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1. **Department of Physiology, University of Ilorin, Ilorin, Nigeria.**
2. **Department of Medical Biotechnology, National Biotechnology Development Agency, Ogbomosho, Nigeria**
3. **C.G. Bhakta Institute of Biotechnology, Uka Tarsadia University, Bardoli, India.**

***Corresponding author**: asufem2017@gmail.com

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# ANDROGENIC EFFECTS OF AQUEOUS SEED EXTRACT OF MORINGA OLEIFERA IN MALE WISTAR RATS

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4. **Department of Physiology, University of Ilorin, Ilorin, Nigeria.**
5. **Department of Medical Biotechnology, National Biotechnology Development Agency, Ogbomosho, Nigeria**
6. **C.G. Bhakta Institute of Biotechnology, Uka Tarsadia University, Bardoli, India.**

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**ABSTRACT**:
Androgenic effects of Aqueous Seed-extract of Moringa oleifera (ASMO) in male Wistar rats were investigated. Eighteen (18) male Wistar rats weighing 200-240g were used for this study. The rats were divided into three (3) groups: Control (Group 1) that received 10 ml/kg of normal saline, Group 2: received low dose of ASMO (200mg/kg), and Group 3: received high dose of ASMO (500mg/kg). The animals were treated for twenty-eight days. On the 29th day, the rats were sacrificed and the testes were carefully removed for semen and biochemical analysis. Body weight, reproductive and vital organ weights were determined. Sperm parameters (motility, morphology, count and viability), tissue testosterone, luteinizing hormone (LH), Malondialdehyde (MDA) and Catalase were also determined using standard methods. Data were analysed using one-way ANOVA followed by Duncan new multiple range post hoc test. The result showed ASMO significantly increased ($p<0.05$) the final body weight, weight of reproductive and vital organs. Moreover, 200mg/kg body weight dose of ASMO significantly increased ($p<0.05$) the sperm parameters but 500mg/kg body weight dose significantly decreased it. ASMO also caused a significant dose dependent increase ($p<0.05$) in testosterone and catalase level but a significant decrease ($p<0.05$) in MDA and LH level compared with the control. In conclusion, this study revealed that ASMO has androgenic effects in male rats and thus provides a basis for the traditional use of Moringa oleifera in the management of male sexual disorders.

**Keywords**: Moringa oleifera, Testosterone, Malondialdehyde, Catalase, Sperm parameters

**INTRODUCTION**:

Every human being has a right to enjoy the highest attainable standard of physical and mental health. Infertility negates the realization of these crucial human rights. It affects about 46 million couples and about 186 million individuals worldwide [1-3]. The contribution of male factor in infertility is now considered as important as the female factor and in some cases account for almost 60% of all cases of infertility [4,5]. Globally, deficient testosterone and low sperm count are major causes of male factor infertility, contributing to the increased incidence of infertility [6,7]. Ageing, toxins, drugs, trauma, infections, ionizing radiation from chemotherapy, environmental stressors like hot weather, air pollution, interfere with androgenesis [8-11]. The need for clinical management of some of these conditions have resulted in the development of a number of available treatment options like Testosterone Replacement Therapy (TRT) [11], drugs like clomiphene, tamoxifen. Others include In vitro fertilisation (IVF) for oligospermia and surgery for hydroceles, varicoceles and vas deference obstruction [12]. Unfortunately, some of these options are quite expensive, not easily accessible and may not blend with the sociocultural life of some people. Some of them are also associated with adverse side effects such as, testicular atrophy, stroke, myocardial infarction, hepatotoxicity, worsening of benign prostatic hyperplasia symptoms and prostate cancer [13].

The natural composition of some approved herbal medicines may actually reduce certain side effects many people experience when taking synthetic drugs, while achieving favourable results [13]. In addition, some herbal supplements may naturally improve the body's ability to synthesize the hormone being targeted, instead of receiving exogenous hormones when the level is low [13]. Some herbal supplements also have systemic effect in providing nutrition [14,15].

Moringa oleifera is one of the World's most useful trees, as almost every part of the tree can be used for food, medicine and industrial purposes [16,17]. It belongs to the Family Moringaceae [18]. The plant has gained a lot of names such as Horseradish tree, Drumstick tree, Ben oil tree, Miracle tree, "Okwe Oyibo" in Igbo language, "Zogale" in Hausa and "Ewe Ile" in Yoruba language [18]. The flowers, leaves, seeds and roots of Moringa oleifera (M. oleifera) has many chemical components, including crude fiber, reducing sugars, resins, alkaloids, flavonoids, saponins, phenols, terpenoids, glycosides, organic acids, sterols, tannins, saponins, proteins, vanillin, carotenoids, ascorbate, tocopherols, beta-sitosterol, kaempferol, and quercetin [19].

In addition, it contains unsaturated fatty acids, especially linoleic, oleic and palmitic acids. M. oleifera is rich in amino acids, vitamins,

minerals particularly iron, and potassium [20]. M. oleifera has been used for many decades as traditional medicine. This has made herbal practitioners lay claims to it having the cure to a myriad of ailments of which, only a few have undergone scientific validation. The leaves of M. oleifera are used as purgative, applied as poultice to sores, rubbed on the temples for headaches, used for piles, fevers, sore throat, bronchitis, eye and ear infections, scurvy and catarrh [21]. The leaf juice is believed to control glucose levels and reduce glandular swelling [22,23]. The seeds are used to decrease liver lipid peroxides, insulin resistance and inflammation. It is also used as an antimicrobial and anti-cancer agent [24-26]. The stem bark is used as an antioxidant and in treating rheumatism [27]. The roots of M. oleifera were shown to possess antilithic, anti-inflammatory, stimulant in paralytic afflictions; act as a blood tonic, used as a laxative, to treat rheumatism, lower back or kidney pain [28,29].

Although some studies have reported the fertility enhancing effect of Moringa oleifera in murine animals [14,24,29,30]. This study aims to shed light on the androgenic potential of Aqueous Seed-extract of Moringa oleifera (ASMO) in healthy male Wistar rats.

**METHODOLOGY**:

Collection of plant material:

They were identified and authenticated in the Department of Plant Biology, University of Ilorin, and allocated the voucher number UILH/001/1275/2020.

Preparation of extract:

M. olifiera seeds were decapsulated and dried in a shaded, well-aerated environment. They were subsequently, grinded into slightly coarse powder to improve percolation of solvent. The powder weighing 300g was macerated in 1.5L of distilled water for 48 hours under room temperature and was stirred every 3 hours.

It was then filtered. The filtrate was concentrated by lyophilisation (Freeze-drying). The concentrate was refrigerated at 4°C for storage [31].

Procurement and acclimatization of the animals:

Eighteen (18) healthy male Wistar rats (about 65 days old) weighing 200-250 gm were procured from the Department of Biochemistry, University of Ilorin. The rats were transported to the animal house of the Faculty of Basic Medical Sciences, University of Ilorin, where they were acclimatized to the animal house environment, for a period of 14 days before the experiment. The rats were housed in well-aerated plastic cages, at normal room temperature. They were fed pellets from Vital feed depot, Sango, Ilorin and tap water ad libitum. The beddings were changed daily to provide a hygienic environment.

Animal grouping and administration

The rats were separated into three (3) groups, of six (6) animals each and kept in separate cages during the experiment as follows:
Group 1: Control Group received 10 ml/kg of normal saline orally daily.

Group 2: Low Dose Treated Group received aqueous seed-extract of Moringa oleifera (ASMO) orally at the dose of 200 mg/kg daily.

Group 3: High Dose Treated Group received aqueous seed-extract of Moringa oleifera (ASMO) orally at the dose of 500 mg/kg daily.
Administration process lasted for 28 days with administration time between 8:00am to 09:00am daily. All the animals were strictly handled in conformation to the Declarations of Helsinki in 1995 (as revised in Edinburgh 2000) and the University's guidelines on Care and Use of Laboratory Animals.

Phytochemical screening:
Preliminary phytochemical screening of Aqueous Seed-Extract of Moringa oleifera (ASMO) was done using the methods of Khadabadi and Deore [32].

Sample Collection:
Organ Weight Measurement
Following 28 days of treatment, the control and experimental groups of male rats were weighed. The animals were completely anaesthetized with ketamine. The following organs; testis, seminal vesicles, epididymis, vas deference, penis and prostate glands alongside vital organs like liver, kidney, adrenal gland, and spleen were carefully removed and weighed using sensitive digital scale (LCD Precision Scale 0.01 g-1000 g).

Semen Analysis:
Sperm count
Upon the elapse of 28 days administration period, the testes from each rat were carefully dissected and excised. The epididymis was identified and removed. Using modified method of Yokoi and Mayi [33], the Spermatozoa in the right epididymis were counted. Briefly, the epididymis was minced with anatomic scissors in 5mL normal saline, placed in a rocker for 10 minutes, and allowed to incubate at room temperature for 2 minutes. After incubation, the supernatant fluid was diluted 1:100 with solution containing 5g sodium bicarbonate and 1mL formalin (35%). Total sperm number was determined using the new improved Neuber`s counting chamber (haemocytometer). Approximately 10µL of the diluted sperm suspension was transferred to each counting chamber of the haemocytometer and was allowed to stand for 5 minutes. This chamber was then placed under a binocular light microscope using an adjustable light source. The ruled part of the chamber was then focused and the number of spermatozoa counted in five 16-celled squares.
The sperm concentration was then multiplied by 5 and expressed as [X] x 106 /ml, where [X]

is the number of spermatozoa in a 16-celled square [34].

Sperm motility:

Sönmez et al. [35] method of analyzing sperm motility was employed. Fluid from the left caudal epididymis was extracted and pipetted, diluted via Tris buffer solution to a volume of 0.5ml. An aliquot of this solution was placed on a glass slide, already resting on a heated table and subsequently introduced to the stage of a light microscope with an adjustable light source. At X400 magnification, percentage motility was evaluated. Motility estimates were performed from three different fields in each sample. The mean of the three estimations was used as the final motility score.

Sperm viability:

40µl of freshly liquefied semen was thoroughly mixed with 10µl of eosin-nigrosin, and 1 drop of this mixture was transferred to a clean slide, to determine viability. At least 200 sperms were counted at a magnification of ×100 under oil immersion. Unstained sperm cells were considered viable, while either red or pink stained sperm cells were nonviable [36,37].

Sperm morphology:

Morphology was assessed at X400 magnification. Caudal sperm were taken from the original aliquot for motility, dilution factor of 1:20 using 10% neutral buffered formalin as diluent (Sigma-Aldrich, Oakville, ON, Canada). 500 sperms from the sample were scored for morphological abnormalities [38]. Spermatozoa were categorized in wet preparations, using phase-contrast optics. A spermatozoon was considered abnormal morphologically if it possessed any of the following features; rudimentary tail, round head and detached head. Result was expressed as a percentage of morphologically normal sperm [34].

Testis Collection:

The scrotums of the rats were carefully dissected and testes were carefully removed. Each testis was separated from the epididymis and was weighed. With a clean mortar and pestle a 10% tissue homogenate was prepared using normal saline as solvent. The homogenate was then transferred into a plain sample bottle for each animal and centrifuged. The centrifugation was done at 4000 rpm for five (5) minutes. The supernatant from the centrifuged homogenate was then transferred using a micropipette into a clean plain bottle and refrigerated.

Measurement of Testosterone:

Testosterone was assayed for by Enzyme-Linked Immunosorbent Assay (ELISA) using Calbiotech's testosterone ELISA kit. The procedures for the assay as contained in the manufacturer's manual were strictly followed [39]. The absorbance was read on ELISA Reader for each well at 450nm within 15 minutes after adding the stop solution.

Measurement of Luteinizing Hormone:

Luteinizing Hormone was assayed for by Enzyme-Linked Immunosorbent Assay (ELISA) using Calbiotech's Luteinizing Hormone ELISA kit. The procedures for the assay as contained in the manufacturer's manual were strictly followed [40]. The absorbance was read with ELISA Reader for each well at 450nm within 15 minutes after adding the stop solution.

Malondialdehyde (MDA):

The level of Malondialdehyde (MDA) was determined indirectly as thiobarbituric acid reactive substances (TBARS) according to the thiobarbitutric acid reaction of Uchiyama and Mihara [41].

Catalase:

The assay buffer, colorimetric assay substrate solution, and color reagent were allowed to equilibrate to room temperature. 25µl of sample and 75µl of assay Buffer was added to a test tube. The reaction was started by adding 25µl of the colorimetric assay substrate solution to the test tube. The solution was mixed by inversion and incubated for 15 minutes. 825µl of stop solution was added and mixed. An aliquot of 10µl of the mixture is added to another test tube to which 1ml of chromogen reagent added and mixed for at least 15 minutes at room temperature for color development. 1ml of the resulting solution is transferred to a cuvette and the absorbance was measure at 520 nm [42].

Statistical Analysis:

All the data were analyzed using one-way analysis of variance (ANOVA) and subjected to Duncan new multiple range post hoc tests. The results are expressed as Mean ± S.E.M using SPSS software (version 23). Values of $p<0.05$ were considered statistically significant

**RESULTS**:

Phytochemical Screening

Qualitative analysis of Aqueous Seed-Extract of Moringa oleifera (ASMO) shows the presence of phenols, saponins, tannins, alkaloids, flavonoids, steroids, terpenoids, glycosides and proteins.

Body Weight:

There was no significant difference ($p<0.05$) in the initial body weight across all groups.

Administration of ASMO caused a significant increase in body weight ($p<0.05$) as shown in Table 1 when compared with the control rats at the end of the 28-day experiment. In addition, there was a significant difference ($p<0.05$) in mean body weight of the treated rats which was dose dependent.

Weights of some organs and tissues:

The mean weight of the testes, caput segment of the epididymis, ventral prostate, seminal vesicle, penis and vas deferens increased

significantly ($p<0.05$) when compared with the control (Table 2). Likewise, there was a significant increase in the mean weights of the liver, adrenal gland, kidney and spleen ($p<0.05$), when compared with that of control (Table 3).

Sperm Analysis:

ASMO significantly increase the spermatic profile of the male rats (Table 4). The treated rats had a significant increase ($p<0.05$) in sperm count, sperm motility, sperm morphology, sperm viability when compared with the control rats. The differences were not dose dependent (Table 4).

Hormone Level:

Table 5 depicts the effect of administration of two different concentrations of ASMO on serum testosterone and Luteinizing Hormone (LH) levels in male Wistar rats. The serum testosterone level in the treated rats was significantly higher ($p<0.05$) when compared with the control rats (Table 5). However, there was no significant difference in the testosterone level between the two groups of treated rats

ASMO decreased serum LH levels significantly ($p<0.05$) in the treated rats when compared with the control (Table 5).

Serum Catalase and Malondialdehyde (MDA) Levels:

The results of administration of ASMO on the serum levels of catalase and MDA are shown in Table 6. ASMO proved to increase the catalase levels in the treated rats significantly ($p<0.05$) when compared with the control rats (Table 6). On the other hand, lipid peroxidation was significantly decreased as shown by the results of MDA level, (Table 6).

The MDA level decrease significantly ($p<0.05$) in the treated groups when compared with the control group.

**Table 1: Effect of ASMO on Body Weight of Male Rats**

| Animal groups | Initial body weight (g) | Final body weight (g) | Differences in body weight |
|---|---|---|---|
| Group 1 (Normal saline) | 220.0±4.38$^{a}$ | 224.2±4.43$^{a}$ | 4.2 ± 0.05 |
| Group 2 (Low dose ASMO) | 227.8±5.0$^{a}$ | 239.0±8.12$^{ab}$ | 11.2 ± 3.12 |
| Group 3 (High dose ASMO) | 232.4±3.26$^{a}$ | 256.0±16.73$^{b}$ | 23.6 ± 13.47 |

Key: Means with different alphabets are significantly different ($p<0.05$) from each other

**Table 2: Effect of ASMO on Reproductive Organs and Tissues of Male Rats**

| Animal groups | Testis (mg) | Epididymis (mg) | Prostate (mg) | Seminal Vesicle(mg) | Penis (mg) | Vas deference (mg) |
|---|---|---|---|---|---|---|
| Group 1 (Normal saline) | 1.27±0.03$^{a}$ | 0.40±0.04$^{ab}$ | 0.25±0.01$^{a}$ | 0.28±0.02$^{a}$ | 0.28±0.01$^{a}$ | 0.22±0.01$^{a}$ |
| Group 2 (Low dose ASMO) | 1.48±0.05$^{b}$ | 0.53±0.04$^{b}$ | 1.48±0.04$^{b}$ | 0.61±0.02$^{bc}$ | 0.38±0.02$^{b}$ | 0.26±0.03$^{ab}$ |
| Group 3 (High dose ASMO) | 1.50±0.06$^{b}$ | 0.77±0.10$^{c}$ | 0.56±0.03$^{b}$ | 0.69±0.05$^{c}$ | 0.48±0.02$^{c}$ | 0.33±0.01$^{b}$ |

Key: Means with different alphabets are significantly different ($p<0.05$) from each other

.

**Table 3: Effect of ASMO on some Vital Organs of Male Rats**

| Animal groups | Liver (%) | Adrenal gland (%) | Kidney (%) | Spleen (%) |
|---|---|---|---|---|
| Group 1 (Normal saline) | 3.41±0.01$^{a}$ | 0.077±0.001$^{a}$ | 0.30±0.01$^{a}$ | 0.32±0.01$^{a}$ |
| Group 2 (Low dose ASMO) | 3.68±0.01$^{ab}$ | 0.084±0.001$^{a}$ | 0.32±0.01$^{a}$ | 0.51±0.01$^{bc}$ |
| Group 3 (High dose ASMO) | 3.70±0.01$_{ab}$ | 0.118±0.001$^{b}$ | 0.40±0.01$^{b}$ | 0.54±0.01$^{c}$ |

Key: Means with different alphabets are significantly different ($p<0.05$) from each other

**Table 4: Effect of ASMO on Spermatic Profile of Male Rats**

| Animal groups | Sperm count (million) | Sperm morphology (%) | Sperm motility (%) | Sperm viability (%) |
|---|---|---|---|---|
| Group 1 (Normal saline) | 50.08±0.97$^{a}$ | 83.38±0.99$^{ab}$ | 82.23±0.74$^{bc}$ | 81.28±4.64$^{a}$ |
| Group 2 (Low dose ASMO) | 66.39±0.94$^{c}$ | 87.83±0.92$^{c}$ | 83.87±0.97$^{c}$ | 88.83±1.33$^{b}$ |
| Group 3 (High dose ASMO) | 56.24±0.36$^{b}$ | 85.83±0.64$^{abc}$ | 82.23±0.74$^{bc}$ | 85.82±1.14$^{ab}$ |

Key: Means with different alphabets are significantly different ($p<0.05$) from each other.

**Table 5: Effect of ASMO on Serum Testosterone and LH Levels in Male Rats**

| Animal groups | Testosterone (ng/ml) | Luteinizing hormone (ng/ml) |
|---|---|---|
| Group 1 (Normal saline) | 4.58±0.06[a] | 107.84±7.62[c] |
| Group 2 (Low dose ASMO) | 5.88±0.54[b] | 70.39±4.95[b] |
| Group 3 (High dose ASMO) | 5.20±0.29[b] | 28.23±3.64[a] |

Key: Means with different alphabets are significantly different ($p<0.05$) from each other

.

**Table 6: Effect of ASMO on Serum Catalase and MDA Levels in Male Rats**

| Animal groups | Malondialdehyde (U/mg Protein) | Catalase (U/mg Protein) |
|---|---|---|
| Group 1 (Normal saline) | 0.82±0.07bc | 2600.44±390.92b |
| Group 2 (Low dose ASMO) | 0.62±0.01ab | 3358.39±518.77bc |
| Group 3 (High dose ASMO) | 0.42±0.01a | 3495.08±389.29c |

Key: Means with different alphabets are significantly different ($p<0.05$) from each other

**DISCUSSION**:

Phytochemical screening of the plant shows that the aqueous seed extract of ASMO contains various constituents, which can be responsible for its androgenic effects. Some of these compounds include steroids, terpenoids, proteins, glycosides, alkaloids, tannins, flavonoids, and saponins. It has been documented from studies that medicinal plants with fertility potentiating effects often contain high amount of these compounds [43-45].

Physiologically, an increase in steroids causes pan-systemic anabolism that resulted in growth and development [46]. The significant increase in the weight of vital organs, reproductive organs as well as body weight may be due to androgenesis caused by phytochemicals in ASMO. This agrees with the work of Varsha et al. [47]. The significant increase in sperm motility, sperm count and sperm morphology and sperm viability in the epididymis of low dose ASMO treated rats which was decreased when compared with high dose ASMO treated rats clearly indicates that it has positive effect on spermatogenesis in rats at low doses, and that it could decrease the quality of spermatozoa at high doses [48]. This might be as a result of the presence of alkaloids which

was more prevalent at dose 500 mg/kg body weight and further gave an impact on spermatogenesis process. Alkaloid compound can suppress the secretion of male reproductive hormone (testosterone) so that it will inhibit spermatogenesis process [49,50]. Moreover, treatment dose of 500 mg/kg body weight decreased spermatozoa morphology significantly when compared with control and 200mg/kg body weight. This may be caused by the tannin compounds of ASMO which were higher in 500mg/kg body weight. The tannin content in ASMO at treatment doses of 200mg/kg body weight did not affect the percentage of spermatozoa morphology compared to the control. High tannin content in the treatment doses of 500 mg/kg body weight impacted the binding of protein and ions in the spermatozoa membrane so that the tyrosine enzyme and phosphorylation process in the spermatozoa membrane were disrupted and eventually resulted in morphological abnormalities of spermatozoa [51].

Reactive oxygen species (ROS) and free radicals are produced in organs of high metabolic activities like the testis, which results in depletion of the antioxidant capacity of spermatozoa, seminal plasma, and testis causing oxidative stress [52,53].

Oxidative stress damages spermatozoa membrane, sertoli cells and leydig cells by lipid peroxidation and molecular damage. Thus, it is a facilitator of sperm cells dysfunction, low testosterone and ultimately infertility [54].

Catalase is one of the important enzymes in the enzymatic antioxidant defense system. It reduces hydrogen peroxide to water and oxygen thus, diminishing the toxic effect caused by the formation of free radical.

The significant increase in the level of the activity of catalase (CAT) following treatment with ASMO is in consonance with previous studies [55-57], reporting antioxidant effects of ASMO. This increase may be as a result of increase in the synthesis of antioxidant molecules or reduced oxidative load due to the presence of different antioxidant compounds in the plant. Similarly, the decrease in MDA (biomarker of lipid peroxidation) in rats treated with ASMO clearly revealed anti-lipid peroxidative effect of the plant. This is also in agreement with previous studies documenting anti – lipid peroxidative effect of M. oleifera [58,59]. The decreased level of MDA in ASMO treated rats may be due to the presence of the antioxidant compounds like flavonoids, phenols, terpenoids reported in this study. Furthermore, antioxidant and anti - lipid peroxidative effects of this plant may also explains the significant increase in the spermatic parameters at low dose (200mg/kg body weight). These findings suggest that ASMO at low doses boost fertility by increasing the antioxidant level similar to findings by Suaskara et al. [48] and Jamalan et al. [60]. Testosterone is the primary male sex hormone.

It plays a key role in growth of reproductive tissues as well as spermatogenesis, libido, growth of muscle and bone mass [61]. The increase ($p<0.05$) in the testicular testosterone level recorded in this study may also be connected to its phytoconstituents (flavonoids, saponins, terpenoids and alkaloids). This finding agrees with the works of Gan [15], Dafaalla et al. [29], Zade et al. [30], Cele et al. [44], Ghosh et al. [62] and Hassan et al. [63] who documented that phytochemicals like alkaloids, flavonoids and saponins play a role in adrogenesis.

The anterior pituitary gland secretes gonadotropins (LH and FSH) which act on the testis to produce testosterone. Physiologically, increased testosterone level inhibits LH production in a negative feedback mechanism. This may explain the significant reduction in LH level in this study. The reduction in the LH level may also be due to the phytochemicals present in the plant extract, because study [64] have shown that some phytochemicals mimic the negative feedback effect of LH on hypothalamus. This in turn inhibits the secretion of hypothalamic gonadotropin releasing hormone, thereby decreasing the level of endogenous LH secretion by pituitary gonadotropes as seen in this study.

**CONCLUSION**:

In conclusion, ASMO increase sperm parameters (at treatment dose 200mg/kg), testosterone, and anti-oxidant capacity of experimental rats. This partly validates the traditional use of Moringa oleifera seed herbal preparation in the management of male infertility. However, treatment dose of 500 mg/kg body weight significantly reduced the sperm parameters; it proved that the higher doses of ASMO decreased the sperm parameters.

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