Malaysian Applied Biology
https://doi.org/10.55230/mabjournal.v54i2.3300

Research Article

Restoring Spermatogenesis Via the Antioxidant Properties of *Moringa oleifera* and *Hibiscus sabdariffa* in Obesity-Induced Male Rats

Nurul Shaqiinah Daaniah Jamaludin[1], Nour Athiroh Abdoes Sjakoer[2], Mahanem Mat Noor[1]*

1. Department of Biological Sciences and Biotechnology, Faculty of Science and Technology, 43600 Bangi, Selangor, Malaysia
2. Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Islam Malang, Malang, Indonesia

*Corresponding author: mahanem@ukm.edu.my

ABSTRACT

This study aimed to assess the impact of aqueous extracts from *Moringa oleifera* (MO), *Hibiscus sabdariffa* (HS), and its combination (MOHS) on testicular cell development in male rats with obesity. Thirty-six male Sprague Dawley rats were divided into control and treatment groups. The control groups comprised normal, negative (obesity-induced rats, without treatment), and positive (obesity-induced rats, receiving 10 mg/kg orlistat) control rats. Meanwhile, the treatment group consisted of obesity-induced rats treated with herbal extracts: the MO400 group (received 400 mg/kg MO), HS150 group (received 150 mg/kg HS), and MOHS group (received a combination of 400 mg/kg MO and 150 mg/kg HS). All groups except the normal control group were fed a high-fat diet (HFD) until the Lee Obesity Index exceeded 310, followed by 38 days of treatment. Histological examination of the testes revealed positive effects of all herbal extracts on treatment, including active spermatogenesis with a high density of spermatogenic cells in the seminiferous tubules. The MOHS treatment significantly increased the diameter of seminiferous tubules compared to the negative control group (P<0.05). Moreover, the MOHS treatment exhibited the highest superoxide dismutase (SOD) enzyme activity compared to all groups (p<0.05) and lower malondialdehyde (MDA) levels compared to the negative control group. Meanwhile, the MO400 and HS150 treatment groups significantly reduced the Lee Obesity Index (p<0.05) compared to the control groups. In conclusion, MO and HS extracts, and their combination have demonstrated significant effects in improving male fertility parameters (spermatogenesis) and reducing the Lee Obesity Index by alleviating oxidative stress associated with obesity, thus supporting their potential as natural supplements to counteract obesity-induced infertility.

Key words: *Hibiscus sabdariffa*, malondialdehyde, *Moringa oleifera*, spermatogenesis, superoxide dismutase

INTRODUCTION

The issue of infertility in adult men constitutes 25% - 30% of all cases of conception failure, with obesity emerging as one of the primary contributing factors (Katib, 2015). This statistic underscores the significant role that obesity plays in reproductive challenges among men. In Malaysia, infertility issues and obesity are interconnected as Malaysia is an Asian country with the highest recorded obesity problem, reaching 45.3% (Azlan *et al*., 2022). Infertility is a condition affecting the male or female reproductive system, characterized by the inability to conceive after 12 months or more of regular, unprotected sexual intercourse (World Health Organisation, 2024). Fertility measures encompass the examination of testicular histology, the measurement of seminiferous tubule thickness, and the assessment of sperm count, motility, and normal morphology.

Obesity is the primary trigger for numerous diseases due to the accumulation of excess fat, leading to oxidative stress induced by reactive oxygen species (ROS) (Manna & Jain, 2015). Oxidative stress, closely linked to inflammation and cell apoptosis (Jubaidi *et al*., 2022), is primarily driven by reactive oxygen species (ROS). While ROS plays a vital role in regulating metabolic pathways and is essential for normal cellular functions, an excess can lead to oxidative damage. This imbalance can harm proteins, lipids, DNA, and RNA, contributing to various pathological conditions, including infertility (Mannucci *et al*., 2022). Mature spermatozoa are enveloped in a protective layer that is susceptible to damage by ROS, thereby impacting spermatogenesis (Dutta & Sengupta, 2018). In chronic cases, this damage can disrupt sperm production, resulting in sperm formation with abnormalities in morphology, motility, and overall quality (Dutta & Sengupta., 2018).

There are numerous uses of alternative medicine utilizing herbs to improve sexual drive and fertility (Kamaruzaman, Aizat & Mat Noor, 2018). *Moringa oleifera* (MO), commonly known as 'Kelor,' is an exceptional botanical with remarkable attributes. It plays a crucial role in reducing weight, alleviating inflammation, and exhibiting antioxidant capabilities, while positively influencing the reproductive system. This botanical marvel is an alternative remedy and significantly improves overall health and well-being (Abidin *et al*., 2023). In a research study, male rats with food containing MO for 60 days exhibited a lower occurrence of abnormal sperm (Zeng *et al*., 2019). The results may be attributed to the presence of flavonoids, recognized as antioxidants, which play a crucial role in mitigating the impact of ROS on spermatogenesis (Kamalrudin *et al*., 2018; Zade *et al*., 2023). The presence of bioactive compounds in MO, such as phenolic acids, flavonoids, isothiocyanates, and various other compounds, greatly enhances overall health (Vergara *et al*., 2017).

Article History
Accepted: 10 April 2025
First version online: 30 June 2025
Cite This Article:
Jamaludin, N.S.D., Sjakoer, N.A.A. & Noor, M.M. 2025. Restoring spermatogenesis via the antioxidant properties of *Moringa oleifera* and *Hibiscus sabdariffa* in obesity-induced male rats. Malaysian Applied Biology, 54(2): 46-54. https://doi.org/10.55230/mabjournal.v54i2.3300

Similarly, *Hibiscus sabdariffa* (HS), also known as "Roselle" or "Asam Paya" in Malaysia (Ali *et al*., 2019), is a widely used herb with numerous health benefits. Its phenolic compounds exhibit strong antioxidant properties and protect against hypercholesterolemia (Amin & Hamza, 2006). In a study involving HS, it was observed that rats administered a higher dose of HS aqueous extract exhibited a higher sperm count compared to rats receiving a lower dose (Beheshti *et al*., 2018). The presence of flavonoids in the HS is recorded to have numerous positive effects on the recovery level of damage resulting from oxidative stress. This is achieved by enhancing antioxidant enzymes such as Superoxide Dismutase (SOD) (Kasim *et al*., 2022).

MATERIALS AND METHODS

Animal sample

Thirty-six male Sprague Dawley rats (12 weeks old, weighing 250–300 g & healthy) were randomly divided into two main groups: a control group and a treatment group. Each leading group was subdivided into three subgroups, with six rats each. The control group included the normal control, positive control, and negative control subgroups, while the treatment group consisted of the MO400 treatment, HS150 treatment, and combined MOHS treatment subgroups. The normal control group was fed standard rat pellets, while the remaining groups were given a High-Fat Diet (HFD). The negative control group did not receive any treatment. In contrast, the positive control group was treated with orlistat at a dose of 10 mg/kg daily, administered via forced oral gavage using a syringe. The treatment groups received their respective herbal doses accordingly.

High Fat Diet (HFD)

The High-Fat Diet (HFD) containing 21.4% fat, 17.5% protein, 50% carbohydrate, 3.5% fiber, and 4.1% ash was prepared following the protocol established by Emir and Noor (2024). Regular rat pellets were ground and combined with blended ghee in a 5:2 ratio, forming a dough into 30 g lumps. These lumps were stored overnight in an airtight container at 4°C before administration to the designated groups. Male rats in the normal control group received standard rat pellets, while those in the positive, negative, and treatment groups had ad libitum access to the HFD for 40 days. The HFD period was based on previous studies indicating that 40 days is sufficient to induce obesity in rats (Emir & Noor, 2024). Rats were classified as obese when their Lee Obesity Index reached 310 g³/cm (Fitriani *et al*., 2016).

Herbal extract preparation

The preparation of the aqueous extract from dried *Moringa oleifera* (MO) leaves followed the method described by Bashah and Noor (2021). The MO leaves were finely ground into a powder. 400 g of the ground MO was extracted with 4800 mL of distilled water in a 1:12 ratio for 3 hr using the reflux method at 60°C. The resulting extract was then filtered through the Whatman filter paper.

Dried *Hibiscus sabdariffa* (HS) calyxes were extracted according to the method outlined by Noor *et al*. (2023). The calyxes were ground into a fine powder and prepared at a dosage of 150 mg/kg, then stored at 4°C until use. The extracts were mixed in a microcentrifuge tube before administration for the group receiving a combination of MO and HS. The doses of 400 mg/kg for MO (as recommended by Bashah & Noor, 2021) and 150 mg/kg for HS (as indicated by Ellis *et al*., 2022) were selected based on previous studies that demonstrated their efficacy in reducing oxidative stress and enhancing sperm quality. Treatments were administered for 40 consecutive days. On day 41, the rats were sacrificed, and the testicles were dissected and weighed before undergoing oxidative stress analysis and testes histology.

Oxidative stress

Testicular tissues were used for the analysis of lipid peroxidation levels (MDA) and the specific activity of the enzyme superoxide dismutase (SOD). All processes for preparing testicular tissue samples for oxidative stress analysis followed the protocols provided by SOLARBIO kit supplier (brand numbers: BC0020 for MDA & BC5160 for SOD). Once prepared alongside testis tissue, each sample was assessed using Nanodrop and spectrophotometer for protein concentration and absorbance value. The absorbance values of 450 nm, 532 nm, and 600 nm were taken for calculations and data preparation.

Histology of the testes

Histological methods were conducted based on Noor et al., 2023. Briefly, testes were washed in 0.9% NaCl and fixed overnight in Bouin's solution, followed by dehydration in a series of graded alcohols and embedded in paraffin wax. Sections of 7 µm were prepared using a microtome, floated in a 39°C water bath, and mounted on slides, which were dried at 37°C overnight. The sections were stained with hematoxylin and eosin (H&E) following standard protocols, and DPX was applied for long-term preservation. The stained sections were examined qualitatively. The mean diameter of round seminiferous tubules was measured under a light microscope (Digital Microscope Carl Zeiss, USA) with the help of an image analyzer (iSolution Lite, IMT i-Solution Inc., USA).

Statistical analysis

Statistical analysis was done using the IBM SPSS Statistics version 22 software (SPSS Inc, USA). Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test for post hoc analysis. Results were expressed as mean ± standard error mean (SEM). *P* value <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Lee Obesity Index

As shown in Table 1, all treatment groups successfully developed obesity after 40 days of high-fat diet (HFD) administration.

After receiving treatment, the groups exhibited a significant (p<0.05) reduction in body weight, reaching Lee Obesity Index (LOI) values below the obesity threshold of 310 g³/cm. After completing 40 days of treatment, LOI showed changes in the MO400 group (307.01 ± 15.35 g³/cm), HS150 group (316.10 ± 15.80 g³/cm), and MOHS treatment group (295.11 ± 14.76 g³/cm). Meanwhile, the normal group recorded an average of (289.51 ± 14.48 g³/cm), and the negative control group averaged (300.73 ± 15.04 g³/cm).

Among the treatment groups, the MO400 group showed the highest reduction in the LOI at 10.85 ± 0.54%, followed closely by the MOHS group with a reduction of 10.69 ± 0.53%. In comparison, the HS150 group demonstrated a significantly smaller reduction of 4.23 ± 0.21%. The MO400 and MOHS groups achieved significantly more significant LOI reductions than the control groups. This difference is likely due to the higher concentration of bioactive compounds in MO, which are more effective in promoting weight loss than those found in HS. The marked reduction observed in the MO400 group can be attributed to the aqueous extract of MO leaves, which is abundant in flavonoids, phenolic acids, carotenoids, and glucosinolates and has a lower caloric content (Kashyap *et al*., 2022). Other bioactive compounds contributing to weight loss in male rats include quercetin, isoquercetin, quercetin-3-0-malonylglucoside, and astragalin, which are key components in the anti-obesity effects of MO leaves (Ali Redha *et al*., 2021).

Table 1. Effects of MO400, HS150, and MOHS on Lee Obesity Index in Obese-Induced Rats

	Average Lee Indeks (g³/cm)		
	After HFD	After treatment	Reduction of Lee Obesity Index (%)
Normal	320.56± 16.03	289.51±14.48	10.17± 0.51
Positive	337.57± 16.89	306.93±15.35*c	9.50±0.47
Negative	332.44± 16.62	300.73±15.04	10.01±0.50
MO400	342.25± 17.11*c	307.01±15.35*c	10.85±0.54
HS150	329.78± 16.49*c	316.10±15.80*c	4.23±0.21
MOHS	328.47± 16.42	295.11±14.76	10.69±0.53

*c Data were expressed as mean ± significantly different from the negative control (p<0.05).

Oxidative stress

Superoxide Dismutase (SOD) is an enzyme crucial for regulating the reactive oxygen species (ROS) homeostasis in the body, helping to mitigate oxidative stress (Wang *et al*., 2018). The analysis results (Figure 1) show that the negative control group exhibited the lowest mean specific SOD activity (281.04 ± 14.05 U/g) compared to the normal control group (497.77 ± 24.89 U/g) and the positive control group (339.01 ± 16.95 U/g). Among the treatment groups, the MOHS group demonstrated the best performance with the highest mean specific SOD activity (1180.52 ± 59.03 U/g), followed by the HS150 group (958.46 ± 47.92 U/g) and the MO400 group (669.71 ± 33.49 U/g).

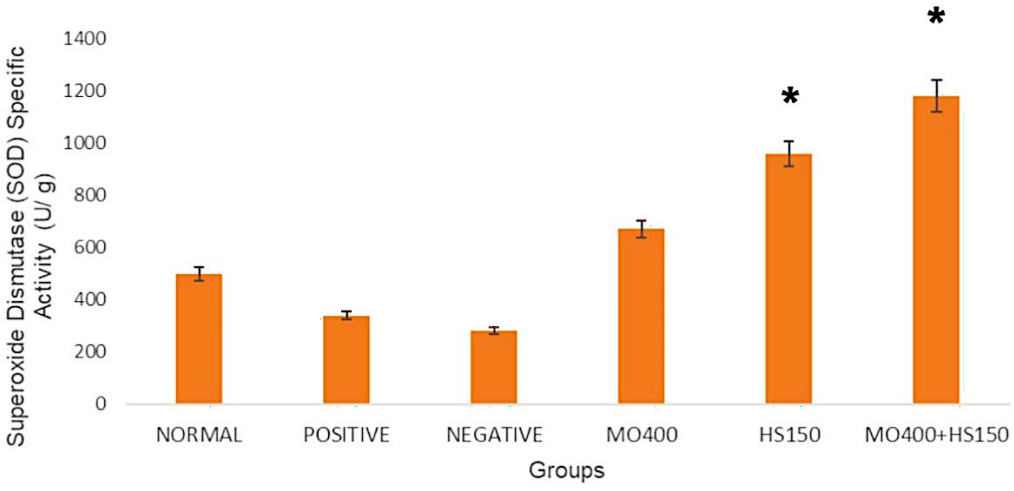

Fig. 1. Superoxide Dismutase (SOD) Specific Activity (U/g) After 40 Days of Herbal Treatment in the Respective Groups. Data were presented as mean ± SEM. MOHS showed significantly higher SOD activity compared to the negative control group (p<0.05, indicated by *).

The observed increase in Superoxide Dismutase (SOD) activity within testicular tissue across all treatment groups (MO400, HS150 & MOHS extracts) can be attributed to the bioactive compounds present in MO dan HS. These compounds play a crucial role in maintaining cellular homeostasis under oxidative stress. Notably, research by Duranti *et al*., 2021, reported that MO extract is rich in phenols and flavonoids, which enhance oxidative metabolism via the SIRT1-PPARα pathway. While SIRT1 is not a direct transcription factor, it promotes the synthesis of SOD enzymes. Additionally, HS is abundant in bioactive compounds such as anthocyanins and phenols, which increase the activity of antioxidant enzymes, including SOD, thereby enhancing

free radical elimination (Amer *et al*., 2022). The significant rise in SOD enzyme activity within the treatment groups highlights the effectiveness of the extracts in reducing oxidative stress. As a key antioxidant enzyme, SOD plays a vital role in protecting cellular structures from the damaging effects of ROS, thus preserving spermatogenesis in obese animal models (Younus, 2018). This study demonstrates the beneficial effects of elevated SOD activity on cell development, as evidenced by the enhanced spermatogenesis observed in the testes of the herbal treatment groups compared to the control groups. ROS activity leads to lipid peroxidation, producing malondialdehyde (MDA), a biomarker for ROS presence (Taib *et al*., 2017).

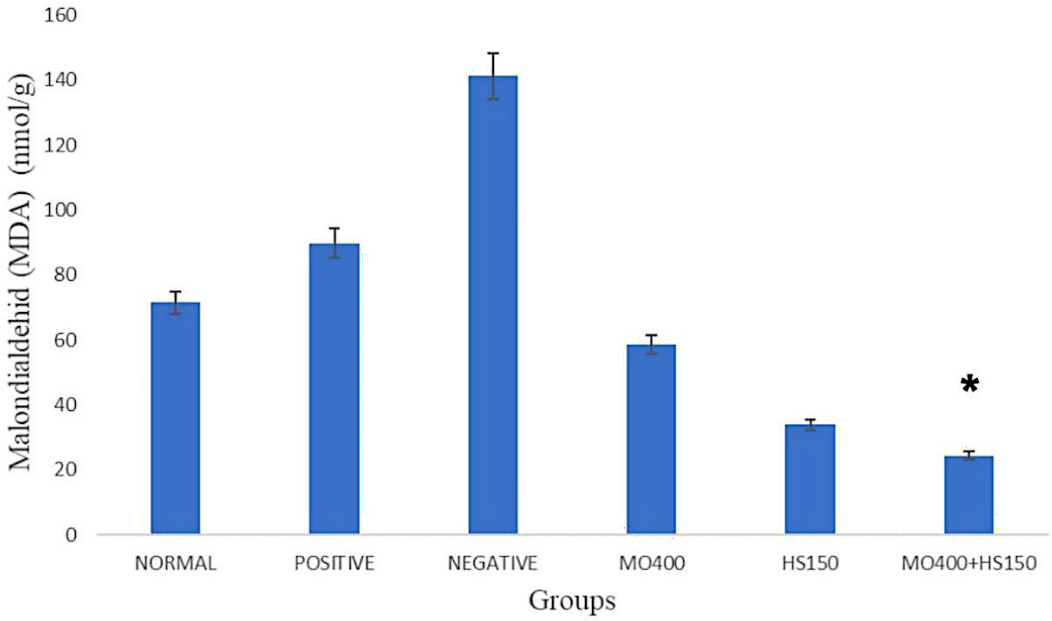

Fig. 2. Malondialdehid (MDA) (nmol/g) After 40 Days of Herbal Administration in Respective Groups. Data were presented as mean ± SEM. MOHS exhibited significantly lower MDA levels compared to the negative control group (p<0.05, indicated by*).

The herbal treatment groups exhibited significantly lower mean MDA values (nmoL/g) compared to the negative control group (p<0.05). The normal control group had an MDA value of 71.43 ± 3.57 nmoL/g, while the positive control group, treated with orlistat, recorded 89.84 ± 4.49 nmoL/g (Figure 2). Notably, the negative control group had the highest MDA levels at 141.26 ± 7.06 nmoL/g, surpassing all other groups. Conversely, the MDA readings for the treatment groups showed that the MOHS had the lowest levels (24.38 ± 1.22 nmoL/g), significantly lower (p<0.05) than those of the negative control. Similarly, the MO400 group (58.69 ± 2.93 nmoL/g) and the HS150 group (33.89 ± 1.69 nmoL/g) also exhibited relatively low MDA levels compared to all control groups.

The reduction in MDA levels across each herbal treatment group highlights the effectiveness of MO and HS extracts. These results align with previous studies, which reported significant decreases in MDA levels following the administration of MO extract at 400 mg/kg (Emir & Noor 2024). Notably, the current results reveal an even more significant reduction in MDA levels in the combined MOHS treatment group compared to either MO400 or HS150 alone. MO extract, rich in flavonoids and phenolic acids, directly scavenges ROS and enhances the activity of antioxidant enzymes, thereby reducing oxidative stress-induced lipid peroxidation in testicular tissues (Jangir & Jain, 2022). This effect is further enhanced by the inclusion of HS calyx extract, which is rich in anthocyanins and flavonoids, compounds known for their potent free radical-scavenging properties (Aju *et al*., 2019). Moreover, the bioactive constituents of the calyx extract contribute to restoring the balance of ROS production within the body (Alyani *et al*., 2021). As a result, the combined MOHS extract exerts a significantly greater impact on reducing MDA levels than the control group.

Histology of the testes (spermatogenesis)

Spermatogenesis is the process of sperm production involving the growth and differentiation of spermatogonial cells through both meiotic and post-meiotic stages. In this study, the normal control group displayed consistent and uniform seminiferous tubule structures (Figure 3). The tubules were rounded, with well-organized layers of spermatogenic cells lining the walls, indicating moderately active spermatogenesis within the lumen of the seminiferous tubules.

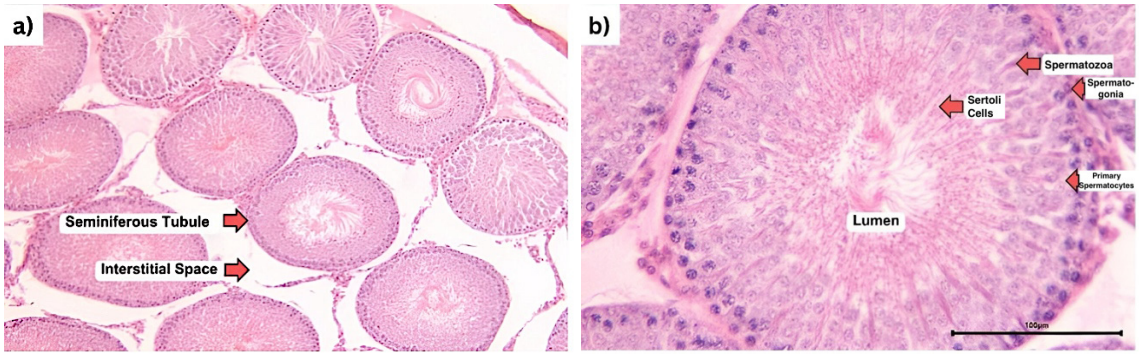

Fig. 3. Seminiferous Tubules of Normal Control Group at a) Magnification x100 and b) Magnification x400.

The positive control group (Figure 4) demonstrates active spermatogenesis and well-developed seminiferous tubules, which are uniform.

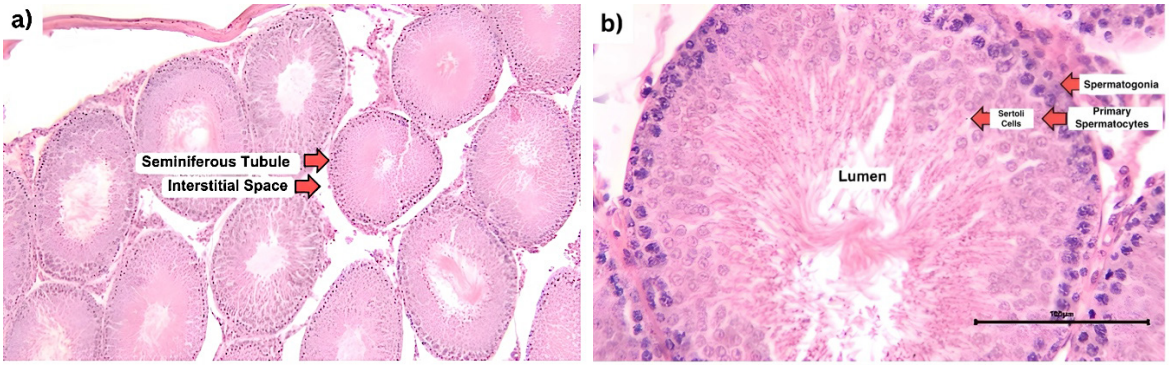

Fig. 4. Seminiferous Tubules of Positive Control Group at a) Magnification x100 and b) Magnification x400.

In contrast, the negative control group exhibited irregularly shaped and poorly formed seminiferous tubules with fragmented walls, lacking the typical round structure (Figure 5). Aberrant spermatogenesis was evident, with disorganized and irregularly arranged spermatogenic cells. The luminal area appeared reduced, with many empty spaces, a clear distinction from the treatment groups that received herb extracts.

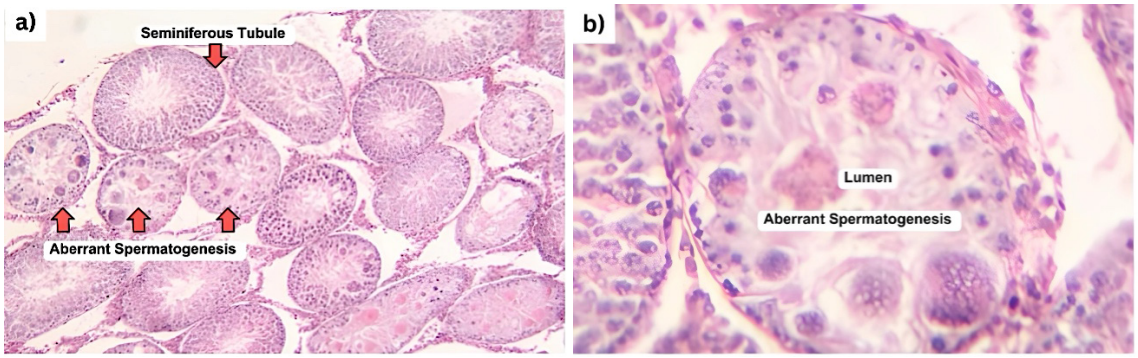

Fig. 5. Seminiferous Tubules of Negative Control Group at a) Magnification x100 and b) Magnification x400.

The treatment groups demonstrated significantly improved testicular histology. The MO400 (Figure 6) and HS150 (Figure 7) groups showed active spermatogenesis within the lumen, with the seminiferous tubules appearing well-rounded and more uniform. These findings suggest enhanced spermatogenic activity compared to the negative control group, reflecting the positive impact of the herbal treatments.

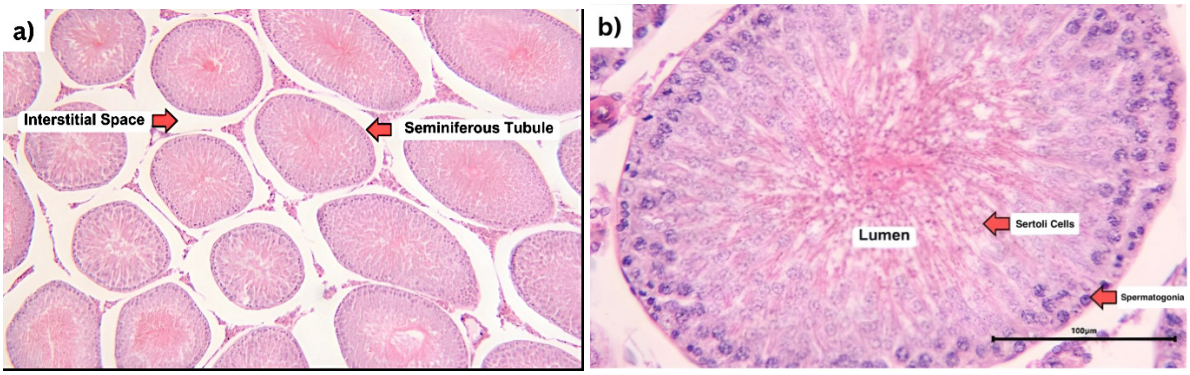

Fig. 6. Seminiferous Tubules of MO400 Group at a) Magnification x100 and b) Magnification x400.

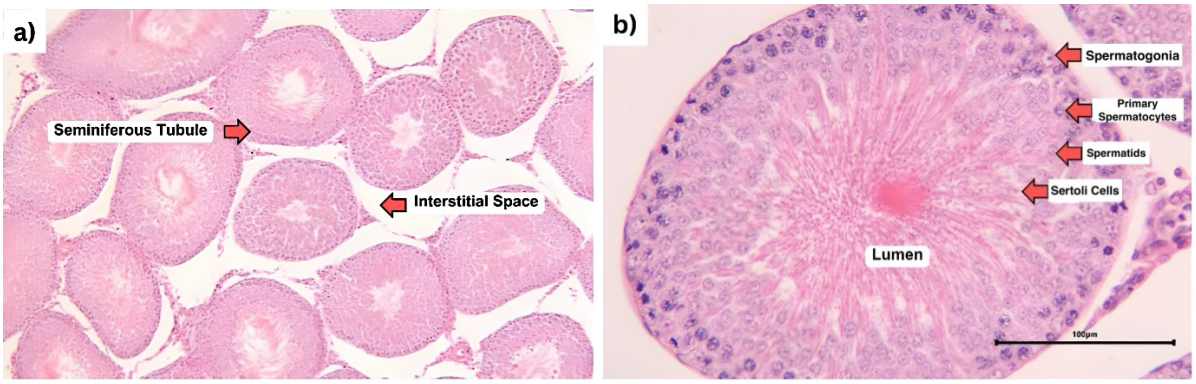

Fig. 7. Seminiferous Tubules of HS150 Group at a) Magnification x100 and b) Magnification x400.

Figure 8 showcases the combined effects of the MO and HS treatments, demonstrating highly active spermatogenesis within the seminiferous tubules. Remarkably, aberrant spermatogenesis is absent, underscoring a significant enhancement in testicular histology attributed to the synergistic benefits of the combined herbal treatments. The combination of MO and HS enhanced spermatogenesis more effectively than individual treatments, likely due to the complementary effects of their flavonoids and anthocyanins, which improve ROS neutralization and androgen regulation.

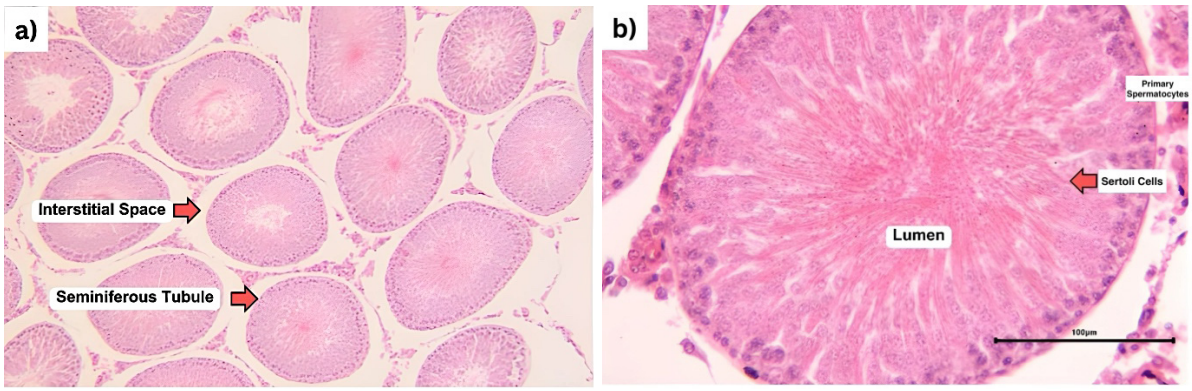

Fig. 8. Seminiferous Tubules of MOHS Group at a) Magnification x100 and b) Magnification x400.

As shown in Figure 5, the negative control group exhibited abnormal testicular histology and aberrant spermatogenesis, which correlated with the highest malondialdehyde (MDA) (Figure 2). These findings suggest a relationship between oxidative stress and impaired testicular function, underscoring the detrimental effects of obesity on spermatogenic activity. Obesity exacerbated these issues by triggering physiological disturbances, particularly in reproductive organs (Hapiz *et al*., 2020). Without any treatment, the negative control group experienced disruptions in hormonal balance, leading to impaired spermatogenesis (Bashah & Noor, 2021), and the presence of lipid-damaging substances further compromised the production of mature sperm (Oliveira *et al*., 2017). In contrast, the herbal treatment groups demonstrated improved histological structures and a significant reduction in MDA levels (Figure 2), indicating that herbal treatments protect against oxidative damage.

The herbal treatment groups demonstrated active spermatogenesis, which can be attributed to the bioactive compounds present in MO. These compounds inhibit ROS production in testicular tissue and promote testosterone expression, leading to

increased cell counts at all stages of spermatogenesis and higher concentrations of spermatozoa in the seminiferous tubule lumen (Mohlala *et al*., 2023). Due to their potent antioxidant properties, the leaves of MO play a significant role in enhancing spermatogenesis by preventing the oxidation of lipids, proteins, and even DNA (Laoung-On *et al*., 2021). Bioactive compounds in MO stimulate the hypothalamic-pituitary-gonadal (HPG) axis, leading to increased luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion, which in turn promotes testicular function and sperm production (Jangir & Jain, 2022). By blocking ROS formation, MO leaf extract protects spermatozoa from continuous damage to their lipid membranes and positively influences hormone levels. Specifically, it aids in regulating testosterone, as demonstrated in our previous study (Bashah & Noor, 2021). The modulation of these hormones is crucial for maintaining reproductive health and optimizing spermatogenesis.

Moreover, HS extract inhibits MDA production by reducing lipid peroxidation in spermatozoa and seminiferous tubules, thereby promoting spermatogenesis and supporting the healthy development of seminiferous tubules (Ifayanti & Abdullah, 2018). The bioactive compounds in HS extract, including alkaloids, flavonoids, phenols, saponins, steroids, and anthocyanins, along with secondary metabolites like polysaccharides, amino acids, and lipids, serve as powerful agents in fertility restoration therapy (Oyewopo *et al*., 2020). Additionally, HS calyx extract helps regulate ROS production levels while enhancing antioxidant enzyme activity (SOD), as shown in Figure 1.

Diameter of the seminiferous tubule

The analysis of seminiferous tubule diameter (Table 2) revealed that the normal control group had a lower average (312.70 ± 15.63 µm) compared to the positive control group (346.44 ± 17.32 µm). However, all treatment groups, except the HS150 group (313.31 ± 15.67 µm), showed a significantly greater average seminiferous tubule diameter (p<0.05) compared to the negative control group, which had the most minor diameter (199.20 ± 9.96 µm). Notably, the MO400 group (371.55 ± 18.58 µm) and the MOHS group (396.34 ± 19.82 µm) demonstrated significantly larger diameters compared to the negative control group.

Although the diameter of the seminiferous tubules showed significant differences between the treatment and control groups, no significant difference (p>0.05) was observed in testis weight between the two groups (Table 2). This lack of difference is not surprising, as sperm mass does not directly contribute to the overall weight of the testes.

Table 2. Average testes weight (g) and diameter of the seminiferous tubule (µm)

	Testes weight (g)	Diameter of seminiferous tubule (µm)
Normal	1.62 ± 0.08	312.70 ± 15.63
Positive	1.45 ± 0.07	346.44 ± 17.32*c
Negative	1.23 ± 0.06	199.20 ± 9.96
MO400	1.31 ± 0.06	371.55 ± 18.58*c
HS150	1.48 ± 0.07	313.31 ± 15.67
MOHS	1.43 ± 0.07	396.34 ± 19.82*c

An asterisk * indicates significant differences at p<0.05. c = negative control group.

The significantly larger seminiferous tubule diameter and increased spermatogenesis observed in the MO400 and MOHS treatment groups, compared to the negative control group, can be attributed to the individual effects of MO and the synergistic interaction of MO and HS extracts in the MOHS preparation. MO promotes the development of the spermatogenic cell layer within the tubules, thereby increasing tubule diameter (Devitasari *et al*., 2023). The aqueous extract from HS calyx enhances androgen hormone levels essential for spermatogenesis, supporting Sertoli cell function and promoting sperm formation within the tubules, further contributing to the increased tubule diameter (Hanis *et al*., 2012). Moreover, the bioactive compounds in MO, including vitamins C, E, and A, as well as carotenoids such as lutein, alpha-carotene, beta-carotene, and quercetin, act as potent antioxidants, protecting cells from oxidative damage and supporting healthy spermatogenesis (Mohammed & Attalla, 2015).

CONCLUSION

In conclusion, this study demonstrates that MO and HS extracts, particularly their combination, significantly enhance testicular health and spermatogenesis. The treatment groups exhibited marked improvements in testis weight, reduced oxidative stress, and heightened spermatogenesis compared to the negative control. The antioxidant-rich MO and HS extracts support spermatogenesis by inhibiting lipid peroxidation and enhancing antioxidant enzyme activity. Their potent antioxidant properties reduce oxidative stress, supporting fertility restoration in obesity-induced male rats. These findings suggest a potential therapeutic strategy for obesity-related male infertility, highlighting the practical benefits of MO and HS as natural health supplements that could be explored in future human trials. The results align with previous pre-clinical studies, reinforcing the potential translational value of MO and HS in fertility treatment. Further clinical studies will be crucial for optimizing their application in human reproductive health.

ACKNOWLEDGEMENTS

We gratefully acknowledge the support of the Faculty of Science and Technology, Universiti Kebangsaan Malaysia. This research was funded by grant GUP-2020-084.

ETHICAL STATEMENT

This study has obtained approval from the Universiti Kebangsaan Malaysia Animal Ethics Committee (Approval Number UKMAEC: FST/2022/MAHANEM/28 SEPT./1272-OCT.-2022-JAN. - 2023).

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

Abidin, Z., Hu, Y.F., Huang, H.T., Huang, C.Y., Wu, Y.S. & Nan, F.H. 2023. Effect of aqueous moringa (*Moringa oleifera*) leaf extract as a prebiotic on growth of the whiteleg shrimp, *Penaeus vannamei* Boone, 1931 (Decapoda, Penaeidae). Crustaceana, 96(2): 139–156. https://doi.org/10.1163/15685403-bja10269

Aju, B.Y., Rajalakshmi, R. & Mini, S. 2019. Protective role of *Moringa oleifera* leaf extract on cardiac antioxidant status and lipid peroxidation in streptozotocin induced diabetic rats. Heliyon, 5(12): article ID e02935. https://doi.org/10.1016/j.heliyon.2019.e02935

Ali, E.A., Kamarudin, K.M., Me, R.C., Sulaiman, R. & Alli, H. 2019. The development of SORREL systematic roselle harvesting system for efficient transfer process. IOP Conference Series: Materials Science and Engineering, 697(1): article ID 012029. https://doi.org/10.1088/1757-899X/697/1/012029

Ali Redha, A., Perna, S., Riva, A., Petrangolini, G., Peroni, G., Nichetti, M., Iannello, G., Naso, M., Faliva, M.A. & Rondanelli, M. 2021. Novel insights on anti-obesity potential of the miracle tree, *Moringa oleifera*: A systematic review. Journal of Functional Foods, 84: article ID 104600. https://doi.org/10.1016/j.jff.2021.104600

Alyani, F.S., Yulianti, R. & Thadeus, M.S. 2021. The effect of roselle (*Hibiscus sabdariffa*) extract on malondialdehyde level in rat liver. Jurnal Gizi Dan Pangan, 16(1): 57–62. https://doi.org/10.25182/jgp.2021.16.1.57-62

Amer, S.A., Al-Khalaifah, H.S., Gouda, A., Osman, A., Goda, N.I.A., Mohammed, H.A., Darwish, M.I.M., Hassan, A.M. & Mohamed, S.K.A. 2022. Potential effects of anthocyanin-rich roselle (*Hibiscus sabdariffa* L.) extract on the growth, intestinal histomorphology, blood biochemical parameters, and the immune status of broiler chickens. Antioxidants, 11(3): article ID 544. https://doi.org/10.3390/antiox11030544

Amin, A. & Hamza, A.E.A. 2006. Effects of roselle and ginger on cisplatin-induced reproductive toxicity in rats. Asian Journal of Andrology, 8(5): 607–612. https://doi.org/10.1111/j.1745-7262.2006.00179.x

Azlan, A., Sultana, S., Huei, C.S. & Razman, M.R. 2022. Antioxidant, anti-obesity, nutritional and other beneficial effects of different chili pepper: A review. Molecules, 27(3): article ID 898. https://doi.org/10.3390/molecules27030898

Bashah, N.A.K. & Noor, M.M. 2021. Antihyperglycemic and androgenic properties of *Moringa oleifera* leaves aqueous extract attenuate sexual dysfunction in diabetes-induced male rats. Malaysian Applied Biology, 50(2):99-105.

Beheshti, R., Bandariyan, E. & Hemati, M. 2018. Effects of *Hibiscus sabdariffa* aqueous extract on spermatogenesis and sperm parameters of mice. Herbal Medicines Journal, 3(3): 109–128.

Devitasari, S., Pesik, R.N., Subandono, J. & Setyawan, N.A. 2023. Effect of ethanolic extract of Moringa (*Moringa oleifera*, Lam.) leaf on seminiferous tubules of Wistar rats (*Rattus norvegicus*) model of metabolic syndrome. Smart Medical Journal, 6(2): 72–81. https://doi.org/10.13057/smj.v6i1.69345

Duranti, G., Maldini, M., Crognale, D., Horner, K., Dimauro, I., Sabatini, S. & Ceci, R. 2021. *Moringa oleifera* leaf extract upregulates nrf2/ho-1 expression and ameliorates redox status in C2C12 skeletal muscle cells. Molecules, 26(16): article ID 5041. https://doi.org/10.3390/molecules26165041

Dutta, S. & Sengupta, P. 2018. Medicinal herbs in the management of male infertility. Journal of Pregnancy and Reproduction, 2(1): article ID 128. https://doi.org/10.15761/jpr.1000128

Ellis, L.R., Zulfiqar, S., Holmes, M., Marshall, L., Dye, L. & Boesch, C. 2022. A systematic review and meta-analysis of the effects of *Hibiscus sabdariffa* on blood pressure and cardiometabolic markers. Oxford University Press.

Emir, I. & Noor, M.M. 2024. Mitigasi *Nigella sativa* dan *Moringa oleifera* terhadap fragmentasi DNA sperma melalui aktiviti antioksidan pada tikus aruhan-obesiti. Sains Malaysiana, 53(11): 3617-3628.

Fitriani, D., Meliala, A. & Agustiningsih, D. 2016. The effect of long-term high-fat diet in ovariectomized Wistar rat on leptin serum levels. Berkala Ilmu Kedokteran, 48(02): 69–80. https://doi.org/10.19106/jmedsci004802201601

Hanis, M., Balkis Budin, S., Osman, M. & Mohamed, J. 2012. Protective role of *Hibiscus sabdariffa* calyx extract against streptozotocin induced sperm damage in diabetic rats. EXCLI Journal, 11: 659-669.

Hapiz, M., Rahman, A., Chenderoh, K.K., Safuraa, I., Abu, B., Kolej, B., Chenderoh, K., Ariffin, N. & Kolej, K. 2020. Kajian nutrisi antara aditif makanan berasaskan herba tempatan Malaysia dan aditif makanan yang mengandungi kandungan monosodium glutamate. Kolej Komuniti Journal of Engineering and Technology, 5(1): article ID 10.

Ifayanti, T. & Abdullah, F. 2018. Pengaruh pemberian ekstrak rosella (*Hibiscus sabdariffa* Linn) terhadap jumlah dan kecepatan spermatozoa, berat testis tikus jantan strain Wistar yang terpapar karbon tetraklorida (CCl4). JIK - Jurnal Ilmu Kesehatan, 2(1): 124–129. https://doi.org/10.33757/jik.v2i1.74

Jangir, R.N. & Jain, G.C., 2022. Ameliorative effect of *Moringa oleifera* Lam. leaves extract on the sex hormone profile and testicular dysfunctions in streptozotocin-induced diabetic wistar rats. Pharmacognosy Research, 14(2).

Jubaidi, F.F., Zainalabidin, S., Taib, I.S., Hamid, Z.A. & Budin, S.B. 2022. Cardiac remodeling in diabetic cardiomyopathy: The role of inflammation, oxidative stress and apoptosis underlying its formation and development. Sains Malaysiana, 51(12): 4043–4057. https://doi.org/10.17576/jsm-2022-5112-14

Kamalrudin, A., Jasamai, M. & Noor, M.M., 2018. Ameliorative effect of Moringa oleifera fruit extract on reproductive parameters in diabetic-induced male rats. Pharmacognosy Journal, 10(6): 54-58. https://doi.org/10.5530/pj.2018.6s.10

Kamaruzaman, K.A., Aizat, W.M. & Mat Noor, M., 2018. Gynura procumbens improved fertility of diabetic rats: Preliminary study of sperm proteomic. Evidence-Based Complementary and Alternative Medicine, 2018(1): p.9201539

Kashyap, P., Kumar, S., Riar, C.S., Jindal, N., Baniwal, P., Guiné, R.P.F., Correia, P.M.R., Mehra, R. & Kumar, H. 2022. Recent advances in drumstick (Moringa oleifera) leaves bioactive compounds: Composition, health benefits, bioaccessibility, and dietary applications. Antioxidants, 11(2): article ID 402. https://doi.org/10.3390/antiox11020402

Katib, A. 2015. Mechanisms linking obesity to male infertility. Central European Journal of Urology, 68(1): 79–85. https://doi.

org/10.5173/ceju.2015.01.435
Kasim, R.M., Jubaidi, F.F. & Budin, S.B. 2022. View of testicular damage and abnormal sperm characteristics due to chronic hyperglycemia exposure restored by polyphenol-rich extract of *Hibiscus sabdariffa* Linn. Journal of Advanced Research in Applied Sciences and Engineering Technology, 23(1): 43–55.
Laoung-On, J., Saenphet, K., Jaikang, C. & Sudwan, P. 2021. Effect of *Moringa oleifera* Lam. leaf tea on sexual behavior and reproductive function in male rats. Plants, 10(10): article ID 2019. https://doi.org/10.3390/plants10102019
Manna, P. & Jain, S.K. 2015. Obesity, Oxidative stress, adipose tissue dysfunction, and the associated health risks: Causes and therapeutic strategies. Metabolic syndrome and related disorders, 13(10): 423–444. https://doi.org/10.1089/met.2015.0095
Mannucci, A., Argento, F.R., Fini, E., Coccia, M.E., Taddei, N., Becatti, M. & Fiorillo, C. 2022. The impact of oxidative stress in male infertility. Frontiers in Molecular Biosciences, 8: article ID 799294. https://doi.org/10.3389/fmolb.2021.799294
Mohammed, M. & Attalla, F.E.-K. 2015. The radioprotective effects of *Moringa oleifera* against mobile phone electromagnetic radiation-induced infertility in rats. International Journal of Clinical and Experimental Medicine, 8(8): 11240–11248.
Mohlala, K., Offor, U., Monageng, E., Takalani, N.B. & Opuwari, C.S. 2023. Overview of the effects of *Moringa oleifera* leaf extract on oxidative stress and male infertility: A review. Applied Sciences, 13(7): article ID 4387. https://doi.org/10.3390/app13074387
Noor, M.M., Zin, N.F.A.M. & Shamsusah, N.A. 2023. Effectiveness of *Hibiscus sabdariffa* calyx aqueous extract as an anti-obesity agent and enhancer of fertility parameters in obese-induced male rats. Malaysian Applied Biology, 52(6): 137–147. https://doi.org/10.55230/mabjournal.v52i6.2838
Oliveira, P.F., Sousa, M., Silva, B.M., Monteiro, M.P. & Alves, M.G. 2017. Obesity, energy balance and spermatogenesis. Reproduction, 153(6): R173–R185. https://doi.org/10.1530/REP-17-0018
Oyewopo, A.O., Olaniyi, K.S., Olojede, S.O., Lawal, S.K., Amusa, O.A. & Ajadi, I.O. 2020. *Hibiscus sabdariffa* extract protects against cadmium-induced ovarian toxicity in adult Wistar rats. International Journal of Physiology, Pathophysiology and Pharmacology, 12(4): article ID 16.
Taib, I.S., Budin, S.B., Ismail, M.N., Zainalabidin, S. & Mohamed, J. 2017. Kesan ekstrak akueus roselle (*Hibiscus sabdariffa* Linn.) terhadap sperma dan testis tikus diadministrasi nikotin. Sains Malaysiana, 46(9): 1611–1616. https://doi.org/10.17576/jsm-2017-4609-33
Vergara, J.M., Almatrafi, M.M. & Fernandez, M.L. 2017. Bioactive components in *Moringa oleifera* leaves protect against chronic disease. Antioxidants, 6(4): article ID 91. https://doi.org/10.3390/antiox6040091
Wang, Y., Branicky, R., Noë, A. & Hekimi, S. 2018. Superoxide dismutases: Dual roles in controlling ROS damage and regulating ROS signaling. Journal of Cell Biology, 217(6): 1915–1928. https://doi.org/10.1083/jcb.201708007
World Health Organisation. 2024. Infertility. https://www.who.int/news-room/fact-sheets/detail/infertility (accessed 09.23.24).
Younus, H. 2018. Therapeutic potentials of superoxide dismutase. Therapeutic Advances in Chronic Disease, 9(9): 123–134. https://doi.org/10.1177/2040622318785470
Zade, V.S., Dabhadkar, D.K., Thakare, V.G. & Pare, S.R. 2023. Effect of aqueous extract of *Moringa oleifera* seed on sexual activity of male albino rats. International Journal of Health Sciences, 7(4): 156–162. https://doi.org/10.53730/ijhs.v7n4.8482
Zeng, B., Luo, J., Wang, P., Yang, L., Chen, T., Sun, J., Xie, M., Li, M., Zhang, H., He, J., Zhang, Y. & Xi, Q. 2019. The beneficial effects of *Moringa oleifera* leaf on reproductive performance in mice. Food Science and Nutrition, 7(2): 738–746. https://doi.org/10.1002/fsn3.918