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Spermicidal Activity of *Azadirachta indica* (Neem) Aqueous Leaf Extract on Male *Albino* Rats

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ABSTRACT: The effect of aqueous leaf extract of *Azadirachta indica* (Neem) on reproduction was studied on male albino rats. The study was divided into three groups of six animals each. The first group (I) received distilled water serve as control. The second and third groups (II and III) of animals were administered the aqueous leaf extract daily at 250mg/kg body wt., and 350mg/kg body wt., respectively for a period of 30days. Significant decreases in the weights of testis, epididymes and seminal vesicle were observed. A dose related reduction in the testicular sperm count, epididymal sperm count and motility and abnormal sperm count were observed. The results showed that *Azadirachta indica* has effects on male rat reproduction, affecting the sexual behavior and epididymal sperm concentration.
Keywords: *Azadirachta indica,* Epididymes, Spermicidal, Seminal vesicle.

INTRODUCTION

The status of herbal medicine has been fast growing all over the world during the last few decades. The World Health Organization (WHO) has set up a Task Force on Plant Research for fertility regulation with an objective to find new orally active non-steroidal contraceptive compounds. In India, phytotherapy has a very long tradition, although proper scientific explanation is relatively new. In our country as well as in the world, there are several medicinal plants associated with antifertility properties [1,2]. Although very few contraceptives have been developed from plant extracts, their potentiality has not been determined accurately, and their mode of action has been beyond our knowledge until now because there are many problems in assessing plant extract including batch to batch variation and a lack of definite active portion of the extract used for the development of herbal contraceptives. Fertility regulation with plants or plant preparations has been reported in the ancient literature of indigenous systems of medicine. A large number of plant species with anti-fertility effects have been screened in China and India beginning about 50 years ago and were subsequently fortified by national and international agencies [3,4]. However, the search for an orally active, safe and effective plant preparation or its compound is yet to be needed for fertility regulation due to incomplete inhibition of fertility or side effects.

Azadirachta indica (Neem) has been extensively used in the Ayurvedic system of medicine for a long time. Various parts of this plant are used for the treatment of various diseases. The neem bark aqueous extract is reported to have therapeutic potential for

controlling gastric hypersecretion and gastroduodenal ulcer (5). A dental gel formulation containing neem extract has been reported to reduce oral infections, plaque index, and bacterial count (6,7). The medicinal utilities have been described especially for neem leaf (8) and its constituents are reported to exhibit immunomodulatory, anti-inflammatory, anti-hyperglycemic, antiulcer, antifungal, antibacterial, antimutagenic, anticarcinogenic, nematicidal, antimalarial, antiviral, insecticidal, and antioxidant properties (8,9).

The present study was carried out to test the efficacy spermicidal activity of aqueous leaf extraxt of *Azadirachta indica* (Neem) on male albino rats.

MATERIALS AND METHODS

Plant material

The leaves of *Azadirachta indica* (Neem) (*Meliaceae*) were collected in and around Vellore District, Tamilnadu, India. The plant materials were cleaned with distilled water and shade dried at room temperature and authenticated (No.CAHC/06/2008) by Dr.B.Annadurai, Department of Botany, C. Abdul Hakeem College, Melvisharam, Vellore Dt, Tamil Nadu., and voucher specimens were kept at the Department of Botany, C. Abdul Hakeem College, Melvisharam. The shade dried plant material was powdered by using electric blender.

Plants extract preparation

100gms of the dried powdered leaves of *Azadirachta indica* (Neem) was taken and mixed with 500ml of distilled water and magnetically stirred in a container for overnight at room temperature. The residue was removed by filtration and the aqueous extracts were lipolization and concentrated under vacuum to get solid yield 10%.

Animals

Adult male Wistar rats weighing around 180-200g were purchased from Tamilnadu Veterinary and Animal Sciences University, Chennai, India. The animals were kept in polypropylene cages (three in each) at an ambient temperature of 25±2^{0}C and 55-65% relative humidity 12±1 hr light and dark schedule was maintained in the animal house till the animals were acclimatized to the laboratory conditions, and were fed with commercially available rat chow (Hindustan Lever Ltd., Bangalore. India) and had free access to water. The experiments were designed and conducted in accordance with the institutional guidelines.

Experimental design

Group I: Control rats.

Group II: The rats were treated with *Azadirachta indica* aqueous leaf extract (250 mg/kg body wt.,) for 30 days.

Group III: The rats were treated with *Azadirachta indica* aqueous leaf extract (350 mg/kg body wt.,) for 30 days.

Estimation of sperm motility and count

The spermatozoa were obtained by making small cuts in caudae epididymis and vasdeferens placed in 1ml of modified Krebs Ringer-bicarbonate buffer (pH 7.4). The sperm suspension was evaluated for sperm content, percent motility. The percent motility was determined by the progressive and non-progressive movements of sperms observed under a compound microscope. The sperm count was determined under a Neubauer haemocytometer (10,11). To evaluate the spermatozoa abnormalities, the sperm suspension was stained with eosin; smears were made on slides, air-dried and made permanent.

Serum testosterone

Serum levels of testosterone were assayed in duplicate using specific RIA method (12). Serum samples were separated by standard procedure and stored at -20^{o}C for subsequent analysis.

RESULTS

During the treatment with *Azadirachta indica* leaf extract, no significant clinical and behavioral changes were observed in both group II and group III animals. The treatment of rats with plant extract caused no effect on the body weight of the animals; weight gain was normal in all the experimental groups. The treatment with *Azadirachta indica* aqueous leaf extract treated rats caused a highly significant ($P<0.001$) decrease in the accessory sex organ weights, namely testis, epididymis and seminal vesicle in all treated groups. The group III animals the sex organ weights were highly reduced ($P<0.001$) when compared to the group II as well as group I (Normal) animals.

A highly significant decline ($P<0.001$) in serum testosterone was observed in both groups when compared to the group I control animals.

The sperm of the control rats had normal counts, motility, and morphology (Table:1). In *Azadirachta indica* aqueous leaf extract treated rats the cauda epididymidal sperm parameters showed evidence of dose dependent toxicity. The sperm counts were significantly ($P<0.001$) decreased in group II and group III animals (Table-1).

Table – 1 : Antifertility effect of *Azadirachta indica* (neem) aqueous leaf extract on male albino rats.

Treatment	Body weight		Reproductive organ weight mg/100g bd wt)			Serum testosterone ng/ml	Total count m/ml	Motile %	Abnormal %	
	Initial	final	Testis	Epididymis	seminal vesicle				HD	Tail
Group- I Control	185±2.55	207±4.02	990±10.2	485±6.2	510±2.73	4.67±0.27	7.8±2.62	94±1.93	1±0.01	1±0.01
Group II (250 mg/kg Body wt.,)	187±3.74	205±5.5 [b]	862.2±4.45[a]	4.28±1.25[a]	478±3.56[a]	2.26±0.40 [a]	5.6±4.61 [a]	65±6.17 [a]	10±1.2 [a]	9±1.02 [a]
Group III (350mg/kg Body wt.,)	192±2.6	202±2.5 [b]	642±6.8 [a]	384±5.8 [a]	424±2.1[a]	0.98±0.14 [a]	3.8±5.78 [a]	35±8.21 [a]	18±4.2 [a]	20±4.7 [a]

Data are expressed as Mean ±S.E.M of 6 individual observations. [a] Significant (P<0.001). [b] non significant. Group II and Group III were compared to Group I (Control).

In group III animals the sperm count were very much reduced when compared to the group II as well as control animals. The sperm motility was very much inhibited both group II and group III animals (Table:1). More than 50% of the sperm had abnormal morphologies of various kinds, which included globose head, coiling of tails, fusion of tails of two or more sperm etc., were observed. The plant extract intoxication exerted a significant (P<0.001) decrease epididymal sperm concentration and sperm progress motility. The live/dead sperm count was increased in both group II and group III animals. The reduction of sperm count and sperm motility were significantly (P<0.001) higher in plant extract 350 mg/kg body wt., (Group III) treated animals when compared to 250 mg/kg body wt., (Group II) and control animals.

DISCUSSION

Treatment with the extract (dose 250 mg/kg body weight and 350 mg/body weight for 30 days) was highly effective in producing reversible functional sterility. The drug treated male rats clearly indicates that the drug caused structural and functional alteration in testes, epididymides and seminal vesicle. Interestingly, it was observed that sperm morphology remained unaltered in placebo- as well as drug treated animals. Depletion of sperm count in the drug treated animals suggests alteration in sperm production in the testes. Decrease in sperm motility suggests alteration of sperm maturation in the epididymides. Changes in both sperm count and motility resulted in complete infertility within 30days. This resulted in abnormal sperm functions which ultimately gave rise to complete male sterility.

Among the plant based contraceptives, inhibition of male fertility after administration of natural substances has been related to decreased spermatozoa density (13). Also, for male contraception, it is not necessary to stop spermatogenesis, but rather to eliminate the fertilizing ability of the spermatozoa by causing changes in the morphology or in the function of the sperm (14). By the treatment employed in this study, no toxic effect was produced in the pituitary gland, which is directly involved on the development and functioning of the male reproductive system (15) nor in the reproductive organs.

In conclusion, our results in suggest that *Azadirachta indica* leaf extract treatment and durations employed in the present study causes marked alterations in the male reproductive organs and that the alterations are reversible after cessation of treatment. Treatment also had a reversible effect on suppression of fertility in males. Further, no toxic effects could be detected in treated rats.

REFERENCES

1. Hiremath SP, Badani S, Hunasagatta SK, Patil SB. Antifertility and hormonal property of flavones of strigaoroleanchioider. Eur J Pharmacol\ 2000;391:193–7.
2. Hiremath SP, Rudresh K, Badani S, Patil SB, Patil SR. Post-coital antifertility of *Aealypha indiac L.* J Ethnopharmacol 1999;67:226–8.
3. WHO, 2000. Reproductive health research at WHO: a new beginning, Biennial Report 1998-99, Special Programme of Research, Development and Research Training in Human Reproduction, World Health Organization, Geneva.
4. Lohiya, N.K., (2000). Plant products for contraception: How to make it a reality? In: Puri, C.P., (Edn.), ISSRF News letter, Vol. 5, Indian society for te study of reproduction and fertility, Mumbai, pp. 9-12.
5. Bandyopadhyay U, Biswas K, Sengupta A, Moitra P, Dutta P, Sarkar D, et al. Clinical studies on the effect of neem (*Azadirachta indica*) bark extract on gastric secretion and gastroduodenal ulcer. Life Sci 2004; 75:2867–78.
6. Pai MR, Acharya LD, Udupa N. The effect of two different dental gels and a mouthwash on plaque and gingival scores: a 6-week clinical study. Int Dent J 2004;54:219 –23.
7. Pai MR, Acharya LD, Udupa N. Evaluation of antiplaque activity of Azadirachta indica leaf extract gel—a six-week clinical study. J Ethanopharmacol 2004;90:99 –103.
8. Subapriya R, Nagini S. Medicinal properties of neem leaves: a review.Curr Med Chem Anti-Cancer Agents 2005;5:149 –56.
9. Sithisaran P, Supabphol R, Gritsanapan W. Antioxidant activity of Siamese neem tree (VP1209). J Ethanopharmacol 2005;99:109 – 12.
10. Srikanth V, Malini T, Arunakaran J, Govindarajulu P, Balasubramanian K. Effects of ethanol treatment on epididymal secretary products and sperm maturation in albino rats. J Pharmacol Exp Ther 1999;288: 509–15.
11. Zaneveld LJD, Polakoski KL. Collection and physical examination of the ejaculate. In: Hafez ESE, editor. Techniques of human andrology. Amsterdam (Holland): North Biomedical Press; 1977. p. 147–56.
12. WHO. Method manual. Programme for the provision of matched assay reagents for the radioimmunoassay of hormones in reproductive physiology fifth editor. Geneva: World Health Organisation: 1987.
13. Sharma N,JocobD.Antifertility investigation and toxicological screening of the petroleum ether extract of the leaves of Menthaarvensis L.in male albino mice.J Ethnopharmacol 2001;75:5-12.
14. Nikkanen V, Soderstrom K-O,Tuusa S,Jaakkola U-M. Effect of local epididymal levonorgestrel on the levorgestrel on the fertilizing ability of male rat,a model for post-testicular contraception 2000;61: 4001-6.
15. Mahony MC, Hodgen GD. Toxic effects on the hypothalamus anterior pituitary-gonadal axis ,control on the male and female reproductive system,and related issues.In:Witorsch RJ,editor.Reproductive toxicology.2nd.New York :Raven press,1995.p.195-213.

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