm)

Figure 18 (A, B & C) showed the different hepatic DNA damage; Tail length, tail DNA and tail moment

3.10. Liver function tests

Figure 19 (A, B, C, D, E and F) showed ALT, AST, ALP, γ -GT activities, albumin and bilirubin contents in control and mice groups. Serum ALT, AST, ALP, γ -GT activities and bilirubin content showed significant reduction in PZQ, *F. carica*-NPCs, Ag-NPCs, and *F. carica*-Ag NPCs compared to the positive control group. Serum albumin content illustrated significant increase in PZQ, *F. carica*-NPCs, Ag-NPCs, and *F. carica*-Ag NPCs compared positive control group.

Figure 19: Liver function parameters in all assessed mice groups; (A) ALT (Alanine aminotransferase), (B) AST (Aspartate aminotransferase), (C) ALP (Alkaline phosphatase), (D) γ -GT (Gamma glutamyl transferase), (E) albumin and (F) bilirubin

3.11. Explanation of the effect of praziquantel drug on hepatic tissues

Consistent with several previous studies, the present study showed that the hepatic injury was induced in liver tissues by *S. mansoni*-infected mice. This was ensured by elevated serum ALT, AST, ALP, and γ -GT activities and an increase in bilirubin content as well as a decrease in albumin content. Mice treated with PZQ showed amelioration in liver functions similar conclusion was recorded by **Tawfeek et al. (2019)**, which was reflected based on a significant decrease in MDA content, a significant increase in γ -GT content as well as SOD and CAT activities. Also, significant decrease in CRP, IL-6, VCAM-1, and ICAM-1 contents were observed when compared with the infected group. Consequently, significant decrease in P53, Bax, Cyt C, caspase 9, 3, and a significant increase in Bcl-2 were observed compared with the +ve control group.

Similar data were recorded by **Giri and Roy (2016)** who clarified that the PZQ drug regulated oxidative stress markers and inflammatory markers. They considered NF-kB and TGF- β 1 which were potential biomarkers for regression of hepatic fibrosis with activation of the related signal pathway of apoptosis which show up-regulation of caspase-3 and P53 markers expression. More obviously, a significant reduction in DNA damage using comet assay in the PZQ group in comparison with the +ve control group.

3.12. Explanation of the effect of *Ficus carica* nanoparticles on hepatic tissues

The observed data showed the same opinion as **Hassan et al. (2023)**; who used Silymarin which has flavonoids, phenolic compounds, organic acids, and vitamin E as fig plant. Its phytochemicals have a hepatoprotective effect by increasing albumin and total protein that represented the synthetic function of liver; it was responsible for their antioxidant properties that aided in preventing and treating oxidative stress related to hepatic diseases (**Lugrin et al., 2014**).

Formononetin (one of the phytochemicals of *F. carica*) was observed to have a positive effect, as it increased the activity of antioxidant enzymes, and reduced the levels of pro-inflammatory markers (TNF- α , IL-1 β , & IL-6) and NLRP3 inflammasome pathways (**Wang et al., 2018**). These results were matched and concluded by **Hu et al. (2022)**; *Ficusdubia* phytochemicals especially phenolic compounds which are rich in *Ficus carica* could suppress the growth and induce apoptosis. The leaves have been shown to regulate the release of Cyt C from mitochondria by affecting the permeability of the mitochondrial

membrane. The data evaluated the expression of Bcl-2 (it controlled mitochondrial membrane permeability) and Transcription Factor 53 (TP53), which essentially up-regulate the pro-apoptotic genes (e.g., Bax, Bak, Fas, etc.) and down-regulate the anti-apoptotic genes (e.g., Bcl-2, Bcl-xL).

The *F. carica*-NPCs group showed a significant reduction in DNA damage compared with the infected mice; it appeared in DNA tail length, (%) tail DNA and tail moment, these results were matched with **Azqueta and Collins (2016)** suggested that polyphenols of *F. carica* had a protective effect against chromosomal aberrations.

3.13. Explanation of the effect of silver nanoparticles on hepatic tissues

Observations of this thesis similar to these results were also recorded by **Reshi et al. (2017)** who explained that Ag-NPCs may restore the GSH in the liver. Alleviated activities of adenosine triphosphatase (ATPase), glucose-6- phosphatase (G6Pase) and antioxidant enzymes. that was explained by **Alkhalaf et al. (2020)**. They assigned their data as anti-inflammatory impact and the role of Ag-NPCs in the process of healing wounds through lowering the levels of tumor necrosis factor (TNF- α), interferons, and interleukin 1, that regulate apoptosis-related genes (caspase-3, Bax, and Bcl-2).

Fehaid and Taniguchi (2019) observed that Ag-NPCs could decrease the TNF α -induced DNA damage response by reducing the surface expression of tumor necrosis factor receptor 1 (TNFR1), which in turn decreased TNF- α signal transduction with a decrease in inflammation. This observed a significant reduction in the Ag-NPCs group compared to the +ve control group.

3.14. Explanation of the effect of *Ficus carica* loaded on silver nanoparticles on hepatic tissues

The illustrated data of *F. carica*-Ag NPCs showed similar data also recorded by **Al-Olayan et al. (2016)**; who used *Ceratonia siliqua* pod extract (CPE) which contains most phytochemicals of *F. carica* as flavonoids, phenols, and tannins; which could restore the activities and expression levels of these antioxidant enzymes by ameliorating CAT and GSH activities for protection from free radicals. Also, they could decrease TNF- α , IL-6, IKK α / β , and NF- κ B contents as anti-inflammatory action.

Moreover, Ag-NPCs could lower the levels of TNF- α , interferons, and interleukin-1 therefore *F. carica*-Ag NPCs has a hepatoprotective effect by regulating the programmed cell death. In the present study, the *F. carica*-Ag NPCs group showed a significant reduction in DNA damage in comparison with the +ve control group. The combined phytochemical nanoparticles *F. carica* and Ag-NPCs showed better amelioration.

Conclusion

The present study proved that the *F. carica*-NPCs and *F. carica*-Ag NPCs have protective and ameliorative effects on oxidative stress markers, inflammatory markers, apoptotic markers, DNA damage, and liver function tests compared with positive control that can enable the hepatic tissue to restore its normal healthy function. It has been shown that the nano samples in the prevention model showed a better

improvement than the PZQ drug in the treatment model and this is what it noted that when using nano samples as a treatment will make the results more preferable

Declarations

Ethical approval:

The experimental protocol was carried out by the guide of the National Institute of Health for the care and use of laboratory animals (NIH publication No. 8523, revised 1996) and was conformed to the local experimental animal ethics committee of the Faculty of Medicine, Mansoura University with approval number **Sc. Ms. 22.12.12. The date of approval: is 16/12/2022.** It was obtained from MU-ACUC for 30 black female C57BL/6 mice in the current experiment.

Funding:

There is no funding.

Author Contribution

By contributing to the research, data analysis, and presentation of results, as well as the drafting and editing of the manuscript by Naira Adel El-Attar and Mamdouh Rashad El-Sawi who worked to create the methodology, prepare figures and run the experiments. Mamdouh Rashad El-Sawi and Eman Ahmed El-Shabasy performed data analysis and interpretation. Naira Adel El-Attar was involved in the study consultation, the conceptualization of the manuscript, and the overall writing and editing. The content was reviewed, discussed and revised by all authors.

Acknowledgement

The resources that Mansoura University offers are greatly appreciated.

References

1. Abd El Hady, W.E., El-Emam, G.A., Saleh, N.E., Hamouda, M.M., Motawea, A., 2023. The Idiosyncratic Efficacy of Spironolactone-Loaded PLGA Nanoparticles Against Murine Intestinal Schistosomiasis. *Int J Nano med.* 2023, 987–1005.
2. Abd El Wahab, W.M., El-Badry, A.A., Mahmoud, S.S., El-Badry, Y.A., El-Badry, M.A., Hamdy, D.A., 2021. Ginger (*Zingiber Officinale*)-derived nanoparticles in *Schistosoma mansoni* infected mice: Hepatoprotective and enhancer of etiological treatment. *PLoS Negl Trop Dis.* 15, e0009423.

3. Abououf, E.A., EL-Hamshary, A.M.S., Nagati, I.M., Eraky, M.A., EL-Kholy, A.A., Ibrahim, A.N., Omar, G.H., 2018. Effect of *Nigella sativa* oil on *Schistosoma mansoni* mature worms in experimentally infected mice. J. Egypt. Soc. Parasitol., 48(1):55–66.
4. Aebi, H., 1984. Catalase *in vitro*. Meth. Enzymol. 105, 121–126.
5. Alkhalaf, M.I., Hussein, R.H., Hamza, A., 2020. Green synthesis of silver nanoparticles by *Nigella sativa* extract alleviates diabetic neuropathy through anti-inflammatory and antioxidant effects. Saudi J. Biol. Sci. 27, 2410–2419.
6. Al-Olayan, E.M., El-Khadragy, M.F., Alajmi, R.A., Othman, M.S., Bauomy, A.A., Ibrahim, S.R., Abdel Moneim, A.E., 2016. *Ceratonia siliqua* pod extract ameliorates *Schistosoma mansoni*-induced liver fibrosis and oxidative stress. BMC Complement Altern. Med, 16, 434.
7. Al-Rajhi, A.M., Salem, S.S., Alharbi, A.A., Abdelghany, T.M., 2022. Ecofriendly synthesis of silver nanoparticles using Kei-apple (*Dovyalis caffra*) fruit and their efficacy against cancer cells and clinical pathogenic microorganisms. Arab. J. Chem. 15, 103927.
8. Amara, R.O., Ramadan, A.A., El-Moslemany, R.M., Eissa, M.M., El-Azzouni, M.Z., El-Khordagui, L.K., 2018. Praziquantel–lipid nanocapsules: an oral nanotherapeutic with potential *Schistosoma mansoni* tegumental targeting. Int. J. Nanomed., 13, 4493–4505.
9. Armitage, P., Berry, G., Matthews, J.N.S., 2008. Statistical Methods in Medical Research, 4th Ed. John Wiley & Sons. Blackwell publishing company. Blackwell Science, Inc., 350 Main street, Malden, Massachusetts 02148–5018, USA. ISBN: 0-632-05257-0.
10. Azqueta, A., Collins, A. 2016. Polyphenols and DNA Damage: A Mixed Blessing. Nutrients. 8, 785.
11. Banerjee, M., Tripathi, L.M., Srivastava, V.M., Puri, A., Shukla, R., 2003. Modulation of inflammatory mediators by ibuprofen and curcumin treatment during chronic inflammation in rat. Immunopharmacol Immunotoxicol. 25,213 – 24.
12. Belfield, A., Goldberg, D.M., 1971. Revised assay for serum phenyl phosphatase activity using 4-amino-antipyrine. Enzyme. 12, 561–73.
13. Beutler, E., Duron, O., Kelly, B.M., 1963. Improved method for the determination of blood glutathione. J. Lab Clin. Med. 61,882–8.
14. Carlos, T.M., Harlan, J.M., 1994. Leukocyte-endothelial adhesion molecules. Blood 84, 2068–101.
15. Cheever, A.W. and Anderson, L.A., 1971. Rate of destruction of *Schistosoma mansoni* eggs in tissues of mice. ASTMH., 20,62–68.
16. Detoni, M.B., Bortoleti, B.T.S., Tomiotto-Pellissier, F., Concato, V.M., Gonçalves, M.D., Silva, T.F., Ortiz, L.S.F., Gomilde, A.C., Rodrigues, A.C.J., de Matos, R.L.N., Bracarense, A.P.F.R.L., de Matos, A.M.R.N., Simão, A.N.C., Endo, T.H., Kobayashi, R.K.T., Nakazato, G., Costa, I.N., Conchon-Costa, I., Oliveira, F.J.A., Pavanelli, W.R., Miranda-Sapla, M.M., 2023. Biogenic silver nanoparticle exhibits schistosomicidal activity *in vitro* and reduces the parasitic burden in experimental schistosomiasis mansoni. Microbes. Infect., 25(7):105145.
17. Dkhil, M.A., Abdel-Gaber, R., Alojyri, G., Al-Shaebi, E.M., Qasem, M.A.A., Murshed, M., Mares, M.M., El-Matbouli, M., Al-Quraishy, S., 2020. Biosynthesized silver nanoparticles protect against hepatic

- injury induced by murine blood-stage malaria infection. Environ. Sci. Pollut. Res., 27, 17762–17769.
18. Doenhoff, M.J., Cioli, D., Utzinger, j., 2008. Praziquantel: mechanisms of action, resistance and new derivatives for schistosomiasis. Curr Opin Infect Dis. 21, 659–67.
 19. Duvall, R.H. and DeWitt, W.B., 1967. An improved perfusion technique for recovering adult schistosomes from laboratory animals. ASTMH., 164, 483–486.
 20. El-Attar, N.A, El-Sawi, M.R, El-Shabasy, E.A, 2024. The synergistic effect of *Ficus carica* nanoparticles and Praziquantel on mice infected by *Schistosoma mansoni* cercariae. Sci. Rep. 14, 18944.
 21. El-Derbawy, M.M., Salem, H.S., Raboo, M., Baiuomy. I.R., Fadil, S.A., Fadil, H.A., Ibrahim, S.R.M., El Kholi, W.A., 2022. *In Vivo* Evaluation of the Anti-Schistosomal Potential of Ginger-Loaded Chitosan Nanoparticles on *Schistosoma mansoni*: Histopathological, Ultrastructural, & Immunological Changes. Life, 12, 1834.
 22. El-Menyawy, H.M., Metwally, K.M., Aly, I.R., Abo Elqasem, A.A., Youssef, A.A., 2021. The Therapeutic role of Thymoquinone Bioactive Compound as Target Natural Product from *Nigella sativa* Loaded with Chitosan Nanoparticles on Schistosomiasis. Egypt. J. Hosp. Med., 84, 1818–1826.
 23. El-Morsy, S.M.A., El-Tantawy, S.A.M., El-Shabasy, E.A., 2022. Antischistosomal effects of *Ficus carica* leaves extract and/or PZQ on *Schistosoma mansoni* infected mice. J Parasit Dis. 46, 87–102.
 24. El-Sayad, M., Abu Helw, S., El-Taweel, H., Aziz, M., 2017. Antiparasitic Activity of Mirazid, Myrrh Total Oil and Nitazoxanide Compared to Praziquantel on *Schistosoma mansoni*: Scanning Electron Microscopic Study. Iran. J. Parasitol., 12(3), 446–452.
 25. El-Shabasy, E.A., El-Morsy, S.M.A., Amer, M.A., 2022. Hepatoprotective, Antioxidant and Immunological Activities of the Ethanolic *Ficus carica* Leave Extract and/or PZQ in *Schistosoma mansoni* Infected Mice. OALib Journal. 9, e9263.
 26. El-Sherif, E.W., 2019. Optimization of Xylazine-Ketamine Anesthetic Dose in Mice with Chronic Liver Injury. Egypt. Acad. J. Biol. Sci. 11, 13–18.
 27. Fehaid, A., Taniguchi, A., 2019. Size-Dependent Effect of Silver Nanoparticles on the Tumor Necrosis Factor α -Induced DNA Damage Response. Int. J. Mol. Sci. 20, 1038.
 28. Feng, Y., Jiang, Y., Zhou, Y., Li, Z., Yang, Q., Mo, Z., Wen, Y., Shen, L., 2023. Combination of BFHY with Cisplatin Relieved Chemotherapy Toxicity and Altered Gut Microbiota in Mice. Int J Genomics. 2023, pp.20.
 29. Frezza, T., Gremião, M., Zanotti-Magalhães, E., Luiz, A., Ana, L., Silmara, M., 2013. Liposomal-praziquantel: Efficacy against *Schistosoma mansoni* in a preclinical assay. Acta. Trop., 128, 70–75.
 30. Giri, B.R., Roy, B. 2016. Praziquantel induced oxidative stress and apoptosis-like cell death in *Raillietina echinobothrida*. Acta Trop. 159,50–57.
 31. Hassan, Z.A., Darwish, Y.H.M., Elmoslemany, A.M., 2023. Potential protective Effect of Sycamore fruits and leaves extracts against diclofenacinduced liver toxicity in male rats. *Journal of Pharmaceutical Sciences* 8690–2356 ISSN-Online: 2974–4423.

32. Hu, R., Chantana, W., Pitchakarn, P., Subhawa, S., Chantarasuwan, B., Temviriyanyukul, P., Chewonarin, T., 2022. *Ficus dubia* latex extract prevent DMH-induced rat early colorectal carcinogenesis through the regulation of xenobiotic metabolism, inflammation, cell proliferation and apoptosis. *Sci. Rep.* 12, 15472.
33. Jeong, W.S., Lachance, P.A., 2001. "Phytosterols and fatty acids in fig (*Ficus carica*) fruit and tree components," *Food Chem. Toxicol.* 66, 278–281.
34. Kamel, I.A., Cheever, A.W., Elwi, A.M., Mosimann, A., Danner, R., 1977. *Schistosoma mansoni* and *S. haematobium* infections in Egypt. I. Evaluation of techniques for recovery of worms and eggs at necropsy. *ASTMH.*, 26(4),696–701.
35. Khan, K., Javed, S., 2021. Silver nanoparticles synthesized using leaf extract of *Azadirachta indica* exhibit enhanced antimicrobial efficacy than the chemically synthesized nanoparticles. *Sci. Prog.*, 104,00368504211012159.
36. Liang, Y.S., Bruce, J.I., Boyd, D.A., 1987. Laboratory cultivation of schistosome vector snails and maintenance of schistosome life cycles. *Proc First Sino Am Sym.* 1, 34–48.
37. Lugin, J., Rosenblatt-Velin, N., Parapanov, R., Liaudet, L., 2014. The role of oxidative stress during inflammatory processes. *Biol Chem.* 395, 203–30.
38. Martinek, R., 1966. Improved micro-method for determination of serum bilirubin. *Clin Chim Acta.* 13, 161–170.
39. Melman, S.D., Steinauer, M.L., Cunningham, C., Kubatko, L.S., Mwangi, I.N., Wynn, N.B., Mutuku, M.W., Karanja, D.M.S., Colley, D.G., Black, C.L., Secor, W.E., Mkoji, G.M., Loker, E.S., 2009. Reduced susceptibility to PZQ among naturally occurring Kenyan isolates of *Schistosoma mansoni*. *PLoS Neglected Tropical Diseases*, 3(8),504–513.
40. Mohamed, S.S., Abdelmksoud, H.F., Sabry, H.Y., Mahmoud, S., El Komi, W., Shousha, T., El-Ashkar, A.M., 2023. Chologogue additive effect of ursodeoxycholic acid to Praziquantel on murine schistosomiasis mansoni: Parasitological and histopathological studies. New therapeutic efficacy against schistosomiasis, 16, 2090–2646.
41. Mokbel, K.M., Baiuomy, I.R., Sabry, A.A., Mohammed, M.M., El-Dardiry, M.A., 2020. *In vivo* assessment of the antischistosomal activity of curcumin loaded nanoparticles versus praziquantel in the treatment of *Schistosoma mansoni*. *Sci. Rep.*, 10(1),15742.
42. Moustafa, M.A., Mossalem, H.S., Sarhan, R.M., Abdel-Rahman, A. A., Hassan, E.M. 2018. The potential effects of silver and gold nanoparticles as molluscicides and cercaricides on *Schistosoma mansoni*. *Parasitol. Res.* 117, 3867–3880.
43. Muema, J., Obonyo, M., Njeru, S., Mwatha, J., 2015. Antischistosomal Effects of Selected Methanolic Plant Extracts in Swiss Albino Mice Infected with *Schistosoma mansoni*. *EJMP*, 9(1),1–11.
44. Nishikimi, M., Roa, N.A., Yogi, K., 1972. *Biochem. Bioph. Res. Common.*, 46,849–854.
45. Ohkawa, H., Ohishi, W., Yagi, K., 1978. *Anal. Biochem.* 95, 351.
46. Pellegrino, J., Oliveira, C.A., Faria, J., Cunha, A.S., 1962. New approach to the screening of drugs in experimental schistosomiasis mansoni in mice. *ASTMH*, 11,201 – 15.

47. Peters, P.A., Warren, K.S., 1969. A rapid method of infecting mice and other laboratory animals with *Schistosoma mansoni*: subcutaneous injection. J. Parasitol. Res. 55, 558.
48. Reitman, A., Frankel, S., 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J. Clin. Pathol. 28, 56–63.
49. Renda, G., Barut, B., Ceren, R., Aydin, E., 2023. *In vitro* tyrosinase inhibitory, DNA interaction studies, and LC-HRMS analysis of *Ficus carica* leaves Turk J Chem. 47, 465–475.
50. Reshi, M.S., Uthra, C., Yadav, D., Sharma, S., Singh, A., Sharma, A., Jaswal, A., Sinha, N., Shrivastav, S., Shukla, S., 2017. Silver nanoparticles protect acetaminophen induced acute hepatotoxicity: A biochemical and histopathological approach. Regul Toxicol Pharmacol. 2017,90 36–41.
51. Saad El-Din, M.I., Gad EL-Hak, H.N., Ghobashy, M.A., Elrayess, R.A., 2023. Parasitological and histopathological studies to the effect of aqueous extract of *Moringa oleifera* Lam. leaves combined with praziquantel therapy in modulating the liver and spleen damage induced by *Schistosoma mansoni* to male mice. Environ Sci Pollut Res Int. 30,15548–15560.
52. Salehi, S., Nori, A., Hosseinzadeh, K., Ganji, D.D., 2020. Hydrothermal analysis of MHD squeezing mixture fluid suspended by hybrid nanoparticles between two parallel plates. Case Studies in Thermal Engineering. Case Stud. Therm. Eng. 21, 100650.
53. Seif el-Din, S.H., El-Lakkany, N.M., Mohamed, M.A., Hamed, M.M., Sterner, O., Botros, S.S., 2013. Potential effect of the medicinal plants *Calotropis procera*, *Ficus elastica* and *Zingiber officinale* against *Schistosoma mansoni* in mice. Pharm Biol, 52(2),144 – 50.
54. Shaaban, A.M., Ibrahim, H.M., Mohamed, A.H., 2019. Effect of *Crocus sativus* aqueous extract (saffron) on *Schistosoma mansoni* worms in experimentally infected mice. Egypt. J. Aquat. Biol. Fish., 23, 391–408.
55. Singh, N.P., McCoy, M.T., Tice, R.R., Schneider, E.L., 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp. Cell Res. 175, 184–191.
56. Tawfeek, G.M., Abdel Baki, M.H., Ibrahim, A.N., Mostafa, M.A.H., Fathy, M.M., Diab, M.S.M. 2019. Enhancement of the therapeutic efficacy of praziquantel in murine Schistosomiasis mansoni using silica nanocarrier. Parasitol. Res. 118, 3519–3533.
57. Theodorsen, L., Strømme, J.H., 1976. Gamma-Glutamyl-3-carboxy4-nitroanilide: the substrate of choice for routine determinations of gamma-glutamyl-transferase activity in serum?. Clin Chim Acta. 72, 205–210.
58. Tice, R.R., Agurell, E., Anderson, D., Burlinson, B., Hartmann, A., Kobayashi, H., Miyamae, Y., Rojas, E., Ryu, J.C., Sasaki, Y.F., 2000. Single cell gel/ Comet Assay: Guidelines for *in vitro* and *in vivo* genetic toxicology testing. Environ. Mol. Mutagen. 35, 206–221.
59. Veberic, R., Jakopic, J., Stampar, F., 2008. Internal fruit quality of figs (*Ficus carica* L.) in the Northern Mediterranean Region. Ital J Food Sci. 20, 255–262.
60. Wang, J., Wang, L., Zhou, J., Qin, A., Chen, Z., 2018. The protective effect of formononetin on cognitive impairment in streptozotocin (STZ)-induced diabetic mice. Biomedicine & Pharmacotherapy. 106. Pp. 1250–1257.

Figures



Figure 1

The experimental preparation of *Ficus carica* nanoparticles



Figure 2

The experimental preparation of silver nanoparticles

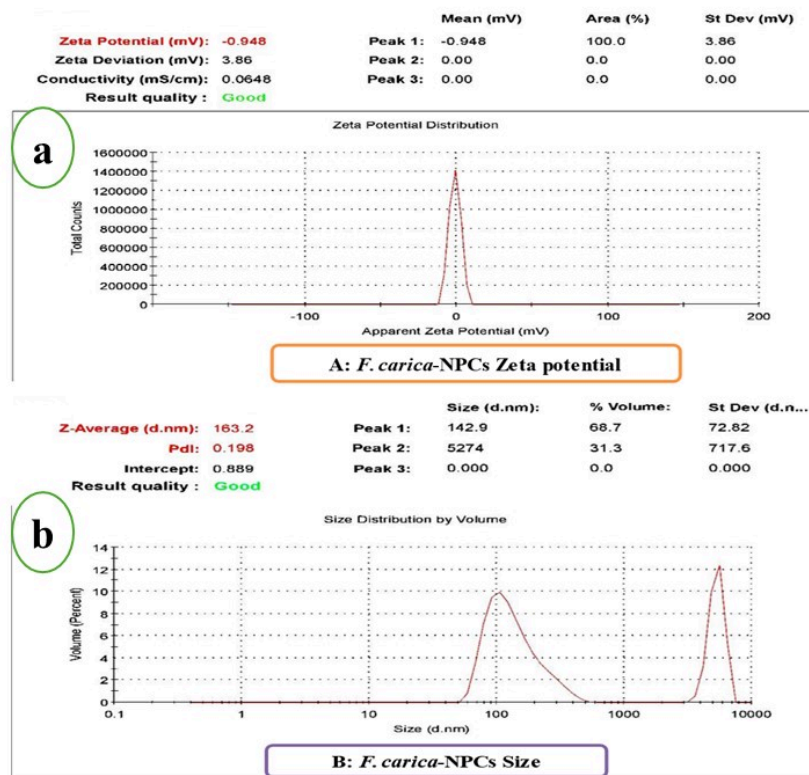


Figure 3

Characterization of *F. carica*-NPCs as A: Zeta potential and B: Size of nanoparticles

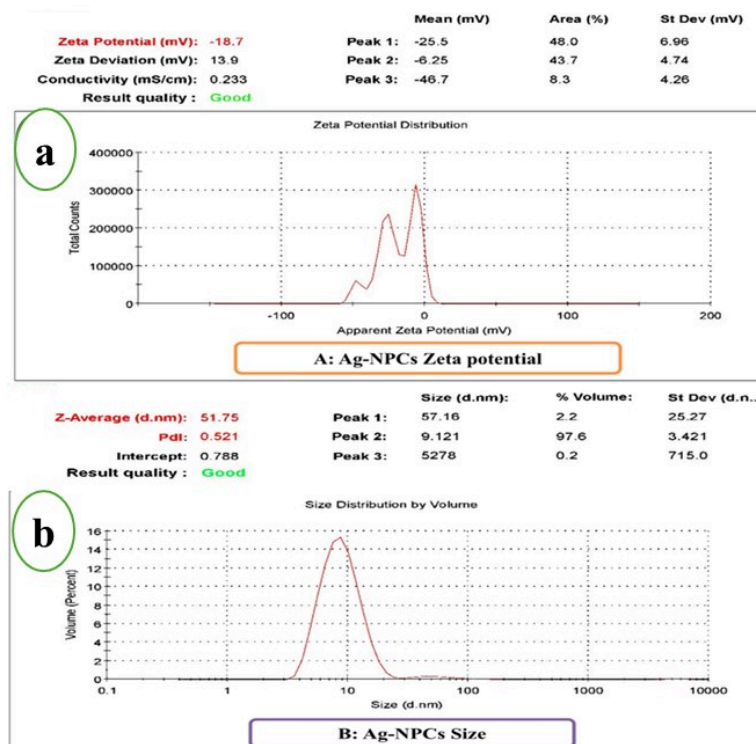


Figure 4

Characterization of Ag-NPCs as A: Zeta potential and B: Size of nanoparticles

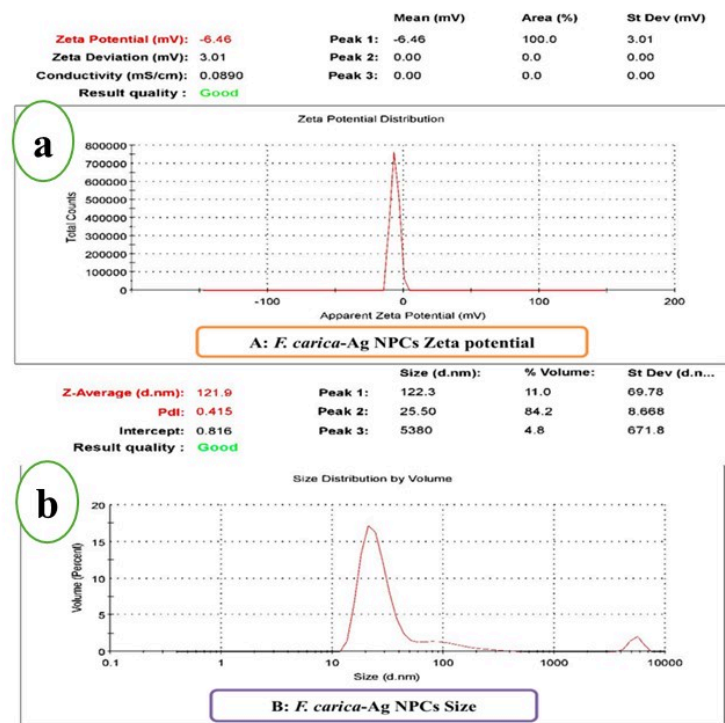


Figure 5

Characterization of *F. carica*-Ag NPCs as A: Zeta potential and B: Size of nanoparticles

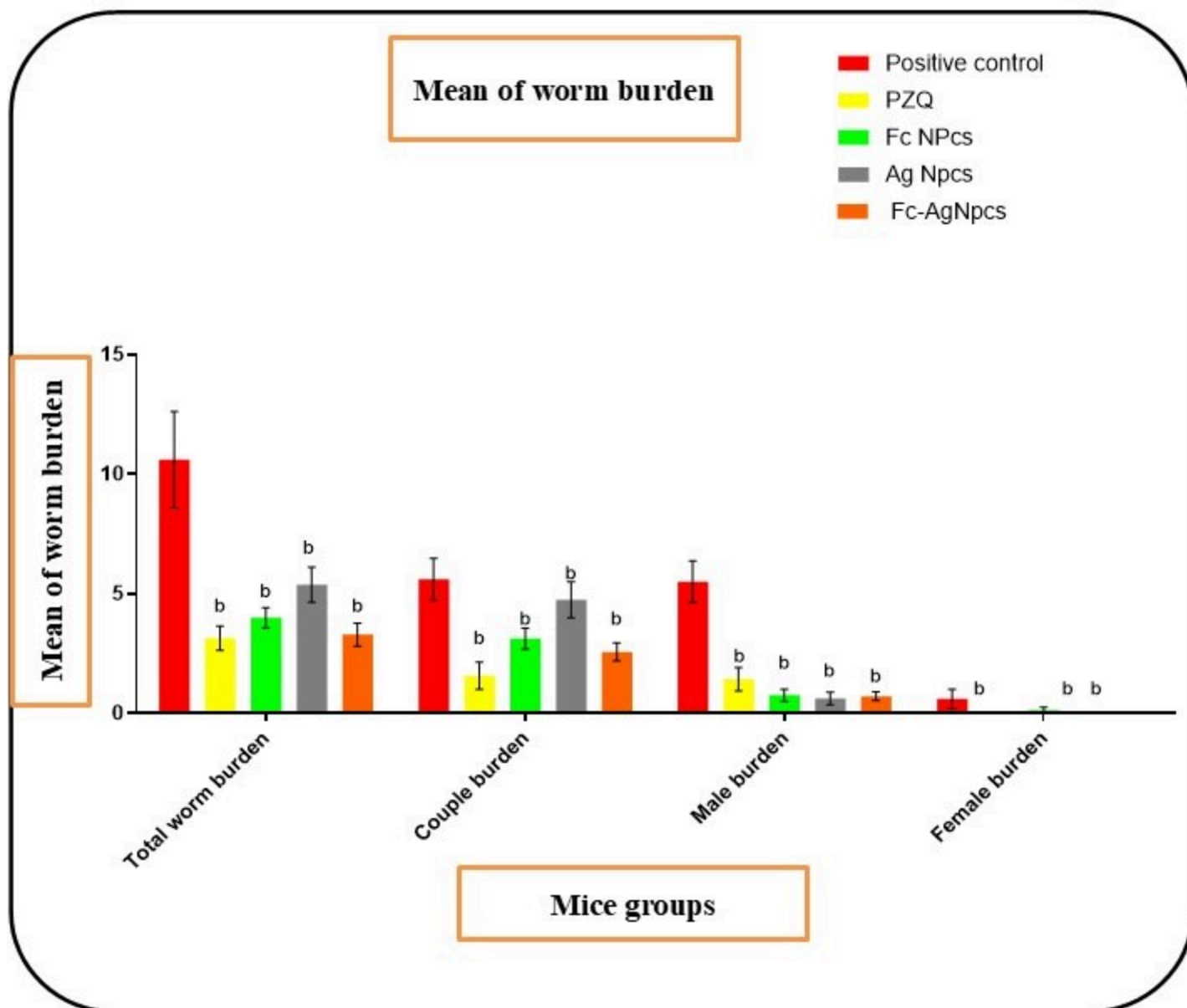


Figure 6

The recovered worms burden in control and different mice groups

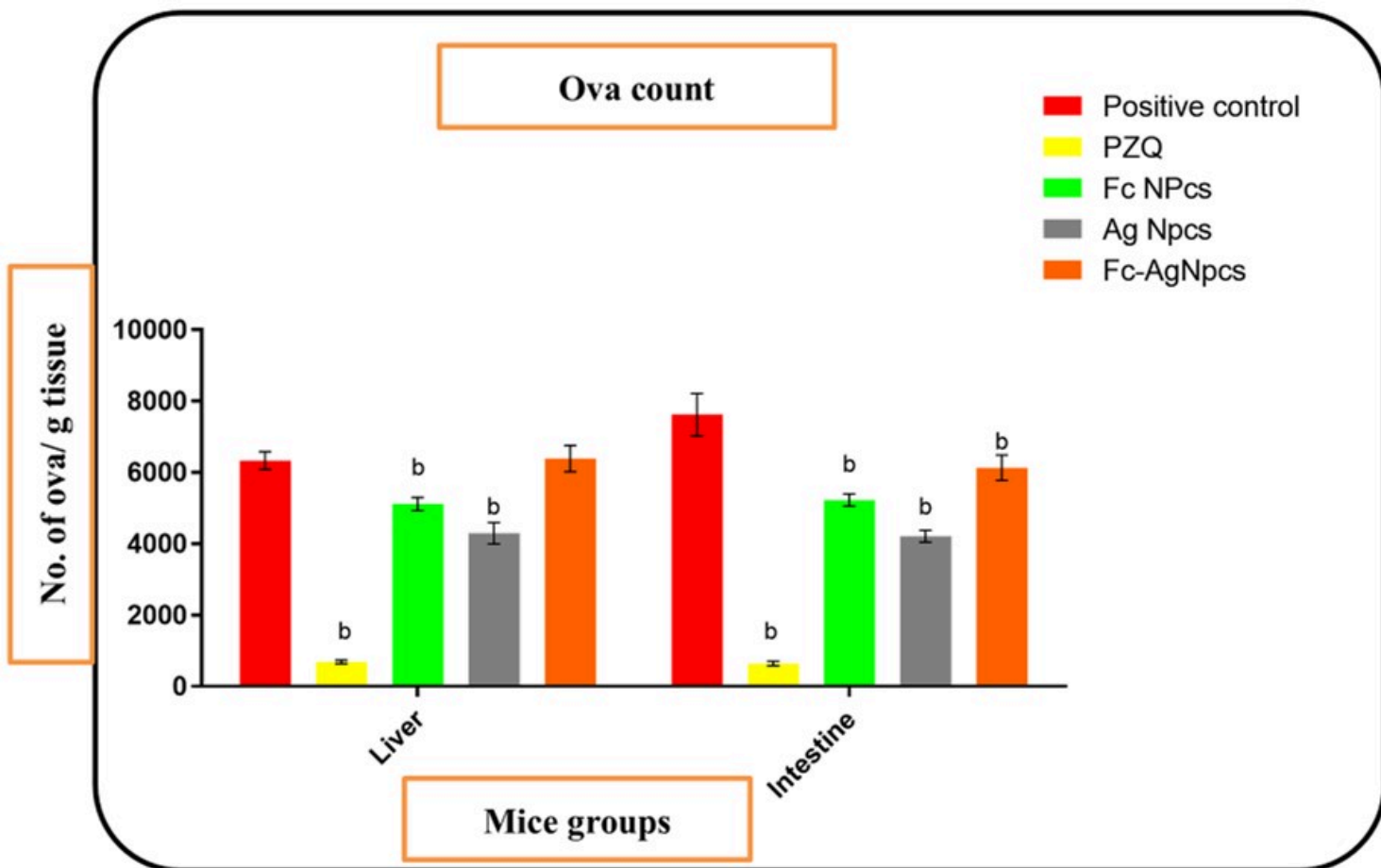


Figure 7

Ova count of control and different mice groups

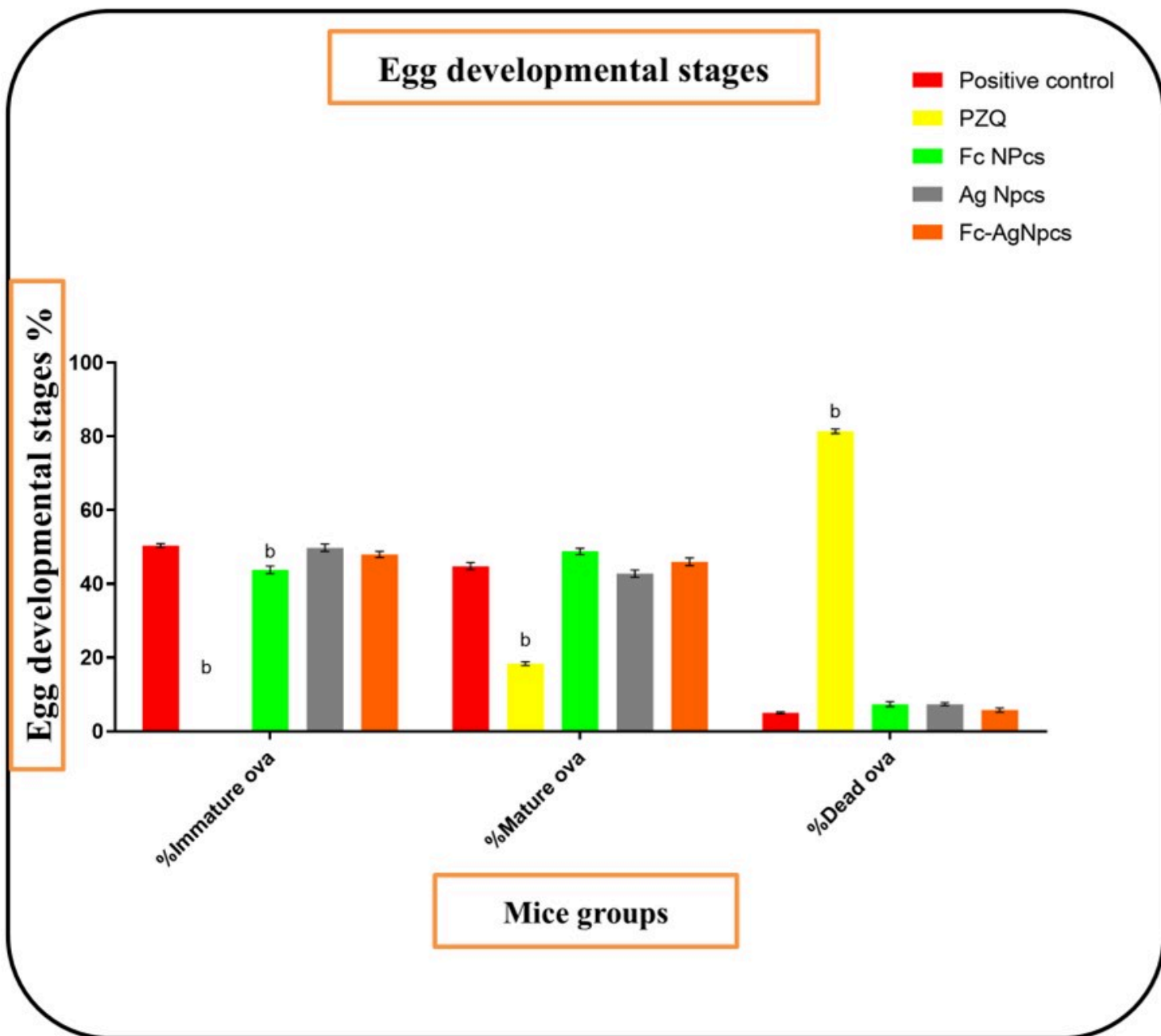


Figure 8

Egg developmental stages% of control and different mice groups

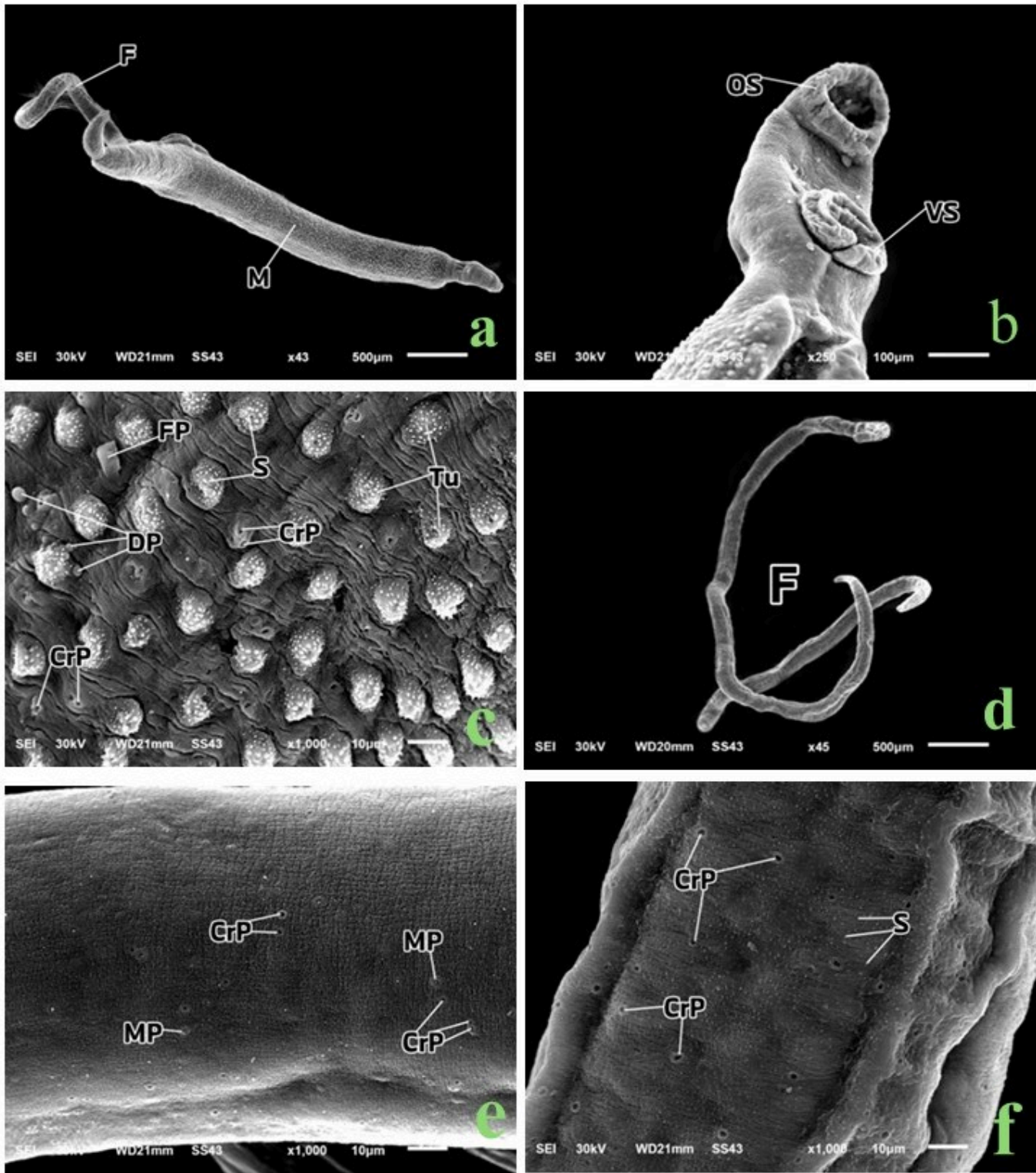


Figure 9

(A-F) Scanning electron micrographs of *Schistosoma mansoni* couple recovered from positive control group

Fig. 9A: Whole mount of male (M) and female (F) couple.

Fig. 9B: Magnified ventral view of the anterior third in Fig. 9A showed normal oral sucker (OS) and ventral sucker (VS).

Fig. 9C: High magnified view in the anterior third of female in Fig. 9A showed normal tubercles (Tu) with spines (S). Appearance of dome (DP), hemispherical (HP), fungiform (FP) and cratered (CrP) papillae.

Fig. 9D: The whole mounts of the female (F) appeared normal.

Fig. 9E & 9F: High magnification of the anterior third and posterior third in the female body beard normal tegmental spines (S). Note: Maculated papillae (MP) and cratered papillae (CrP) appeared on tegument.

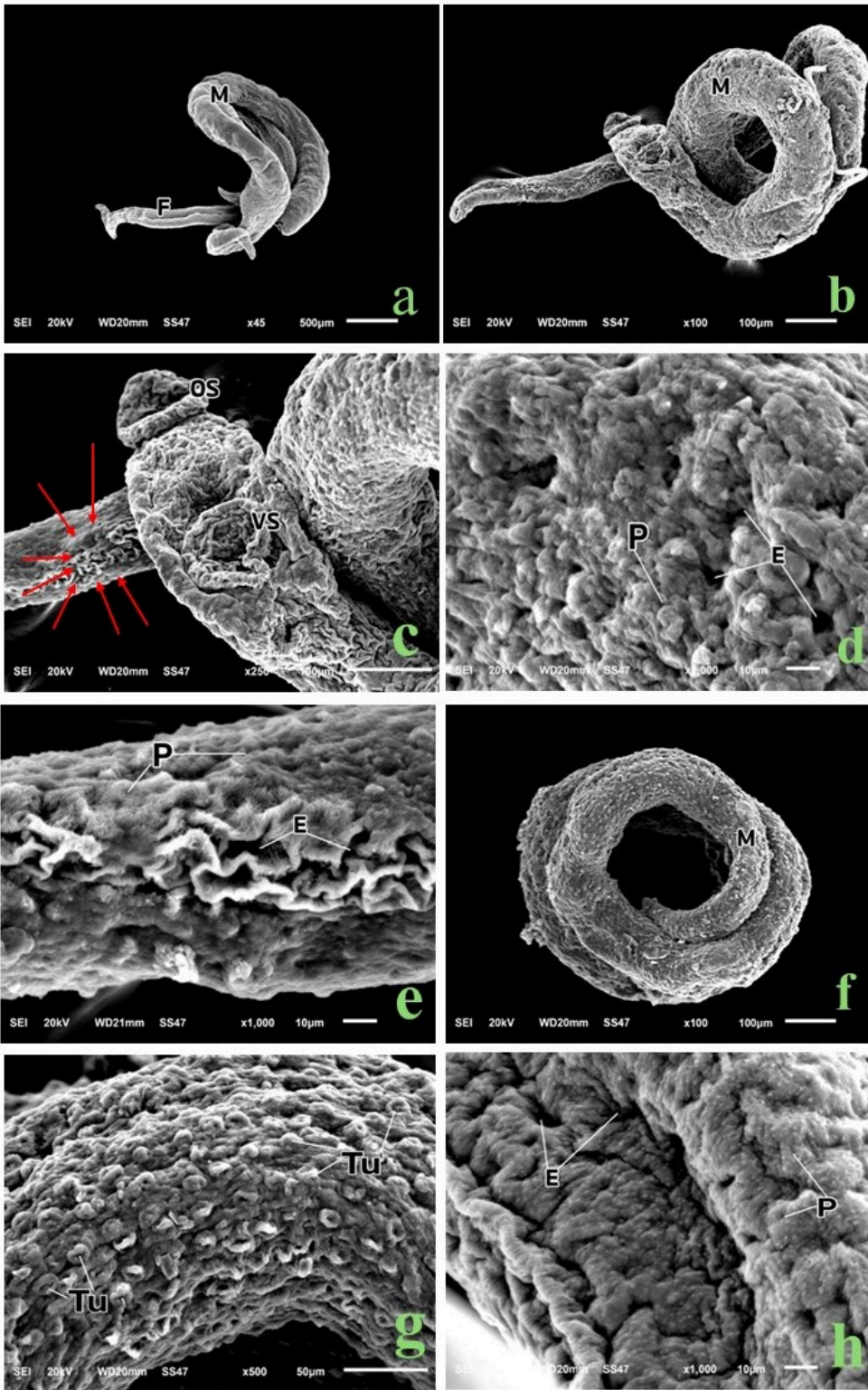


Figure 10

(A-H) Scanning electron micrographs of *Schistosoma mansoni* male recovered from mice treated with praziquantel group

Fig. 10A: Male (M) and female (F) worms in copulation.

Fig. 10B: Whole mount of coiled male (M) worm.

Fig. 10C: Magnified view of Fig. 10B; in the anterior third showed highly flattened triangular-shaped oral sucker (OS) and pulled shrinkage ventral sucker (VS).

Fig. 10D: High magnification of dorsal tegument highly corrugated showed peeling (P) and erosion (E).

Fig. 10E: Highly magnified (end third) of Fig. (10C) as red arrows showed deformed tegument with erosion (E) as continuous lines that resulted from peeling (P).

Fig. 10F: The male worm (M) appeared coiled.

Fig. 10G: Dorso-lateral surface of Fig. 10F; it observed naked tubercles (Tu) which appeared like blebs.

Fig. 10H: High magnification of Fig. 10A showed a peeled ventral surface (P) of the female tegument that had eroded areas (E).

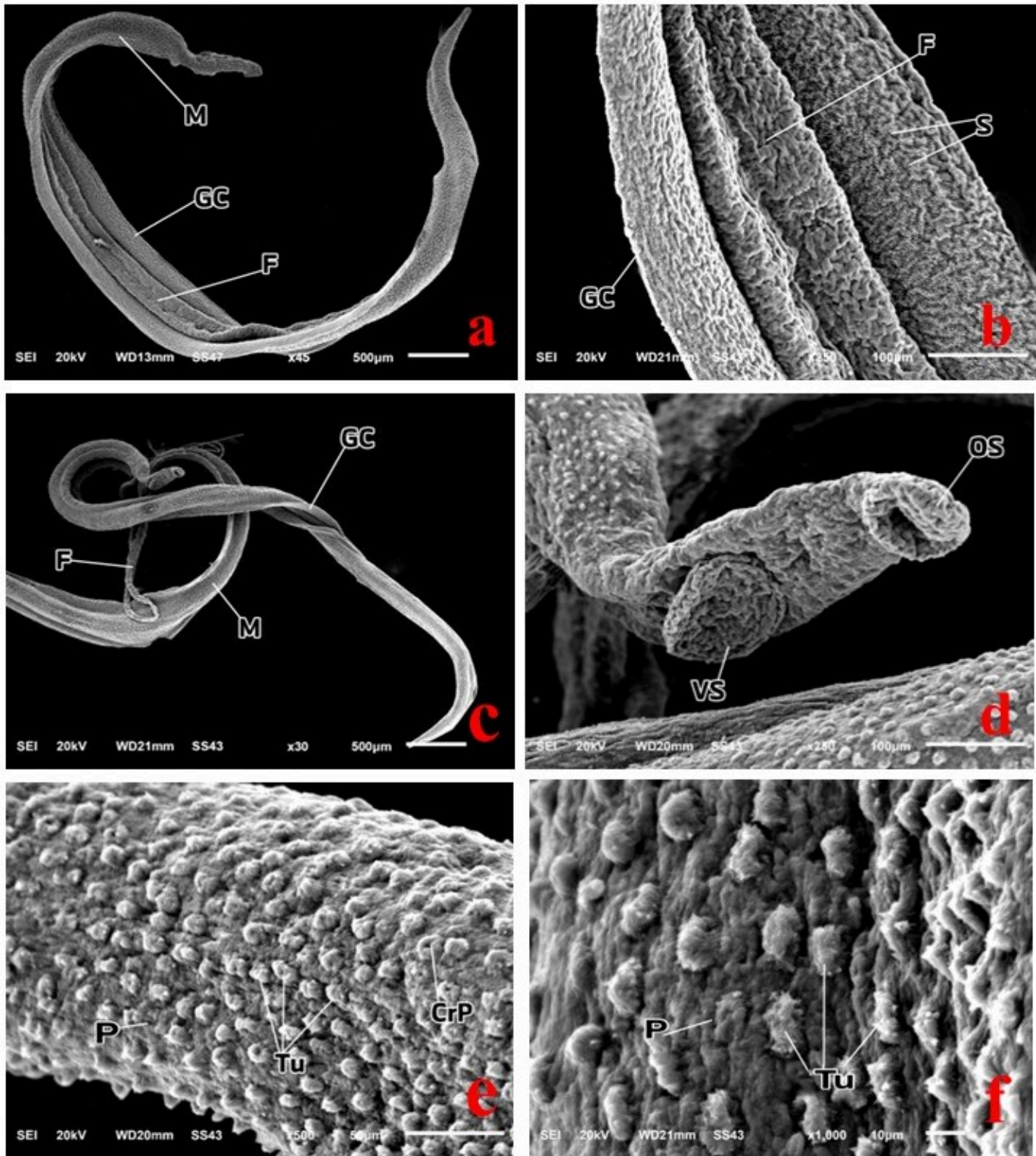


Figure 11

(A-F) Scanning electron micrographs of *Schistosoma mansoni* couples recovered from mice protected by *Ficus carica* leaves extract nanoparticles group

Fig. 11A: The whole mount of the male (M) carrying the female (F) inside a completely opened gynaecophoric canal (GC). Note: The couples had elongated bodies.

Fig. 11B: The female (F) appeared peeled and shrinkaged inside opened the gynaecophoric canal (GC).

Note: Appearance gynaecophoric canal spines (S).

Fig. 11C: The appearance of highly elongated couples' bodies illustrated twisting in the middle third.

Note: An opened male (M) carrying the female (F) in gynaecophoric canal (GC).

Fig. 11D: Dorsal view of male showed deformed suckers; shrunk oral sucker (OS) and flabby ventral sucker (VS).

Fig. 11E: A magnified view of Fig. 11C showed deformed peeled (P) tegument with damaged tubercles (Tu) like-blebs. Note: Presence of cratered papillae (CrP) between tubercles.

Fig. 11F: High magnification of Fig. 11E showed flattened appearance of tubercles that appeared naked (Tu) on peeled (P) tegument.

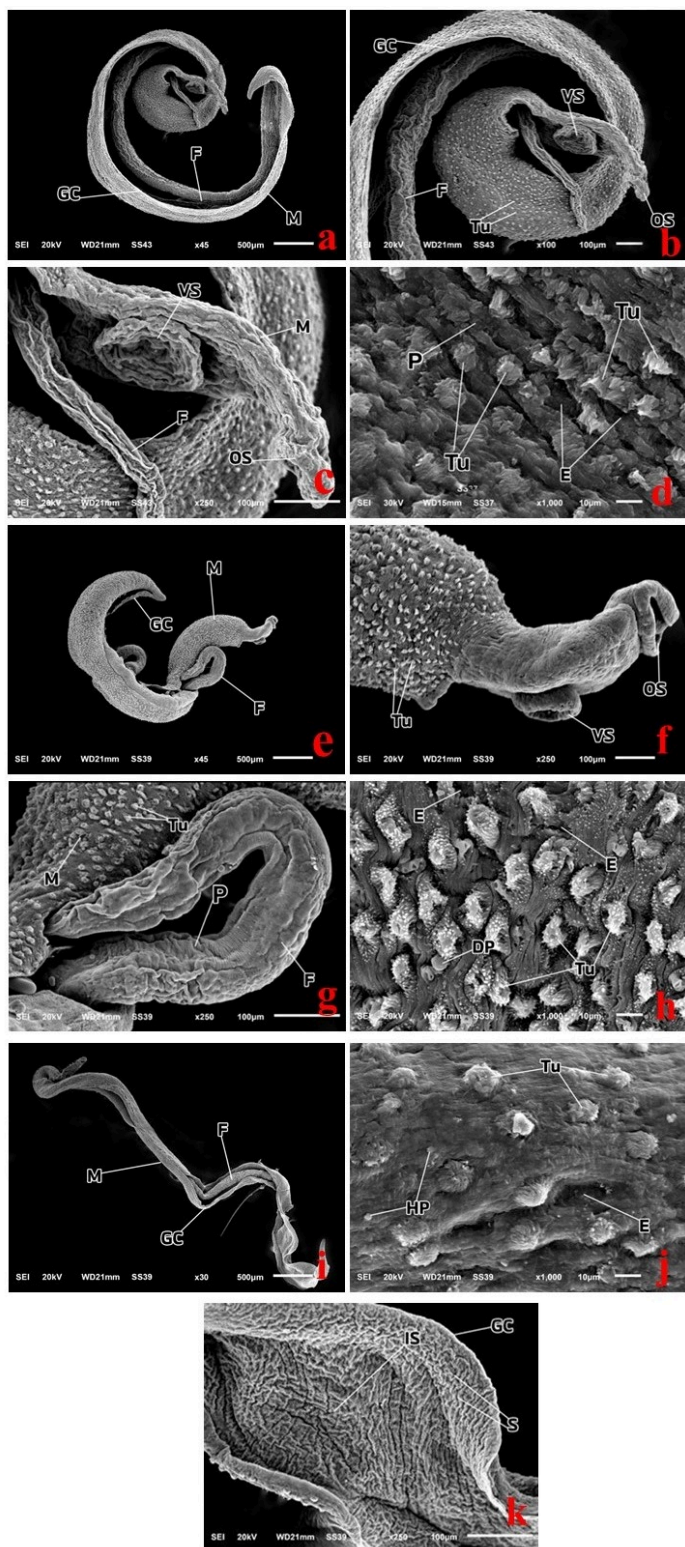


Figure 12

(A-K) Scanning electron micrographs of *Schistosoma mansoni* couple adult worms recovered from mice protected by silver nanoparticles group

Fig. 12A: The couple showed elongated partially coiled male (M) body. The gynaecophoric canal (GC) had a highly open appearance carrying female (F) inside.

Fig. 12B: A magnified view of Fig. 12A in the anterior third showed tegument bearing tubercles (Tu), oral sucker (OS) and ventral sucker (VS) appeared shrinkage. Note: The female (F) body exited from opened male's gynaecophoric canal (GC).

Fig. 12C: A magnified view of Fig. 12A showed shrinkage of body tegument with peeling appearance in both; female (F) and male (M). Note: Oral sucker (OS) and ventral sucker (VS) had shrinkage indulged architecture.

Fig. 12D: High magnification showed alteration in tegmental layer; deformed naked tubercles with loss of spines (Tu), peeling (P) and erosion (E).

Fig. 12E: Another male (M) and female (F) in the copulation position had twisting that showed protrusion of the female body from the gynaecophoric canal (GC). Note: Swollen of the male body was remarkable.

Fig. 12F: Magnified view of Fig. 12E showed a ventrolateral side (anterior third) in the male body with a deformed pulled shrinkage oral sucker (OS) and swollen ventral sucker (VS). Note: Tubercles (Tu) appeared like spots.

Fig. 12G: Magnified view through the second third in male (M) body showed protrusion of female (F) from the copulatory canal. Tubercles (Tu) appeared as blebs. Note: Deformed peeled (P) tegument of a female with absence of spines.

Fig. 12H: High magnification of male body showed erosion (E) of tegument; deformed tubercles (Tu) without spines and appearance of dome papillae (DP) in between tubercles.

Fig. 12I: The male (M) and female (F) couple had an extremely elongated body with semi-opened gynaecophoric canal (GC).

Fig. 12J: High magnification of dorsolateral surface showed eroded (E) tegument; mostly naked tubercles (Tu). Note: Appearance of hemispherical papillae (HP).

Fig. 12K: High magnified view of the inner surface (IS) of the gynaecophoric canal (GC) showed a deformed corrugated surface with minute spines (S).

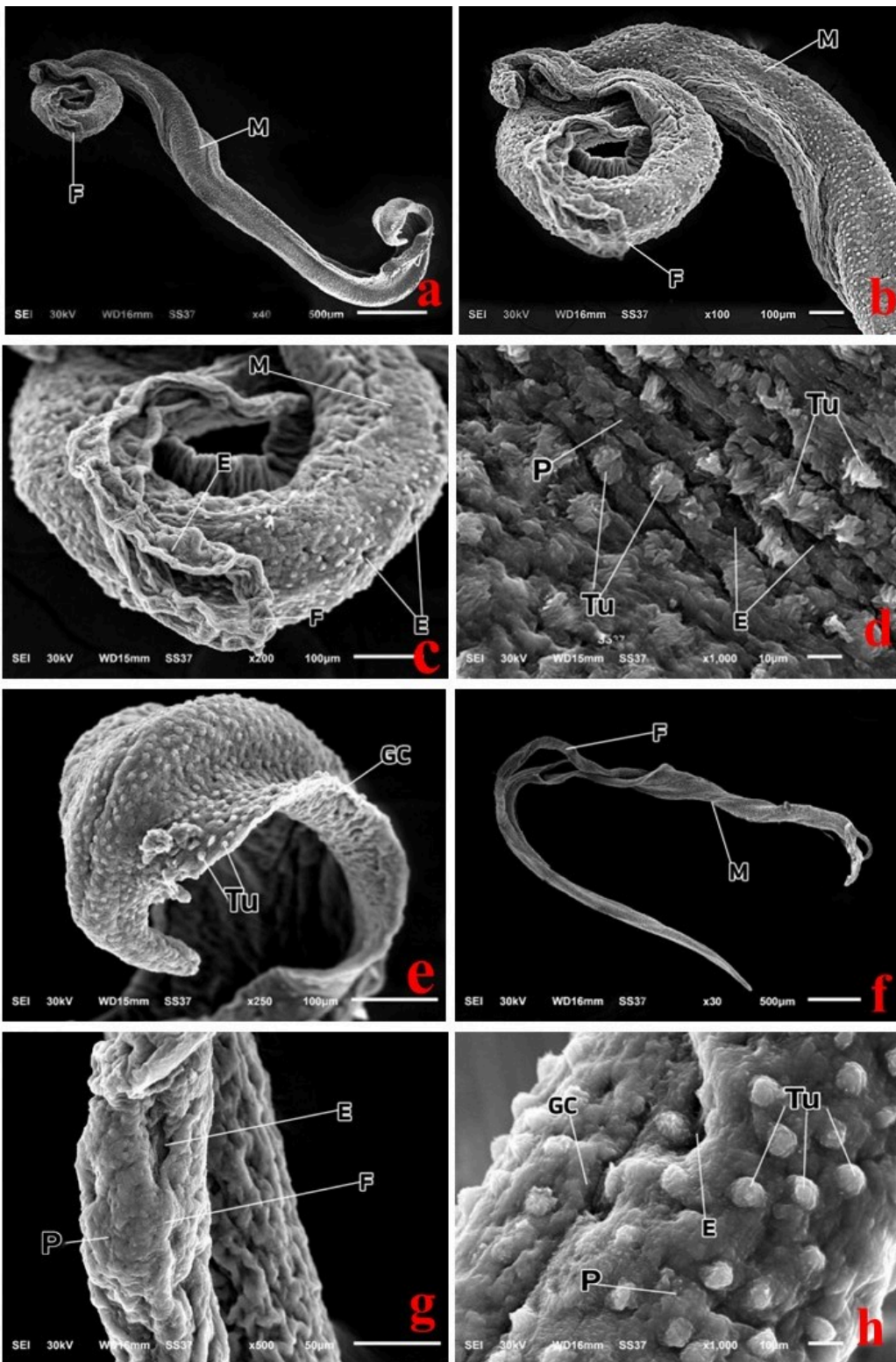


Figure 13

(A-H) Scanning electron micrographs of *Schistosoma mansoni* couple recovered from mice protected by Ficus carica loaded on silver nanoparticles group

Fig. 13A: Whole mount of elongated, partially coiled conjugated male (M) and female (F).

Fig. 13B: The magnified view in the anterior third of Fig. 13A showed a coiled male body (M) and emerged female (F).

Fig. 13C: The magnified view of Fig. 13B showed a protruded shrunk female body (F) from the gynaecophoric canal (GC), and in the tegument had erosion (E).

Fig. 13D: High magnification of tegument in anterior third showing highly deformed tegument with erosion (E), peeling (P), and damaged tubercles (Tu) without of spines that appeared naked. Note: Appearance of hemispherical papillae (HP).

Fig. 13E: Dorsal magnified view at last third showing that the tegumental surface of the male appeared a leaf-like shape with naked tubercles (Tu). Note: The gynaecophoric canal (GC) was opened till the end of the body.

Fig. 13F: Whole mount of highly elongated twisted male body (M) bearing female body (F). Note: The gynaecophoric canal had partially opened appearance.

Fig. 13G: Magnified view of Fig. 13F in the middle third of the couple's body showing protruded swollen female body (F) with peeled (P) tegument. Note: Presence of erosion (E) in some areas of tegument.

Fig. 13H: Magnification of ventral side of posterior third of couple body of Fig. 13F showing closed gynaecophoric canal (GC). Note: Erosion (E) of tegument with naked tubercles (Tu) caused by peeling (P).

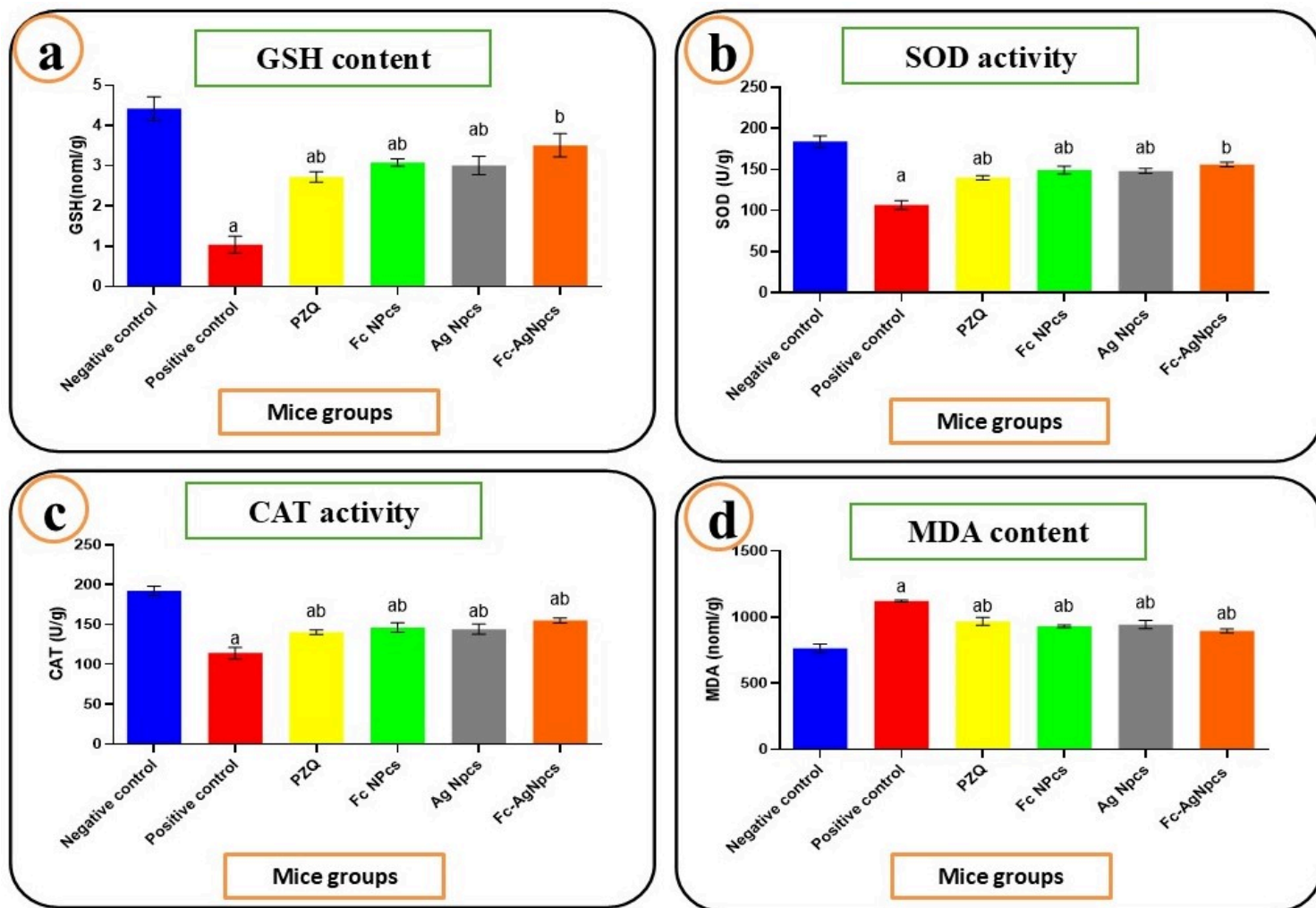


Figure 14

Antioxidant and oxidative stress biomarkers in control and all other tested mice groups; (A) GSH (Reduced glutathione), (B) SOD (Superoxide dismutase), (C) CAT (Catalase) and (D) MDA (Malondialdehyde)

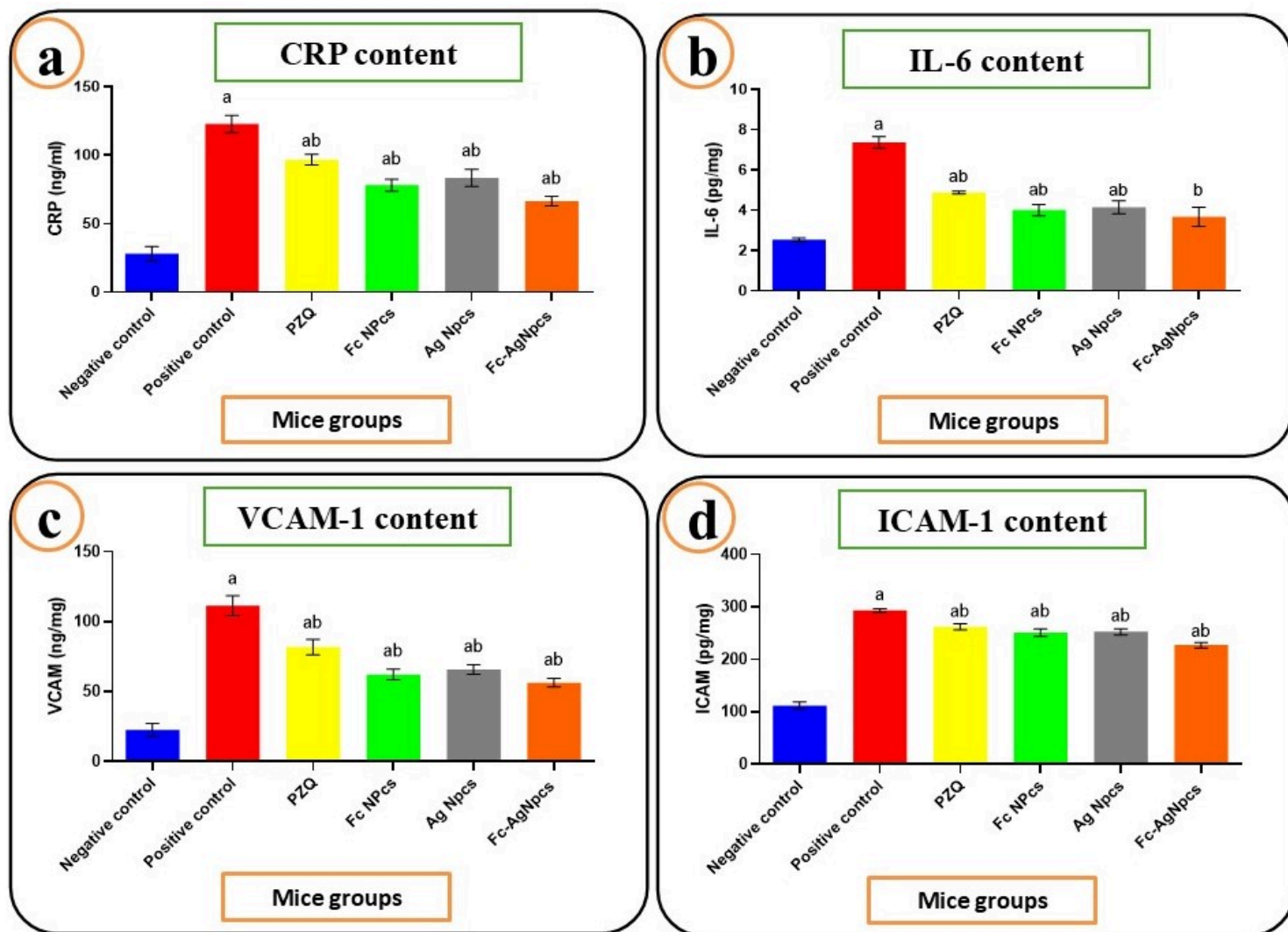


Figure 15

Inflammatory markers in control and all other tested mice groups; (A) CRP (C-reactive protein), (B) IL-6 (Interleukin-6), (C) VCAM-1 (Vascular cellular adhesive molecule-1) and (D) ICAM-1 (intercellular adhesion molecule-1)

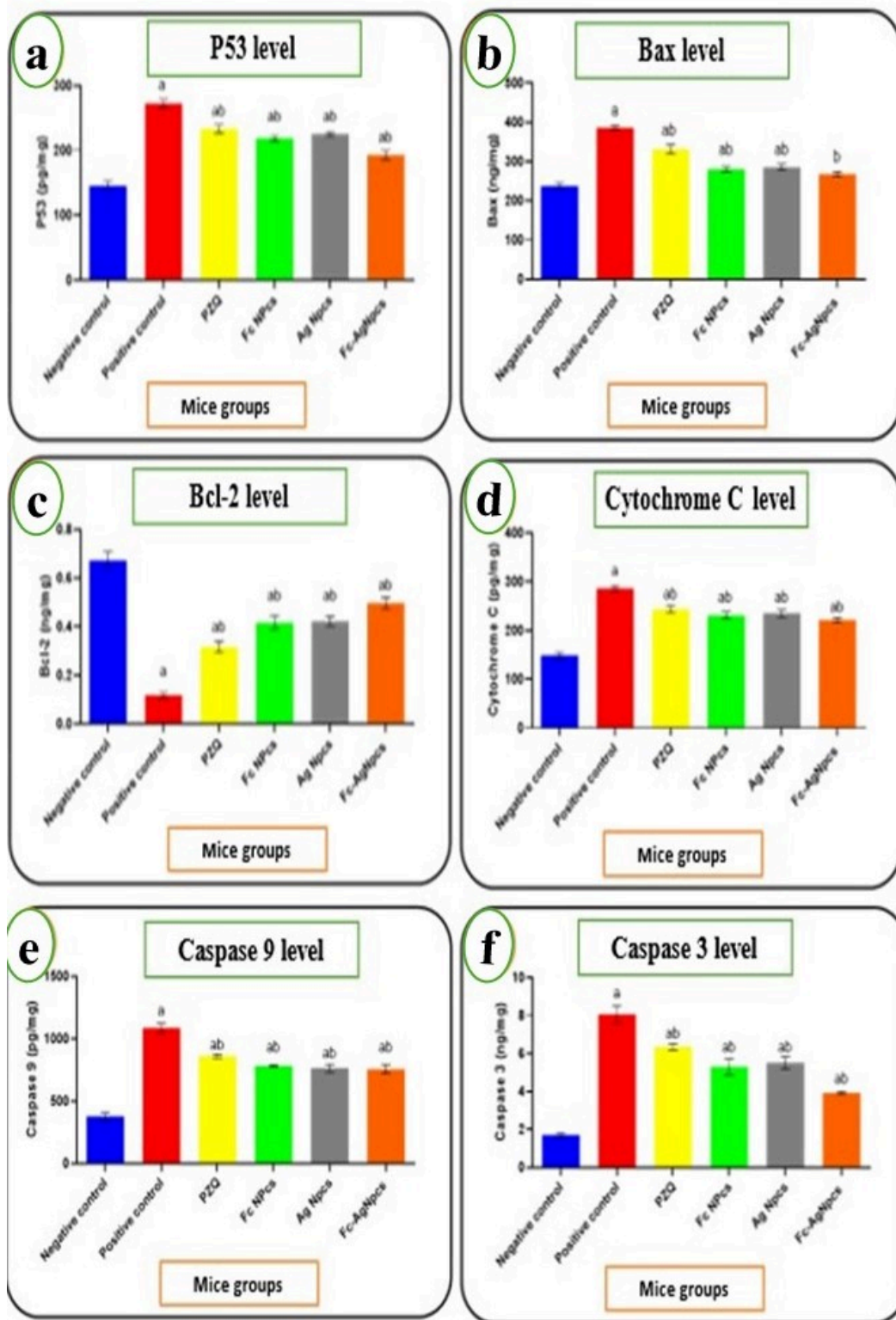


Figure 16

Hepatic apoptotic and anti-apoptotic markers in control and all other assessed mice groups; (A) P53, (B) Bax, (C) Bcl2, (D) cytochrome C, (E) caspase 9 and (F) caspase 3

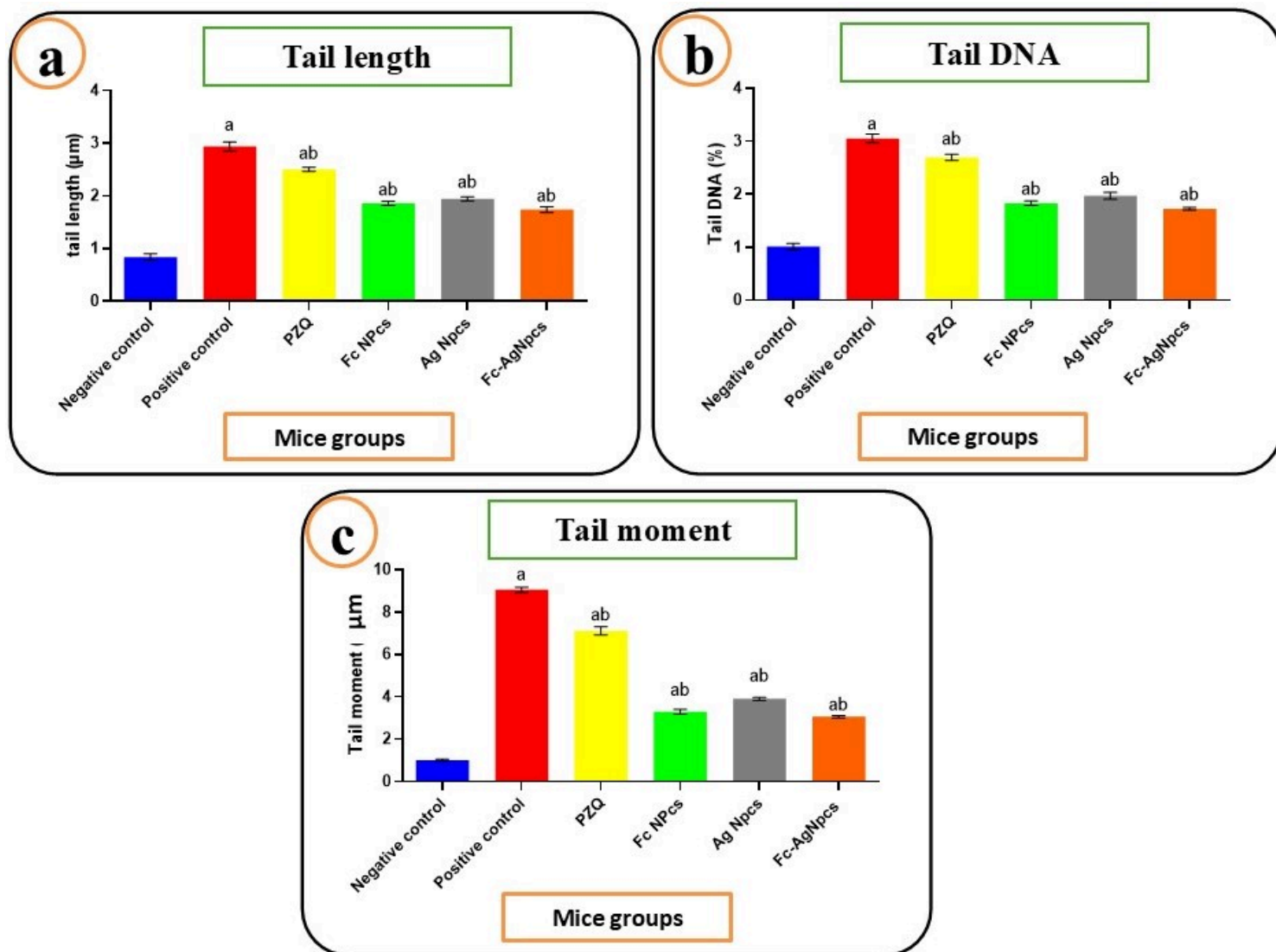


Figure 17

Photomicrographs of DNA damage of all studied groups (Scale bar = 100 μm)

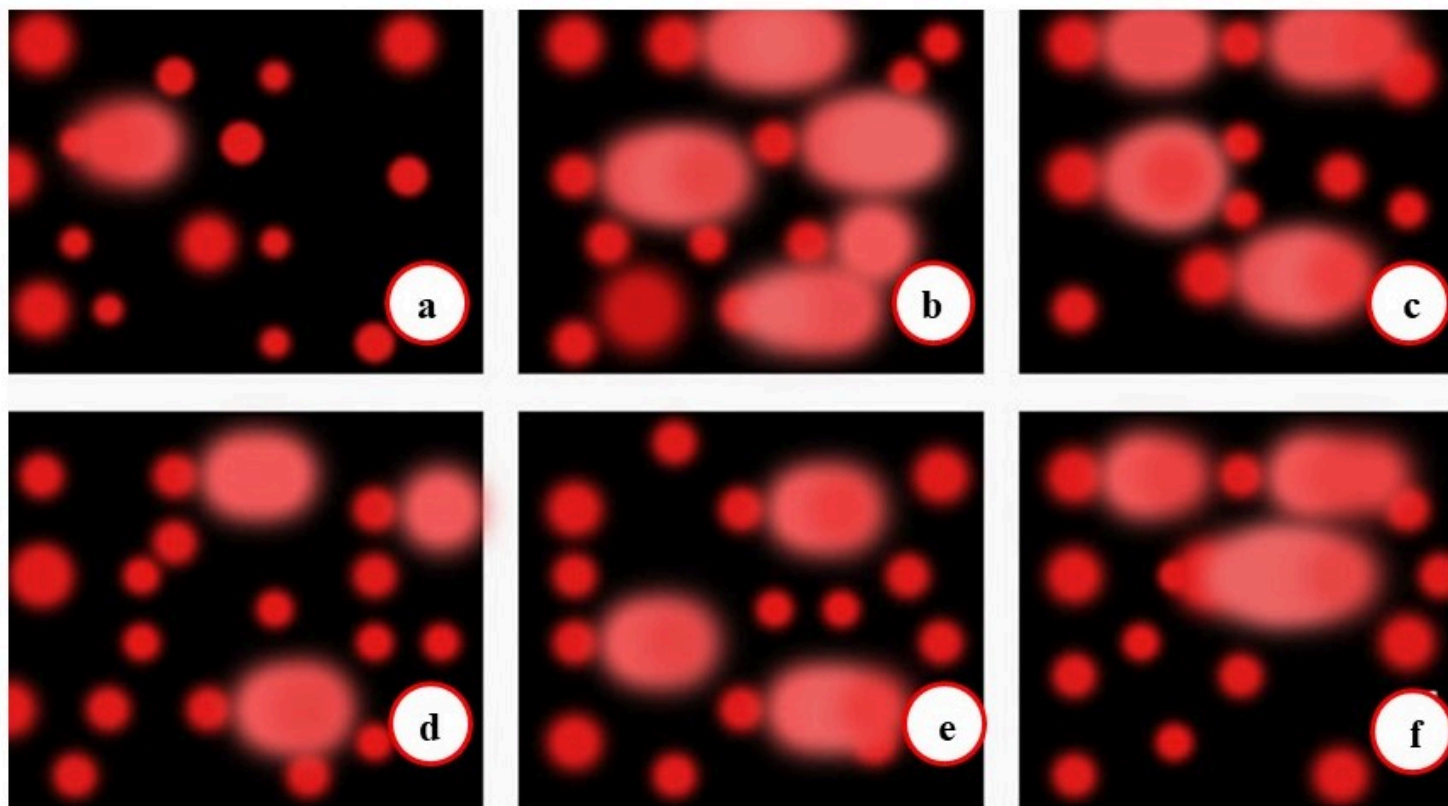


Figure 18

(A, B & C) showed the different hepatic DNA damage; Tail length, tail DNA and tail moment

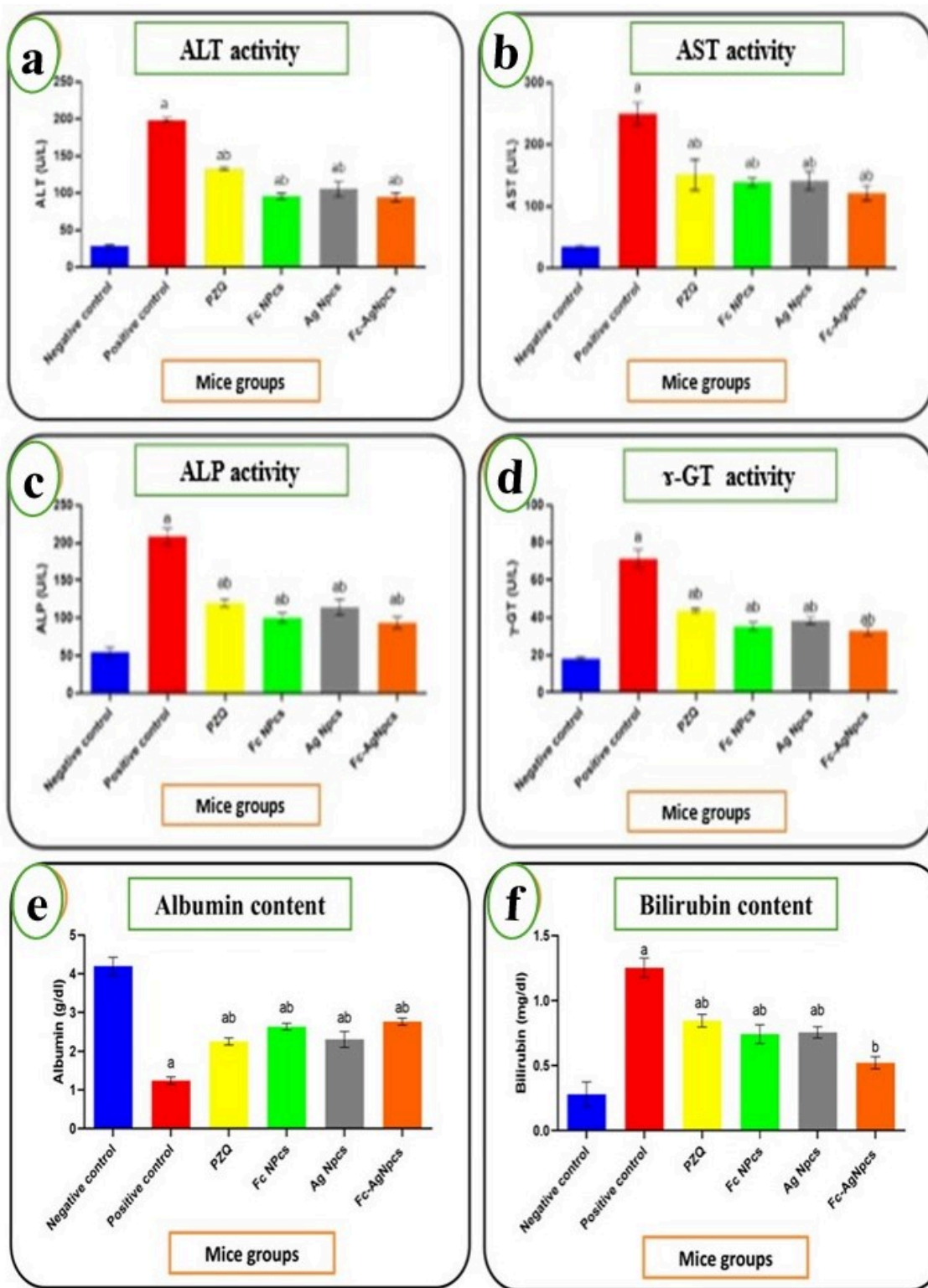


Figure 19

Liver function parameters in all assessed mice groups; (A) ALT (Alanine aminotransferase), (B) AST (Aspartate aminotransferase), (C) ALP (Alkaline phosphatase), (D) γ-GT (Gamma glutamyl transferase), (E) albumin and (F) bilirubin

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementarymaterial.docx](#)