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Evaluation of Reversible Contraceptive Efficacy of Methanol Extract of *Momordica dioica* Root in Male Albino Rats

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Abstract: The purpose of this study was to evaluate the reversible contraceptive efficacy of methanol extract of *Momordica dioica* (Cucurbitaceae) root at the dose level of 20mg/kg.b.wt./rat/day for 60 days. The treatment caused significant reduction in number of spermatozoa in cauda epididymides as well as in testis with a decreased in the motility of sperm collected from the cauda epididymides. The weights of testes, epididymides, seminal vesicle and ventral prostate were also significantly decreased. Marked decline was also found in the testicular germ cell population, Leydig cell nuclear area and the number of mature Leydig cells however, no morphological changes were observed in Sertoli cells as well as in their counts. Serum gonadotropins LH and FSH as well as testosterone levels were also altered after *Momordica dioica* treatment. The protein, glycogen, sialic acid, acid phosphatase and alkaline phosphatase content of testes, protein and sialic acid in cauda epididymis and fructose in seminal vesicle decreased significantly, while cholesterol content of testes increased significantly. Moreover, *M. dioica* extract did not alter the blood & serum parameters, which show its nontoxic nature. All the parameters were reversible after withdrawal of the drug. In conclusion methanol extract of *Momordica dioica* roots have reversible contraceptive activity in male rats.

Key words: *Momordica dioica* · Sperm density · Sperm motility · Leydig cells · Testosterone · Antifertility

INTRODUCTION

The search for an effective, safe and reversible male antifertility agent with minimum side effects remains a challenge. To date, a number of plants with antifertility effects have been screened [1-3]. The plant *Momordica dioica* Roxb. (Cucurbitaceae) fruit pulp exhibited moderate and concentration dependent antifeedant activity against *Spodoptera litura* [4]. Shreedhar *et al.* [5] suggested that aqueous and ethanol extracts of *Momordica dioica* root possess postcoital antifertility activity in female rats. Two aliphatic constituents, characterized as 6-methyl tritriacont-50on-28-of and 8-methyl hentracont-3-ene, have been isolated from the fruit of *Momordica dioica* along with the known sterol pleuchiol. Momordicaursenol, a pentacylic triterpene was isolated from its seeds [6]. Antifertility effects of various triterpenes isolated from a range of medicinal plants have also been reported [7,8].

In our research laboratory the antifertility effect of methanol extract of *Momordica dioica* root being investigated to explore the possibilities of contraceptive efficacy in male albino rats emphasizing reversible fertility after withdrawal of the extract.

MATERIALS AND METHODS

Animal Model: Colony bred, healthy adult (4-5 months old) male albino rats of the Wistar strain, weighing between 250 and 280 g were used. The rats were housed in plastic cages under standardized condition (12h light/12h dark) rat feed (Hindustan Lever Ltd.) and tap water provided *ad libitum*. Body weight of each animal in all groups was measured weekly to see the possible weight loss throughout the experiments.

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Ethical Aspects: The study was approved by the ethical committee of the Department of Zoology, University of Rajasthan, Jaipur (India). Indian National Science Academy, New Delhi [9] guidelines were followed for maintenance and use of the experimental animals.

Test Material: The roots of *Momordica dioica* were collected in the month of October from Jhalawar district of Rajasthan (India) and were identified from the herbarium, Department of Botany, University of Rajasthan, Jaipur, India. The shade, dried roots (250 g) were powdered and extracted with 100% methanol in a Soxhlet for 24 hours. The methanol was removed under reduced pressure to obtain a viscous brown material, which has been referred to as a drug in the present report. For oral administration to rats, the material was dissolved in distilled water.

Three triterpenes α-spinasterol octadecanonate (I), α-spinasterol-3-*O*-β-d-glucopyranoside (II), 3-*O*-β-d-glucuronopyranosyl gypsogenin (III) and two steroidal compounds 3-*O*-β-d-glucopyranosyl gypsogenin (IV) and 3-*O*-β-d-glucopyranosyl hederagenin (V) were isolated from the dry root of *Momordica dioica* [10].

Dose and Duration of Treatment: The male rats of the experimental group were divided in to two groups. First group of 10 rats served as vehicle treated control, while other group of 20 rats, were treated with methanol extract of *Momordica dioica* root at the dose level of 20mg/kg.b.wt./rat/day. The second group was subdivided in to two groups IIA and IIB at the 60th day of experiment. Group IIA rats were autopsied after 60 days period of dosing while IIB group rats were allowed to recover for another 60 days without any drug administration.

Autopsy Schedule: The rats were autopsied within 24 h of the last dose and at the end of withdrawal period, under ether anesthesia. The testes, epididymides, seminal vesicle and ventral prostate were excised, dissected and freed of fat/blood vessels and weighed. Half tissues were kept at -20°C and remaining halves were fixed in Bouin's fluid, dehydrated in graded ethanol, cleared in xylene and embedded in paraffin wax. Sections were cut at 6 μm, stained with Harris hematoxylin and eosin for histopathological examination.

Fertility Test: Mating tests were performed before the commencement of the treatment and at the end of the experiment between days 55 to 60 (6 days). The male rats were cohabited with proestrous females at a ratio of 1:2. The presence of sperm in vaginal smear in the next morning were considered positive matings. The inseminated females were separated and numbers of litters delivered were recorded.

Sperm Density and Motility: Sperm motility was assessed in cauda epididymides and the sperm density was assessed in cauda epididymides and testes [11]. Spermatozoa samples were obtained by the cauda epididymal puncture in physiological saline (0.9% NaCl). Sperm density and motility were determined by using Neubauer haemocytometer.

Toxicology Investigation: Blood samples of each animal were collected by cardiac puncture. Total RBC, WBC, haematocrit, haemoglobin, blood sugar and blood urea values were recorded. Serum protein, cholesterol, triglycerides, phospholipids, HDL-cholesterol, creatinin, bilirubin, glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were also recorded simultaneously using reagent kits (Sigma Diagnostics, Baroda).

Tissue Biochemistry: Tissues were kept at -20°C until assayed. Protein [12], sialic acid [13], glycogen[14], cholesterol [15], acid phosphatase [16] and alkaline phosphatase [16] in testis, protein and sialic acid in cauda epididymides, fructose [17] in seminal vesicle were estimated.

Quantitative Analysis: Testicular cell dynamics was based on the counts of each cell type per cross-tubular sections. Various cell components were quantitatively analyzed using spherically appearing sections. Abercrombie [18] correcting factor was introduced to correct for the better chance a big cell has to be counted. Interstitial cell types such as fibroblast, degenerating and mature Leydig cells were estimated applying a differential count. Mean tubular diameter of 100 selected seminiferous tubules was determined by tracing. Leydig cell nuclear area and Sertoli cell area were measured at X800 magnification.

Radioimmunoassay of Hormones: Blood samples were also collected for estimations of serum testosterone, FSH and LH. Serum samples were separated by standard procedures and stored at -20°C for subsequent analysis.

Statistical Analysis: The data are expressed as mean ± SEM. Statistical analysis was carried out by one-way analysis of variance (ANOVA) and the comparisons between the groups were done by Student's *t*-test. Differences were considered to be statistically significant when $p<0.01$.

RESULTS

Body and Organ Weights: The oral administration of *Momordica dioica* extract to male rats for 60 days did not cause any significant change in the body weight of treated rats as well as in recovery group. Further results revealed that the weights of the reproductive organs of the treated rats decreased in comparison to the control group, however organ weights, after withdrawal of drug, were found to be measured significantly (Table 1).

Sperm Density, Motility and Fertility: The treated rats showed significant reduction in the sperm concentration of testes and cauda epididymides. The sperm motility of the cauda epididymides was also reduced significantly. Following withdrawal of treatment the values were comparable to those of control (Table 1). The *Momordica dioica* root extract treatment reduced, the fertility of male rats by 15% with 60 days drug treatment, whereas control animals exhibited normal fertility. The most significant observation was the return of fertility after 60 days of withdrawal of the drug (Table 2).

Toxicological Investigation: Total RBC, WBC, haematocrit, haemoglobin, blood sugar and blood urea (Table 3) as well as serum protein, cholesterol, triglycerides, phospholipids, HDL-cholesterol, creatinin, bilirubin, GOT and GPT levels (Table 4) did not show appreciable changes throughout the course of investigation.

Tissue-Biochemistry: The protein and sialic acid contents of testes and epididymides were reduced significantly. Glycogen contents in the testes and seminal vesicular fructose were also decreased significantly. Testicular cholesterol levels increased after drug treatment. However, all the parameters were found almost unaltered with respect to the treated group after withdrawal of drug (Table 5).

Table 1: Effect of methanol root extract of *M. dioica* on the body weight, organ weight, sperm motility, density and seminiferous tubular diameter in rats

		Organ weight (mg/100 g.b.wt)					Sperm density(million/ml)		
Treatment	Body Weight (g)	Testis	Epididymides	Seminal vesicle	Ventral prostate	Sperm motility (%) (Cauda epididymides)	Testis	Cauda epididymides	Seminiferous tubular diameter (μm^2)
Group-I Control	260.47±6.88	1548.45±22.78	456.14±8.49	799.08±3.59	404.31±11.64	76.06±4.31	6.92±0.53	73.83±3.94	278.40±2.55
Group-IIA *M. dioica* root extract 20mg/kg. b.wt./rat/day	274.11±5.48	934.38±15.66**	341.59±6.27**	605.40±10.27**	332.84±10.79**	43.52±4.27**	2.47±0.36**	18.55±4.01**	163.23±1.97**
Group-IIB Recovery group	295.09±6.21	1486.73±19.98$^{ns\,\dagger\dagger}$	438.47±6.46$^{ns\,\dagger\dagger}$	745.98±12.85$^{ns\,\dagger\dagger}$	399.00±9.40$^{ns\,\dagger}$	73.17±4.63$^{ns\,\dagger}$	6.44±0.42$^{ns\,\dagger\dagger}$	70.99±3.62$^{ns\,\dagger\dagger}$	260.55±3.41**†

Values are in mean ± SEM (n=10)

Levels of significance - ns: non-significant $^{*}P\leq 0.01$; $^{**}P\leq 0.001$ compared with Group I (control)

$^{\dagger}P\leq 0.01$; $^{\dagger\dagger}P\leq 0.001$ compared with Group IIA

Table 2: Effect of methanol root extract of *M. dioica* root on the percent fertility in females mated with the males

Treatment group	No. of mated males/females	No. of female delivered	Percent fertility
Group-I Control	10/20	20	100
Group-IIA *M. dioica* root extract 20mg/kg.b.wt./rat/day	10/20	3	15
Group-IIB Recovery group	10/20	20	100

Table 3: Effect of methanol root extract of *M. dioica* on the blood biochemical parameters in male rats

Treatment	RBC (million/mm^3)	WBC (-/mm^3)	Haematocrit (%)	Haemoglobin (g%)	Blood sugar (mg/dl)	Blood urea (mg/dl)
Group-I Control	5.93± 0.50	8763.43± 123.07	14.74± 1.03	46.38± 1.93	97.32± 2.45	45.20± 1.28
Group-IIA *M. dioica* root extract 20mg/kg.b.wt./rat/day	5.63± 0.63^{ns}	8633.16±102.43^{ns}	14.21±1.14^{ns}	44.89±1.68^{ns}	92.11±2.60^{ns}	45.77±1.39^{ns}
Group-IIB Recovery group	5.70±0.61^{ns}	8700.81±108.29^{ns}	13.99±1.00^{ns}	45.89±1.60^{ns}	96.73±2.97^{ns}	43.60±1.06^{ns}

Values are in mean ± SEM (n=10)

Levels of significance - ns: non-significant $P\leq 0.001$ compared with Group I (control)

Table 4: Effect of methanol root extract of *M. dioica* on the serum biochemical parameters in male rats

Treatment	Protein (mg/dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	Phospholipids (mg/dl)	HDL-cholesterol (mg/dl)	Creatinine (U/L)	Bilirubin (U/L)	GOT (U/L)	GPT (U/L)
Group-I Control	14444.33± 149.67	156.36± 4.48	109.71± 3.92	124.00± 3.60	36.50± 1.21	0.97± 0.09	0.90± 0.10	36.81± 1.44	39.74± 1.02
Group-IIA *M. dioica* root extract 20mg/kg.b.wt./rat/day	14333.88±130.89^{ns}	153.60± 4.63^{ns}	104.81±3.63^{ns}	120.60±4.13^{ns}	34.96±1.29^{ns}	0.94±0.06^{ns}	0.92±0.08^{ns}	36.49±1.43^{ns}	37.51±1.20^{ns}
Group-IIB Recovery group	3889.98±132.49^{ns}	150.22±5.12^{ns}	105.60±3.58^{ns}	120.33±4.00^{ns}	34.23±1.23^{ns}	0.96±0.07^{ns}	0.90±0.08^{ns}	32.37±1.69^{ns}	36.47±0.99^{ns}

Values are in mean ± SEM (n=10)

Levels of significance - ns: non-significant P≤ 0.001 compared with Group I (control)

Table 5: Effect of methanol stem extract of *M. dioica* on tissue biochemical parameters of male rats

Treatment	Glycogen (mg/g)	Acid phosphatase (µg/h/mg)	Alkaline phosphatase (µg/h/mg)	Cholesterol (mg/g)	Protein (mg/g)		Sialic acid (mg/g)		Fructose (mg/g)
	Testis	Testis	Testis	Testis	Testis	Cauda epididymides	Testis	Cauda epididymides	Seminal vesicle
Group-I Control	2.92±0.13	1.32±0.05	1.47±0.04	9.28±0.36	236.17±4.09	270.03±3.31	5.08±0.20	5.67±0.22	5.74±0.22
Group-IIA *M. dioica* root extract 20mg/kg.b.wt./rat/day	1.63±0.09^{a*}	1.01±0.05^{a}	1.18±0.03^{a*}	12.03±0.38^{a*}	181.36±2.28^{a*}	201.14±2.44^{a*}	3.83±0.13^{a*}	3.60±0.19^{a*}	4.07±0.14^{a*}
Group-IIB Recovery group	2.98±0.15$^{ns b*}$	1.30±0.03$^{ns b*}$	1.36±0.03$^{ns b}$	10.04±0.26$^{ns b}$	228.63±3.59$^{ns b*}$	263.45±2.98$^{ns b*}$	5.11±0.17$^{ns b*}$	5.60±0.16$^{ns b*}$	5.63±0.18$^{ns b*}$

Values are in mean ± SEM (n=10)

Levels of significance - ns: non-significant ^{a}P≤ 0.01; ^{a*}P≤ 0.001 compared with Group I (control)

^{b}P≤ 0.01; ^{b*}P≤ 0.001 compared with Group IIA

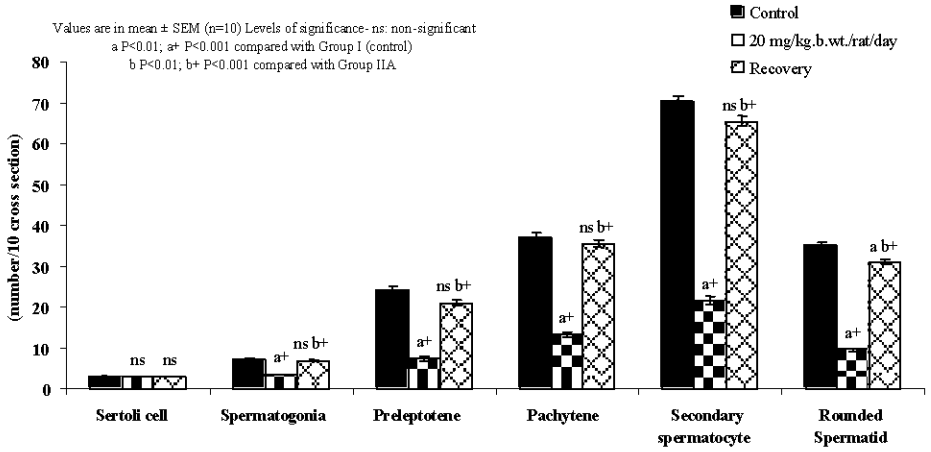


Fig. 1: Effect of *M. dioica* methanol root extract on testicular cell counts

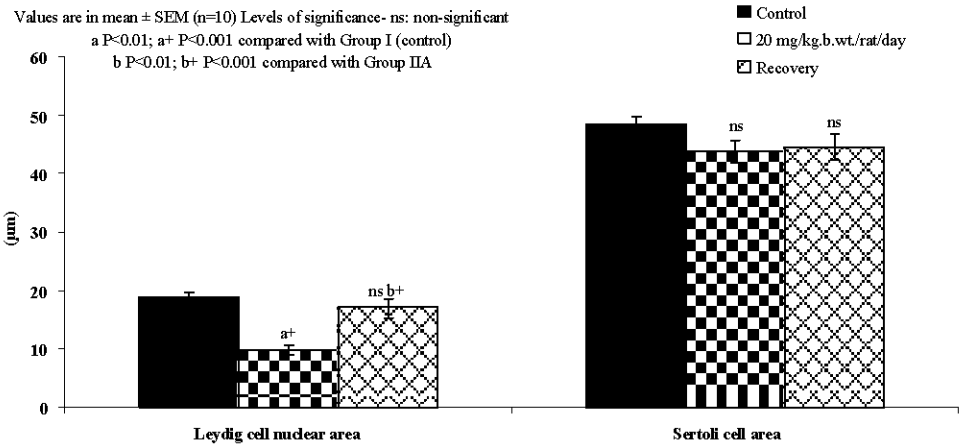


Fig. 2: Effect of *M. dioica* methanol root extract on testicular histometery

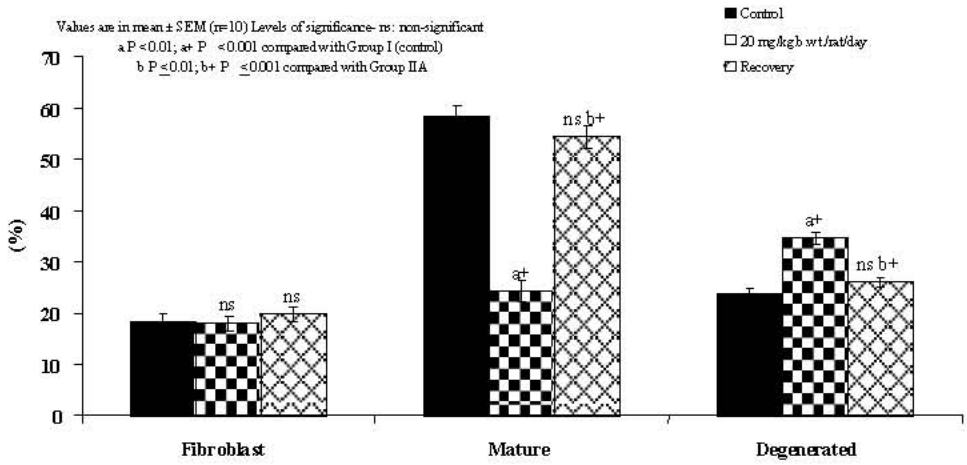


Fig. 3: Effect of *M. dioica* methanol root extract on Leydig cell differential counts

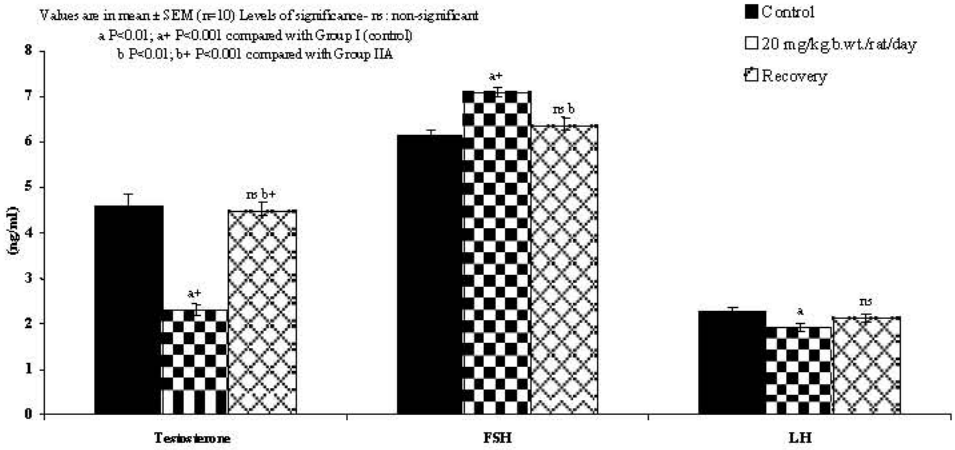
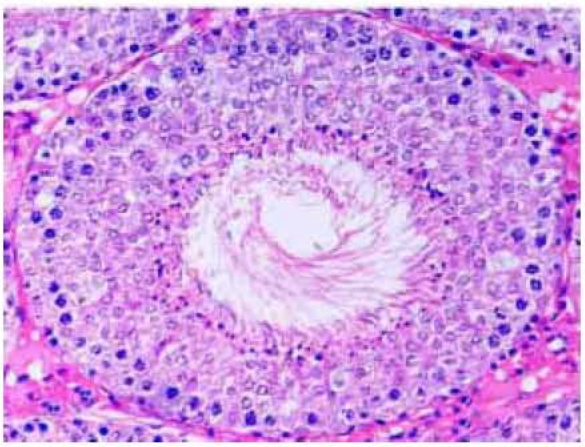
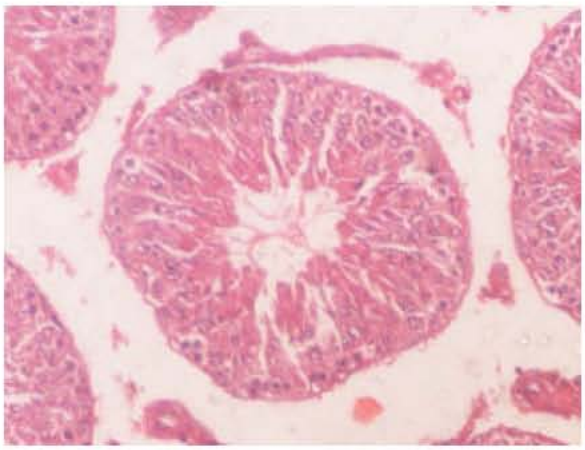


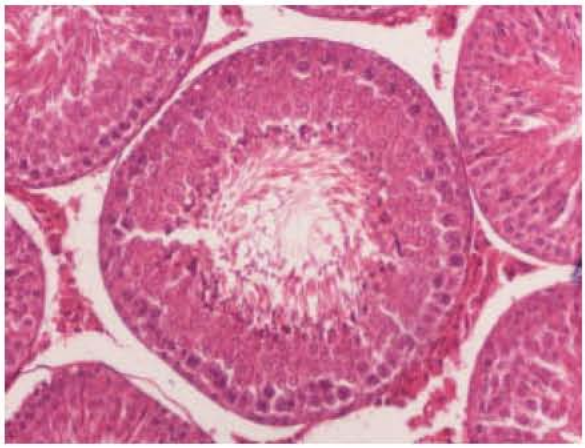
Fig. 4: Effect of *M. dioica* methanol root extract on serum hormonal levels



Microphotograph 1: Control rat testis showing normal size of seminiferous tubule with all successive stages of spermatogenesis. Lumen is filled with sperm.



Microphotograph 2: Rats treated with *M. dioica* showing regressed size of seminiferous tubule. Decreased number of germ cells and the lumen is filled with less number of spermatozoa.



Microphotograph 3: After recovery of drug treatment seminiferous tubular size restored almost normal. Sperm seen in lumen

Cell Population Dynamics: The diameter of seminiferous tubules reduced markedly after treatment of *Momordica dioica* root extract ($P<0.01$) (Table 1). It also brought about a significant reduction in most of the germinal cell types. The spermatogonia, preleptotene and pachytene spermatocytes were reduced in significant manner ($P<0.01$). A highly significant reduction was seen in secondary spermatocytes and round spermatids (Fig. 1). The Sertoli cell count (Fig. 1) and their surface area (Fig. 2) showed a non-significant change in treated rats. The number of mature Leydig cells decreased significantly ($P<0.01$), whereas degenerating Leydig cells were increased (Fig. 3). After recovery period all testicular cell counts restored in the normal range.

Hormonal Changes: A significant ($p<0.01$) reduction in the serum testosterone as well as in LH levels of *Momordica dioica* root extract treated animals were observed in comparison to controls, however FSH levels of treated rats were increased, which were found within the normal range after recovery period (Fig. 4).

DISCUSSION

The presence of triterpenes in the *Momordica dioica* root extract suggested a possible role of this plant as a potential agent in the field of male fertility regulation. Treatment with *M. dioica* root extract was highly effective in producing reversible functional sterility. Weight reduction of the reproductive organs of treated male rats clearly indicate that the drug caused structural and functional alteration in testes, epididymides, seminal vesicle and ventral prostate and also lowered the testosterone as these organs are androgen-dependent [19]. The reduced protein content may be another reason as the growth rate of any organ is proportional to its protein content [20]. Androgen deprivation effects in present study, not only suppress spermatogenesis, leading to low sperm concentration, but alters the epididymal milieu also, which renders it hostile for maturation and survival of the spermatozoa [21,22]. At the testicular level, the absence of stimulation by LH would cause Leydig cell dysfunction, thereby resulting in testosterone depletion, which is responsible for diminished spermatogenesis and hence, reduction in sperm counts [23, 24].

Significant reduction in the acid and alkaline phosphatase content of the testes also suggest the decreased testicular steroidogenesis activities as free lysosomal enzymes have been shown to rise when testicular steroidogenesis is increased [25].

Reduced testicular and epididymal protein content could be correlated with absence of spermatozoa in the lumen [26], since the luminal fluid of epididymis contains a number of proteins [27]. Decreased number of spermatozoa or reduced androgens production may affect the level of sialic acid in testes. The reduced sialic acid content might alter the structural integrity of acrosomal membrane, ultimately affects the metabolism, motility and fertilizing capacity of spermatozoa [26], which could not penetrate the cervical mucus and thus failed to fertilize the ova [28, 29]. All these factors thus brought about functional sterility in the extract treated rats. However, the induced infertility was completely reversed after withdrawal of treatment of another period of 60 days. Also no apparent abnormality was observed in the litters delivered by the females mated with the males of recovery group. Non-toxicity of methanol extract of *Momordica dioica* is further supported by the data obtained after examination of hematological parameters, which remain unaltered. Thus, it can be concluded that methanol extract of *Momordica dioica* root possible exert a reversible antifertility effect mediated through testes and/or epididymis. The effects may have an inhibitory influence on gonadotropin release, which may be responsible for the decline in testosterone production, leading to changes in spermatogenesis. It was concluded that *M. dioica* possess contraceptive activity in male rats which is reversible after drug withdrawal without any adverse toxicological effects. Isolation and purification of the active compound/fraction of methanol extract is under progress.

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REFERENCES

1. Sarkar, M.P., B. Gangopadhyay, K. Basak, J. Chakrabarty, P. Banerji and A. Chatterjee, 2000. The reversible antifertility effect of *Piper betle* Linn on Swiss albino male mice, Contraception, 62: 271-274.
2. Watcho, P., P. Kamtchouing, S. Sokeng, P.F. Moundipa, J. Tantchou, J.L. Essame and N. Koueta, 2001. Reversible antispermatogenic and antifertility activity of *Mondia whitei* Linn. in male albino rats, Phytotherapy Res., 15: 26-29.
3. Gupta, R.S., J.B.S. Kachhawa and R. Chaudhary, 2006. Antispermatogenic and antiandrogenic activities of *Albizia lebbeck* (L.) Benth bark extract in male albino rats, Phytomedicine, 13: 277-283.
4. Narasimhan, S., S. Kannan, K. Ilango and G. Maharajan, 2005. Antifeedant activity of *Momordica dioica* fruit pulp extracts on *Spodoptera litura*, Fitoterapia, 76: 715-717.
5. Shreedhar, C.S., K.S.R. Pai and V.P. Vaidya, 2001. Postcoital antifertility activity of the root of *Momordica dioica* Roxb., Indian J. Pharmaceutical Sci., 63(6): 528-531.
6. Ali, M. and V. Srivastava, 1998. Characterization of phytoconstituents of the fruits of *Momordica dioica*, Indian J. Pharmaceutical Sci., 60: 287-289.
7. Yuan, J.L., W.P. Ding, J.P. Shi, Z.Z. Lu, B.N. Zhou and C.A. Erdelmeier, 1991. Studies on the antifertility compounds from *Marsdenia koi*, J. Tongji Medical University, 11: 165-168.
8. Shivalingappa, H., N.D. Satyanarayan and M.G. Purohit, 2001. Antiimplatation and pregnancy interruption efficacy of *Rivea hypocratiriformis* in rat, J. Ethnopharmacol., 74: 245-249.
9. I.N.S.A., 2000. Guidelines for care and use of animals in scientific research. Indian Natural Science Academy, New Delhi.
10. Luo, L., Z. Li, Y. Zhang and R. Huang, 1998. Triterpenes and steroidal compounds from *Momordica dioica*, Yao Xue Xue Bao., 33(11): 839-842.
11. Prasad, M.R.N., N.J. Chinoy and K.M. Kadam, 1972. Changes in succinate dehydrogenase levels in the rat epididymis under normal and altered physiological conditions, Fertility and Sterility, 23: 186-190.
12. Lowry, O.H., N.J. Rosenbrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with folin-phenol reagent, J. Biological Chemistry, 192: 265-275.
13. Warren, L., 1959. The thiobarbituric acid assay of sialic acid, J. Biological Chemistry, 234: 1971-1975.
14. Montgomery, R., 1957. Determination of glycogen, Archieves of Biochemistry and Biophysics, 67: 378-381.
15. Oser, B.L., 1965. Hawks Physiological Chemistry, 14th Edition. Tata McGraw Hill, Bombay and New Delhi, pp: 1054.
16. King, E.J. and K.N. Jagathesan, 1959. Method for estimation of acid and alkaline phosphatase, J. Clinical Pathol., 12: 95-99.
17. Mann, T., 1964. Fructose, polyols and organic acids. In: Biochemistry of semen and of the male reproductive tract, Methuen and Co. (London), pp: 237-249.
18. Abercrombie, M., 1946. Estimation of nuclear population from microtome sections, Anatomical Records, 94: 239-247.
19. Dohle, G.R., M. Smit and R.F.A. Weber, 2003. Androgens and male fertility, World J. Urology, 21: 341-345.
20. Vijay, K.B., I. Sangamma, A. Sharanabasoppa and P.B. Saraswati, 2004. Antispermatogenic and hormonal effect of *Crotalaria juncea* Linn. seed extracts in male mice, Asian J. Androl., 6: 67-70.
21. Rao, M.V. and K.D. Shah, 1988. Endocrine approach to male fertility control by steroid hormone combination in rat *Rattus norvegicus*, Indian J. Experimental Biol., 36: 775-779.
22. Sharma, N. and D. Jacob, 2000. Assessment of reversible contraceptive efficacy of methanol extract of *Mentha arvensis* L. leaves in male albino mice, J. Ethnopharmacol., 80: 9-13.
23. Toney, T.W. and B.J. Danzo, 1989. Estrogen and androgen regulation of protein synthesis by the immature rat epididymis, Endocrinol., 125: 231-242.
24. Verhoeven, G., 1992. Local control systems within the testis, Clinical Endocrinology and Metabolism, 6(2): 313-333.
25. Mathur, P.P. and S. Chattopadhyay, 1982. Involvement of Lysosomal Enzymes in Flutamide Induced Stimulation of Rat Testis Andrologia, 14: 171-176.
26. Chinoy, N.J. and S. Bhattacharya, 1997. Effect of chronic administration of Aluminium chloride on reproductive functions of testis and some accessory sex organs of male mice, Indian J. Environment and Toxicol., 7(1): 12-15.

27. Brooks, D.E. and S.J. Higgins, 1980. Characterization and androgen dependence of proteins associated with luminal fluid and spermatozoa in the rat epididymis. J. Reproduction and Fertility, 59: 363-375.
28. Amelar, R.D., L. Dubin and C. Schoenfeld, 1980. Sperm motility. Fertility and Sterility, 34: 197-215.
29. Sharma, S., M. Kumar, R.B. Goyal, B. Manivannan and N.K. Lohiya, 1999. Reversible antispermatogenic effect of gossypol in langur monkey (*Presbytis entellus entellus*), Advances in Contraception, 15: 15-27.