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Research Article

Effect of high-fat diet on oxidative stress and testicular function in male rabbits and protective effect of silver nanoparticles and *Moringa oleifera*

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Abstract
This study aimed to evaluate the effect of silver nanoparticles and *Moringa* leaf extract on testicular histology and antioxidants parameters after a high-fat diet exposure in male rabbits. The experiment was conducted on 30 male white rabbits, which were assigned into 6 experimental groups. The levels of Malondialdehyde (MDA) and the diameter of seminiferous tubules and germinal epithelium thickness were examined and compared. In blood serum of rabbits fed a high-fat diet noted an increased level of MOD. The addition of silver nanoparticles and *Moringa* leaf extract to the diet decreased the MOD level showed a beneficial effect. Rabbits fed a high-fat diet had a significant reduction in the diameter of seminiferous tubules and a decrease in the thickness of the germinal epithelium. The reduction was induced by supplementation with silver nanoparticles and *Moringa* leaf extract. Considering these results, it was possible to assume that exposure to silver nanoparticles and *Moringa* leaf extract have a possible protective effect on the reproductive system of male rabbits.
Keywords: Food, *Moringa*, Nanoparticles, MDA.

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Introduction

High-fat diet consumption is considered one of the main reasons for the development of obesity which is one of the growing worldwide health problems (Woods et al. 2003). Studies have shown a direct association between mean nutritional fat consumption and the prevalence of obesity and its associated problems and risk issues (Black et al. 2013; Askari et al. 2020). Obesity is broadly linked with comorbidities such as diabetes, certain types of cancer, cardiovascular diseases, hypertension, and obstructive sleep apnea as well as affects the reproductive system and fertility (Zhang et al. 2014; Roushandeh et al. 2015). The excess calorie intake of both fat and carbohydrates stimulates the production of reactive oxygen species (ROS). The accumulation of ROS results in oxidative stress (Bojková et al. 2021). Prolonged exposure of tissues to oxidative stress can lead to tissue damage. At the level of the testes, oxidative stress can disrupt the steroidogenic capacity of Leydig cells (Hales et al. 2005), besides, oxidative stress can also interrupt the ability of the germinal epithelium to differentiate normal sperm (Naughton et al. 2001). There are countless weight-loss strategies, including lifestyle modification strategies, mainly dietary modification, behavior change, exercise behaviors, and bariatric surgery (Kushner 2014).

The use of nanotechnology in medicine provides a wide-range of new tools and promises for disease detection, treatment, and prevention. Nanotechnology-based therapies have the potential to be used as an alternative strategy for the treatment of obesity and prevent its comorbidities (Ash et al. 2019; Sibuyi et al. 2019). Despite their potential toxicity, silver nanoparticles (AgNPs) are commonly used in various applications, including biomedical devices, health care, and consumer products due to their exceptional physical and chemical properties (Zhang et al. 2017).

Similarly, the beneficial effects of numerous plants and herbs in curing many human diseases have been emphasized in many countries (Lawal et al. 2016; Azez & Hamza 2020). *Moringa oleifera* is one of these plants. The seed extracts have been confirmed as a rich source of vitamins, minerals, phenolic, ascorbic acid, and isothiocyanate derivatives, which may be attributed to their antioxidant activity (Flora & Pachauri 2011). As a result, it is equitable to increase the research to improve healthier options from such plant species to address metabolic disorders. Based on above mentioned background, this study aims to elucidate the effect of dietary high-fat consumption on the reproductive system of male rabbits and the general oxidative activity in terms of Malondialdehyde (MOD) and the possible protective effect of silver nanoparticles and *M. oleifera* leaf extract.

Materials and Methods

Experiment animals: A total of 30 male white rabbits (red eyes) weighing 1860 to 1086g were were purchased from local breeding facilities in Baghdad and Al-Qadisiya provinces. Animals were kept in cages under a stable temperature (25-30°C) with free access to food and water and in a regular light-dark cycle at the animal house of the University of Kerbala, Veterinary College. The study started in January 2020 and last for 4 months. Animals were acclimatized to the animal house conditions for 2 weeks before the experiment.

Experiment design: The Rabbits were randomly divided into 6 groups. Each group was housed in aluminum cages (50×40×50 cm). Animals in each group were subjected to the following treatment: (1) an untreated or negative control where animals received only normal feed and water daily, and (2) a positive control group (Hypercholesterolemia) where animals fed a high-fat diet enriched with cholesterol powder 2.5 % for 3 months. After 3 months, the animals were further subdivided into 4 groups of 5 animals, including (I) animals received a high-fat diet with the addition of 5mg/kg body weight of silver nanoparticles for 21 days , (II) animals received a high-fat diet with the addition of 10 mg/kg body weight of silver nanoparticles for 21 days, (III) animals received a high-fat diet with the addition of 15mg/kg body weight of silver nanoparticles for 21 days, and (IV) animals received a high-fat diet with the addition of 200mg/kg body weight of *M. oleifera* leaves extract for 21 days.

Dissection of animal: The animals were kept overnight fast at the end of the experiment and sacrificed under light chloroform anesthesia. The blood samples were drawn from the ventricles, centrifuged and the serum was frozen at -20°C until used for biochemical tests. Testis was removed immediately and stored in 10% formalin for histological study.

Measurement of the diameter and the germinal layer thickness of seminiferous tubules: After histological tissue preparation, the paraffinized tissue was cut into 5µm thick sections and the sections were stained with hematoxylin and eosin. The diameter of seminiferous tubules and germinal epithelium thickness were measured using a light microscope (Olympus, Japan) equipped to a digital camera. In each group, 5 fields/rabbits (randomized 5 seminiferous tubules/field) were counted (Jalili et al. 2015; Salahshoor et al. 2018).

Biochemical analyses: The level of serum malondialdehyde (MDA) was detected following the method of Al-Daamy & Al-Zubiady (2020).

Statistical analysis: A one-way analysis of variance (ANOVA) method was used to compare data. The results were expressed as the mean ± standard deviation (SD), and a P-value≤0.05 was considered significant.

Results

The effect of a high-fat diet (HFD) on the histology

Table 1. Represent the means of diameters of seminiferous tubules and germinal layer thickness of seminiferous tubules in the testis of experimental rabbits' groups.

Parameters	Diameters of seminiferous tubules (µm)	The germinal layer thickness of seminiferous tubules (µm)
Control group	386.16±34.74[a]	182.48±19.68[a]
Hypercholesterolemia group	248.29±30.78[b]	50.87±16.81[b]
Hypercholesterolemia Treated with 5% silver nanoparticles	300.25±15.64[c]	127.21±20.11[c]
Hypercholesterolemia Treated with 10% silver nanoparticles	359.33±25.93[a]	170.57±12.25[a]
Hypercholesterolemia Treated with 15% silver nanoparticles	305.18±10.6[c]	130.55±17.24[c]
Hypercholesterolemia Treated with *Moringa oleifera*	372.5±18.28[a]	169.19±13.54[a]

Values represent mean ± SD, different letters represent significant differences ($P \leq 0.05$).

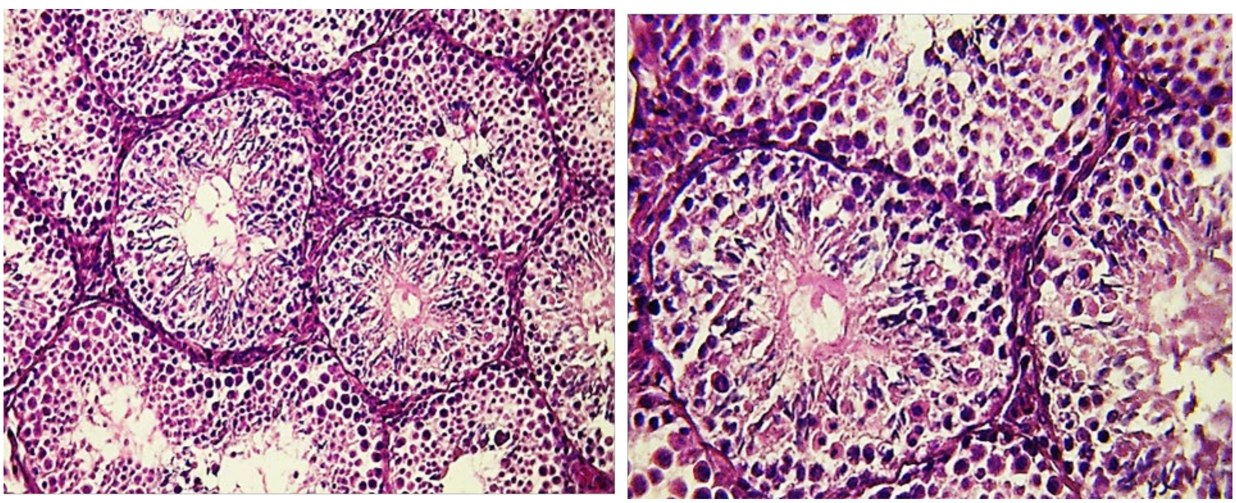

Fig.1. (Left and Right) cross-section in testes of the control group showed the normal structure of seminiferous tubules, all stages of spermatogenesis are present, normal structure of basement membrane, sperm appear in the lumen of seminiferous tubules (H&E) (200 and 400x).

of the testes of the male rabbits was studied and in male rabbits fed with HFD (Hypercholesterolemia group), there were a significant decrease ($P<0.05$) in the diameter of seminiferous tubules and in the thickness of the germinal epithelium (Table 1). The average luminal diameter of seminiferous tubules and thickness of the germinal epithelium in treated animals with 5 % silver nanoparticles record a significant increase ($P<0.05$) by 17 and 60%, respectively compared with HFD. Moreover, animals supplemented with 10 and 15% of silver nanoparticles resulted in a significant increase ($P<0.05$) in the luminal diameter of seminiferous tubules and thickness of the germinal epithelium by 31 and 70%, and 19 and 61%, respectively relative to those of HFD. However, a greater increase of 33% ($P<0.05$) in the diameters of seminiferous tubules was reported between *M. oleifera* leaf extract and HFD. Similarly, the thickness of the germinal epithelium was significantly increased by 70% compared with HFD.

The testicular tissue from negative control rabbits A and B showed the testicular tissue with normal characteristics (Fig. 1). However, the absence of normal structure of seminiferous tubules, degeneration of most cell types of spermatogenesis

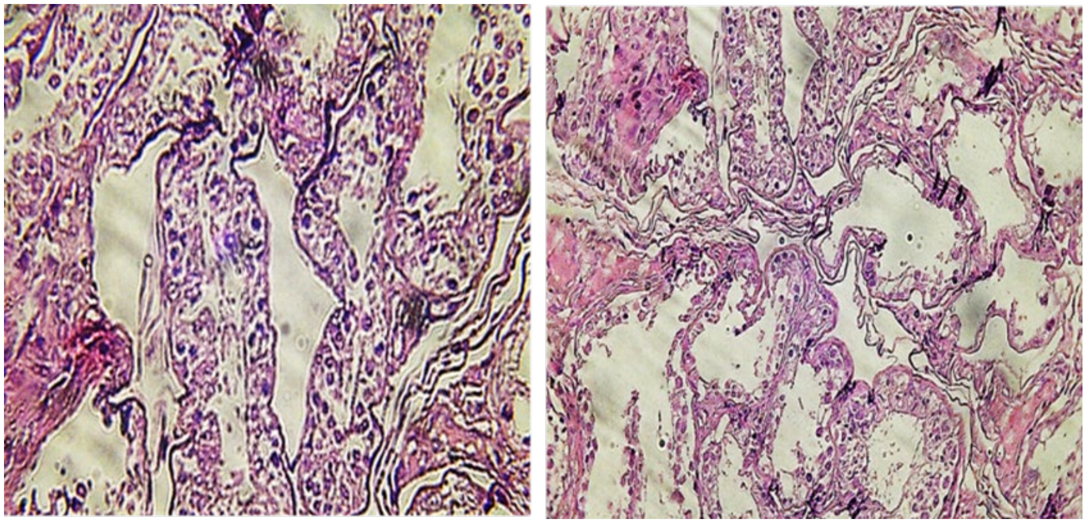

Fig.2. (Left and Right) cross-section in testes of the positive control group (hypercholesterolemic group) showed absence of normal structure of seminiferous tubules, degeneration of most cell types of spermatogenesis (H&E) 200 and 400x.

Table 2. Represent the mean of MDA in serum of experimental rabbit's groups.

Groups	Malondialdehyde (MDA) (µmol/L)
Control group	1.86±0.32[a]
Hypercholesterolemia group	4.6±0.52[b]
Hypercholesterolemia Treated with 5%silver nanoparticles	3.05±0.45[c]
Hypercholesterolemia Treated with 10%silver nanoparticles	2.01±0.62[a]
Hypercholesterolemia Treated with 15%silver nanoparticles	2.95±0.4[c]
Hypercholesterolemia Treated with *Moringa oleifera* 200mg/kg	2.12±0.52[a]

Values represent mean ± SD, different letters represent significant differences ($P \leq 0.05$).

was observed in the hypercholesterolemia group (Fig. 2). Experimental animals fed a high-fat diet for 21 days showed the following pathological signs: sections of the testis in groups 3-5 treated with 5, 10, and 15% silver nanoparticles, respectively, return the normal structure of seminiferous tubules with presence of degeneration in some cell types of spermatogenesis, and the thickness of basement membrane (Figs. 3-5). In contrast, animals treated with *M. oleifera* leaves extract demonstrated improvement of spermatogenesis cell types and normal structure of basement membrane of seminiferous tubules (Fig. 6).

The levels of Malondialdehyde (MDA) in different experimental categories were shown in Table 2. The level of MD increased significantly in the HFD and treated groups ($P<0.05$). While the levels of MDA remained significantly lower in silver nanoparticles and *M. oleifera* leaf extract-treated groups than that in the HFD group.

Discussion

Our data demonstrated that exposure to high–fat diet for 21 days decreases the thickness of the germinal epithelium and the diameter of seminiferous tubules as well as alters the testicular morphological structure. In agreement with our findings, rats exposed to high–fat diet for 6-7 and 12 weeks, respectively showed histopathological alteration in testicular tissue (Demirci & Sahin 2019;

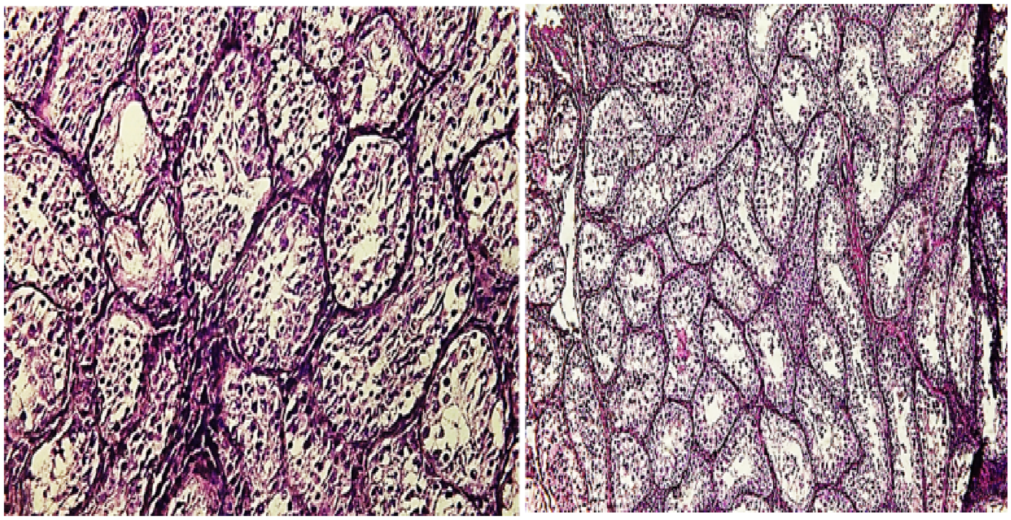

Fig.3. (Left and Right) cross-section of testes of the group (3) hypercholesterolemic treated with 5% silver nanoparticles showed return of the normal structure of seminiferous tubule with the presence of degeneration of some cell types of spermatogenesis and thickness of basement membrane (H&E) 100 and 200x.

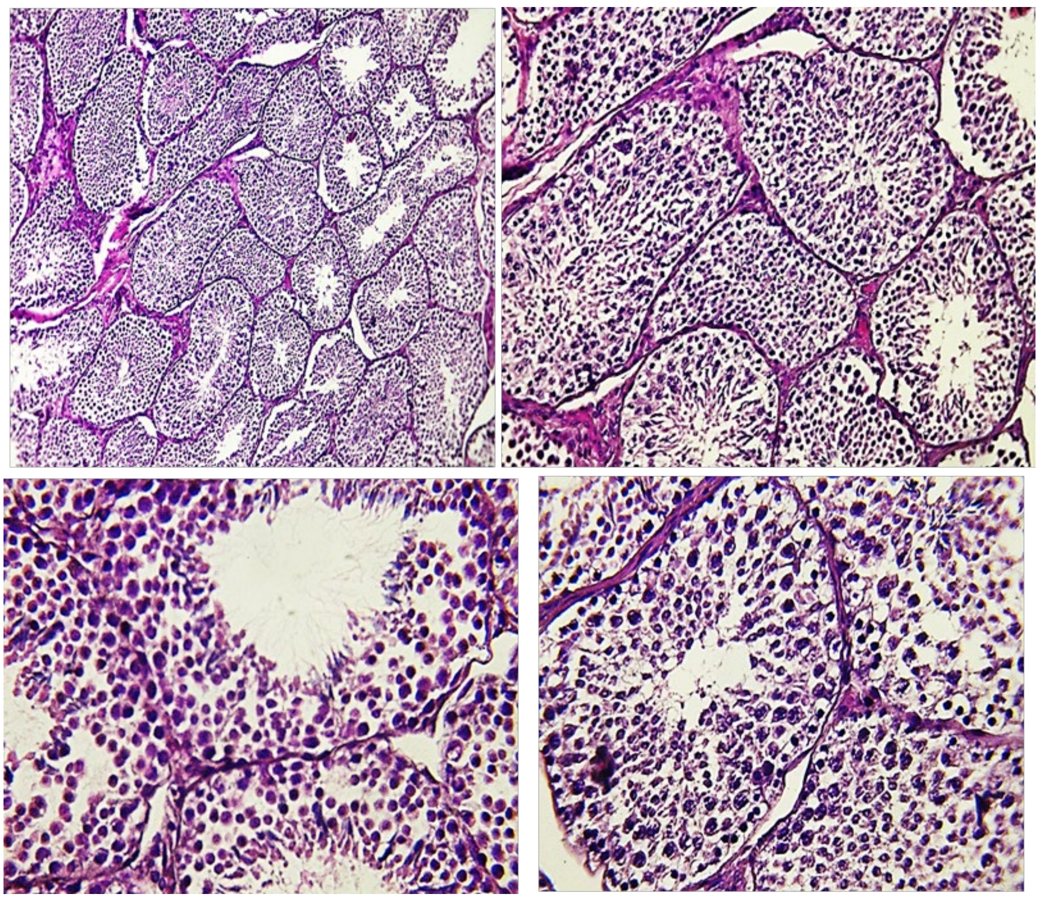

Fig.4. (Left and Right) cross-section of testes of the group (3, upper) (4, below) hypercholesterolemic treated with 10% silver nanoparticles showed return of the normal structure of seminiferous tubule with the presence of degeneration of some cell types of spermatogenesis and thickness of basement membrane (H&E) 400x.

Matuszewska et al. 2020). A possible harmful mechanism could be related to the abnormal fat accumulation around testes in obese animals which might be the cause of structural alterations and the subsequent lack of fertility (Campos-Silva et al. 2015; Funes et al. 2019). The results of histological

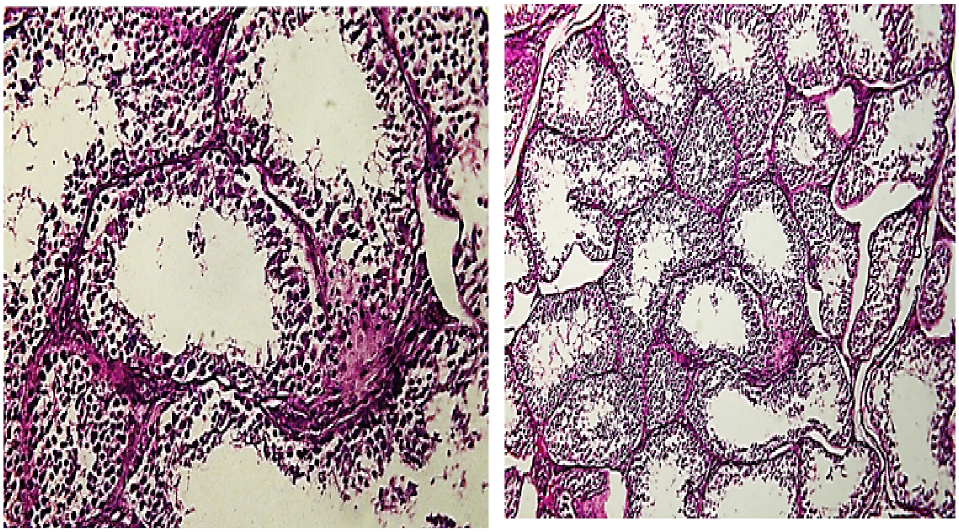

Fig.5. (Left and Right) cross-section of testes of the group (5) hypercholesterolemic treated with 15% silver nanoparticles showed return of the normal structure of seminiferous tubule with the presence of degeneration of some cell types of spermatogenesis and the thickness of basement membrane (H&E) 100 and 200x.

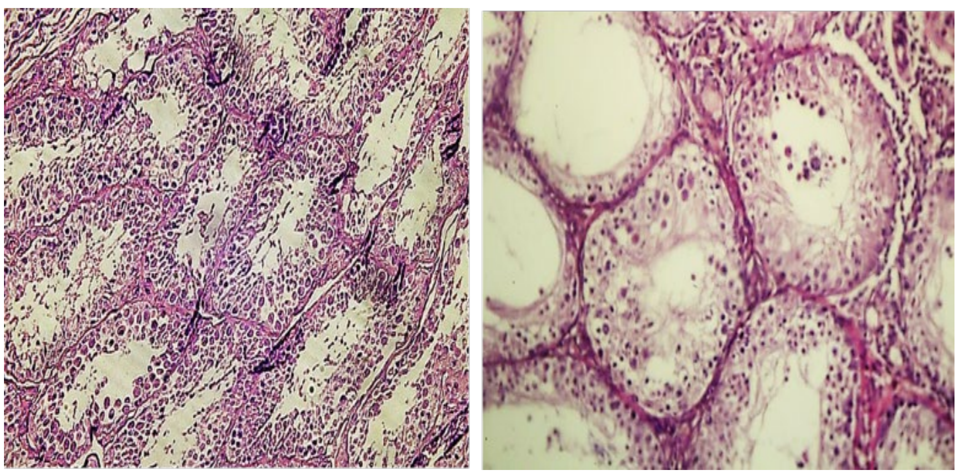

Fig.6. (Left and Right) cross-section of testes of hypercholesterolemic rabbits treated with Moringa leaves extract showed improvement of spermatogenesis cell types and normal structure of basement membrane of seminiferous tubules (H&E), 200x.

examination of sections of the rabbits' testes treated with silver nanoparticles displayed mild changes in testicular tissues at different concentrations in comparison to the control group. This finding conflicts with other previous studies which reported a negative effect of silver nanoparticles on the reproductive tissue of male rabbits. Silver nanoparticles can cross the blood-testis barrier and affect sperm production (Giulia et al. 2020). This affinity was probably due to the persistence of silver nanoparticles in the testes for a long period. However, other works did not recognize any morphological change in the testes (Foote & Carney 2000). Treatment with *M. oleifera* leaves extracts for 21 days at 200mg/kg was able to partially prevent the damage caused by high-fat diet exposure. This result was demonstrated by the improvement of the histological structure of the testicular tissue and decrease in serum MDA levels as compared to high–fat diet group. Previous studies have revealed a protective effect of *M. oleifera* leaves extracts on testicular function in rats (Sadek 2014) and mice (Nayak et al. 2016). Oxidative stress is also suggested as a potential mechanism by which high-fat diet could affect reproductive function. A growing of evidence has linked obesity with increased oxidative stress and the consequent formation of free radicals and diminished antioxidant capacity, mainly the inhibition of antioxidant enzymes (Rahman et al. 2017). An increase in oxidative stress and a significant decrease in the level of antioxidants parameters in the liver of animals fed a high-fat diet have been reported in previous studies (Matsuzawa et al. 2008; Jarukamjorn et al. 2016; Othman et al. 2019). The current study revealed a significant increase in MDA level in the high-fat diet group. Our results were consistent with the results of Sozen et al. (2021) who indicates an increase in MDA levels in the testes tissue of rabbits. This result proposed a high fat diet-induced systemic oxidative stress as an inducer of inflammatory reaction that leads to a negative impact on male fertility. The results of this study showed that the treatment with silver nanoparticles in combination with the high-fat diet ameliorated the activity of Malondialdehyde when compared to the high-fat diet group. Silver nanoparticles can raise oxidative stress which consequently causes oxidative damage to the cells (Zhang et al. 2014). Reduced activities of antioxidant enzymes were reported by Govindasamy & Rahuman (2012), when fish were exposed to silver nanoparticles at 25, 50, and 75mg/L for eight days.

However, Glutathione peroxidase mRNA level in chickens was significantly increased after the dietary administration of silver nanoparticles for 12 days. This gene encodes the antioxidant enzyme glutathione peroxidase which is required to decline reactive oxygen species generation and inhibit cellular damage prompted by oxidative stress (Saleh & El-Magd 2018). Further, the dietary supplementation of silver nanoparticles at a dose of 0.5mg/kg significantly enhances the anti-oxidative status, yet feeding with a higher concentration demonstrated a negative impact (Kumar et al. 2018). Various factors such as dose, exposure time, and cell types play vital roles in controlling the toxicity of silver nanoparticles (Zhang et al. 2014).

The polyphenol-rich plants might be applied as dietary antioxidants to inhibit oxidative stress-induced damage. Our results showed that the supplementation of *M. oleifera* extracts at 200mg/kg diet has a definitive role against oxidative stress. A similar effect was reported by Mabrouki et al. (2020) who found that the oral administration of *M. oleifera* extracts at doses of 200 and 400mg/kg/bw in the diet of high fat diet-treated rats for 12 weeks significantly inhibit the lipid peroxidation levels and balances the antioxidant enzyme activities (Zainab & Maytham 2021; Shabgah et al. 2021). The presence of phenolic compounds in *M. oleifera* has an antioxidant action that is involved inhibiting and scavenging lipid-free radicals (Qasim & Al-Mayali 2019; Tahmasebi et al. 2021). In most studies, the action of *M. oleifera* extract appears to be responsible for the reduction of oxidative stress, consequently improving reproductive features (Hamed & El-Sayed 2019; Mabrouki et al. 2020).

Conclusion

The consumption of a high-fat for 21 days, caused oxidative stress in rabbit and the serum MDA activity increased. The histological examination of testes showed alteration in the testicular tissue. *Moringa* leaf extract and silver nanoparticles supplementation to HFD exposed rabbits partially minimized all these factors demonstrating a possible protective effect on rabbit reproductive tissue.

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