Effect of methanol seed extract of *Strychnos potatorum* on accessory sex organs of male albino rats

R. S. Gupta*, M. Kanwar and J.B.S. Kachhawa

Center for Advanced Studies, Reproduction Physiology Section, Department of Zoology, University of Rajasthan, Jaipur 302002, India

Abstract:

To control the problem of population explosion, investigations are undertaken to discover an effective contraceptive drug. In our previous study *Strychnos potatorum* seeds have shown contraceptive efficacy in male albino rats. The present study is an attempt to evaluate the effect of 70% methanol seed extract of *Strychnos potatorum* at the dose level of 100 mg/rat/day on sex accessory organs of male rats. For this, body and accessory reproductive organ weight, serum testosterone level, biochemical analysis and histological examinations were carried out to assess the effect of *S. potatorum*. Treatment did not bring any significant change in the body weight, whereas the weights of epididymides, seminal vesicles, ventral prostates and vas deferens decreased significantly ($P<0.01$). Testosterone concentration of serum depleted by 56.60%. Biochemical parameters also showed significant decline in treated group. Disorganization of epithelial layer of cauda epididymis and reduced concentration of secretory fluid in seminal vesicle and in ventral prostate could also be seen. In conclusion *S. potatorum* seed extract also affects the accessory reproductive organs with its contraceptive efficacy of male rats.

Key words: *S. potatorum*, testosterone, accessory sex organs, and histology.

Introduction

Fertility regulation with plants or plant preparations have been reported in the ancient literature of indigenous systems of medicine. A large number of plant species with antifertility effects have been screened. A part of this *Strychnos potatorum* seeds also shows its contraceptive efficacy in male albino rats (Gupta et al., 2005). Further more, the present study deal with the effect of 70% methanol seed extract of *Strychnos potatorum* on sex accessory organs of male rats.

Materials and methods

Seeds of *Strychnos potatorum* were dried, crushed and powdered. This powder of plant material was subjected to soxhlet for extraction, with 70% methanol for 24-30 hours. The extract was filtered and methanol was removed under reduced pressure to obtain dark viscous brown mass. A part of this extract was fed to rats as crude drug.

The present study was carried out on healthy and sexually mature male albino rats (*Rattus norvegicus*) of Wistar strain weighing about 150-180 g obtained from Jamia Hamdard, Hamdard University, New Delhi (India). Animals were housed in polypropylene cages measuring 12”X10”X8” and maintained under standard conditions (12 hrs. light / 12 hrs. dark cycle; 25±3°C, 30-60% relative humidity).

Strychnos potatorum seeds extract was dissolved in distilled water and administered orally to animals. Animals were divided into 2 groups with 5 animals in each.

Group I : Intact control or vehicle treated (Dist. water) for 60 days
Group II : *S. potatorum* (seed) 100 mg/rat/day for 60 days

*Corresponding Author: Dr. R.S. Gupta, Teacher’s Hostel, University of Rajasthan, Jaipur-302004. INDIA. Ph. No. +91-141-2711228

Autopsy schedule

The male rats were weighed and sacrificed under ether anesthesia after 24 hours of the last drug administration of 60 days duration. Blood was collected by cardiac puncture and serum was separated for testosterone analysis (Be'langer, 1980). Accessory reproductive organs i.e. Epididymis, seminal vesicle, ventral prostate and vas deferens were removed, cleared off fats, connective tissues and weighed on an electronic balance and kept at -20°C for biochemical estimation.

Biochemical analysis

The estimation of protein (Lowry, 1951) and sialic acid (Warren, 1959) were performed in epididymis, seminal ventral prostate and vas deferens. Fructose in seminal vesicle was also estimated (Mann, 1964).

Histological preparation

For examination of pathological changes, tissues were fixed in Bouin's fluid, dehydrated in graded ethanol, cleared in xylene and embedded in paraffin wax, sections cut at 5 µm stained with hematoxylin and eosin for the discrimination of the stages of spermatogenesis.

Statistical analysis

Mean and standard error of mean [SEM] were calculated and the significance of difference was analyzed by applying student's 't' test.

Results:

Body and organ weight

Table 1: Effect of *Strychnos potatorum* extract on the body weight and the organs weight

Treatment	Body weight (g)	Organ weight (mg/ 100g b.wt)				Testosterone level
		Epididymis	Seminal vesicle	Ventral prostate	Vas deferens	
Group-I Control	210.00 ±9.56	476.82 ±3.09	482.90 ±7.03	397.12 ±9.47	113.68 ±3.12	4.37 ±0.08
Group-II *S. potatorum* **100 mg/rat/day**	200.00^{ns} ±5.00	411.11** ±5.61	413.25** ±7.46	190.85** ±6.64	98.56** ±9.47	1.94** ±0.06

values are mean ± SEM (n=5); ns non significant, ** P > 0.01 highly significant

Biochemical findings

Table 2: Effect of *Strychnos potatorum* stem bark extract on tissue biochemistry

Treatment	Protein (mg/g)				Sialic acid (mg/g)				Fructose (mg/g)
	Cauda epididymis	Seminal vesicle	Ventral prostate	Vas deferens	Cauda epididymis	Seminal vesicle	Ventral prostate	Vas deferens	Seminal vesicle
Group-I Control	204.34 ±6.62	192.44 ±3.35	177.53 ±3.13	166.76 ±0.90	5.31 ±0.21	5.30 ±0.10	5.89 ±0.12	4.92 ±0.09	5.04 ±0.19
Group-II *S. potatorum* **100 mg/rat/day**	167.77** ±1.67	148.59** ±1.02	164.19** ±1.73	153.03** ±1.28	3.62** ±0.06	3.58** ±0.05	3.69** ±0.07	3.65** ±0.02	3.55** ±0.05

values are mean ± SEM (n=10); ns non significant, ** P > 0.01 non significant

Body weights of rats, after the treatment did not alter significantly, however there is significant reduction in the weight of accessory reproductive organs i.e. epididymis, seminal vesicle, ventral prostate and vas deferens. Testosterone concentration of serum depleted by 56.60% (Table-1).

Biochemical parameters showed significant alteration after treatment. Protein content of all the accessory sex organs were reduced significantly ($P \leq 0.01$) after seed extract treatment. Content of sialic acid also depleted significantly ($P \leq 0.01$). Fructose content of seminal vesicle also showed significant ($P \leq 0.01$) reduction (Table-2).

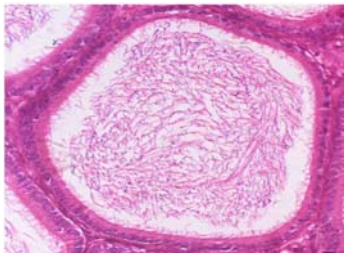

Fig. 1: Cauda epididmis (Control)

Photograph showing mature and conspicuous spermatozoa in the lumen. Epithelial lining with long stereocilia.

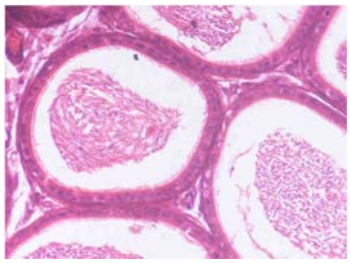

Fig. 2: Cauda epididmis (*S.potatorum* 100 mg/rat/day)

Microphotograph depicting debris in lumen of some tubules. Normal epithelial lining with disrupted stereocilia.

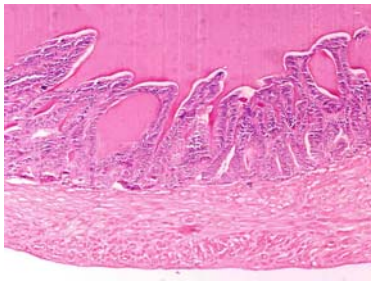

Fig. 3: Seminal vesicle (control)

Photograph showing secretory layer with crypts of mucosa and secretory fold. Muscular coat is well developed. Lumen filled with secretion.

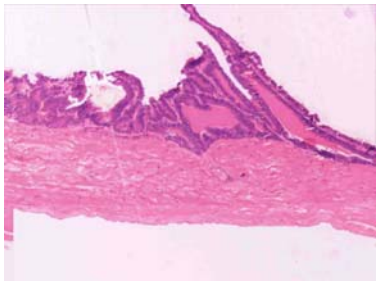

Fig. 4: Seminal vesicle (*S.potatorum* 100 mg/rat/day)

Seminal vesicle exhibiting degeneration of secretory layer. Height of muscular coat is reduced. Lumen devoid of secretion.

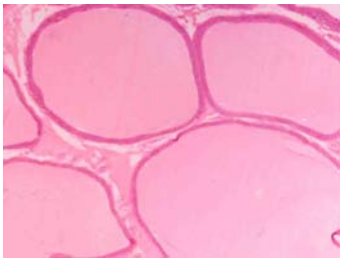

Fig. 5: Ventral prostate (*S.potatorum* 100 mg/rat/day)

Section of ventral prostate with tubular-alveoli glands embedded in a frame work of bio-muscular tissue.

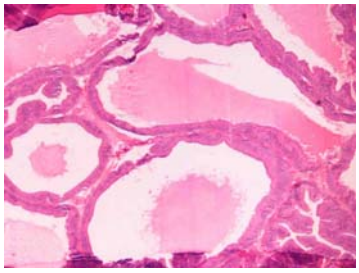

Fig. 6: Ventral prostate (*S.potatorum* 100 mg/rat/day)

Amount of secretion is reduced, prostate gland exhibiting less secretion in the tubular-alveoli.

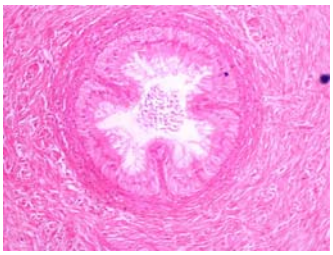

Fig. 7: Vas deferens (*S.potatorum* 100 mg/rat/day)

Microphotograph showing normal epithelium with stereocilia, surrounded by muscle layers. Lumen with spermatozoa.

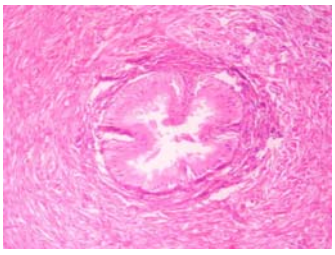

Fig. 8: Vas deferens (*S.potatorum* 100 mg/rat/day)

Vas deferens showing disorganization of epithelium. Folds of epithelium projected into lumen. Lumen without spermatozoa.

Discussion

Statistically significant reduction in the weight of accessory sex organs were observed in the rats treated with 100 mg/rat/day dose level of *Strychnos potatorum* (seed) extract. Structural and functional alteration in epididymides, seminal vesicle, ventral prostate and vas deferens which, is reflected by the histological and biochemical results of treatment is also a clear indication of reduction in weight of these organs. The relationship was also concluded by Chaturvedi *et al*. (2003) in their treatment with ethanolic extract of *Citrullus colocynthis* schrad fruit in male rats.

The *S. potatorum* (seed) extract and their isolated fraction treatment causes reduction in weight of accessory sex organs which indicates the atrophy of glandular tissue and also a reduction in secretory ability (Mathur and Malarvizhi, 1995).

Reduction in concentration of spermatozoa in epididymal lumen could be the cause of decrease protein level as luminal fluid of epididymis contain number of proteins. Some of which remain bounded to spermatozoa (Brooks and Higgins, 1980). The decreased level of protein in seminal vesicle and ventral prostate is due to reduced secretion, which might be due to reduction in number of secretory granules and golgi apparatus (Wong and Tam, 1988).

Hormonal deficiency causes a decrease or even disappearance of seminal fructose and a compensatory treatment with androgen restores the ability of the accessory glands to produce this sugar (Kempinas and Lamano-Carvalho, 1988). Deficiency of androgen caused a marked reduction in tubular diameter, regression of epididymal epithelium and changes in composition of epididymal plasma (Brooks, 1981; Kasturi *et al*., 1995).

Seminal vesicle secrete substances which directly stimulate sperm motility and antigen that seems to prevent immune response against spermatozoa (Gonzales, 1989; Gonzales *et al*., 1992). Adverse effect of plant extract resulted in reduced weight, epithelial thickness, pycnotic nuclei and reduced secretion of seminal vesicle (Neumann *et al*., 1969; Spring-Mills, 1980). Differentiated stroma cells of prostate gland are involved in a network of intercellular signaling pathways to ensure a balanced growth of the prostate gland and maintain its normal steady state (Tuxhorn *et al*., 2002). Decrease in androgen level will affect functions and histology of prostate gland as observed in the present study (Prins *et al*., 1991; Arnold *et al*., 2002). Histoarchitecture of vas deference with slight degenerative changes in the epithelium and muscular layer along with the absence of spermatozoa in the lumen also may be due to low supply of androgen to vas deferens (Simm and Gleeson, 1999; Leinonen *et al*., 2001). In conclusion S. *potatorum* seed extract possess contraceptive activity as reported earlier as well as affect the accessory sex organs of male reproductive system.

References:

Arnold JT, Isaacs JT, et al. Mechanisms involved in the progression of androgen-independent prostate cancers: it is not only the cancer cell's fault. Endocr-Relat Cancer 2002; 9: 61-73.

Be'langer A, Caron S, Picard V. Simultaneous radio-immuno assay of progestins, androgens and estrogens in rat testis. J Steroid Biochem 1980; 13(2): 185-190.

Brooks DE. Androgen effects on epididymal metabolism. Physiol Rev 1981; 61: 515-555.

Brooks DE, Higgins SJ. Characterization and androgen dependence of proteins associated with luminal fluid and spermatozoa in the rat epididymis. J Reprod Fertil 1980; 59: 363-375.

Chaturvedi M, Mali PC, Ansari AS. Induction of reversible antifertility with a crude ethanol extract of *Citrullus colocynthis* (Schrad) fruit in male rats. Pharmacol 2003; 68: 38-48.

Gonzales GF. Function, structure and ultra structure of seminal vesicles. Arch Androl 1989; 22: 1-4.

Gonzales GF, Kortebani G, Mazzolli AB. Leukocytospermia and function of the seminal vesicles on semen quality. Fertil Steril 1992; 57(5): 1058-1065.

Gupta RS, Choudhary R, Yadav RK, Verma SK, Dobhal MP. Effect of Saponins of *Albizia lebbeck* (L.) Benth bark on the reproductive system of male albino rats. J Ethnopharmacol 2005; 96(1-2): 31-36.

Kasturi M, Manivannan B, Ahamed RN, Shaikh PD, Pathan KM. Changes in epididymal structure and function of albino rat treated with *Azardirachta indica* leaves. Ind J Expt Biol 1995; 33(10): 725-729.

Kempinas WG, Lamano-Carvalho TL. A method for estimating the concentration of spermatozoa in the rat cauda epididymides.

Lab Anim 1988; 22: 154-156.

Leinonen J, Lovgren T, Vornanen T, Stenman UH. Vas deferens role in sperm transportation and reproduction. Zygote 2001; 11: 65-79.

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with Folin-Phenol reagent. J Biol Chem 1951; 192: 265-275.

Mann T. Fructose, polyols and organic acids. In: Biochemistry of semen and of the male reproductive tract. Methuen and Co. (London). 1964; pp. 237-249.

Mathur PP, Malarvizhi D. Effect of Cisplatin on physiological status of normal rat testis. Ind J Expt Biol 1995; 33: 281-283.

Neumann F, Van Berswardt-Wattrabe R, Elger W, Steinback H, Hahn JD. Effect of antiandrogens. Excerp Med Int Cong 1969; 184: 823-836.

Prins G, Birch L, Greene G. Androgen receptor localization in different cell type of the adult rat prostate. Endocrinology 1991; 129: 3187-3199.

Simm B, Gleeson M. Role of androgens in vas deferens development. Endocrinology 1999; 118: 93-117.

Spring-Mills E. The seminal vesicle. In: Male Accessory Sex Glands. Spring-Mills, E. and Hafez, E.S.E. (eds.) Elsevier/North Holland Biomedical Press, New York. 1980; pp. 63-77.

Tuxhorn JA, Ayala GE, Smith MJ, Smith VC, Dang TD, Rowley DR. Reactive stroma in human prostate cancer: induction of myofibroblast phenotype and extracellular matrix remodeling. Clin Cancer Res 2002; 8: 2912-2923.

Warren L. The thiobarbituric acid assay of sialic acid. J Biol Chem 1959; 234: 1971-1975.

Wong, V.C. and Tam, C.C. (1988): A structural and cytochemical study of the effects of gossypol on epithelial cells of the guinea pig seminal vesicle. J Reprod Fertil 84: 659-668.