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EFFECTS OF *ACTINIOPTERIS DICHOTOMA* (SW.) ON REPRODUCTIVE FUNCTION OF MALE RAT

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SUMMARY

Oral administration of *Actiniopteris dichotoma* (whole plant) crude extract (50% ethanol) and its isolated chromatographic fraction 50:50 ($CHCl_3$: CH_3OH) at the dose level of 50 mg/kg b.wt/day for 60 days, induced infertility in male rats. The reduction in fertility is coincided with suppressed sperm production and reduced sperm motility. The treatment caused degeneration and vacuolation in spermatogenic cells and reduced seminiferous tubule dimensions. The spermatids were declined by 98.0% and 95.6%. A marked diminition in the germ cell population specially step-19 spermatids and mature Leydig cell number was noticed. Probable androgen deprivation could be explained by decreased testicular glycogen, protein and sialic acid contents. Reversibility could be achieved after 8-10 weeks of cessation of the treatment. *A. dichotoma* can be used to develop a safe male contraceptive pill.

Key words : *Actiniopteris dichotoma*; Androgen; Leydig cell; spermatogenesis.

INTRODUCTION

The development of new fertility regulating drug from medicinal plants is an attractive preposition. In recent years, number of plant materials have been tested to evaluate their antifertility properties (1-5).

A. dichotoma (Sw.) Link is a xerophytic fern (family : actiniopteridaceae) commonly known as 'morepankhi' or 'mayurshikha'. *A. dichotoma* possesses anthelmintic properties (6), the leaves of the plants are used to cure tuberculosis, diarrhoea and spermatorrhoea by tribals of Rajasthan. Garasias (A tribal community), make a paste of fresh leaves and give both to males and females as a birth control remedy (7). Quercettin-3-rutinoside (rutin) and β-sitosterol are chief compounds present in *A. dichotoma*. Quercetin-3-rutinoside has been used to

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decrease the fragility of blood capillaries, and also added in multivitamin preparations and possesses antibiotic properties (8). Present investigation was undertaken to understand the mechanism of action of *A.dichotoma* in fertility regulation.

MATERIALS AND METHODS

Actiniopteris dichotoma plants were collected from the hillocks near university campus, Jaipur. Shade dried, crushed whole plants were extracted with 50% ethanol in a soxhalet apparatus to obtain a solid viscous brown mass, that is "crude extract" (yield : 2.9%).

Fractionation and isolation of the compounds :

The crude extract was subjected to column chromatographic resolution by using silica-gel as an absorbent and elution was done with 50:50 ($CHCl_3$: CH_3OH). The isolated fraction termed Fr. I and yield was 34.9%. On the basis of physical (m.p.) and chemical characterizations (TLC, PC, colour reactions and spectral analysis) the two compounds quercetin-3-rutinoside (rutin) and β-sitosterol were identified.

Screening Procedure :

Male albino rats of Sprague-Dawley strain weighing between 170-200 gm purchased from AIIMS, New Delhi were used. Animals were housed in plastic cages with proper aeration and temperature, maintained on standard rat feed (Hindustan Lever Ltd) and water *ad libitum*. Proven fertile male rats were weighed and grouped into 3 groups of 10 animals each.

Group I - Control or vehicle treated (1 ml distilled water)
Group II - *A. dichotoma* crude extract 50 mg/kg b.wt./day for 60 days.
Group III - *A. dichotoma* Fr.I – 50 : 50 ($CHCl_3$: CH_3OH) 50 mg/kg b.wt./day for 60 days.

Crude extract as well as Fr. I was dissolved in 1 ml distilled water and administered orally. After 55 days of treatment, males were cohabited with proestrous females in the ratio of 1:2 for fertility test. The vaginal plug or the presence of sperms in the vaginal swab was a proof of mating. The mated females were separated and implantation sites were recorded on day 16th of the pregnancy through laparotomy. On day 61, half of the animals were sacrificed using light ether anaesthesia. Blood was collected through cardiac puncture and serum was separated, RBC and WBC count (9), haemoglobin (10), glucose (11) and urea (12) were estimated in blood and serum was analysed for cholesterol (13), phospholipid (14) and triglyceride (15). Testes and accessory sex organs (epididymes, seminal vesicle and prostate gland) were discussed out, freed off fat and connective tissues and half of the organs were frozen for biochemical estimation of glycogen (16), protein (17) and sialic acid (18). For the histological study the remaining organs were fixed in Bouin's fluid, embedded in paraffin wax, sectioned and stained with haematoxylin and eosin. Sperm motility and density in cauda epididymidis and testes were assessed by method of Prasad *et al* (19). The evaluation of cell

population dynamics was based on the calculations made for each cell type per cross tubular section. All raw counts were transformed to nuclear points by using *Abercrombie's* formula (20). Interstitial cell types such as fibroblast, mature and degenerating Leydig cells [mature Leydig cells containing agranular clear cytoplasm, degenerating Leydig cells are those with residual bodies or dense bodies in abundance and fibroblastic cells which are peritubular myoid cells (21)] were estimated by applying a differential count over 200 cell and statistically verified by the binomial distribution (22). Seminiferous tubule diameter, area of Leydig cell nuclei with cytoplasm and Sertoli cell nuclei were calculated. The results were analysed using Student's '*t*' test.

RESULTS

Weight response

The weight of testes, epididymes, seminal vesicle and prostate gland were decreased significantly ($P<0.001$) after both the treatment (Table –1).

Sperm dynamics and fertility

The sperm motility in cauda epididymidis was decreased by 71.8% and 81.7% after crude extract and Fr. I treatment, respectively. A severe impairment of sperm density in testes and cauda epididymidis were observed after crude extract and Fr. I treatment as compared with controls (Table –1). Fertility test showed 100% negative fertility in both the treatment groups.

Biochemical findings

A marked reduction ($P<0.01$ to $P>0.001$) in protein and sialic acid contents of testes and sex accessory organs were observed after crude extract administration. In Fr. I treated rats sialic acid content of prostate gland did not alter significantly. However, testicular glycogen decreased significantly in both the treated groups (Table 2). No significant change was observed in blood and serum parameters (Table 5).

Testicular histopathology and cell population dynamics (Fig. 1-3)

Administration of crude extract and Fr. I caused an effective inhibition of spermatogenesis in male rats. The production of round sprmatids was decreased by 98.0% and 95.6%, respectively in both Gr. II and III as compared to control rats (Gr. I). The number of preleptotene and pachytene spermatocytes decreased significantly ($P<0.001$) (Table-4). The mature Leydig cell number was depleted significantly ($P<0.001$), whereas population of fibroblast like cells remain unaffected. The area of Leydig cells and Sertoli cell nuclei reduced by 37.1% and 56.3%, respectively in Gr. II and by 89.4% and 54.0% in Gr. III. Normal seminiferous tubule and tubules containing step-19 spermatids were decreased significantly. Seminiferous tubule diameter was also reduced (Table-3).

Table 1 : Effects of *A. dichotoma* on sperm dynamics and fertility of male rats

Treatment	Sperm Motility(%)	Sperm Density (million/ml)		Fertility	Reproductive Organ Weights mg/100gm b.wt			
	(cauda Epididy-midis	Testes	Cauda Epididy-midis	Fertility (%)	Testes	Epidymes	Seminal vesicle	Ventral prostate
Group I Intact Control (Vehicle treated)	72.4 ± 0.81	4.62 ± 0.11	42.5 ±0.92	100%(+ve)	1292.6 ± 54.0	510.9 ± 12.3	677.2 ± 25.1	347.1 ± 2.4
Group II Intract + *A. dichotoma* 50 mg/kg b.wt/day orally for 60 days	20.4 ±7.38**	0.17 ± 0.06 **	2.76 ±0.92 **	100%(-ve)	659.4 ± 134.3*	312.9 ± 34.1 **	356.7 ± 72.0 *	134.1 ± 25.9 *
Group III Intact + Fr.I 50:50 ($CHCL_3$: CH_3OH) 50 mg/kg b. wt/day orally for 60 days	27.7 ± 3.15**	0.62 ± 0.12 **	7.6 ± 1.45 **	100%(-ve)	1071.5 ± 30.5*	407.1 ± 12.0 **	551.3 ± 12.4 *	165.1 ± 18.3 **

(Mean ± SEM of 5 animals)

* = P<0.01 Significant

** = P<0.001 Highly Significant

Group II, III compared with Group I

Table 2 : Tissue biochemistry of *A. dichotoma* treated male rats.

Treatment	Glycogen (mg/gm)	Protein (mg/gm)				Sialic Acid (mg/gm)			
	Testes	Testes	Cauda Epididymidis	Seminal Vesicle	Ventral Prostate	Testes	Cauda Epididymidis	Seminal Vesicle	Ventral Prostate
Group I Intact Control (Vehicle treated)	2.63 ± 0.14	195.3 ± 3.21	235.0 ± 6.02	214.7 ± 4.10	185.4 ± 1.25	4.50 ± 0.07	5.81 ± 0.07	5.34 ± 0.18	4.91 ± 0.06
Group II Intact + A. dichotoma 50 mg/kg b. wt/day orally for 60 days	1.84 ± 0.06**	141.2 ± 2.44**	173.6 ± 5.03**	143.3 ± 4.20*	136.3 ± 3.20**	3.45 ± 0.21*	4.35 ± 0.12**	3.96 ± 0.05*	4.45 ± 0.12*
Group III Intact + Fr.I ns 50:50 ($CHCL_3$: CH_3OH) 50 mg/kg b.wt/day orally for 60 days	1.18 ± 0.04**	99.3 ± 1.92**	178.8 ± 5.9**	144.4 ± 3.76*	123.1 ± 1.92**	3.14 ± 0.22**	4.38 ± 0.03**	3.90 ± 0.2**	4.48 ± 0.17

(Mean ± SEM of 5 Animals)

ns = Non – significant

* = P<0.01 Significant

** = P<0.001 Highly Significant

Group II, III compared with Group I

Table 3 : Testicular cell dynamics of *A. dichotoma* treated rats.

Treatment	Seminiferous Tubules/Cross Section				Leydig Cell				
	Diameter (µm)	Normal tubules (%)	Abnormal tubules (%)	Step - 19 Spermatids (%)	Aea (µm²)		Differential Counts (%)		
					Cytoplasmic	Nuclear	Mature	Degenerating	Fibroblast like cells
Group I Intact Control (Vehicle treated)	2.61 ± 3.83	78.9 ± 0.63	21.0 ± 2.23	24.2 ± 0.39	59.4 ± 5.69	15.7 ± 0.73	41.2 ± 1.19	29.3 ± 0.81	29.0 ± 0.79
Group II Intact + *A. dichotoma* 50 mg/kg b. wt/day oralyl for 60 days	167.7 ± 17.6 **	5.4 ± 3.28 **	94.5 ± 3.30 **	4.1 ± 2.45 **	29.3 ± 1.93 *	9.87 ± 0.69 *	21.0 ± 3.80 **	47 ± 3.08 **	31.8 ± 4.35 **
Group III Intact + Fr.I 50:50 ($CHCL_3$: CH_3OH) 50 mg/kg b.wt/day orally for 60 days	195.2 ± 9.63 **	12.5 ± 1.61 **	87.3 ± 1.69 **	7.3 ± 0.62 **	26.5 ± 1.34 **	9.5 ± 0.18 **	17.9 ± 0.67 **	47.1 ± 0.92 **	36.0 ± 1.73 *

(Mean ± SEM of 5 Animals)

ns = Non – significant

* = P<0.01 Significant

** = P<0.001 Highly Significant

Group II, III compared with Group I

Table 4 : Quantitative analysis of the testicular cell population of *A. dichotoma* treated rats.

Treatment		Spermatogenic Cell Counts/Cross Section					Sertoli Cell	
	Sperma-togonia	Prelep-totene	Pachytene	Secondry Spermato-cyte	Round spermatid	Elongated spermatid	Number/10 cross section	Nuclear area (μm^2)
Group I Intact Control (Vehicle treated)	80.6 ±0.30	14.8 ± 0.47	35.3 ±0.76	57.8 ± 3.82	23.1 ± 0.76	36.5 ± 2.47	48.2 ± 0.50	60.7 ± 2.53
Group II Intract + *A. dichotoma* 50 mg/kg b.wt/day orally for 60 days	1.56 ±0.39**	3.76 ± 0.56**	6.60 ± 1.76**	4.90 ± 2.61*	0.44 ± 0.26*	1.78 ± 0.75**	16.3 ± 4.20**	26.5 ± 1.73**
Group III Intact + Fr.I 50:50 ($CHCL_3$: CH_3OH) 50 mg/kg b. wt/day orally for 60 days	1.89 ±0.14**	4.0 ± 0.53**	31.1 ± 1.04*	13.2 ± 1.06**	1.0 ± 0.43**	2.3 ± 3.37**	16.7 ± 1.43*	27.9 ± 1.76**

(Mean ± SEM of 5 animals)

* = P<0.01 Significant

** = P<0.001 Highly Significant

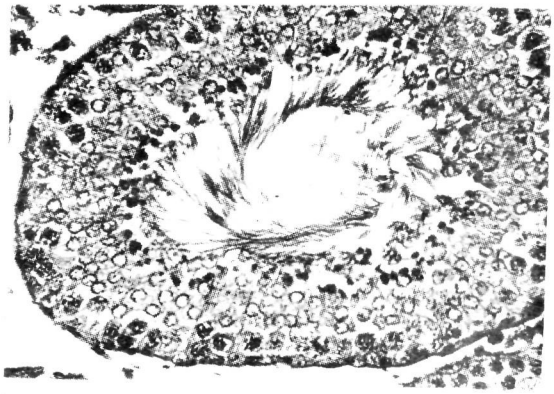
Group II, III compared with Group I

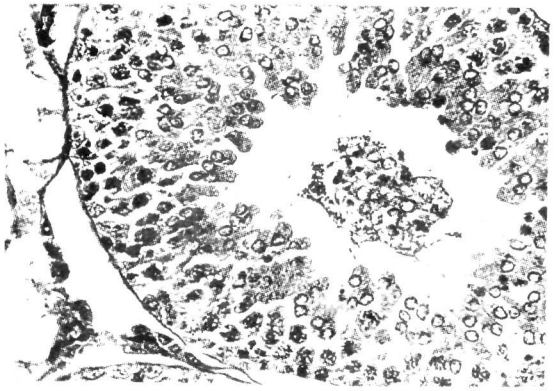
Table 5 : Blood and serum analysis of *A. dichotoma* treated rats.

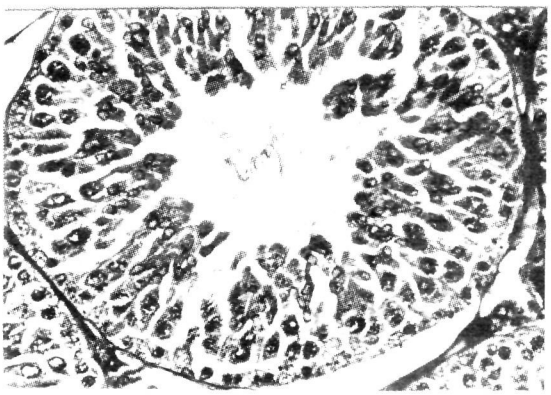
Treatment	Blood Analysis					Serum Analysis		
	R.B.C. Count (million/mm³)	W.B.C. Count (per mm³)	Haemo-globin (gm%)	Sugar (mg/dl)	Urea (mg/dl)	Cholesterol	Phospho-lipid (mg/dl)	Trigly-ceride
Group I Intact Control (Vehicle treated)	5.38 ± 0.43	8825.0 ± 140.4	35.3 ± 0.76	57.8 ± 3.82	23.1 ± 0.76	36.5 ± 2.47	48.2 ± 0.50	60.7 ± 2.53
Group II Intract + *A. dichotoma* 50 mg/kg b.wt/day orally for 60 days	4.82 ± .04 ns	7850.0 ± 275.3	12.15 ± 0.68 ns	82.07 ± 5.1 ns	33.3 ± 1.84 ns	85.9 ± 3.92 ns	108.8 ± 3.09 ns	71.4 ± 3.63 ns
Group III Intact + Fr.I 50:50 ($CHCL_3$: CH_3OH) 50 mg/kg b. wt/day orally for 60 days	4.2 ± 0.29 ns	7808.0 ± 351.5	11.7 ± 0.65 ns	85.2 ± 2.73 ns	131.3 ± 2.78 ns	76.5 ± 9.67 ns	92.3 ± 7.42 ns	70.1 ± 8.4 ns

(Mean ± SEM of 5 animals)

ns = Non Significant Group II, III compared with Group I







Figures 1 -3 : Effect of A.dichotoma on rat testis

Fig. 1 : Control testes showing normal spermatogenesis x200he.

Fig. 2 : *A. dichotoma* crude extract. Note the spermatogenic arrest. Cellular debris present in lumen. x200he

Fig. 3 : *A. dichotoma* Fr. I Photograph showing arrest of spermatogenesis at spermatocyte level. Lumen in empty. x200he.

Recovery Phase

Normal spermatogenesis was seen after 8-10 weeks of cessation of drug administration. The organ weights, testicular cell number and dimensions restored to near normal.

DISCUSSION

Antispermatogenic effect of *A. dichotoma* whole plant was conspicuous showing severe reduction in sperm production. Decrease in the testes weight may be due to reduction in the number of spermatogenic elements and spermatozoa (23, 24). Reduction in weights of accessory sex structures indicate the atrophy of glandular tissues, reduction in secretory ability and reflecting the decreased level of testosterone as these organs are androgen dependent (25, 26). Sperm count is considered to be one of the important factors that effect fertility. Low cauda epididymidal sperm density may be due to alteration in androgen metabolism. The physiological

and biochemical integrity of epididymis are dependent on androgens (27). The principal cells of epididymal epithelium synthesize proteins which are androgen dependent and forms one of the constituents that ensures the maturation of spermatozoa (28).

The reduction in number of spermatogenic cells may be due to non-availability of testosterone. Spermatogenesis is activated by testosterone which is synthesized by Leydig cells and acts on Sertoli cells and peritubular cells (29). Depletion in spermatids together with secondary spermatocytes reflect non-availability of ABP from Sertoli cells. (30, 31). Since the ABP is required for maintaining intratubular androgen concentration and the transformation particularly meiotic / post-meiotic level and highly sensitive to available androgen concentration (32), the alteration in androgen level in testes may affect the transformation of primary spermatocytes to spermatids thus resulted in less number of spermatids.

The impairment of Leydig cell function was evinced by its reduced area, nuclear dimensions and fewer number of mature Leydig cells. Reduction in volume and number of mature Leydig cells indicate low production of androgen, affecting fertility (33). Structural changes (reduction in area) of Sertoli cell nuclei may be due to lack of androgens and exogenous testosterone (34). Reduced testicular weight and decreased serum level of FSH are associated with the malfunction of Sertoli cells (35). Further, the reduced glycogen level was correlated with diminished post-meiotic germ cells (spermatid), a site of glucose metabolism (36). Low levels of sialic acid might be due to loss of viability and fertilizing ability of spermatozoa (37). Bedwal *et al* (38) reported that the reduced testicular and epididymal protein content could be correlated with absence of spermatozoa in the lumen. It has been observed that blood and serum parameters were within normal range, indicating non-toxic nature of the plant material.

Our results suggested that crude extract of *A. dichotoma* is more effective to suppress spermatogenesis than its isolated fraction. Further studies are in progress and shall be reported elsewhere.

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