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FERTILITY ENHANCING EFFECT OF *Asparagus racemosus* ROOT METHANOL EXTRACT ON ORNIDAZOLE-INDUCED SPERMATOTOXICITY IN MALE WISTAR ALBINO RATS

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Abstract

Drug-induced spermatotoxicity and male infertility are significant issues related to reproductive health. In this work, male Wistar albino rats were exposed to ornidazole-induced spermatotoxicity, and the fertility-enhancing impact of *Asparagus racemosus* methanolic root extract was assessed. Thirty rats were split into five groups: extract-treated (50 and 100 mg/kg body weight), control, untreated, and standard drug-treated. Ornidazole (600 mg/kg body weight) was used to produce spermatotoxicity. While decreasing sperm DNA fragmentation, the extract markedly increased body weight, reproductive organ weight, sperm count, motility, viability, morphology, plasma membrane integrity, hematological parameters, and reproductive hormone levels. There was more protective action at the higher dosage. According to the study, *Asparagus racemosus* has important spermatoprotective and fertility-boosting qualities.

1. INTRODUCTION

Male factors play a big role in infertility, a serious global reproductive health disease that affects about 15% of couples. Male infertility is mostly caused by oxidative stress, hormonal imbalance, infections, environmental contaminants, and long-term medication therapy (Agarwal *et al*., 2014). Because a number of antimicrobial medicines negatively impact spermatogenesis and sperm function, drug-induced reproductive

toxicity has garnered increased attention. A nitroimidazole derivative, ornidazole is frequently used to treat anaerobic bacterial and protozoal infections. However, extended use of ornidazole has been linked to hormonal imbalance, decreased sperm count, seminiferous tubule degradation, and compromised reproductive function (Raji *et al*., 2005). One of the main causes of drug-induced spermatotoxicity is thought to be oxidative stress produced by reactive oxygen species (Aitken and Roman, 2008).

Due to their therapeutic and antioxidant qualities, medicinal plants have become more important in reproductive medicine. *Asparagus racemosus* is one of the medicinal herbs that Ayurveda uses extensively to treat reproductive issues and enhance general health. The plant, which is rich in steroidal saponins, flavonoids, alkaloids, tannins, and glycosides, is a member of the Asparagaceae family (Goyal *et al*., 2003). According to earlier research, *Asparagus racemosus* has immunomodulatory, adaptogenic, antioxidant, and reproductive-enhancing properties. According to Singh et al. (2009), the plant increases hormonal release and lowers oxidative stress to improve reproductive function. Similarly, *A. racemosus* roots have been shown to boost spermatogenesis and reproductive capacity in experimental mice (Sharma and Bhatnagar, 2011).

Asparagus racemosus's phytochemical components, such as shatavarins, which have gonadotropic and androgenic properties, may be responsible for the plant's ability to promote fertility (Thakur *et al*., 2009). There is little scientific proof of *Asparagus racemosus*'s preventive function against ornidazole-induced reproductive toxicity, despite the fact that numerous publications have detailed its pharmacological properties. Thus, by measuring sperm parameters, reproductive organ weights, hematological profile, and reproductive hormone levels, the

current study was conducted to assess the spermatoprotective and fertility-enhancing effect of methanolic root extract of *Asparagus racemosus* against ornidazole-induced spermatotoxicity in male Wistar albino rats.

2. MATERIALS AND METHODS

2.1 Collection of *Asparagus racemosus*

Asparagus racemosus roots and leaves were gathered from Agasthyarkoodam. According to Kirtikar and Basu (2006), established taxonomic descriptions were used to identify the plant samples. The roots were properly cleaned under running water, rinsed with distilled water, let to dry in the shade at room temperature, and then ground into a fine powder using a mechanical grinder. Before being extracted, the powdered samples were kept in sealed containers.

2.2 Preparation of Root Methanol Extract

Using methanol in a Soxhlet device, the powdered root sample was extracted using the procedure outlined by Harborne (1998). For use in additional experiments, the resulting methanolic extract was concentrated under low pressure and kept at 4°C.

2.3 Ethical Approval

The Dale View College of Pharmacy and Research Center's Institutional Animal Ethics Committee granted ethical approval for the animal experiment under CPCSEA Registration Number 1118/PO/Re/S/07/CPCSEA. The CPCSEA criteria for the care and management of laboratory animals were followed when conducting animal experiments (CPCSEA 2003).

2.4 Experimental Animals

Thirty male Wistar albino rats in good health, weighing between 150 and 250 grams and

between eight and ten weeks old, were obtained from the Dale View College of Pharmacy and Research Center's animal house. The animals were kept in regular laboratory settings at 22±2°C with a 12-hour light/dark cycle. In accordance with OECD norms, rats were given standard meal and unlimited access to water for seven days prior to research (OECD 2008).

2.5 Experimental Design and Induction of Spermatotoxicity

Five groups of six rats each were randomly selected from among the experimental animals. Using the technique outlined by Raji et al. (2005), oral administration of ornidazole at a dose of 600 mg/kg body weight for 30 days caused spermatotoxicity in all groups except the normal control. For the duration of the experiment, Group I was the normal control and was given regular saline. Rats in Group II were untreated after being provoked with ornidazole and were given regular saline. The conventional medication, clomiphene citrate (1.04 mg/kg body weight), was administered to ornidazole-induced rats in Group III. Ornidazole-induced rats in Groups IV and V were given methanolic root extract of *Asparagus racemosus* orally for 28 days at doses of 50 mg/kg and 100 mg/kg body weight, respectively. The plant extract's spermatoprotective and fertility-boosting properties against ornidazole-induced reproductive toxicity were assessed using the experimental design.

2.6 Collection of Blood and Organ Samples

Prior to sacrifice, the experimental animals' body weights were noted at the conclusion of the treatment period. To acquire serum for hormonal analysis, blood samples were obtained by ocular puncture into anticoagulant-free vials and centrifuged for 15 minutes at 400 rpm. According to Ugwah

et al. (2019), reproductive organs such as the testis, epididymis, vas deferens, seminal vesicle, and prostate were removed and weighed for organosomatic examination.

2.7 Semen Collection and Evaluation of Sperm Parameters

For sperm analysis, the cauda epididymis was removed and submerged in regular saline. A hemocytometer was used to count the sperm using the Clegg et al. (2001) method. WHO laboratory guidelines were used to assess sperm motility (WHO 1999). The eosin-nigrosin staining method, as outlined by Wells and Awa (1970), was used to evaluate sperm viability. Sperm morphology was assessed in accordance with WHO guidelines (WHO 1999). According to Ranjan et al. (2009), the hypo-osmotic swelling test was used to evaluate the integrity of the plasma membrane. Using the aniline blue staining method, DNA fragmentation analysis was carried out in accordance with Wong et al. (2008).

2.8 Evaluation of Hematological Parameters

Standard hematological techniques outlined by Coles (1986) were used to examine packed cell volume (PCV), hemoglobin concentration (Hb), red blood cell count (RBC), and white blood cell count (WBC).

2.9 Determination of Hormonal Profile

Enzyme-linked immunosorbent assay (ELISA) was used to measure serum levels of testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estrogen in accordance with Uotila et al. (1981).

2.10 Statistical Analysis

Mean ± SEM was used to express the experimental data. SPSS version 20 was used for statistical analysis. According to Snedecor and Cochran (1989), one-way

analysis of variance (ANOVA) and Duncan's multiple range test were employed to identify significant differences between groups at $P<0.05$.

3. RESULTS AND DISCUSSION

3.1 Effect on Body Weight

When *Asparagus racemosus* methanolic root extract was administered to treated rats, their body weight increased significantly in comparison to the untreated spermatotoxic group (Table 1). The largest rise in body weight was observed in Group V, which received treatment with 100 mg/kg extract. The increased metabolic and anabolic activity generated by the phytoconstituents in the extract may be responsible for the improvement in body weight. Sharma et al. (2010) revealed similar findings, showing that *Asparagus racemosus* has adaptogenic qualities that enhance physiological performance and nutrient utilization in test animals. Ornidazole-induced oxidative stress and tissue deterioration may be linked to the reduction in body weight seen in untreated rats.

Table 1. Effect of methanol root extract of *Asparagus racemosus* on body weight in Ornidazole-induced rats

Body weight (gm)	Treatment groups				
	Group-1	Group-2	Group-3	Group-4	Group-5
Before	187.0±1.28	186.1±1.02	189.0±1.01	185.1±0.12	192.0±1.21
After	230.1±1.12	239.1±1.06	248.1±0.18	265.2±2.11	271.1±1.01

Values are expressed as Mean ± SEM (n=6).

3.2 Effect on Reproductive Organ Weight

When compared to rats that were not treated, the methanolic root extract considerably raised the weights of the testis, epididymis, seminal vesicle, vas deferens, and prostate (Table 2). Group V treated with 100 mg/kg extract showed the most increase.

Degeneration of seminiferous tubules and suppression of androgen production may be the cause of the reduction in organ weight after ornidazole therapy. Testicular function and androgenic stimulation are restored when reproductive organ weight is restored. Mishra et al. (2013) found similar results, showing that treatment with *Asparagus racemosus* extract boosted reproductive organ weights and spermatogenic activity.

Table 2. Effect of methanol root extract of *Asparagus racemosus* on reproductive organ weight in Ornidazole-induced rats

Organ weight	Treatment groups				
	Group-1	Group-2	Group-3	Group-4	Group-5
Testis (gm)	1.03±0.02	0.61±0.28	1.07±0.04	1.26±0.03	1.29±0.02
Epididymis (mg)	517.0±1.10	491.1±1.01	555.1±0.18	672.1±0.10	669.0±1.02
Vas deferens(mg)	106.0±1.02	104.1±2.12	110.0±0.02	129.1±1.02	134.1±0.21
Seminal Vesicle (mg)	259.1±1.02	212.1±0.11	284.0±0.06	307.2±0.01	313.1±0.02
Prostrate (mg)	243.1±0.02	220.1±1.02	261.0±0.17	279.0±2.01	275.1±1.01

Values are expressed as Mean ± SEM (n=6).

3.3 Effect on Sperm Parameters

When compared to untreated rats, the methanolic root extract dramatically increased sperm count, motility, viability, morphology, and plasma membrane integrity while decreasing DNA fragmentation in extract-treated groups (Table 3, Plate 1). Group V showed the highest level of fertility-enhancing activities. Ornidazole-induced oxidative damage may be the cause of the sharp decline in sperm quality seen in untreated rats. Reactive oxygen species can damage DNA and compromise the integrity of sperm membranes, which can result in infertility (Aitken and Roman, 2008). The antioxidant activity of the flavonoids and steroidal saponins found in *Asparagus*

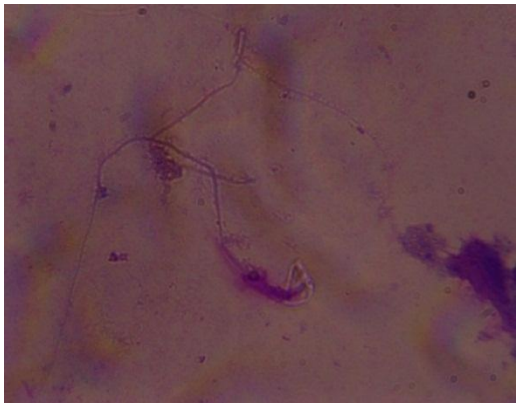
racemosus may be responsible for the improvement in sperm parameters after extract administration. According to Gupta et al. (2005), antioxidant-rich medicinal herbs enhance spermatogenesis and sperm membrane integrity. Similarly, Singh et al. (2009) showed that *Asparagus racemosus* improves reproductive function and sperm quality by lowering oxidative stress.

Table 3. Effect of methanol root extract of *Asparagus racemosus* on sperm parameters in Ornidazole-induced rats

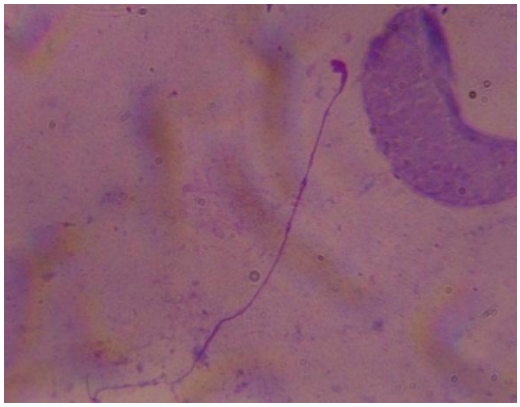
Sperm characters	Treatment groups				
	Group-1	**Group-2**	**Group-3**	**Group-4**	**Group-5**
Sperm Count (x10^6)	91.0±0.10	01.0±0.11	121.0±1.02	152.0±1.01	159.0±0.02
Sperm motility (%)	72.0±1.04	01.0±0.02	76.0±2.10	90.1±1.02	93.1±0.03
Sperm viability (%)	61.0±0.16	01.0±0.01	75.0±0.10	91.0±0.01	93.0±0.02
Sperm morphology (% normal)	66.0±1.01	01.0±0.01	76.0±1.01	84.0±1.02	88.0±0.01
Plasma membrane Integrity (%)	52.0±0.02	01.0±1.02	64.1±1.02	81.0±1.01	88.1±0.01
DNA Fragmentation (%)	13.0±0.01	99.8±1.02	10.1±0.14	03.0±0.02	02.0±0.01

Values are expressed as Mean ± SEM (n=6).

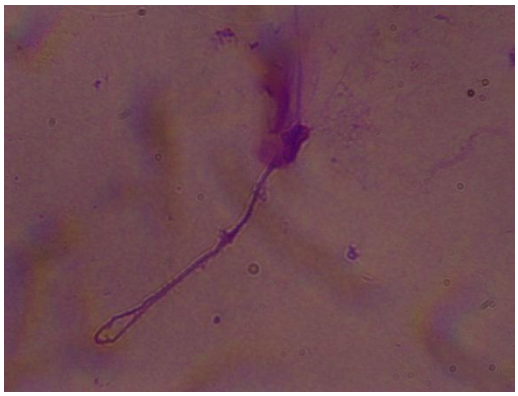
Plate 1. Photomicrographs showing DNA fragmentation in experimental groups



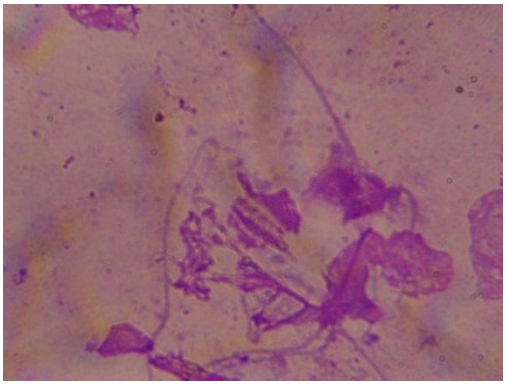
(A) Normal control showing intact sperm chromatin



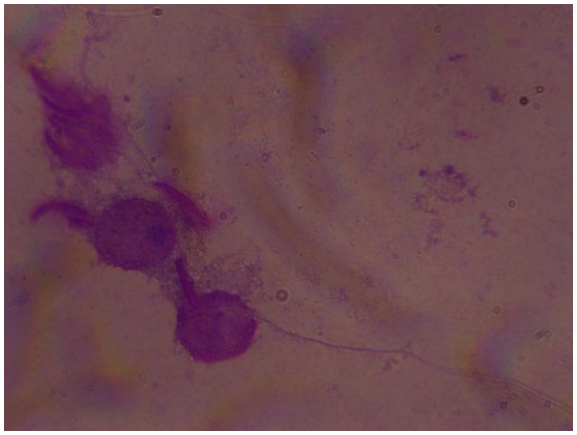
(B) Ornidazole-induced untreated group showing severe DNA fragmentation



(C) Standard drug-treated group showing reduced DNA damage



(D) *Asparagus racemosus* extract treated group (50 mg/kg)



(E) *Asparagus racemosus* extract treated group (100 mg/kg)

3.4 Effect on Hematological

The hematological profile showed that the untreated animals' PCV, Hb, RBC, and WBC counts were significantly lower than those of the treated groups (Table 4). Hematological parameters returned to normal when the methanolic root extract was administered. *Asparagus racemosus*'s immunomodulatory and antioxidant properties may be connected to the improvement in hematological indicators. According to Patel et al. (2011), the plant enhances immunological function and promotes hematopoiesis in test animals.

Table 4. Effect of methanol root extract of *Asparagus racemosus* on hematological parameters in Ornidazole-induced rats

Parameters	Treatment groups				
	Group-1	Group-2	Group-3	Group-4	Group-5
Packed cell volume (%)	36.01±1.01	33.10±0.01	39.02±1.02	41.01±1.01	40.01±1.20
Haemoglobin conc. (g/dL)	10.10±0.01	08.01±0.01	11.01±1.10	12.18±0.01	13.01±0.01
Red Blood cell count (10^6µl)	6.11±1.04	03.01±0.02	05.01±0.02	05.19±0.02	06.01±0.02
Total WBC count (10^3mm^3)	07.03±0.11	05.40±0.02	07.01±2.01	07.14±0.01	07.28±0.02

Values are expressed as Mean ± SEM (n=6).

3.5 Effect on Hormonal Profile

When rats were given with methanolic root extract, their levels of testosterone, FSH, LH, and estrogen were considerably higher than those of untreated rats (Table 5). The hypothalamic-pituitary-gonadal axis is stimulated and endocrine function is enhanced when hormone levels are restored. According to Thakur et al. (2009), *Asparagus racemosus* contains steroidal saponins that increase androgen synthesis and enhance reproductive function. This study's increased hormonal profile confirms the plant extract's ability to promote fertility.

Table 5. Effect of methanol root extract of *Asparagus racemosus* on hormonal profile in Ornidazole-induced rats

Parameters	Treatment groups				
	Group-1	Group-2	Group-3	Group-4	Group-5
Follicle stimulating hormone (µIU/mL)	05.12±1.01	0.15±0.01	3.01±1.02	5.24±0.01	06.02±1.01
Luteinizing hormone(µIU/mL)	04.11±0.01	0.01±0.01	05.03±1.10	07.26±0.01	08.01±0.11

Testosterone (ng/mL)	6.10±1.03	01.01±0.06	06.48±0.01	07.01±0.02	07.04±1.02
Estrogen (pg/ml)	12.01±0.31	02.21±0.02	14.31±1.01	17.11±0.01	18.14±0.02

Values are expressed as Mean ± SEM (n=6).

4. CONCLUSION

The current study showed that *Asparagus racemosus* methanolic root extract significantly improves fertility and protects sperm from ornidazole-induced reproductive damage in male Wistar rats. When the extract was administered, it decreased sperm DNA fragmentation and increased body weight, reproductive organ weight, sperm quality, plasma membrane integrity, hematological indices, and hormonal profile. When compared to the lower dose and conventional treatment, the higher dose (100 mg/kg body weight) demonstrated superior protective benefits. The antioxidant, androgenic, and regenerative qualities of the phytoconstituents found in *Asparagus racemosus* may be responsible for the positive benefits. These results indicate the plant's potential as a natural medicinal agent for the treatment of spermatotoxicity and infertility, and they scientifically verify its traditional use in male reproductive diseases.

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