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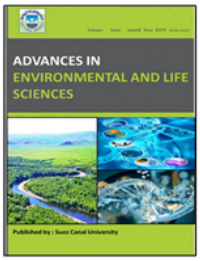

Biochemical study on the effects of silver nanoparticles on male albino rats

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Abstract

Silver nanoparticles represent a superior nano product with potential applications in medicine and hygiene because of their antibacterial effects**,** antiviral actions, and antifungal activity**.** The use of silver nanoparticles is not only restricted to medical applications but also extended to various issues related to the environment and consumer products. These particles accumulate in liver cells and lead to oxidative stress by generating reactive oxygen species. In addition, these particles decrease mitochondrial activity and cause morphological changes and toxicity. This study investigates the possible protective effect of Rutin and Morin in treating silver nanoparticle toxicity in rats' livers. 70 White Sprague-Dawley male rats were randomly assigned to seven groups. The first group was the normal groupG1 (control); G2 (treated with Rutin 100 mg/kg body wt). G3 (treated with Morin 30 mg/kg body wt) G4 (fed with AgNPs at 125 mg/kg body wt). G5 (AgNPs + Rut). G6 (AgNPs + Mor). G7 (AgNPs + Rut + Mor). For 30 days. Venous blood was taken to obtain serum for liver function test. In (AgNPs) treated group, AST and ALT activity, total and direct bilirubin, ALP, levels were significantly increased while Alb, total protein were significantly decreased compared to the control group. Treatment with Rutin and Morin reduced serum AST, ALT, total and direct bilirubin, and ALP; increased the level of Alb and Total protein activity compared to the AgNPs group.

Keywords: silver nanoparticles, Albino rats, Rutin, Morin, liver

1. Introduction

Nanotechnology characterizes an emerging field of science that incorporates the synthesis and development of various nanomaterials. Nanoparticles can be known as objects ranging in size from 1-100 nm. With the rapid development of nanotechnology, nanoparticle applications have been applied in a wide range of fields, from medical applications to environmental science. Moreover, now silver is the most used engineered nanoparticle in a consumer product**.** There are numerous applications of nanoparticles, among which are industrial applications in the construction industry in cement, coatings, paints, and insulating materials, as electrocatalysts, for advanced energy conversion and storage, in functionalized textiles, in food contact material, in cosmeceutical treatments for conditions such as photo aging, hyperpigmentation, and wrinkle [1] **.**

Several researchers have studied the hepatotoxic effect of silver and discovered that AgNPs interact with cellular functions, cause toxic effects, and, in addition, may interfere with specific biological systems in vitro [2]. Several studies in animals have demonstrated that AgNPs can be translocated in the blood circulation and extended to several organs, including the liver, kidney, and lung, after exposure via subcutaneous injections [3] and inhala-

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tion [4] and oral administration [5] **.** In most cases, the liver is proposed to be the main target organ for AgNPs [4, 5]

Morin (2´, 3, 4´, 5, 7-pentahydroxyflavone) is a phenolic compound in vegetables and plants**.** Many beneficial effects have been described, including anticancer**,** anti-inflammatory, and cardiovascular protective effects [6] **.**

Treatment by Rutin is abundantly found in onions, apples, tea, and red wine [7]. Rutin shows multiple pharmacological activities, including antitumor, anti-inflammatory, antidiarrheal, antiulcer, ant mutagenic, myocardial protecting, vasodilator, immunomodulation, and hepatoprotective activities [8]**.**

2. Materials and methods

2.1. Chemicals

AgNPs powder was purchased from Sigma-Aldrich, ≤ 100 nm, Morin hydrate powder was purchased from Alfa easer, Rutin powder, yellow, was purchased from Alfa Aesar, liver enzymes kits were obtained by Spectrum, Obour city industrial, Egypt

2.2. Experimental animals

Seventy white male Sprague-Dawley rats (4-6) weeks old and weighting (150-200 gm) were obtained from the Laboratory Animal Research Center, Faculty of Veterinary Medicine, Zagazig University. The animals were housed in standard metal cages at 22°C ± 3°C, 55% ± 5% humidity and were supplied with a standard laboratory diet and water ad libitum and artificially illuminated (12 h dark/light cycle). Rats were kept in constant environmental and nutritional conditions throughout the experiment. The animals were left for 14 days for acclimatization before the beginning of the experiment. The Animal Ethical Committee of Faculty of Veterinary Medicine, Zagazig University, approved the experimental study and was by their rules.

2.3. Experimental design

The rats were allowed to acclimatize for 14 days before treatment. Rats were randomly divided into seven groups, each containing ten rats (n = 10) G1 (Control): Rats were given 1ml/day of normal saline orally using a stomach tube. G2 (Rutin): Rats were given 1ml/day saline orally containing rutin (100 mg/kg body weight) [9], G3 (morin): Rats were given 1ml/day saline orally containing morin (30 mg/kg body weight),G4 (AgNPs): Rats were given 1ml/day saline orally containing AgNPs (125 mg/kg body weight). (Kim et al., 2010).

G5 (AgNPs +Rutin): Rats were given 1ml/day saline orally containing AgNPs (125 mg/kg body weight) + (100 mg/kg body weight) Rutin ,G6 (AgNPs + morin): Rats were given 1ml/day saline orally containing AgNPs (125 mg/kg body weight) + (30 mg/kg body weight) Morin ,G7 (AgNPs + rutin + morin): Rats were given 1ml/day saline orally containing AgNPs (125 mg/kg body weight) + (100 mg/kg body weight) rutin + (30 mg/kg body weight) morin using stomach tube. All treatments were given daily for 30 days.

2.4. Blood samples:

At the end of the experiment, blood was collected from the retro-orbital venous plexus located at the medial canthus of the eye in dry, clean Wasserman tubes and incubated for 1/2 hr. Clear serum was collected by centrifuging blood at 3000 rpm for 15 minutes and utilized for biochemical analysis. The activity of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), Alkaline phosphatase, Albumin, Total protein, and total and direct billirubin levels level were estimated for liver function test.

3. Results

3.1. Evaluation of ALT& AST enzymes:

The obtained results presented in tables (1,2,3) and illustrated in Figures (1,2,3) showed a significant increase in serum ALT, AST, and ALP activity in group 4 (AgNPs) when compared to group 1 (control). But in group 5 (AgNPs + Rut) and group 6 (AgNPs + Mor), the activity of serum ALT, AST, and ALP decreased when compared to group 4 (AgNPs) but was still higher than in the control. In treated group 7 (AgNPs + Rut + Mor), ALT, AST, and ALP activity returned to normal values.

Table 1: Effect of Morin and/or Rutin administration onserum ALT activity (U/L) against AgNPs induced toxicity inrats.

Groups/ Parameter's	G1	G2	G3	G4	G5	G6	G7
ALT (U/L)	43.75±0.81	41.53±0.45^{cd}	38.33±1.61^{d}	83.17±3.05^{a}	56.70±1.71^{b}	59.83±3.06^{b}	40.70±4.76^{cd}

Data expressed as(mean±SE).
Different alphabet letters indicate significant difference(with at least<0.05).

Table 2: Effect of Morin and/or Rurin administration on serumAST activity (U/L) against AgNs induced toxicity in rats

Groups/ Parameter's	G1	G2	G3	G4	G5	G6	G7
AST (U/L)	146.57±3.9^{b}	143.17±2.31^{b}	139.17±2.62^{b}	190.33±3.18^{a}	154.50±1.44^{ab}	172.50±2.52^{a}	142.10±2.61^{b}

Data expressed as(mean±SE).
Different alphabet letters indicate significant difference(with at least<0.05).

Table 3: Effect of silver and/or morin and/or rutin administration on serum ALP (U/L) against AgNPs induced toxicity in rats.

Groups/ Parameter's	G1	G2	G3	G4	G5	G6	G7
ALP (U/L)	216±4.16^{bc}	229±25.5^{bc}	230±15.27^{bc}	332±24^{a}	250±11.55^{bc}	259.67±7.75^{b}	203.67±4.18^{c}

Data expressed as(mean±SE).
Different alphabet letters indicate significant difference(with at least<0.05).

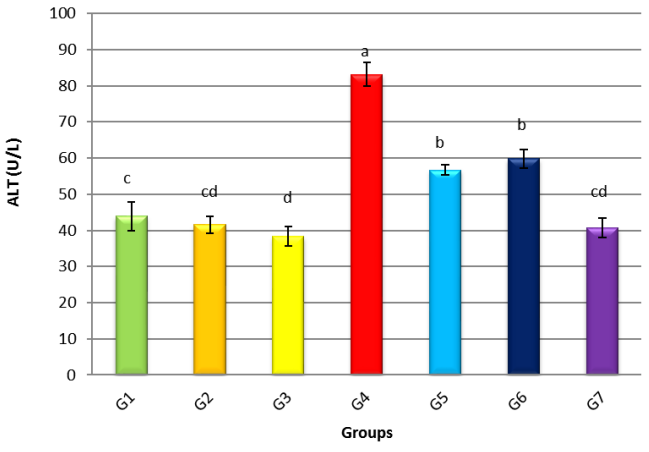

Figure 1: Effect of silver and/or murin and/or rutin administration on serum ALT level (U/L) against AgNPs induced toxicity in rats.

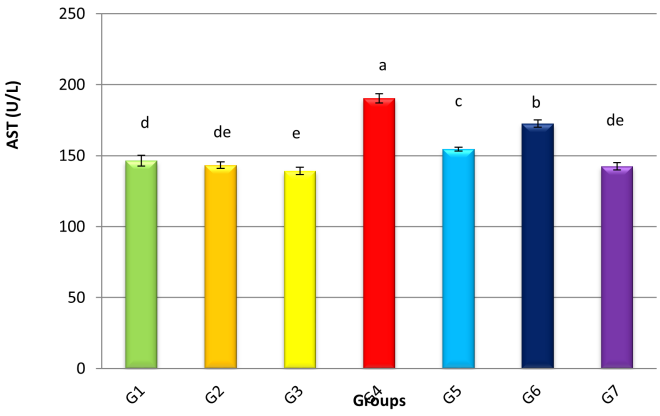

Figure 2: Effect of Morin and/or Rurin administration on serum AST activity (U/L) against AgNPs induced toxicity in rats

3.2. Evaluation of ALB& of D Bil & Total Bil:

The obtained result presented in table (4) and illustrated in Figure (4) showed that Total protein and ALB levels significantly decreased in group 4 (AgNPs) when compared to all other groups. No significant difference was observed between groups 2 and 3 compared to the control group. Mor & Rut's administration eased the Total protein and

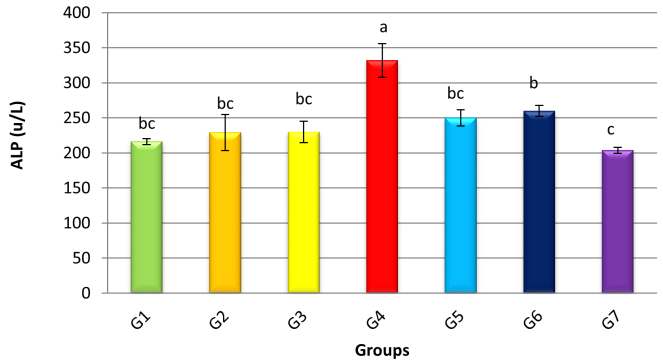

Figure 3: Effect of silverand/or morin and/or rutin administration on serum ALP level (U/L) against AgNPs induced toxicity in rats

ALB level and restored it to the average value.

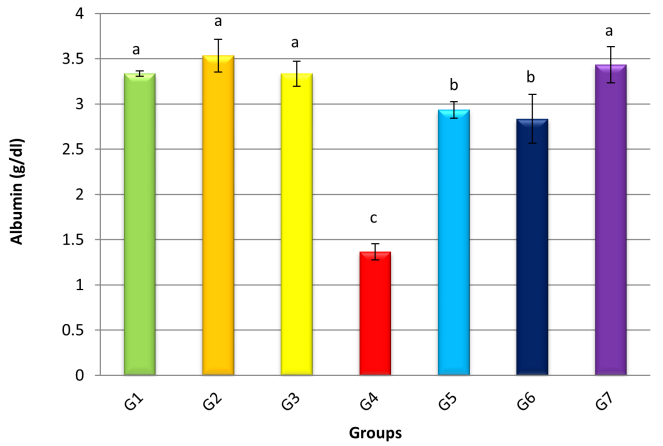

Figure 4: Effect of Morin and/or Rurin administration on serum albumin concentration (g/dl) against AgNPs induced toxicity inrats

3.3. *Evaluation of Direct and total Billirubin:*

The obtained result presented in table (5,6) and illustrated in Figures (5,6) showed a significant decrease in the level of serum Direct and total Billirubin in group 4 (AgNPs) when compared to the control group. But in group 5(AgNPs + Rut), group 6 (AgNPs + Mor) and group 7 (AgNPs + Mor + Rut), the levels of serum Total Bil, D.Bil significantly decreased when compared to group 4 (AgNPs) but still higher than control.Discussion

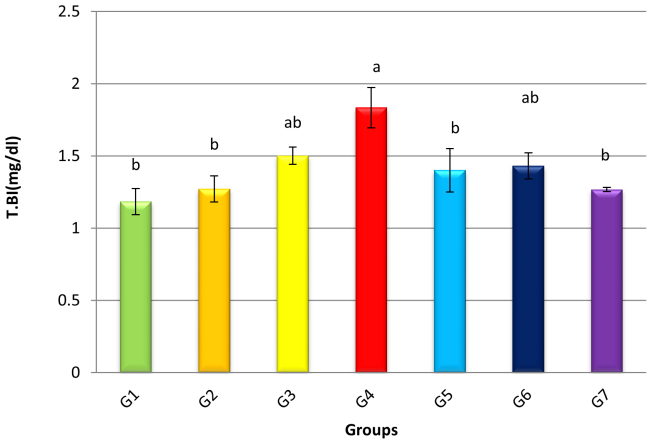

Figure 5: Effect of Morin and/or Rutin administration on serumtotal bilirubin level (mg/dl) against AgNPs induced toxicity inrats

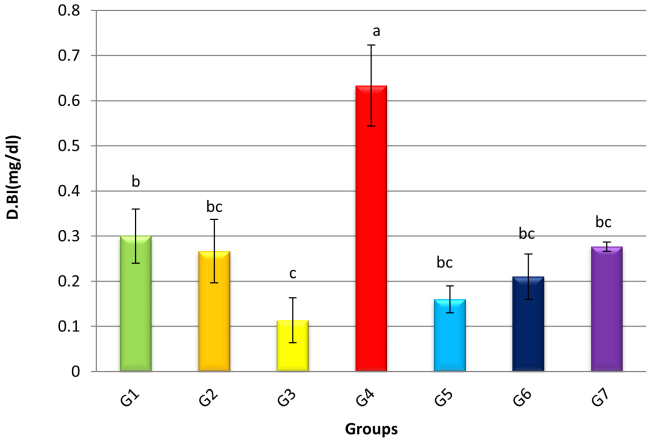

Figure 6: Effect of Morin and/or Rutin administration onserum direct bilirubin Level (mg/dl) against AgNPs induced toxicity in rats

4. Discussion

AgNPs are the most broadly connected nanomaterial in the biomedical and pharmacological fields. Besides injection, human exposure can occur via inhalation, ingestion, and dermal contact [10]. The liver is one of the most critical targets for exposure routes involving translocation to the bloodstream [11]**.** Sadauskas showed that Kupffer cells are essential for particle removal following intravenous administration [12].

Kim et al**.** revealed that small-sized AgNPs (10 nm size) had a more prominent capacity to incite

Table 4: Effect of Morin and/or Rurin administration on serumalbumin concentration (g/dl) against AgNs induced toxicity in rats

Groups /Parameter's	G1	G2	G3	G4	G5	G6	G7
ALB (U/L)	3.60±0.06a	3.50±0.06^{a}	3.60±0.06^{a}	1.33±0.09^{c}	3.90±0.06^{b}	4.00±0.06^{b}	3.90±0.06^{a}

Data expressed as (mean±SE).
Different alphabet letters indicate significant difference(with at least<0.05)

Table 5: Effect of Morin and/or Rutin administration on serumtotal bilirubin level (mg/dl) against AgNPs induced toxicity in rats

Groups /Parameter's	G1	G2	G3	G4	G5	G6	G7
TB (U/L)	1.18±0.09^{b}	1.27±0.18^{b}	1.50±0.06^{ab}	1.83±0.14^{a}	1.40±0.15^{b}	1.43±0.09^{ab}	1.27±0.014^{b}

Data expressed as (mean±SE).
Different alphabet letters indicate significant difference(with at least<0.05).

Table 6: Effect of Morin and/or Rutin administration onserum direct bilirubin Level (mg/dl) against AgNPs induced toxicity in rats.

Groups /Parameter's	G1	G2	G3	G4	G5	G6	G7
DBI (mg/dl)	0.30±0.06^{b}	0.27±0.07^{bc}	0.11±0.05^{c}	0.63±0.09^{a}	0.16±0.03^{bc}	0.21±0.05^{bc}	0.28±0.01^{bc}

Data expressed as (mean ± SE).
Different alphabet letters indicate significant difference(with at least<0.05).

apoptosis in MC3T3-E1 cells than large-sized AgNPs (50 and 100 nm) [5]. Previous studies have shown that small-sized AgNPs are more toxic than large ones [13].

Sung and etal indicated that most nanoparticles either accumulated into the organ and tissue or were taken out from the body through the liver and kidney by the renal system. Thus, the remaining amount of nanoparticles was decreased in the blood. Specific adverse effects can occur due to AgNPs accumulation, such as pathological changes in liver morphology, generation of ROS, DNA damage, and liver enzyme activities [14]. Several studies confirmed that the liver is the target organ for the effect of silver nanoparticles [4, 5]. Several studies on the biodistribution of AgNPs after injection demonstrated that the liver is the primary site of AgNPs deposition [10]. Rutin is abundant in onions, apples, tea, and red wine [7]**.** Rutin exhibits multiple pharmacological activities, including antibacterial, antitumor, anti-inflammatory, and hepatoprotective [8]**.** Morin (2′,3,4′,5,7-pentahydroxyflavone), a flavonoid isolated from Maclura tinctoria, Maclura pomiferaand from leaves of Psidium guajava, has a wide range of pharmacological properties including, anti-inflammatory, anti-oxidant, anti-autophagy, and anti-apoptosis [15]**.** In the present investigation, the physicochemical properties of AgNPs were determined using X-ray Diffraction (XRD). The purpose of the current study was to evaluate AgNPs toxicity and oral treatment with Morin and/or Rutin rats. Our Results on the effect of silver nanoparticles on liver enzyme Activity indicate that silver nanoparticles caused a significant increase in the activity of the enzymes in group 4 (silver nanoparticles) compared with group 1 (control), While group (2,3), which was treated with Morin, Rutin showed the lowest significant increase we can conclude that the Morin, Rutin

may exert a little pit toxic effect on liver but the upper hand of toxicity appeared on liver cells were caused by SNPs adversely affect liver functions on the hepatocyte. Assay of aspartate aminotransferase (AST/SGOT) and alanine aminotransferase (ALT/SGPT) activities have long been considered sensitive hepatic injury indicators. Injury to the hepatocytes alters their transport function and membrane permeability, leading to leakage of enzymes from the cells. This leakage causes a decrease in levels of AST and ALT in hepatic cells but an increase in levels of AST/SGOT with AST and ALT/SGPT with ALT in the blood .The liver is an important site for nanoparticles accumulation [5]. Our results correlated with the data obtained by [16], revealing that AgNPs induce a significant increase in symptoms of hepatic damage, such as elevation in the level of ALP, ALT, and AST enzymes. The level of particular enzymes in blood serum is a good indicator of liver injury [14]. Our data shows that AgNPs could increase plasmatic AST, ALT, ALP, and T. Bil activities. The AgNPs exposure could induce cell shape, reduce cell viability, and increase reuse. The LDH release is related to cell membrane damage and increased plasmatic AST activity; a promising biomarker of hepatocellular level damage suggests hepatocellular injury induced by AgNPs exposure. Serum total bilirubin concentration exhibited a relative increase. This higher value may be attributed to defense mechanisms against free radical-induced oxidative damage, including reducing free radicals by increasing electron donors, such as bilirubin. [14] reported that high concentrations of AgNPs in the bloodstream may lead to severe toxicity, as and the l function enzymes (including ALT, AST, ALP, GGT, and T. Bil) were increased when injected. Our results on liver enzymes agree with [14] . In contrast, Rathore et al. indicated that serum ALT and AST levels didn't significantly change, which may be due to the shallow dose of SNPs used in this study (3mg/kg).

On the other hand, [17] showed that the intraperitoneal administration of small-sized AgNPs (10 nm) in mice led to a significant increase in AST with an increasing tendency in ALT. At the same time, [18] reported that only AST was significantly increased in rats after oral administration of 0.5 and 1 mg/ kg AgNPs daily for 28 days, in addition to minor pathological changes in the liver and kidneys. Contrary to our results [19], oral administration of AgNPs orally to experimental rats for 28 days did not show considerable changes in the serum level of AST and ALT when given in different doses. Our finding coincides with an earlier study in which Rutin has been reported to restore the level of serum aspartate transaminase, alanine transaminase, alkaline phosphatase, and gamma-glutamyl transpeptidase in serum raised tetrachloride trichloride-induced hepatotoxicity [20]. Moreover, our finding also coincides with those of an earlier study in which morin has been reported to restore the level of ALT, AST, ALP in serum .

Our finding coincides with an earlier study in which Rutin has been reported to restore the level of serum aspartate transaminase, alanine transaminase, alkaline phosphatase, and gamma-glutamyl transpeptidase in serum-raised tetrachloride trichloride-induced hepatotoxicity [19]. Moreover, our finding also coincides with an earlier study in which morin has been reported to restore the activities of ALT, AST, and ALP in serum.

5. Conclusion:

Our results show that using a high concentration of Silver nanoparticles could cause undesirable effects on the liver, evidenced by damage to hepatocytes and elevation of liver enzyme activity. However, Rutin and Morin drug therapy is beneficial for treating AgNPs toxicity.

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