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FERTILITY REGULATION IN MALE RATS WITH THE HELP OF *ECHINOPS ECHINATUS* (ROXB) ROOT EXTRACT

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Echinops echinatus, a pure anti-androgenic drug, when administered orally to male albino rats at dose level of 50, 100 and 200 mg/Kg b.wt./day/rat orally for 60 days, significantly decreased the weight of testes, epididymides, ventral prostate, vas deferens and seminal vesicle. Sperm motility and sprem density also showed significant reduction. The concentration of protein, sialic acid, ascorbic acid, fructose, acid/alkaline phosphatase were significantly decreased in *Echinops echinatus* treated groups. *Echinops echinatus* inhibits spermatogenesis in many animals. Its antifertility effect may be a combination of the effect on the developing spermatids as well as that on spermatozoal motility.

Keywords : Androgen; *Echinops echinatus;* Spermatogenesis.

Introduction

Ayurveda has a tradition of using herbs and considers all plants as potentially medicinal. Many plants and their products are studied in the hope that a search among traditional medicinal plants may provide universal, effective, cheap and safer contraceptive agents.[1-3] The plant *Echinops echinatus* (Roxb) is used as a diuretic, nerve tonic, used in hoarse cough, hysteria, dyspepsia, scrofula and opthalmia[4]. In the present investigation, the antifertility activity of *E.echinatus* has been studied to discover an ideal oral contraceptive for male albino rats.

Materials and Methods

The roots of *E.echinatus* were collected from in and around the Jaipur, shade dried, powdered and soxhlated with 50% ethanol at 55 - 60°C for 36 hours. The extract was collected after evaporating the ethanol and washed with petroleum ether, benzene, chloroform and acetone for removing impurities. The extract was dissolved in distilled water and administered orally at 3 doses - 50, 100 and 200 mg/Kg b.wt./rat/day to male rats for 60 days.

Chemical analysis of *E.echinatus* showed the presence of echinopsine, echinopsidine and echinozolinone as 3 (2 hydroxy ethyl) -4(3 H) quinozolinone alkaloids[5].

Adult healthy fertile (150 - 200 gms) male albino rats of sprague daweley strain from inbred colony were used. The animals were housed in well ventilated animal house at 25 ± 5°C. They were divided into four groups, each containing five animals and were fed with standard pellet diet (Hindustan Lever Ltd., India) soaked wheat and black gram and water *ad libitum*.

Experimental Design

Group I : Vehicle treated control

II : 50% EtOH extract of *E.echinatus* (Root 50 mg/kg b. wt./day orally, 60 days)

III : 50% EtOH extract of *E.echinatus* (Root) (100 mg/Kg b.wt./day orally, 60 days)

IV : 50% EtOH extract of *E.echinatus* (Root) (200 mg/Kg b.wt./day orally, 60 days)

After completion of the experiment on day 61, the animals were sacrificed by

using ether anaesthesia. Blood was collected from the heart, allowed to clot at room temperature. Seperated serum was submitted for biochemical analysis.

Motility of epididymal spermatozoa was counted in haemocytometer chamber. The density of cauda epididymal sperm suspension was assessed by the method of Prasad *et.al*[6]. The weights of reproductive organs were recorded.

Fresh frozen tissues were analysed for glycogen[7], ascorbic acid[8], cholesterol[9], protein[10], sialic acid[11], fructose[12] and acid/ alkaline phosphatase[13].

Results and Discussion

E.echinatus treated rats showed non-significant change in body weight. Sperm motility was significantly reduced ($P \leq 0.001$) in cauda epididymis of *E.echinatus* treated rats. Similar type of reduction ($P \leq 0.001$) was noted in sperm density in testes and cauda epididymis, following treatment with *E.echinatus* extract (Table 1).

The weights of reproductive organs viz. testes, epididymides ventral prostate, vas deferens and seminal vesicle were significantly reduced in *E.echinatus* treated rats.

Protein, sialic acid, glycogen, fructose, ascorbic acid, acid/alkaline phosphatase were significantly reduced in all the reproductive organs. Testicular cholesterol was increased significantly in *E.echinatus* treated rats ($P \leq 0.01$) (Table 2A & B).

The results revealed that oral administration of *E.echinatus* extract showed anti-spermatogenic and anti-androgenic effect. The decreased weight of testis and accessory sex organs by the plant extract is due to androgen imbalance suggested by several workers[14]. Dixit[15] has observed decreased motility of dog and rat spermatozoa after Solasodine treatment. According to him the extract of *Solanum xanthocarpum* may be interfering with enzymatic reactions including uncoupling oxidative phosphorylation. Kanwar *et al*[16] reported

Table 1. Histometric changes in control and *E. echinatus*·(root extract) treated male rats.

Treatment	Sperm Motility %	Sperm Density mill/ml	
	Cauda	Testes	Cauda
Control (received vehicle only) Gr.I	57.2 ±1.8	4.60 ±0.3	49.3 ±3.6
E. echinatus (50 mg/day) Gr.II	37.7[b] ±0.5	1.9[b] ±0.6	10.0[a] ±0.4
E. echinatus (100 mg/day) Gr.III	26.4[b] ±1.3	1.70[b] ±0.05	5.0[b] ±0.1
E. echinatus (200 mg/day) Gr. IV	22.6[b] ±0.9	0.50[b] ±0.06	4.5[b] ±0.5

$P \leq 0.01$ - a : Significant Gr.II compared with Gr.I
$P \leq 0.001$ - b: Highly significant Gr. III compared with Gr.I
P ≤ ns - c : Non-significant Gr. IV compared with Gr. I

Table 2 A. Tissue Biochemistry of control and *E. echinatus* (root extract) treated male rats.

Unit : mg/gm

Treatment	Ascorbic acid	Fructose		Glycogen		Cholesterol			Protein					
	Adrenal	Seminal Vesicle	Liver	Heart	Testis	Liver	Heart	Testis	Adrenal	Testis	Cauda epididymis	Seminal vesicle	Ventral prostate	Vas deferenes
Control (received vehicle only) Gr. I	3.92 ±0.15	5.18 ±0.20	5.72 ±0.36	2.30 ±0.15	2.90 ±0.26	14.26 ±0.26	7.52 ±0.40	7.26 ±0.32	23.25 ±0.23	196.2 ±0.2	256.4 ±2.0	212.3 ±1.6	184.3 ±1.2	182.4 ±0.2
E. echinatus (50 mg/day) Gr. II	2.44[b] ±0.12	2.86[b] ±0.32	5.69[c] ±0.32	1.96[c] ±0.10	1.78[a] ±0.06	14.03[c] ±0.16	7.11[c] ±0.49	10.31[a] ±0.17	22.81[c] ±0.17	194.6[b] ±0.2	239.4[a] ±4.5	204.3[a] ±1.6	180.2[a] ±0.3	180.2[a] ±0.6
E. echinatus (100 mg/day) Gr. III	2.36[b] ±0.10	2.47[b] ±0.02	5.28[c] ±0.24	1.96[c] ±0.10	1.43[a] ±0.27	13.05[c] ±0.80	6.89[c] ±0.35	12.12[a] ±0.30	22.80[c] ±1.01	182.5[b] ±2.3	237.2[a] ±4.6	202.2[b] ±1.5	176.3[a] ±1.6	176.4[a] ±1.5
E. eachinatus (200 mg/day) Gr. IV	2.12[b] ±0.08	2.32[b] ±0.25	5.13[c] ±0.16	1.72[c] ±0.20	1.25[b] ±0.03	12.80[c] ±0.84	6.87[c] ±0.36	12.63[b] ±0.41	21.25[c] ±0.72	178.3[b] ±4.4	236.4[a] ±4.5	202.2[a] ±1.6	172.4[a] ±2.7	172.5[a] ±2.3

Table 2 B. ...Continuation of Table 2A

Treatment	Sialic acid					Alkaline phosphatase					Acid phosphatase				
	Testes	Cauda epididymides	Seminal vesicle	Ventral prostate	Vas deferens	Testes	Cauda epididymides	Seminal vesicle	Ventral prostate	Vas deferens	Testes	Cauda epididymides	Seminal vesicle	Ventral prostate	Vas deferens
Control (received vehicle only) Gr. I	4.62 ±0.01	6.72 ±0.02	5.23 ±0.01	4.82 ±0.02	4.37 ±0.05	5.48 ±0.03	6.76 ±0.05	5.42 ±0.03	5.66 ±0.09	5.28 ±0.02	2.89 ±0.02	3.12 ±0.02	2.44 ±0.05	2.62 ±0.03	3.36 ±0.05
E. echinatus (50 mg/day) Gr. II	4.56[a] ±0.01	6.52[a] ±0.06	5.13[a] ±0.02	4.62[a] ±0.04	4.17[a] ±0.04	5.36[a] ±0.01	6.52[a] ±0.03	5.32[a] ±0.02	5.23[a] ±0.06	5.12[b] ±0.02	2.62[b] ±0.04	3.02[a] ±0.01	2.25[a] ±0.01	2.42[a] ±0.06	3.12[a] ±0.04
E. echinatus (100 mg/day) Gr. III	4.36[b] ±0.05	6.46[b] ±0.04	5.01[b] ±0.04	4.61[a] ±0.06	4.07[a] ±0.06	5.28[a] ±0.06	6.25[a] ±0.10	5.15[a] ±0.04	5.06[a] ±0.30	1.98[a] ±0.36	2.17[b] ±0.15	2.98[a] ±0.04	2.20[a] ±0.05	2.16[b] ±0.08	2.96[a] ±0.09
E. echinatus (200 mg/day) Gr. IV	4.06[b] ±0.11	6.16[b] ±0.12	4.86[a] ±0.08	4.21[a] ±0.14	3.96[a] ±0.10	5.20[a] ±0.08	6.10[a] ±0.24	5.05[a] ±0.38	4.98[a] ±0.27	4.92[b] ±0.10	2.07[b] ±0.16	2.80[a] ±0.08	2.02[a] ±0.96	2.06[a] ±0.12	2.82[a] ±0.12

P≤0.01 - a : Significant Gr. II Compared with Gr. I
P ≤0.001 - b : Highly significant Gr. III compared with Gr. I
P ≤ ns - c : Non-significant Gr. IV Compared with Gr. I

that sperm motility was decreased significantly in solasodine treated buffalo bull spermatozoa and this decrease was dose and time dependent.

The protein concentration reduced in the testis is probably due to the absense of stages of spermatogenesis in seminiferous tubules[17]. Reduced concentration of protein in cauda epididymis is probably due to the absence of spermatozoa in the epididymal lumen[18,19]. Marked increased in concentration of cholesterol in testis implies inhibition of androgenesis and impairment of spermatogenesis[20]. Decline in acid phosphatase activity of ventral prostate was probably due to decline in the endogenous androgen production induced by anti-gonadotropic effect of the extract.

Sen Gupta *et al.*[21] reported that glycogen content was reduced during spermatogenesis and also during maturation of spermatozoa. *E.echinatus* treatment also reduced testicular glycogen levels which may be attributed to interference in glucose metabolism. Reduction in ascorbic acid content of adrenal gland indicates the supressive role of the plant extract of steroidogenesis.

In conclusion *E.echinatus* treatment gave excellent, reversible antifertility results with no adverse toxic side effects.

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