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Research Article

Synergistic Effect of Yemeni Sesame Oil and Squalene on Hyperlipidemia-induced Reproductive Damage in Male Rats

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Abstract: This study was purposed to explore the synergistic amelioration effect and optimal feeding time of sesame oil and squalene on hyperlipemia-induced sexual dysfunction rats. We established the hyperlipidemia-induced reproductive damage model, the three groups of test substances (sesame oil, a mixture of sesame oil and squalene, and sildenafil) were orally administrated to those hyperlipidemic rats on day 30 and day 60. The results showed that compared with the pure sesame oil, the mixture of sesame oil and squalene can synergistically decrease concentration levels of TG, TC, and LDL-C, significantly increasing the serum testosterone level and sperm count of the epididymal tail, which the 30 days' effect was better than the day 60. Compared with the model control (MC) group, the Organ Coefficient of penile increased significantly in the sesame oil (SO), sesame oil+ Squalene (SOS), and Sildenafil (SN) group, and no pathological changes were found in the penile and testis in above three groups at the day 30 and the day 60. In conclusion, the present results demonstrated that sesame oil and squalene have a synergistic amelioration effect on lowering blood lipid and promoting the recovery of erectile and sexual function on hyperlipemia-induced reproductive damage rats at day 30. However, further studies should be carried out to deeply elucidate the molecular mechanisms of Sesame oil and squalene in lowering blood lipids and improving sexual function *in vivo*.

Keywords: Sesame oil, squalene, Ameliorating effect, hyperlipidemia model, sexual dysfunction rats

1. INTRODUCTION

Hyperlipidemia, one of the common metabolic syndromes [1], is usually expressed as the abnormal elevation of any or all lipids or lipoproteins in the blood [2]. Hyperlipidemia is a critical damage-inducing factor for cardiovascular disease and frequently brings about many complications, such as cardiac damage [3], sexual dysfunction [4], cognitive impairment [5], inflammation, and insulin resistance [6]. Strong associations are seen between hyperlipidemia and sexual dysfunction especially erectile dysfunction (ED). Experimental studies have shown that the reduction of arterial blood flow induced by hyperlipidemia directly can affect the organ functions of the cortical center, pituitary-testis axis, and corpus cavernosum of the penis [7], and inhibit the production of testosterone [8], which all caused a decline of sexual function. Nowadays, many synthetic pharmaceuticals like sildenafil are widely used for the management of ED. However, their long-term use always causes many serious side effects, including vasodilatation, dizziness, indigestion, stuffy nose, heartburn, and headaches, indigestion, stuffy nose, heartburn, and headaches. For these reasons, investigating efficient

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and safe candidates is of great value.

Medicinal plants are promising sources in the regulation and management of ED [9]. sesame oil is a common edible oil in Yemen and contains a variety of active ingredients [10,11], such as tocopherols, polyphenols, flavonoids, phenolic ligands, Squalene, sesamol, sesamin, sesamolin, which can raise the activity of internal Superoxide Dismutase (SOD) and blood oxygen content [12], stimulate blood circulation [13], improve sexual function [14]. Vitamin E can promote the secretion of sex hormones and maintain the normal function of the Genital organs [8]. Sesame tocopherols and polyphenols can reduce serum cholesterol and prevent cardiovascular diseases [15]. The research group observed in the previous study that the proper amount of sesame oil can decrease blood lipids, promote the secretion of the sex hormone testosterone and improve sexual function [14]. However, It's not effective enough. Based on this research, Squalene, which is one of the active ingredients of sesame oil, was proposed to be added to feed the hyperlipemia-induced sexual dysfunction rats as a combining substance in this research, and then observe the changes in blood lipid and testosterone levels, epididymal sperm count, testis and corpus cavernosum tissue structure. This study aims to explore the synergistic amelioration effect and optimal feeding time of sesame oil and squalene on hyperlipemia-induced sexual dysfunction rats and then provide a basic experimental basis for the later development of sesame oil health products.

2. MATERIALS AND METHODS

2.1 Materials and chemicals

High-purity sesame oil was purchased from Yassin laboratories for Yemeni oils and spices (Sana'a, Yemen). Its nutritional ingredients contain 39.7 g/100 g of monounsaturated fatty acid, 6.72.0 g/100 g of linoleic acid, 1.9 g/100 g of linolenic acid, 5 g/100 g of stearic acid, 8g/100g of palmitic acid, 1.40 mg of vitamin E, 13.6 μg of vitamin K, 0.0 g/100 g of protein, 0.0 g/100 g of cholesterol and 0.0 g/100 g of sugar.

Basal feed and high-fat feed were provided by Al Shamel Trading Co., Ltd., Al Qasr Street (Sana'a, Yemen). 100 g of high-fat feed consists of 78.8 g basal feed, 10.0 g animal fats, 10.0 g yolk powder, 1g cholesterol, and 0.2 g cholate.

Sildenafil was bought from Shaphaco Pharmaceutical Ind. Attan, (Sanaa- Yemen). Squalene was bought from Beijing InnoChem Science and Technology Co., Ltd (Beijing, China). Total cholesterol (TC), triglyceride (TG), and low-density lipoprotein cholesterol (LDL-C) test kits were obtained from Spinreact (Spain). A testosterone ELISA kit was purchased from Roche-Diagnostic LTD (Germany). All other used reagents were of analytical grade.

2.2 Animals

Pathogen-free male albino rats weighing 160-190 g were purchased from the Sana'a Zoo in Yemen. Prior to the trials, the animals were housed in cages in a pathogen-free room with controlled temperature (18-26 °C), relative humidity (40-60 percent), and 12/12 h of light-dark intervals with ad libitum food and water. All animal experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institute of Health (NIH, 1978). Animal handling and all related procedures were carried out by the procedures approved by the Animal Experiment Ethics Committee of the faculty of medicine and health science, Al-Razi University, Yemen (021/FMHS/2022).

2.3 Animal experiment design

Rats were fed a high-fat diet to establish the hyperlipidemia-induced reproductive damage model. Rats were randomly divided into the normal control (NC) group (12 rats) and model control (MC) group (48 rats), and were respectively supplied with basal feed high-fat fat feed for 4 weeks. The serum TC, TG, LDL-C, and testosterone levels of rats were measured by the corresponding kits, according to the manufacturer's instructions. Compared with the NC group, rats showed significant increments in serum TC, TG, and LDL-C levels and a prominent reduction in serum testosterone content in the MC group were identified to be successfully modeled.
Then, the successfully modeled rats were randomly classified into four groups (12 rats per group): Converted by recommended intake of human oils and fats, squalene, sildenafil and "equivalent dose ratio table of human and animal body surface area conversion", MC group received 3 mL/

kg•bw•d of 0.9 % normal saline; sesame oil (SO) group treated with 3mL/kg•bw•d of sesame oil; sesameoil+Squalene (SOS) group supplemented with 3mL/kg•bw•d of suspension solution (sesame oil: squalene=30:1) composed by sesame oil and squalene; The Sildenafil (SN) group was supplemented with 3 mL/kg·bw·day suspension solution (SO: sildenafil = 3:1) containing SO and sildenafil. Meanwhile, rats in the NC group were orally administrated with 3 mL/kg•bw•d of 0.9 % normal saline. During the experimental period, the blood of rats was collected on the 30th day (Day 30) and the 60th day (Day 60). Half of the rats in each group were sacrificed on day 30 and day 60 respectively, and the testis, epididymis, and penis of rats were gained.

2.4 Sexual organ index determination

The obtained testis, epididymis and penis were rinsed with 0.9 % normal saline and blotted with filter paper. Then, they were weighed and the organ indexes were calculated as the weight of organ/ the weight of the rat.

2.5 Serum lipid and testosterone levels measurement

The blood of rats was centrifuged at 3000 rpm/min for 10 min under 4 °C to collect the serum. TC, TG, LDL-C, and testosterone levels of serum were detected by assay kits referring to the instructions provided by the manufacturer.

2.6 Sperm count metering in epididymitis

The cauda of epididymitis was cut off and put into 4 mL of 0.9 % normal saline, followed by a cut up. It was incubated at 37 °C for 20 min to allow the running out of sperm. After that, the supernate was obtained by filtration using a 100 mesh strainer. The supernatant 10 μL was transferred into the sperm count board, and the sperm number was counted under microscopy.

2.7 HE staining observation of testis and penis

A part of the testis and penis was respectively fixed in 10 % formalin for over 24 h and embedded in paraffin. Then, paraffin sections (5 μm thick) were stained with HE dye and followed by histopathological observation under light microscope.

2.8 Statistical analysis

The mean and standard deviation were used to represent all data values (SD). Software called SPSS 23.0 was used to do the statistical analysis. To compare the significant differences across all the groups using Tukey's technique, one-way analysis of variance (ANOVA) was used. At p 0.05, differences were deemed significant.

3. RESULTS

3.1 Hyperlipidemia-induced reproductive damage in high-fat diet model

As shown in Figure 1(a), the TC, TG, and LDL-C levels of rats were significantly elevated in high-fat diet treatment groups, including MC, SO, SOS, and SN groups, compared with the NC group. It indicated that the hyperlipidemia model was successfully established. The serum testosterone level of rats was determined to evaluate whether hyperlipidemia induces reproductive damage, as illustrated in Figure 1(b). Compared with the NC group, the serum testosterone level was prominently decreased in hyperlipidemia rats.

3.2 Synergistic effect of Sesame oil and squalene on hyperlipidemia-induced reproductive damage in male rats

The sexual organ (testis, epididymis, and penis) indexes of rats were revealed in Table 1. There were no significant differences in testis and epididymis organ indexes of rats among the NC, MC, SO, SOS, and SN groups. While compared with the MC group, the penis organ index of rats increased significantly in the NC, SO, SOS, and SN groups on day 30 and day 60, suggesting that the SO, SOS, and SN groups can improve the relaxation and atrophy of the Corpus cavernosum penis in rats.

The serum lipid (TG, TC, and LDL-C) levels of normal and hyperlipidemia rats were shown in Figure 2. Compared with the NC group, the serum lipid levels of rats were notably increased in the MC group on day 30 and day 60. Treatments of test substances (Sesame oil, mixture of Sesame oil and squalene, and sildenafil) could reverse this phenomenon. As illustrated in Figure 2(a-b), on day 30, the serum TG and TC levels of rats in the SOS and SN groups observably declined in comparison with that in the MC group. Whilst, no remarkable

Table 1. Sexual organ indexes of hyperlipidemia-induced reproductive damage rats

Group	Testis index (g/100 g)		Epididymis index (g/100 g)		Penis index (g/100 g)	
	Day 30	Day 60	Day 30	Day 60	Day 30	Day 60
NC	0.79±0.08[a]	0.76±0.03[a]	0.24±0.02[a]	0.22±0.02[a]	0.08±0.010[a]	0.08±0.02[a]
MC	0.80±0.08[a]	0.75±0.09[a]	0.25±0.02[a]	0.22±0.04[a]	0.07±0.011[b]	0.06±0.01[b]
SO	0.78±0.08[a]	0.73±0.08[a]	0.25±0.01[a]	0.22±0.03[a]	0.08±0.008[a]	0.07±0.01[a]
SOS	0.80±0.10[a]	0.75±0.08[a]	0.24±0.02[a]	0.23±0.03[a]	0.08±0.008[a]	0.08±0.01[a]
SN	0.76±0.07[a]	0.73±0.05[a]	0.25±0.02[a]	0.23±0.03[a]	0.08±0.011[a]	0.07±0.01[a]

NC, normal control; MC, model control; SO, *Sesame* oil; SOS, *Sesame* oil+ squalene; SN, sildenafil. Values in the same column or row with different letters(a-b) represent significantly different (p<0.05) from each other.

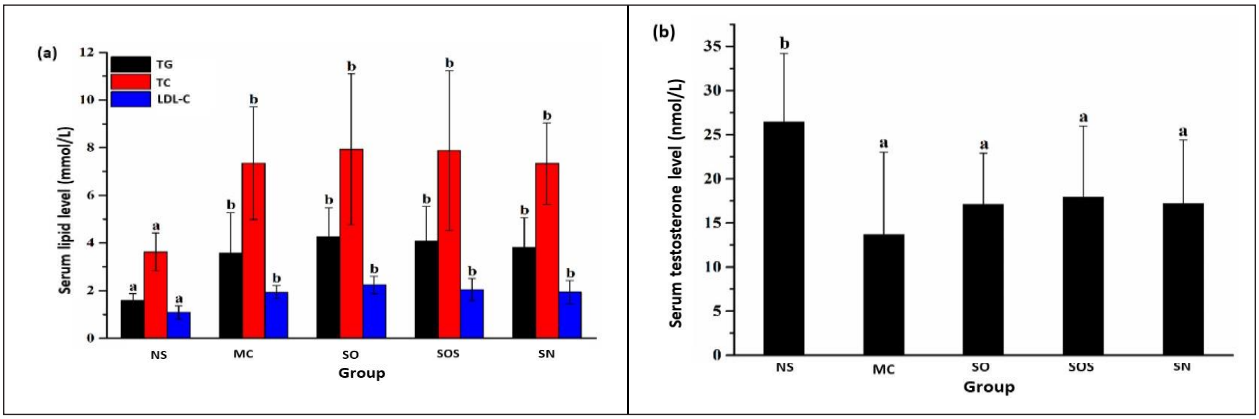

Fig. 1. Serum lipid (a) and testosterone (b) levels of rats in hyperlipidemia-induced reproductive damage model. TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol. NC, normal control; MC, model control; SO, *Sesame* oil; SOS, *Sesame* oil+ squalene; SN, sildenafil. The mean±SD was used to express all values. Different letters (a-c) indicated statistically significant differences (p<0.05).

distinction was observed between the SO group and the MC group, indicating Sesame oil and squalene could synergistically decrease the serum TG and TC levels of hyperlipidemia rats. On day 60, the serum TG and TC levels of rats were notably decreased in the CO, COS, and SN groups. Figure 2(c) reveals that the LDL-C level of rats supplemented with the above test substances was markedly lower than that in the MC group, on day 30 and day 60.

The serum testosterone levels of rats were detected in Figure 3. Compared with the NC group, the serum testosterone level of rats was significantly decreased in the MC group on day 30 and day 60. On day 30, the serum testosterone level of rats was dramatically increased in the SOS and SN groups, while no outstanding increment was seen in the SO group. It suggested that Sesame oil and squalene exerted a synergistic effect against hyperlipidemia-induced reduction in serum testosterone levels of rats. On day 60, rats in the SO, SOS, and SN groups showed significant elevation in serum testosterone levels in comparison with those in the MC group. While, compared with day 30, the serum testosterone level showed varying degrees of decrease in the SOS and SN group.

The sperm counts in the epididymitis of rats were revealed in Figure 4. Compared with the NC group, the sperm counts in the epididymitis of rats were prominently reduced in the MC group. Oral administration of the test substances (Sesame oil, mixture of Sesame oil and squalene, and sildenafil) could significantly increase the sperm counts in epididymitis of rats on day 30 and day 60, as compared to the MC group. On day 60, there was no significant difference in the sperm counts in epididymitis of rats in the SO, SOS, and SN groups, as compared to day 30.

The histomorphological structure of the testis and penis is shown in Figure 5-6. Compared with the NC group, no pathological changes were found in the Corpus cavernosum penis and testes of rats

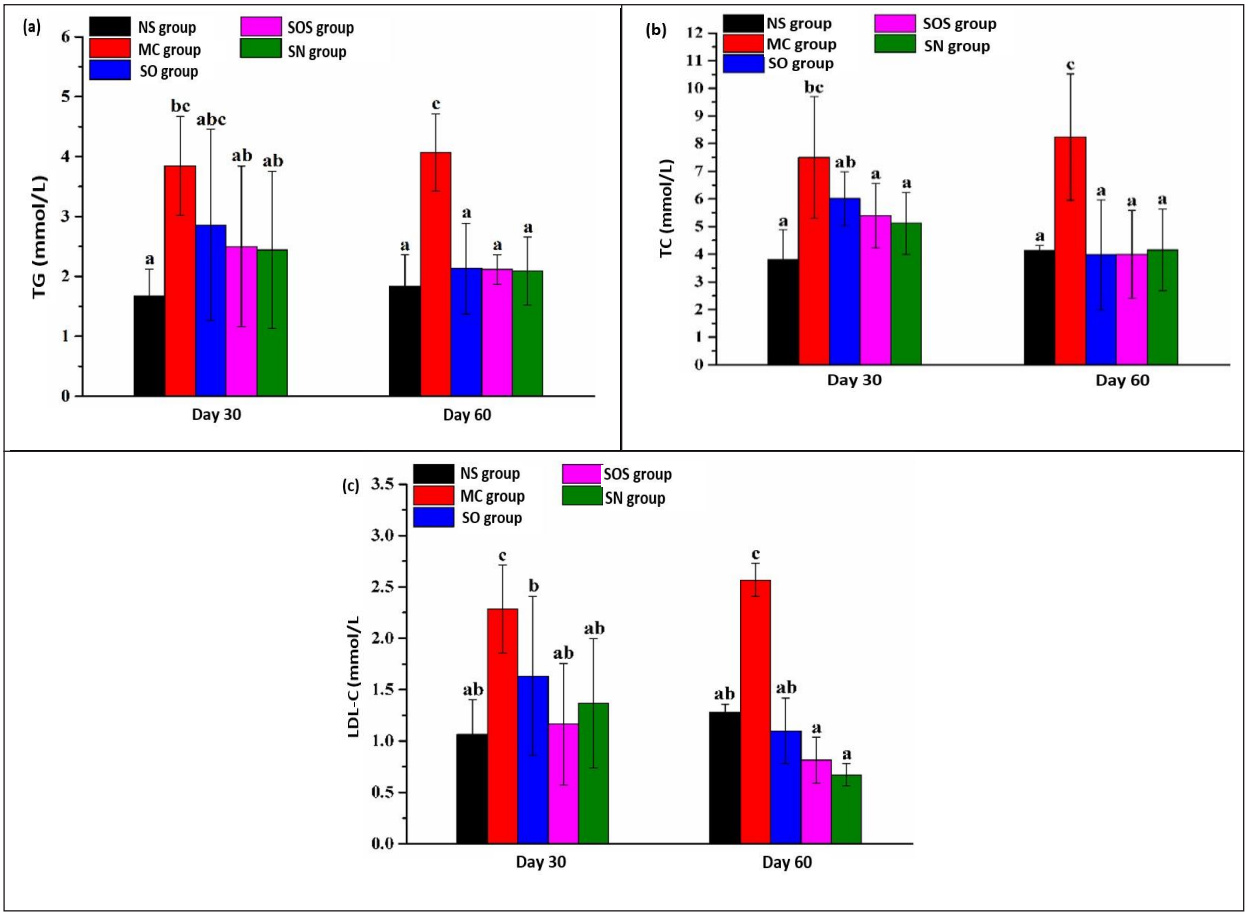

Fig. 2. Serum lipid levels of hyperlipidemia-induced reproductively damaged rats. (a) TG level; (b) TC level; (c) LDL-C level. TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol. NC, normal control; MC, model control; SO, *Sesame* oil; SOS, *Sesame*oil+squalene; SN, sildenafil. The mean±SD was used to express all values. Different letters (a-c) indicated statistically significant differences ($p<0.05$).

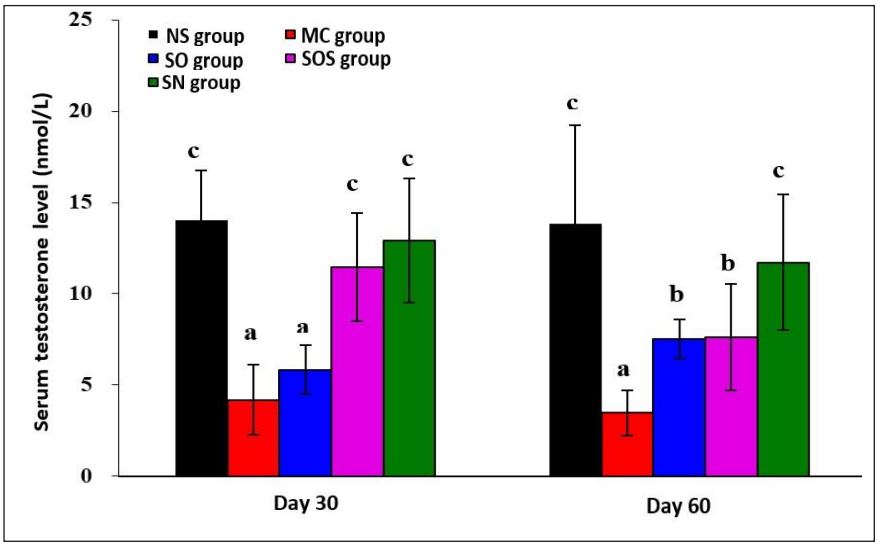

Fig. 3. Serum testosterone level of rats with reproductive damage due to hyperlipidemia. NC, normal control; MC, model control; SO, Sesame oil; SOS, Sesame oil+squalene; SN, sildenafil.Themean±SD was used to express all values. Different letters (a-c) indicated statistically significant differences ($p<0.05$).

in the SO, SOS and SN groups. While in the MC group, the number of smooth muscle cells and cavernous sinus decreased significantly in the Corpus Cavernosum of the Penis, and the smooth muscle of the Cavernous Body was distributed unevenly, arranged disorderly, and loosely. At the same time, all levels of spermatogenic cells in the testis were arranged disorderly, some spermatogenic cells showed necrosis, apoptosis, and nuclear pyknosis on day 30 and day 60.

4. DISCUSSION

The present study was designed to explore the amelioration effect and optimal feeding time of sesame oil and synergists squalene in hyperlipemia-induced sexual dysfunction rats. As expected, the hyperlipemia rat model was successfully induced by feeding a high-fat diet for 4 weeks [16]. Then, when the three groups of test substances (sesame oil, mixture of sesame oil and squalene, and sildenafil) were orally administrated to those hyperlipidemic rats, Serum levels of TC, TG and LDL-C were significantly decreased compared with the MC group, at the day 60. The above results indicate that sesame oil can reduce blood lipids significantly with rich in monounsaturated fatty acids, oleic acid, linoleic acid, linolenic acid, etc. [10]. However, only SOS and SN groups significantly reduced the level of blood lipids at day 30, suggesting that sesame oil mixed with squalene could play a synergistic role in enhancing the effect of reducing blood lipids, which the effect was better than pure sesame oil. According to studies, squalene likely contributed to the inhibition of intestinal cholesterol absorption or the activity regulation of key enzymes involved in the production of endogenous cholesterol, including hepatic acyl-CoA oxidase, fatty acid synthase, and hydroxyl-3-methylglutarylcoenzyme A reductase (HMG-CoA) [17,18].

Testosterone is the main male hormone in the body, which can promote the development of male reproductive organs and sperm, and maintain sexual function [19]. This study indicated that the level of testosterone and sperm count of the epididymal tail in SO, SOS, and SN groups were significantly higher than those in the MC group at day 60. We infer that the three groups of test substances significantly increase testosterone levels and sperm counts in the epididymal tail of male rats with hyperlipidemia. However, only SOS and SN groups significantly increased testosterone levels at day 30, suggesting that sesame oil mixed with squalene also could play a synergistic role in enhancing the effect of promoting the level of testosterone and the number of epididymal tail sperm, which the effect was better than pure sesame oil. It may be because squalene can participate in cholesterol biosynthesis and various biochemical reactions in the body, accelerate the synthesis of steroid hormones such as testosterone [20], increase the activity of superoxide dismutase (SOD) and blood oxygen content [21], promote blood circulation and improve sexual function. Meanwhile, on day 60, the testosterone level and sperm count in the epididymis tail did not increase compared with day 30, suggesting that the testosterone level and sperm count in the epididymis did not increase with feeding time. Therefore, we consider that the optimal feeding time of sesame oil and squalene on hyperlipemia-induced sexual dysfunction rats is day 30.

Current animal studies have shown that hyperlipidemia could impair erectile function by changing morphological structures of sexual organs, for example, penile corpus cavernosum lesions [22, 23]. The Organ Coefficient and pathological results of this study showed that the Organ Coefficient of the penile decreased significantly, and the penile and testis appeared pathological changes to different degrees in the MC group, which was consistent with the findings previously reported [22, 24]. Compared with the MC group, the organ coefficient of the penile increased significantly and no pathological changes were found in the penile and testis in the SO, SOS, and SN groups, suggesting that the three groups of test substances could improve the damage to the penis and testis, and promote the recovery of erectile function.

5. CONCLUSION

The present results demonstrated that the addition of squalene to sesame as a combining substance to feed hyperlipidemia-induced reproductive damage rats for 30 days could play an important role in combining the effect of lowering blood lipids, promoting the level of testosterone and the number of epididymal tail sperm, improving the damage of penis and testis, and promoting the recovery of erectile and sexual function. However, further studies should be carried out to deeply elucidate the molecular mechanisms of Sesame oil and squalene in lowering blood lipids and improving sexual function *in vivo*.

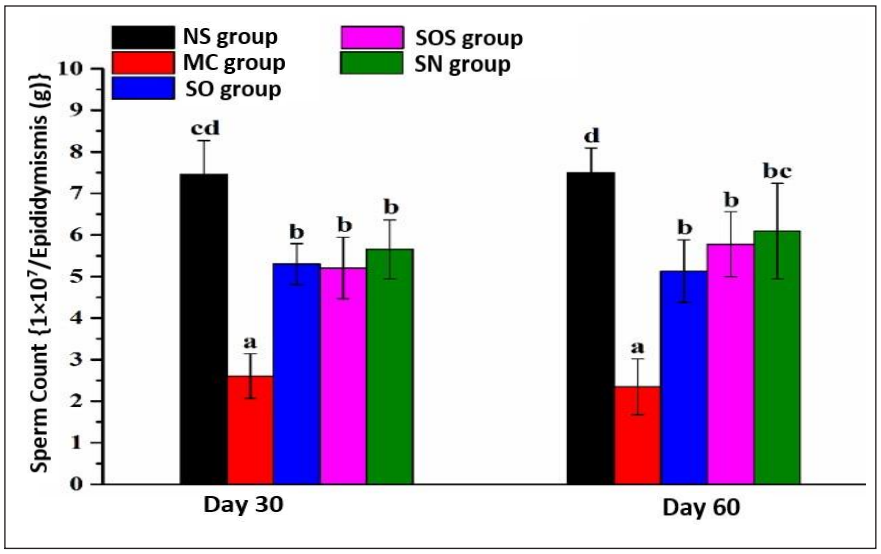

Fig. 4. Sperm count in epididymitis of rats with reproductive damage due to hyperlipidemia. NC, normal control; MC, model control; SO, Sesame oil; SOS, Sesame oil+ squalene; SN, sildenafil. The mean±SD was used to express all values. Different letters (a-c) indicated statistically significant differences ($p<0.05$).

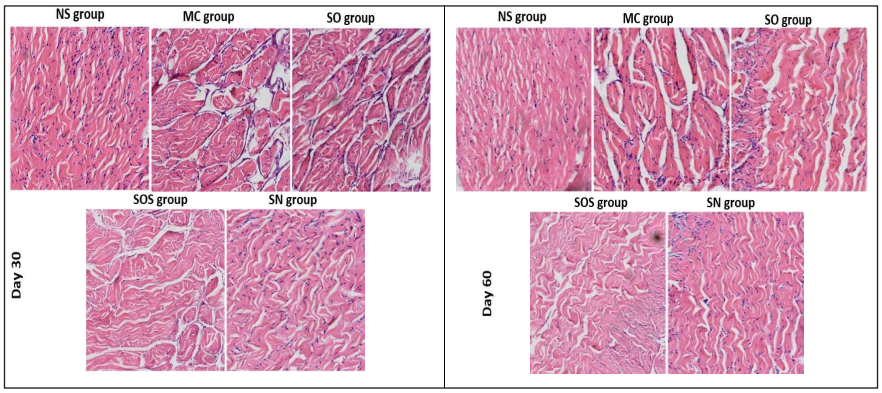

Fig. 5. Penis histomorphology of hyperlipidemia-induced reproductive damage rats. NC, normal control; MC, model control; SO, *Sesame* oil; SOS, *Sesame*oil+squalene; SN, sildenafil.

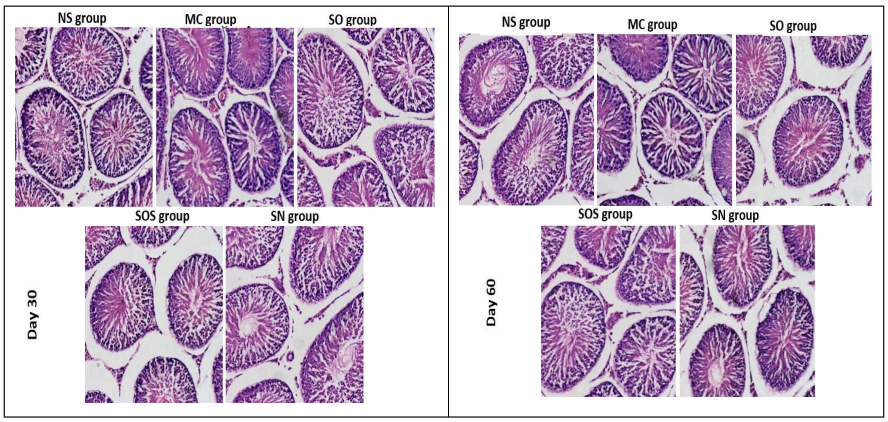

Fig. 6. Testis histomorphology of hyperlipidemia-induced reproductive damage rats. NC, normal control; MC, model control; SO, *Sesame* oil; SOS, *Sesame*oil+squalene; SN, sildenafil

6. CONFLICT OF INTEREST

The authors declared no conflict of interest.

7. REFERENCES

1. S.Medina, andJ.C. Rodríguez. A review of the pathophysiological factors involved in urological disease associated with metabolic syndrome.Actas Urológicas Espaolas 40: 279-287(2016).
2. J.S. Wang, H.H. Dai, Y.B.Yan, X.H.Gong, X. Li, andH.S.Li. Research of stroke combined hyperlipidemia-induced erectile dysfunction in the rat model.Aging Male 22: 278-286(2019).
3. Z.W.Pei, Y. Guo, H.L. Zhu, M. Dong, Q. Zhang, andF. Wang. Thymoquinone Protects against Hyperlipidemia-Induced Cardiac Damage in Low-Density Lipoprotein Receptor-Deficient (LDL-R-/-) Mice via Its Anti-inflammatory and Antipyroptotic Effects. Hindawi BioMed Research International 1-9(2020).
4. M.L. Schulster, S.E. Liang, and B.B. Najari. Metabolic syndrome and sexual dysfunction. Current Opinion in Urology, 27 435-440 (2017).
5. V. Uppin, P. Acharya, B.B.Kempaiah, and R.R. Talahalli. Zerumbone augments cognitive enhancement potentials of EPA+DHA: insight from a hyperlipidaemic rat model. British Journal of Nutrition, 124: 1353-1360(2020).
6. T.W. Jung, J. Park, J.L. Sun, S.H. Ahn, A.M.A. EL-Aty, A. Hacimuftuoglu, H.K. Kim, J.H. Shim, S.S. Shin, andJ.H. Jeong. Administration of kynurenic acid reduces hyperlipidemia-induced inflammation and insulin resistance in skeletal muscle and adipocytes. Molecular and Cellular Endocrinology, 518: 110928 (2020).
7. H.G. Jing, K.K. Szpunar, andD.L. Schacter. Interpolated testing influences focused attention and improves integration of information during a video-recorded lecture. Journal of Experimental Psychology Applied 22(3): 305 (2016).
8. Y.J. Dong, C. Zheng, Y.E. Li, C.M. Zhang, S.L. Shen, andJ.H. Liang. Effects of serum total testosterone and sex hormone-binding globulin levels on erectile function in hyperlipidaemia rats. Journal of Guangxi Medical University34: 1699-1702(2017).
9. N.P. Masuku, J.O. Unuofin, and S.L. Lebelo. Promising role of medicinal plants in the regulation and management of male erectile dysfunction. Biomedicine & Pharmacotherapy130: 110555 (2020).
10. R.C.A. Guimarães, M.L.R.Macedo, C.L.Munhoz, , W. Filiu, L. Viana, V.T. Hand Nozaki. Sesame and flaxseed oil: nutritional quality and effects on serum lipids and glucose in rats. Food Science and Technology (Campinas) 33(1): 209-217 (2013).
11. S.S.A. Ali, and M.S.A. Al-Janabi. Comparative Study on Therapeutic Effect of Wheat Germ Oil and Sesame Oil with Some Lipid-lowering Drugs in Local Male Rabbits with Hyperlipidemia, induced by Triton x-100: Physiological and Histological Study. International Journal of Pharmaceutical Quality Assurance12(2): 76-83(2021).
12. E. Hsu, andS. Parthasarathy. Anti-inflammatory and Antioxidant Effects of Sesame Oil on Atherosclerosis: A Descriptive Literature Review. Cureus 9(7): e1438 (2017).
13. P. Jayaraj, C.A.Narasimhulu, S. Sanjay Rajagopalan, S. Parthasarathy, and R.DesikanSesamol: a powerful functional food ingredient from sesame oil for cardioprotection. Journal Food and Function. 11: 1198-1210. (2020).
14. A. GowederFawzia, T.M. Abu El-Nasr and A.A. El-Sayed. The effect of sesame intake on adult albino rat testis during sildenafil long-term administration. Journal of Basic and Applied Zoology82(2): 2-7 (2021).
15. D. Hota, A. Srinivasan, J.P. Sahoo, K.K. Behera, B.K. Patro, and D. BandyopadhyayPossible Anti-Diabetic and Anti-HyperlipidemicEfficacy of Blended Rice Bran Oil with Sesame Oil in Comparison with Soybean Oil: A Clinical Investigation in Pre-Diabetic and Diabetic Individuals. Journal of Clinical Trials 10: 419 (2020).
16. T.T. Shen, and S.X. Wu. Effects of Tea Seed Oil on Hyperlipidemic Rats Induced by High-fat Diet. Food Science and Technology 23: 101-109 (2017).
17. M.N. Woo, S.H. Bok, and M.S. Choi. Hypolipidemic and body fat lowering effects of Fat clean in rats fed a high-fat diet. Food and Chemical Toxicology 47: 2076-2082(2009).
18. Y.S. Kwak, J.S. Kyung, J.S. Kim, J.Y. Cho, and M.H. Rhee. Antihyperlipidemic effects of red ginseng acidic polysaccharide from Koreanred ginseng. Biological and Pharmaceutical Bulletin 33: 468-472 (2010).
19. Y.H. Zhang, Y. Li, D. Cao, T. Yu, J.Y. Gao, and C.Y. Pan. Research progress on the effects of testosterone on animal reproduction and growth and development. Journal of Animal Ecology 39: 1-7(2018).
20. S. Li, Y. Liu, and C.L. Wang. The health benefits

and application of Squalene. Food Research and Development. 14: 206-209 (2016).

21. C. Y. Liu, M.H. Ma, G.F. Zhan, F.Geng, Q.L. Wang, and S.G. Sun. Research process on squalence and bioactivities. Journal of Chinese Institute of Food Science and Technology 15: 147-156 (2015).
22. R. Li, K. Cui, T. Wang, S. Wang, X. Li, J.Qiu, G. Yu, J. Liu, B. Wen, and K. Rao. Hyperlipidemia impairs erectile function in rats by causing cavernosal fibrosis. Andrologia 49: 12693 (2017).
23. J.L. Hu, H.X. Chen, H.R. Chen, Y. Wu, X.W.Sun, Z. Li, and J.F. Xing. Novel noninvasive quantification of penile corpus cavernosum lesions in hyperlipidemia-induced erectile dysfunction in rabbits by two-dimensional shear-wave elastography. Asian Journal of Andrology 21: 143-149 (2019).
24. L.L. Xia, X.J. Wang, T.Y. Xu, L. Qin, X. Zhang, Z.W. Zhu, X.H. Zhang, S. Zhong, M.G. Zhang, and Z.J. Shen.) Androgen combined with phosphodiesterase type 5 inhibitor for the treatment of erectile dysfunction in diabetic rats. Journal of Modern Urology 5: 347-352 (2019.