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Contraceptive Efficacy of *Strychnos potatorum* Seed Extract in Male Albino Rats

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Abstract : Present study was undertaken to evaluate the contraceptive efficacy of 70% methanolic extract of *Strychnos potatorum* seeds. The aqueous solution of extract (100 mg/rat/day) was administered orally to male rats of proven fertility for 60 days. Sperm motility, sperm density, serum testosterone level, biochemical analysis and testicular cell population dynamics were carried out to assess the contraceptive effect of *S. potatorum.* The treatment did not bring any body weight loss, whereas, the weights of testes, epididymides, seminal vesicle and ventral prostate were decreased significantly ($P<0.01$). Reduced sperm count and motility resulted in suppression of fertility by 91.81%. Significant reduction was noticed in protein and sialic acid contents in reproductive organs. Number of spermatogonia and Sertoli cells were decreased. The population of preleptotene, pachytene and secondary spermatocytes were decreased by 55.72%, 63.40% and 49.81%, respectively. The seminiferous tubular diameter and Leydig cell nuclear area were reduced significantly ($P<0.01$) as compared to the controls. *Strychnos potatorum* seed possesses suppressive effects on male fertility and could be useful in development of male contraceptive agent. However, further studies are needed.

Keywords : Leydig cell**,** Sialic acid, *Strychnos potatorum***,** Sperm motility, Testes, Testosterone.

Introduction :

Herbal medicines are popular as remedies for diseases by vast majority of world's population. *Strychnos potatorum* (Linn.) belongs to family Loganiaceae, is commonly known as Nirmali and the plant is native of India. According to Ayurveda, its seeds are acrid, alexipharmic, lithotriptic and cure strangury, urinary discharges and head diseases (Agharkar, 1991). In Unani system of medicine, seeds are used in liver and kidney complaints, gonorrhea and for colic (Oudhia, 2004). Biswas *et al.* (2002 a,b) studied on the diuretic and antidiarrhoeal activities of *Strychnos potatorum* Linn. seed extract in albino rats and found it quite worthy.

Due to polysaccharide gum its seeds are utilized in paper and textile industries (Adinolfi *et al.*, 1994). Roots cure leucoderma, whereas fruits are useful in eye diseases, thirst, poisoning and hallucinations. Its seeds are used to purify water for drinking (Willis, 1973). Compounds norharmane, akuammidine, nor- C-fluroiocuraine, ochrolifuanine, bisnordihydrotoxiferine, 11-methoxy-henningsamine, 11-methoxy-12 hydroxydiabolin and 11-methoxydiabolin were isolated from crude extract and structures of these compounds were established by spectral analysis with authentic data (Massiot *et al.*, 1992).

An oral herbal contraceptive would allow couples control their fertility without

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consulting a health worker, which in turn would likely markedly increase the number of couples practicing family planning. Other advantages of such a contraceptive would include the familiarity rural people have with herbal medicines, the fewer side effects associated with herbal preparations, their ready availability from local sources, and protection of privacy. There are many references to plants in India with antifertility properties. Since 1966, the Indian Council of Medical Research (ICMR) has been conducting research to identify a herbal contraceptive, as have other organizations. Plants that have exhibited antifertility activity in clinical trials include *Hibiscus rosasinensis* (benzene extract of the flower petals suppresses implantation); Rudrapushpaka (extract of the flower petals prevents pregnancy); *Embelia ribes* (pregnancy prevention); *Davcus carota, Butea monosperma,* and *Sapindus trifoliatis* (seeds have an anti-implantation effect); and *Mentha arvensis* (leaves have anti-implantation effect). The Central Drug Research Institute in Lucknow, India, in collaboration with the US National Institutes of Health, the World Health Organization, and the ICMR confirm anti-implantation activity in *Ferula jaeschkeana, Bupleurum marginatum, Lepidium capitatum, Caesalpinia sepiaria, Lonicera japonica, Juniperus communis, Lotus corniculatus, Lamium allum,* and *Acacia farnesiana*. In China, scientists have evaluated the cotton-seed extract gossypol as a male contraceptive. They are now studying the possible antifertility effect on men of the plant *Tripterygium wilfordii*. From all the aforementioned plants as well as others under investigation, three possible types of contraceptives could be developed: an anti-ovulatory contraceptive; a postcoital contraceptive; and a male contraceptive. Some obstacles to their development include difficulties in obtaining adequate quantities of the herbs, a shortage of clinical pharmacologists and clinicians interested in conducting clinical trials, and lack of long-term financial support (Chaudhury, 1993). Hence present study had been attempted on the possible contraceptive efficacy of ***Strychnos potatorum*** seed extract in male albino rats as the properties of this herb is manifold and obstacles to the development and clinicians interested in conducting clinical trials would prove smoother in long-term with an emergence of a potent herbal male contraceptive.

Materials and Methods :

Plant material : Seeds of S*trychnos potatorum* were crushed and powdered. This powder was subjected to soxholet extraction with 70% methanol for 24-30 hrs. The viscous brown mass obtained after filtration that was used as crude extract.

Animal model : Male albino rats of Wistar-strain, weighing 150-170 g (90-100 days old) were used for experiment. Animals were acclimatized under standard conditions, rat feed and water were provided *ad libitum*.

Treatment protocol : Male rats of proven fertility were divided into two groups of five in each :

Group I : Rats were given distilled water 0.5 ml/rat/day orally for 60 days, treated as control.

Group II : Rats were treated orally with *S. potatorum* seed extract at the dose level of 100 mg orally/rat/day for 60 days.

Fertility test and autopsy schedule : Fertility test of individual rat was done before the experiment and after 55 days of treatment. Each male rat was cohabitated with proestrous females in 1 : 2 ratio. Vaginal smear was examined every morning for positive mating and number of litters delivered were recorded.

The rats were sacrificed within 24 hrs. after the last administration of the extract i.e. on 61 day of experiment. The testes, epididymides, seminal vesicles, ventral prostate were removed, cleared off fat, blood vessels and connective tissue before weighing.

Sperm count and motility : Sperm motility and sperm density were assayed in cauda epididymides and testes by the method of Prasad *et al.* (1972).

Serum testosterone level : Serum testosterone was assessed by Radio Immuno Assay (Belanger *et al.*, 1980).

Tissue biochemistry : The parts of testes, epididymides, seminal vesicles and ventral prostates from each rat were kept at -20°C until assayed for protein (Lowry *et al*., 1951) and sialic acid (Warren, 1959).

Quantitative analysis : The evaluation of cell population 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's correcting factor was introduced (Berndtson, 1977).

Statistical analysis : Student's "t"-test was used for assessment of significance of variation. Data are presented as Mean ± S.E.M.

Results and Discussion :

The administration of *S. potatorum* seed extract to rats did not cause any significant change in the body weight and on the libido of treated rats, whereas weights of testes and other accessory sex organs were decreased significantly ($P<0.01$) [Table 1]. Reduction in the weights of testes and other accessory sex organs might be due to low level of androgen, which was not enough to maintain the weight of gonads and accessories (Sharma and Jacob, 2001). Sperm motility in cauda epididymides, and sperm density in cauda epididymides and testes decreased by 56.87%, 63.15% and 58.82%, respectively [Table 2]. These depletions suggest alterations in sperm maturation and sperm production (Sarkar *et al.*, 2000). Inadequate concentration, sluggishly motile or immotile spermatozoa could not penetrate the cervical mucus and thus failed to fertilize the ova (Sharma *et al.*, 1999). Testosterone is produced by Leydig cells in the testes and decreased number of Leydig cells and their nuclear area in the treated rats diminished the production of testosterone (Hulethel and Lunenfeld, 2004), which might have affected the fertility (91.81%) in treated rats [Table 2].

In present investigation, protein content in testes and other sex organs significantly decreased following *Strychnos potatorum* seed extract administration [Table 3] probably due to the absence of the spermatogenic stages (Chinoy and Bhattacharya, 1997) in the testes. Low level of sialic acid in testes, cauda epididymides, seminal vesicles and ventral prostates in treated rats [Table 3] may be correlated with loss of androgen (Gupta *et al.*, 2001).

Table 1 : Effect of *S. potatorum* on body and organ weights (Mean ± SEM of 5 animals)

Treatment	Body wt. (g.)		Organ weights (mg/100 g. body weight)			
	Initial	Final	Testes	Epididy-mides	Seminal vesicle	Ventral Prostate
Group-I Control	170.00 ± 4.50	210.00 ± 9.56	1429.79 ± 9.73	476.82 ± 3.09	482.90 ± 8.05	377.00 ± 9.47
Group-II Treated	165.00^{ns} ± 4.50	200.00^{ns} ± 5.00	1015.42** ±11.42	411.11** ± 9.61	413.25* ± 17.46	190.85** ± 6.64

ns = Non-significant
* = P = 0.01 significant
** = P = 0.001 highly significant

Table 2 : Effect of *S. potatorum* on sperm motility, concentration and fertility in rats (Mean± SEM of 5 animals)

Treatment	Sperm motility (%) cauda epididymides	Sperm density (Million/ml)		Fertility	Testosterone (ng/dl)
		Testes	Cauda epididymides		
Group-I Control	73.42 ± 1.25	4.25 ± 0.18	48.20 ± 3.52	100% (+ Ve)	4.37 ± 0.08
Group-II Treated	31.66** ± 1.66	1.75** ± 0.13	9.10** ± 0.50	91.81 (-Ve)	1.94** ± 0.06

** = P = 0.001 highly significant

Shrinkage in seminiferous tubular diameter [Table 4] may be attributed possibly due to decline in testosterone production (Raji and Bolarinwa, 1997). The population of Sertoli cells and spermatogonia got decreased by 31.80% and 38.19%, respectively [Table 4]. Reduction in number of Sertoli cells resulted in decreased number of spermatogonia, as Sertoli cells play a critical role in spermatogenesis by providing the physical support, nutrients and hormonal signals, necessary for successful spermatogenesis (Okamura *et al.*, 2004). Sertoli cells can seriously reduce their supportive capacity and result in increased elimination of germ cells (Richburg, 2000). Number of preleptotene, pachytene and secondary spermatocytes has been noticed to be decreased by 55.72 %, 63.40% and 49.81%, respectively in *S. potatorum* treated rats [Table 4]. The reduction might be due to antiandrogenic nature of drug as these stages are completely androgen dependent (Purohit and Daradka, 1999) and *S. potatorum* possess antiandrogenic properties.

Table 3 : Effect of *S. potatorum* on tissue biochemistry in experimental rats. (Mean± SEM of 5 animals)

Treatment	Protein (mg/g)				Sialic acid (mg/g)			
	Testes	Cauda epididymides	Seminal vesicles	Ventral prostates	Testes	Cauda epididymides	Seminal vesicles	Ventral prostates
Group- I Control	225.00 ±6.12	204.34 ±6.62	192.44 ±3.35	177.53 ±3.13	4.85 ±0.11	5.31 ±0.31	5.30 ±0.84	5.89 ±0.42
Group- II Treated	185.13** ±5.09	157.77** ±6.67	153.65** ±6.02	154.19** ±1.73	3.63** ±0.03	3.45** ±0.13	3.48** ±0.30	3.76** ±0.07

** = P< 0.001 highly significant

Table 4 : Effect of *S. potatorum* on testicular cell population in rats and seminiferous tubular and Leydig cell nuclear diameter. (Mean± SEM of 5 animals)

Treatment	Testicular Cell Counts (Number/10 cross section)					Seminiferous tubular diameter (µm)	Leydig Cell nuclear area (μm^2)
	Sertoli cell	Spermatogonia	Preleptotene	Pachytene	Secondary spermatocytes		
Group-I Control	3.05 ± 0.07	8.09 ± 0.41	18.79 ± 1.65	31.79 ± 0.66	47.09 ± 2.18	270.01 ± 8.98	22.76 ± 1.85
Group-II Treated	2.08** ± 0.10	5.00** ± 0.30	8.32** ± 1.29	11.56** ± 0.97	23.63** ± 2.98	215.57** ± 8.04	12.38** ± 0.81

** = P< 0.001 highly significant

Conclusion :

From present study it can be concluded that *Strychnos potatorum* is capable to suppress male fertility without altering general metabolism. Hence the possible male contraceptive efficacy of ***Strychnos potatorum*** seed extract can not be ignored paving way to the smooth development for the clinicians interests in clinical trials towards emergence of a potent herbal male contraceptive.

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