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Antispermatogenic Activity of *Momordica Dioica* Methanolic Root Extract

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Abstract:- Population explosion is a global phenomenon and has robust impact on various global issues. It is a major concern for public policy makers and governments. Hence, there is a need for an ideal contraceptive with maximum efficacy and safety. Many plant based products have antispermatogenic activity that needs to be investigated. In this context, the current study evaluates the antispermatogenic potential of methanolic crude extract of roots of *Momordica dioica* on experimental rats. Experiments were carried out by administering the root extract of *M. dioica* at different dose levels of 10, 20 and 50 mg/kg body weight/day for 60 days on male albino rats. The reproductive organ weights of experimental rats were analyzed manually of different dose levels. Effect of the drug was examined by serum Testosterone, LH and FSH levels. Sperm morphology and motility was observed in semen of cauda epididymis. Histological sections of rat testis and epididymis were studied. The effect of root extract of *M. dioica* showed significant decrease in the weights of reproductive organs and hormonal levels.There was a significant change and decrease in sperm morphology and motility respectively in the experimental rats after administering with the above drug. Histological examination of testis and epididymis of experimental rats showed degeneration as compared to the control group. The present research concludes the effectiveness of *M. dioica* root in posing the antispermatogenic activity.

Keywords:- *Antispermatogenic, Sperm Morphology, Sperm Motility, Momordica dioica, Contraception.*

I. INTRODUCTION

Population in India is a case study for those who still think that population explosion is only theoretical. India is pacing towards becoming the world's most populous country leaving China behind. China has already managed to neutralize its population growth (0% growth rate). At present India's population is 1.4 billion and predicted to grow uninterrupted until 2060 reaching a peak of 1.7 billion[1]. Commendably, Indian policy makers have done remarkable achievement in the last 5 decades to restrict population growth. A multi-dynamic approach including literacy, promotion of contraceptives, awareness of STDs, advertising advantages of small families, banning child marriages, and by linking villages with working policies was challenging indeed. However, the current rate of population growth is still challenging and would take another 40-50 years before initial decline.

Contraception is an act of preventing pregnancy. Use of contraceptives is not recent; it has been around since ancient times. Contraception allows families to delay, space, and/or limit the number of children. Contraception not only control population growth but save billions of dollars spent on unintended pregnancies and unsafe abortions. Modern methods of contraception provide a range of products that can be made available to all age groups, of any financial background and communities. The reason for demanding contraceptive use is due to unmet needs and possibilities of improvement in available methods. Use of contraceptives have risen from previous estimates as developing countries and women of all reproductive ages have significantly increased uses in the last 40-50 years[2]. It is reported that vaccines, hormone releasing devices, vaginal chemicals, injectable and biodegradable implants for men and immunocontraceptives will possibly replace latex condoms in future[3].

Although much of the contraceptive methods in use are safe with variable efficacy rate, nonetheless, with maximum output, chances of side-effects also arise. Side-effects cannot be compromised as these remedies and surgical intervention may have a long-lasting impact on the health of the subjects. Most modern methods have either some drawbacks or side-effects, which lead researchers to continue searching for an ideal contraceptive with maximum efficacy and are absolutely safe. Researchers are working towards the invention of hormonal modulators that suppress spermatogenesis while retaining androgenic activities[4].

Since most available contraceptive methods are for females, men's contribution towards family planning is limited. One of the major causes for such a huge difference in contribution is lack of safe, reversible and effective methods. Thus, an alternative method of contraception for men would provide additional effort in regulating population control and improve the role of men in family planning.

Plant or herbal medicines are used culturally around the globe. Historians have recorded many plant and plant products that have been used as contraceptives and abortifacients since ancient times[5]. Many researchers have examined herbs and parts of plants for contraceptive properties in both men and women.To be a successful male contraceptive the candidate agent should be reversible and effective. Since plant and plant products are non-hormonal, its approach is by blocking either of the four or combination of two or all at specific phases 1) mitotic phase of spermatogonia, 2) meiotic phase-doubling of chromosome number in spermatocytes 3) spermatid condensation stage, and 4) spermiation[6]. Sperm maturation and capacitation in epididymis can also be a good target for novel agents[7].Since

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plant based contraceptives have advantage over another allopathic alternative due to perception of low side-effects. Due to this many researchers are observing local remedies for potential antifertility effects. Therefore, more plant based contraceptives must be investigated to increase the number of options for men. There are studies that have investigated poly herbal formulation, which means extracts of more than one plant was used to enhance antifertility effect[8,9].

M. dioica commonly known as spiny gourd is a perennial plant, a climber of the family Cucurbitaceae. The plant originally grows in Indo-Malayan region but is efficiently grown in distant regions such as; Japan, Polynesia, South East Asia, South America, and Tropical Africa. *M. dioica* fruit, leaves and roots are used as medicine by traditional medicinal practitioners. The extracts of plant are considered anti-hyperglycemic[10], renoprotective[11], hepatoprotective[12], antidiabetic[13], anticancer[14]. It is reported that alcoholic extract of *M. dioica* is a strong antioxidant[15]. A study reported that ethanolic extract of *M. dioica* root could act as a strong abortifacient[16]. Similarly, 250 mg/kg of fruit ethanolic extract could impose antifertility in female rats, though no such evidence was present in male rats[17]. Based on the previous work of this plant extract and proven medicinal property with vague information on its potential role in male fertility, the - study was carried out to evaluate the antispermatogenic activity of methanolic crude extract of *M. dioica* roots.

II. EXPERIMENTAL SECTION

The roots of *M. dioica* were collected from Sikar district of Rajasthan and identified by the Department of Botany, University of Rajasthan, Jaipur. The root of *M. dioica* was shade dried and powdered which was extracted through methanol solvent in 5 to 1 ratio. Dried concentrated methanolic extract was stored at 20 degree C for a maximum of 21 days. Male Wistar albino rats (*Rattus norvegicus*) were used as animal models in the study weighing in the range of 150-200 g. It ensured that animals were receiving a 12:12 h light and dark routine. Breeding of rats and procedural methods to carry out research was strictly supervised by the veterinary expert and followed according to CPCSEA guidelines (CPCSEA, 2010). The experiments in this study were designed based on OECD's Fixed Dose Procedure-Reproductive Toxicity Guidelines. The doses of extracts were followed according to method/s explained in OECD for testing chemical through oral administration (OECD/OCDE-420, 2001).

The experiment was focused on assessment of crude methanolic extract of *M. dioica* root on male reproductive health. Three groups of animals were investigated for the experiments with the control group. Each group consisted of 10 healthy male rats. Three doses were selected to carry out the experiment, these were 10, 20 and 50 mg/kg body wt./day for 60 days (Table 1).

EXPERIMENTAL GROUP	SPECIFICATION
Group I (Control)	Vehicle treated
Group II	Rats were treated with *M. dioica* root extract- 10 mg/kg B.wt./day for 60 days
Group III	Rats were treated with *M. dioica* root extract- 20 mg/kg B.wt./day for 60 days
Group IV	Rats were treated with *M. dioica* root extract- 50 mg/kg B.wt./day for 60 days

Table 1: Groups and their respective specifications (n=10)

Initial and final body weights were noted at the commencing and termination of experiments, respectively. On the termination day, rats were sacrificed, following which blood and reproductive organs were dissected out. Main and accessory reproductive organs such as testis and epididymis were examined, weighed and stored in Bouin's fluid for further investigation.

Matings were designed on each 15 days interval for 60 days of investigation. In which, an individual male rat was cohabitated with two fertile females to record fertility rate. Successful mating was confirmed through vaginal plug or with presence of sperms in the vaginal smear. At the termination of the experimental schedule, rats were sacrificed and sperm samples were collected from cauda epididymis by chipping off the organ in 1 ml normal saline. Likewise, vaginal smear following confirmation of mating was examined through Papanicolaou staining[18]. Blood samples were collected through a 'V' shaped cut made in the abdomen and a needle was inserted via diaphragm into the heart. Blood collected in EDTA tubes for hormonal assays. Serum Testosterone, LH and FSH levels were measured by ELISA kit. Procedures to carry out the experiment were instructed in the manual. Optical densities were noted spectrophotometrically at 450 nm wavelength. Portions of dissected organs fixed in 10% formalin, dehydrated, cleared and were embedded in paraffin wax. Later H&E staining was used to observe the changes in tissue under the microscope.

Mean of all numeric values were calculated and represented with their respective standard error (SE). One way ANOVA (Analysis of Variance) was applied to compare multiple parameters for the assessment of variables in conjunction with Tukey's multiple comparison tests with 95% CI. For quantitative variation, $p<0.05$, 0.01 and 0.001 were considered significant, highly significant and extremely significant.

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III. RESULTS AND DISCUSSION

The present study investigated the antispermatogenic activity of methanolic crude extract of *M. dioica* roots. Oral administration of the drug at different dose levels to male rats did not show any significant difference in the body weight and weight of epididymis while there was a significant reduction in the weight of testis at 50 mg/kg b.wt./day dose level ($p<0.05$) (Table 2). Significant reduction in testis weight may be due to decreased levels of serum Testosterone and interference in the formation and maturation of spermatozoa[19,20]. Testosterone has a pivotal role in the process of spermatogenesis[21]. The sperm motility rate of treated group 20 mg and 50 mg/kg b.wt./day showed significant reduction ($p<0.001$) with compared to control rats (Table 3). The caudal epididymal sperm count significantly reduced ($p<0.001$) that were treated with methanolic root extract of *M. dioica* at 50 mg/kg b.wt./day in comparision to control rats (Table 3). Abnormality of sperm morphology at 50 mg/kg b.wt./day was observed by 59% when compared to control rats (Table 3). Due to androgen imbalance and reduced supply of Testosterone to epididymis attributes reduction of sperm motility and sperm count in epididymis[22]. Hence, the abnormal sperm dynamics and morphology suggests the antispermatogenic activity of *M. dioica* roots. Oral treatment of methanolic crude extract at different dose levels caused decreased fertility rate by 65%, 55% and 40% at 60th day at 50mg, 20mg and 10mg/kg b.wt./day respectively (Table 4). The present study reveals decreased fertility rate of experimental rats which may be due to decreased sperm motility that correlates with alteration in the sperm axoneme engine required for ATP supply to maintain motility of sperm[23].

The serum testosterone levels significantly reduced after administration of the drug at 20mg/kg b.wt./day and 50mg/kg b.wt./day when compared to the control group ($p<0.01$) (Table 5). It is evident that reduction in testis weight and abnormality of sperm morphology may be attributed to a decline in the testosterone production[24,25].

The present study shows significant reduction in the levels of LH and FSH in serum after administering the experimental rats with the drug ($p<0.05$) at 50 mg/kg b.wt./day (Table 5). Previous studies suggest that FSH and LH are necessary for spermatogenesis by stimulating the development of spermatogonia to spermatocytes[26]. Reduced sperm count may indicate the lowered levels of FSH and LH[24].

Histological examination of testis of control rat showed normal seminiferous tubules with active spermatogenesis (Figure 2a). There was slight decrease in the sperm count in the lumen of seminiferous tubule in group II (Figure 2b). Histological study of testis of group III and IV showed degeneration of spermatogonia lining the seminiferous tubules and reduced levels of sperms in the lumen (Figure 2c and 2d). The epididymis of control rat showed normal convoluted tubule while the experimental group showed deterioration in the epithelial lining of tubular part of epididymis (Figure 3a, 3b, 3c and 3d). Histological study of sperm dynamics and morphology was normal in the control group whereas the sperm morphology in the experimental group showed sperm head and tail distortion suggesting the antispermatogenic and antifertility activity of the *M. dioica* root extract (Figure 4a, 4b, 4c and 4d).

Groups	Initial Body Weight (g)	Final Body Weight (g)	Testis weight (mg/100g)	Epididymis weight (mg/100g)
I	158.5±1.52	234.00±2.53	991.10±5.97	224.70±6.54
II	159.7±1.14	235.50±1.91	1000.43±4.28^{ns}	230.70±5.73^{ns}
III	159.60±0.96	248.70±3.43	1001.64±7.40^{ns}	233.24±4.91^{ns}
IV	159.90±0.89	242.20±3.37	958.65±8.69*	212.26±6.07^{ns}

Table 2: Body weight and Organ weight of experimental rats administered orally with *M. dioica* methanolic crude root extract

GROUPS	Sperm Motility (%)	Sperm count (million/ml)	Sperm abnormality (%)
I	78.68±1.20	65.34±2.09	21.39±1.39
II	75.92±1.42ns	63.90±1.99ns	24.51±1.04ns
III	73.05±1.87*	55.00±2.32**	28.74±1.25**
IV	61.67±1.85***	36.78±1.49***	34.09±1.54***

Table 3: Sperm dynamics and Sperm characteristics of rats with oral administration of *M. dioica* methanolic crude root extract

Fertility (%)	Group I	Group II	Group III	Group IV
0th day	100	100	100	100
6th day	100	100	95	90
12th day	100	95	100	85
18th day	100	95	95	85
24th day	100	90	90	80
30th day	100	90	90	65
36th day	100	85	80	55
42nd day	100	80	75	50
48th day	100	70	70	40
54th day	100	60	55	35
60h day	100	60	45	35

Table 4: Fertility test of male rats treated with *M. dioica* methanolic crude root extract

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GROUPS	Testosterone (mg/ml)	FSH (m/u/ml)	LH (m/u/ml)
I	7.66±0.13	3.17±0.06	4.22±0.10
II	7.14±0.06ns	3.06±0.10ns	4.03±0.15ns
III	7.05±0.23**	2.85±0.11*	3.71±0.17*
IV	6.51±0.30**	2.74±0.18*	3.41±0.19*

Table 5: Serum Hormonal assay of male rats treated with *M.dioica* methanolic crude root extract

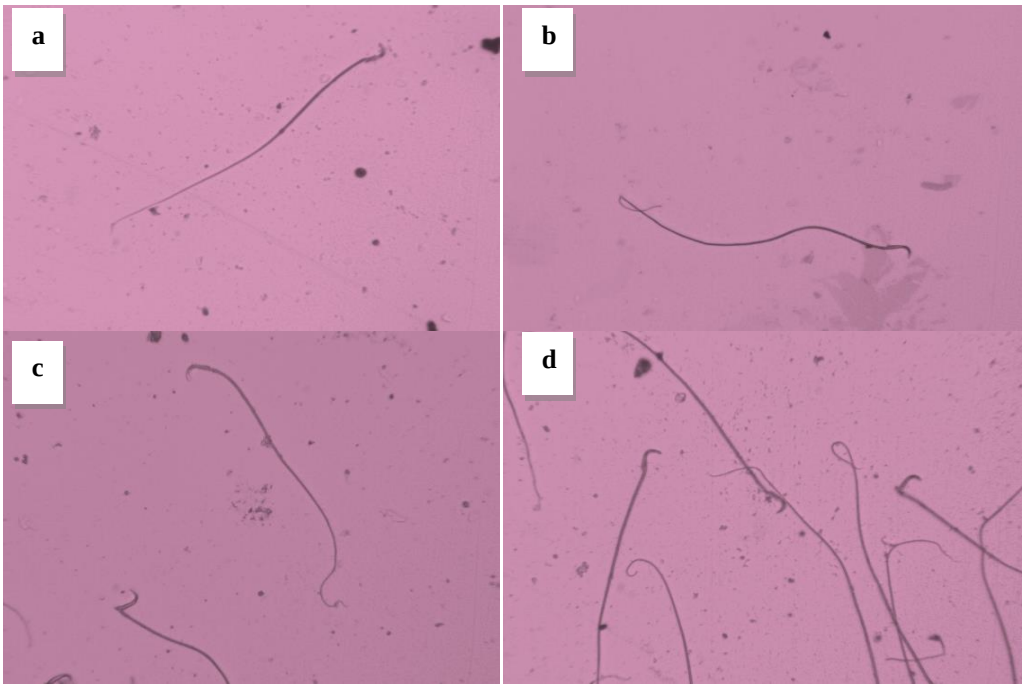

Fig. 1: Photomicrograph of Sperm dynamics and sperm morphology of experimental rats administered with *M. dioica* root extract

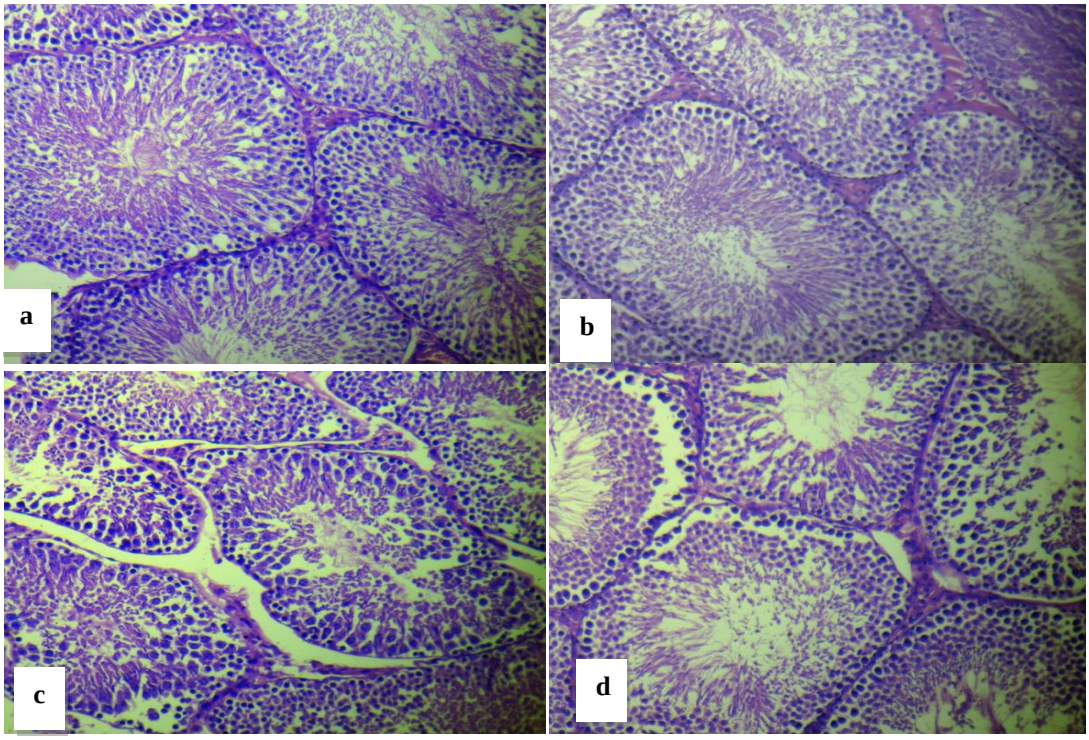

Fig. 2: Photomicrograph of rat testis administered orally with *M. dioica* root extract

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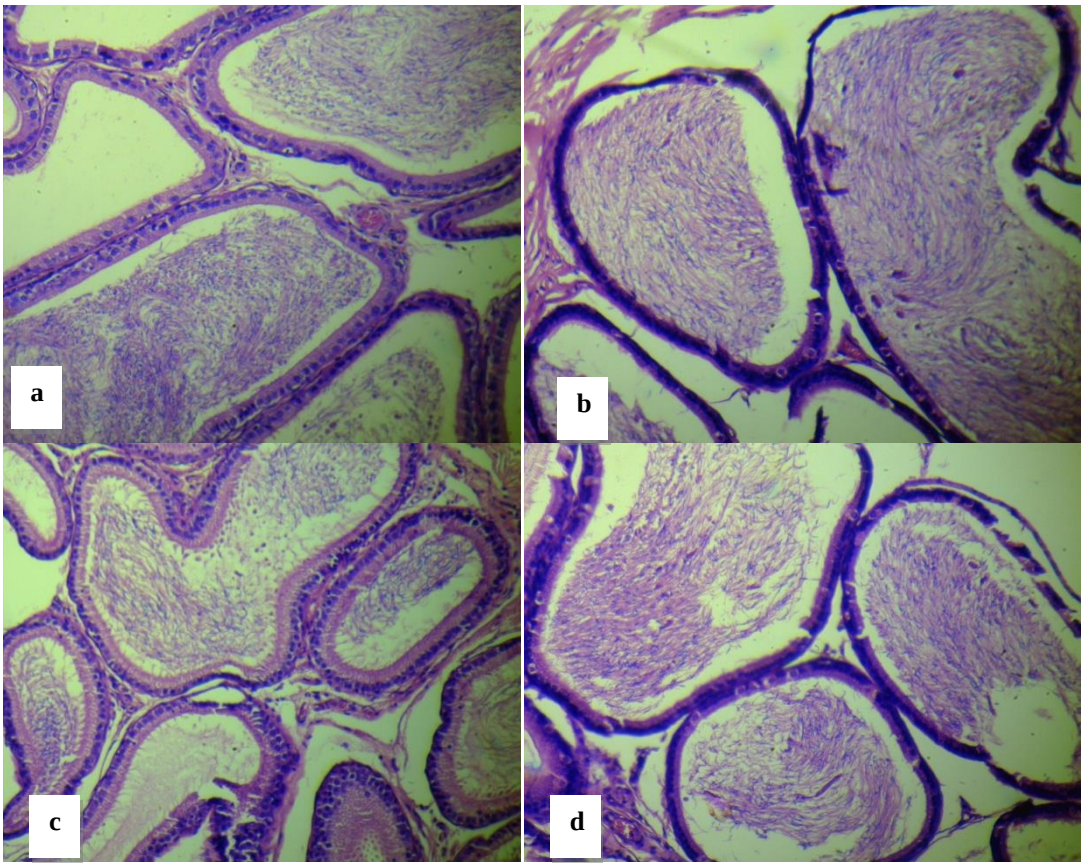

Fig. 3: Photomicrograph of rat epididymis treated with methanolic crude extract of *M. dioica* root

IV. CONCLUSION

The result of the current study concludes that oral treatment of *M. dioica* root methanolic crude extract has potent effect on fertility rate of experimental rats due to intervening androgen levels and abnormal sperm dynamics and its morphology. The drug can be proved to be a potent herbal agent for reduced spermatogenic activity and can endorse the participation of males in family planning.

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