Antispermatic activity of *Azadirachta indica* leaves in albino rats

Parveen D. Shaikh, B. Manivannan, K. M. Pathan, M. Kasturi* and R. Nazeer Ahamed*

Department of Zoology, Karnatak Science College, Dharwad 580 001, India
*Department of Zoology, Karnatak University, Dharwad 580 001, India

Dry powder of the leaves of *Azadirachta indica* at the dose level of 20 mg, 40 mg and 60 mg/rat/day for 24 days results in a decrease in the weight of seminal vesicle and ventral prostate, a decrease in the sperm count, sperm motility and relative percentage of normal sperm and an increase in the forward velocity of the sperm. The observations suggest that the leaves of *A. indica* are deleterious to the fertilizing ability of the sperm.

EARLIER studies revealed that *Azadirachta indica*, a common neem tree of India, possesses emmenagogue, antiimplantation, spermicidal and antifertility properties[1-4]. The purpose of the present study is to investigate the effect of *A. indica* leaves on some of the sperm parameters, viz. total sperm count, sperm motility, relative percentage of normal and abnormal sperms and forward velocity of the sperm in albino rats.

The leaves of *A. indica* were dried in shade and finely powdered for oral administration (gavage) to rats. Adult male albino rats of Wistar strain (230 to 250 g body weight, 4 to 5 months old) were acclimatized to laboratory condition with standard rat pellet diet and water *ad libitum*. The rats were divided into four groups, each consisting of five animals. The animals belonging to group I were administered 1 ml of distilled water by gavage for 24 days and served as control. The animals of groups II, III and IV were administered orally 20 mg, 40 mg and 60 mg of *A. indica* leaf powder suspended in 1 ml of distilled water respectively per rat/day for 24 days. Twenty-four hours after the last dose, the rats were sacrificed by cervical dislocation. The testis, epididymis (caput, corpus and cauda), seminal vesicle and ventral prostate were dissected out, blotted free of mucus and weighed. The cauda epididymis was chipped into phosphate buffered glucose saline (PBGS) (composition: NaCl 50 mM/l; Na_2HPO_4 200 mM/l; glucose 200 mM/l and KH_2PO_4 26 mM/l). The debris was removed and a clear suspension, viz. the epididymal plasma was used for the analysis of total sperm count, sperm motility, relative percentage of normal and abnormal sperms and the forward velocity of the sperm. The total sperm count and motility were calculated according to the method of Besley *et al.*[5] using Neubauer haemocytometer. The relative proportion of the normal and abnormal sperms was analysed by smears stained with Ziehl Neelson's carbol fuchsin and counter stained with Loeffer's methylene blue[6] and the forward velocity of the sperm was calculated according to the method of Ratnasoorya[7].

The results are presented in Tables 1 and 2. Table 1 shows a decrease in the weight of seminal vesicle and ventral prostate which reflects an interference of treatment on the testosterone output. Table 2 shows an adverse effect on the various sperm parameters, as based on the reduction in the sperm count, motility relative percentage of normal sperm and the increase in the percentage of abnormal sperm.

Oral administration of the leaves of *A. indica* leads to antispermatogenic and antiandrogenic effects[8,9]. The antispermatogenic effect is reflected in the arrest of spermatogenesis as seen by the adverse effect on the spermatocytes, spermatids, cytolytic lesions in the

Table 1. Effect of treatment of dry leaf powder of *Azadirachta indica* on the weights of reproductive organs (mg/100 g body weight) of male albino rats. (Values are expressed as standard error of mean of five animals).

Group	Testis	Change (%)	Epididymis	Change (%)	Seminal vesicle	Change (%)	Ventral prostate	Change (%)
I (Control)	572.00 ± 29 86	—	168 00 ± 9.68	—	81.00 ± 0.02	—	84.00 ± 0.01	—
II (20 mg)	584.20 ± 29 31	2%	168 00 ± 2.56	—	68.00* ± 0.009	16%	56.00* ± 0.009	34%
III (40 mg)	601 00 ± 42.20	5%	164.00 ± 0 07	2%	48.00* ± 0.05	41%	45 00* ± 0.006	47%
IV (60 mg)	590.40 ± 23.99	3%	163 00 ± 0 02	3%	43 62* ± 0 004	46%	43 00* ± 0 009	49%

*$P < 0.001$. (The doses of leaf powder are given in parenthesis).

Table 2. Effect of treatment of dry leaf powder of *Azadirachta indica* on sperm parameters of the male albino rats. (Values are expressed as standard error of mean of five animals)

Group	Sperm count (total no. × 10^4/ml)	Change (%)	No. of motile sperm (total no. × 10^4/ml)	Change (%)	Forward velocity	Change (%)	Percentage of normal sperm
I (Control)	38.04 ± 1.07	—	34 60 ± 3.39	—	129.40 ± 3.58	—	73.07 ± 2.72
II (20 mg)	32 26 ± 2.24	15%	32.56 ± 3.54*	6%	100 00 ± 2.09**	23%	53.72 ± 1 31**
III (40 mg)	16.02 ± 0.94**	58%	10 98 ± 0.41**	69%	75.20 ± 2.09**	42%	22.64 ± 2.34**
IV (60 mg)	7.34 ± 0.77**	81%	3.62 ± 0.38**	90%	64.00 ± 3.58**	51%	21 87 ± 2 86**

*$P < 0.05$, **$P < 0.001$, (The doses of leaf powder are given in parenthesis).

germinal layers and cell debris in the lumen of the seminiferous tubules[8]. The antiandrogenic effect is evident in the reduction in the weight of accessory reproductive glands, regressive and degenerative changes in the seminal vesicle and ventral prostate[9]. It is known that sperm reproduction cannot preceed optimally to completion without a continuous androgen supply[10]. Studies on hypo-physectomy, castration and androgen replacement therapy reveal that androgen is essential for the maturation, motility and survival of the spermatozoa in the epididymis[11,12]. As the *A. indica* leaves have been reported as antispermatogenic and antiandrogenic, the adverse effect on the various sperm parameters would have resulted from the alterations in the epididymal milieu and the reduction in the sperm count might be due to the reduced output of the spermatozoa from the testis.

Sperms have two principal attributes, viz, the motility and the fertilizing ability which are the prerequisites for fertilization. Any negative impact on motility would seriously affect the fertilizing ability[13]. Sperm sample/ejaculate containing more than 20% of abnormal spermatozoa is considered to be more doubtful in fertility[6]. The negative fertility rate with reference to the implantations[8] supports this view. The recovery on the process of spermatogenesis, 24 days after the withdrawal of the treatment[8], suggests the possibility of recovery of sperms to normalcy after the withdrawal of the treatment. By increase in the relative percentage of abnormal sperm and reduced motility of sperm, *A. indica* leaf powder appears to be deleterious to the fertilizing ability of the sperm.

1. Sinha, K C., Riar, S C., Bardhan, J., Thomas, P., Kain, A. K and Jain, R. K., *Indian J. Med. Res.*, 1984, **80**, 708–710.
2. Sinha, K. C., Riar, S. S., Tiwary, R. S., Dhawan, A. K., Bardhan, J., Thomas, P., Kain, A. K. and Jain, R. K., *Indian J Med. Res.*, 1984, **79**, 131–136.
3. Deshpande, Y., Mendulkar, K. N. and Sadre, N. L., *J. Postgrad. Med.*, 1980, **26**, 167–170.
4. Choudhary, C. N., Singh, J. N., Verma, S. K. and Singh, B. P., *Indian J. Exp. Biol., 1990*, **28**, *714–716.*
5. Belsey, M. A., Eliasson, R., Gallegos, A. J., Moghissi, K. S., Paulsen, C. A. and Prasad, M. R. N., *Laboratory Manual for the Examination of Human Semen and Semen–Cervical Mucus Interaction*, WHO Press Concern, Singapore, 1980.
6. Bauer, J. D., Ackerman, P. G. and Toro, G., *Clin. Lab. Methods*, C. V. Mosby Co., St. Louis, 1974
7. Ratnasoorya, W. D., *Indian J. Exp. Biol.*, 1984, **22**, 463–466.
8. Parveen, D. S., M Phil. Dissertation, Karnatak University, Dharwad, 1990.
9. Kasturi, M., M Phil Dissertation, Karnatak University, Dharwad, 1990.
10. Brooks, D. E., *Physiol. Rev.*, 1981, **61**, 515–518.
11. Dyson, A. L. M. B. and Orgebein-Crist, M C., *Endocrinology*, 1973, **93**, 391–402.
12. Setty, B S., Rair, S. S. and Kar, A. B., *Fertil. Steril.*, 1977, **28**, 674–681.
13. Murugavel, T., Ruknudin, A., Thangavelu, S. and Akbarsha, M. A., *Curr. Sci.*, 1989, **58**, 1102–1103.

Received 10 August 1992; revised accepted 7 December 1992

MEETINGS/SYMPOSIA/SEMINARS

International Symposium on Tropical Tuber Crops

Place: Trivandrum, India
Date: 13–15 November 1993

The theme is—Tropical Tuber Crops: Problems, Prospects and Future Strategies. The Symposium will consist of seven sessions, viz. Crop improvement, Crop production, Crop protection, Processing and utilization, Biotechnology, Social sciences and Plenary session.

Contact: The Secretary,
Indian Society for Root Crops,
Central Tuber Crops Research Institute,
Sreekariyam, Trivandrum 695 017, India

International Conference on Physics & Industrial Development: Bridging the Gap

Date: January 17–19, 1994
Venue: The Taj Palace Intercontinental Hotel, New Delhi
Accommodation will be arranged in this hotel at $135 (Rs 2750 for Indian citizens) per room per night, single or double.
Registration fee: $300
$100 for delegates from developing countries

Tentative list of plenary speakers includes:
Leo Esaki, Nobel Laureate, President, Tsukuba University, Japan; C. Hilsum, FRS, Director of Research, GEC, London; Song Jian, Chairman, State Science & Technology Commission, China, C. K. N. Patel, Executive Director, AT&T Bell Labs, NJ, USA; Praveen Chaudhari, IBM Thomas J. Watson Centre, New York; P. Rama Rao, Secretary, DST, J. J. Irani, President, CII and Managing Director, TISCO,

Contact: Prof. S. Chandrasekhar, Centre for Liquid Crystal Research
P. B. No 1329, Jalahalli, Bangalore 560 013, India

Telephone: +91 80 340124, Extn. 226 337123 Telefax +91 80 340492