Ancient Science of Life Vol. No XX (1&2) July, August, September, October 2000 Pages 77 - 81

SPERMICIDAL ACTIVITY IN VITRO OF BARK EXTRACT OF AZADIRACHTA INDICA IN RATS

VINITA BHARGAVA and A.O. PRAKASH
Laboratory of contraceptive research & Reproductive Health
School of studies in Zoology, Jiwaji University, Gwalior – 474011

Received: 24.4.2000 **Accepted: 26.7.2000**

ABSTRACT: *Ethanolic extract of bark of Azadirachta indica showed potent spermicidal activity when tested on rat spermatozoa in vitro. When the extract was applied in 1:1 and 1:2 ratio at tree different concentration using wet drop method, cent percent mortality of spermatozoa was observed within 10 seconds. 10% concentration of the extract was considered as an effective dose.*

INTRODUCTION

Azadirachta indica commonly known as Neem is a popular plant and cosmopolatian in distribution. Neem is having rich medicinal properties (1). Various fractions of different extract of A. indica have been tested for spermicidal activity. Neem oil has been reported as a good spermicidal agent when tested through intravaginal administration (2,3,4) praneem, a herbal preparation containing Neem oil and saponins form soap nut sowed good spermicidal activity in rats (5). Inspite of extensive research on Neem, no safe and potent spermicidal agent has been developed so far from this plant. Moreover, most of these preparations belong to oil and no other part has been studied, bark of Neem has been studied a little in this area. Therefore, the present investigation has been carried out to evaluate the spermicidal activity of 50% ethanolic extract of bark of A indica A Juss. In rat spermatozoa through in-vitro Studies.

MATERIALS AND METHODS

(I) Animal; Adult healthy male rats of Wistar strain (160 ± 10 g) were selected for the present study and were maintained under uniform husbandry conditions of light and temperature and were given rat palleted diet and water ad libitum.

(ii) Preparation of Extract: The stem bark of Neem shade, grinded to powder form. Extract of this portion was prepared using dumping method (6). The powdered form of plant material was dumped with 50% ethanol in big air tight glass jar. Before putting into glass jar, the dried powder of stem bark was weight . After regular stirring for 2-3 weeks, the supernatant was collected and evaporated to dryness under reduced pressure and low temperature. Obtained dried mass was kept in sealed bottles and stored in a refrigerator till its use. Different dilutions were made in Ringer's Loke's solution whose pH was adjusted to 7.4.

(iii) Preparation of spermatozoa suspension: Healthy albino male rats form Wistar strain (160 ± 10g) were anaesthetized using light there anesthesia and with the help of fine scissor the skin of the scrotum was cut and a mild scrotal incision was made to pose tunica vaginalis. Small incision as then

made on the upper part infornt of tunica vaginalis to make a rent s the at testes and the epididymis can come out through this rent. After pulling out the tests, contents of cauda epididymis were removed and flushed in Locks Ringer solution buffer to make suspension of spermatozoa. The testes were returned back tough the rent and the wound was sutured.

(iv) Assessment of the Motility of spermatozoa: Wet drop method was adopted to evaluate the motility of spermatozoa (7) Under this method two different protocols were adopted.

(i) After missing the spermatozoa suspension with the preparation: In this experiment 2 µl of spermatozoa suspension and 2 µl of bark extract (1:1) was placed on a clean glass slide and it was mixed quickly and gently and then it was covered by glass cover slip. Similarly, in another experiment, spermatozoa suspension and extract was taken in 2:1 ration was placed on a glass slide, mixed and covered with cover slip. It was examined under microscope and motility of spermatozoa was observed at various time intervals upto 60 seconds

(ii) Spermatozoa suspension with the preparation at junction: In this experiment a drop of 2 µl of spermatozoa suspension was placed on a clean glass slide and just nearest to it a drop of the same volume of extract was placed and ten both of these drops were covered with a common glass cover slop by which a junction of spermatozoa and extract was formed. It was examined under microcope and the motility of spermatozoa and extract was formed. It was examined under microcope and the motility of spermatozoa was observed at various time intervals upto 150 seconds

RESULTS

Table – 1 revealed that control rats showed initial motility of about 80 % which remained almost static upto 60 seconds as observed in present study. When extract and spermatozoa suspension was applied in 1:1 ratio at 10% of concentration of extract, 100% mortality of spermatozoa was observed within 10 seconds, when the spermatozoa suspension was prepared in the 1:2 ratio at 10% concentration all spermatozoa were found dead with in 20 seconds.

DISCUSSION

Number of approaches are being used to control fertility in males and females however it is yet difficult to suggest a specific method which is quite safe, effective and economic. Use of chemical contraceptives by a women is effective but it has some limitations owing to some undersired effects, since last decades, efforts are being made to work on other areas such as spermicidal agents. In order to achieve this goal spermicidal agents are being used but most of them belong to synthetic group and cause irritation and itching to vaginal epithelium. Herbal preparation have also been tested and praneem is one of them (5). Although this preparation has been clinically tested in number of countries (5), but has not been accepted uniformly. Sodium nimbidinate, and nimbinate have also been studied long back for their spermicidal activity on human spermatozoa, but these agents could not be developed further (8,9). Neem oil when administered intravaginally caused spermicidal activity (2). Neem rich

and neem 76 have also been reported as a good spermicidal agents (3,4) Garg and co-workers (10) have conducted studies on rats fed with neem seed kernel cake (NSKC), water washed NSKC solvent extracted NSKC, or solvent extracted cum water washed NSKC along with other conventional feeds for 50 days. The result showed tat none of the treatment could make NSKC free from bitter/toxic responsible for adverse effects on growth and reproductive development in rats. Bark extract of A. indica having the active Constituents has been tested in the present investigation and is found very effective to immobalize the rat spermatozoa through in-vitro studiese. Under the wet drop method, the application of 50% ethanolic extract of the bark of A. indica and spermatozoa suspension in the ratio of 1:1 caused immediate morality, however the diluted extract took about 20 seconds. The finding of the present investigation clearly suggest tat it is not only the Neem oil or saponins but bark extract (50% ethanolic) too ma be used as a potent spermicidal agent.

ACKNOWLEDGEMENTS

The authors are thankful to prof R. Mathur, school of studies in Zoology for providing necessary facilities.

REFERENCE:

1. Kirtikar, K,R. and Baus, B.D. “Indian Medicinal Plants” Vol 1, Lalit Mohan Basu 49, Leader Road, Allahabad, 536,541,(1935).

2. Sinha , K.C., Rair S.S., Tiwari, R.S., Dhawan, A.K. Bardhan, J., Thomas, P.,. Kain, A.K. and Jain , R.K. Neem Oil as vaginal contraceptive, Indian J, Med Research, 79,136 (1984).

3. Rair S.S., Dev Kumar, C., Iiavazagen G., Bardhan, J., Kain, A.K., Thomas, P., Singh B. Volatile fraction of Neem oil as a spermicidal. Contraception, 42(4), 479, (1990).

4. Rair, S.S., Sawhney, R.C., Tiarahagan, G., Roy J.B. Kain A.K., Thomas P., Singh, R., Singh, B. and Dev kumar, C. Neem as a contraceptive, world Neem Conference 85, Bangalore (1993).

5. Talwar, G.P., Garg, Taluja, V., Kaur, R. and Upadhyay, S Dual approaches for contraceptive and reproductive health. Department of zoology, University of Rajasthan, Jaipur, Feb 6-4,19, (1994).

6. Prakash, A.O. Saxena, V., Shukla, S. and Mathur, R. Contraceptive potency of Puraria tuberosa D.C and its hormonal status, Acta Europea fertililatis (1985).

7. W.H.O. Laboratory manual for the examination of human semen and semen cervical mucus interaction, Published by press concern, Singapore (1980).

8. Sharma Y.N. and saksena, K.P. Sodium ninbidinate in vitro study of its spermicidal action. Indian J. Med Sci., 13,1038,(1959a)

9. Sharma Y.N. and saksena, K.P. Spermicidal activity of sodium ninbidnate. Indian J. Med. Research, 47,322,(1959 b).

10. Garg A.K., Agrawal, D.K., Singh, S.D. and Nath, K. Growth and spermatogenesis in rats fed solvent extracted. Water washed and untreated Neem. Indian J, Animal Sci., 7(2) 223, (1992).

Table -1
EFFECT OF ETHANOLIC EXTRACT OF BARK OF AZADIRACHTA INDICA ON THE MOTHILITY OF RAT SPERMATOZOA (IN-VITRO)
(Values are Mean ± S.E.., N=6)
(Using wet drop method after mixing)

Extract: Semen Suspension	Initial motility %	Extract %	Motility of spermatozoa (%) at various time intervals (sec)					
			10	20	30	40	50	60
Control	80.2 ± 7.9	-	80.2 ± 7.9	80.2 ± 7.9	80.2 ± 7.9	80.2 ± 7.9	80.2 ± 7.9	78.3 ± 29
1:1	-	1	22.5±2.7	6.2 ± 1.2	All dead			
1:1	-	5	3.5±1.8	All dead				
1:1	-	10	All dead					
1:2	-	1	40.1± 7.9	33.1 ± 5.1	27.2 ± 5.1	11.5 ± 3.0	4.1 ± 1.1	All dead
1:2	-	5	11.2 ± 1.9	4.8 ± 1.9	All dead			
1:2	-	10	81 ± 0.7	All dead				

Table -2
EFFECT OF ETHANOLIC EXTRACT OF BARK OF AZADIRACHTA INDICA ON THE MOTHILITY OF RAT SPERMATOZOA (IN-VITRO)
(values are Mean ± S.E.., N=6)
(Using wet drop method after mixing)

Extract: Semen Suspension	Initial motility %	Extract%	Motility of spermatozoa (%) at various time intervals (sec)							
			10	20	30	40	60	90	120	150
Control	80.2 ± 7.9	-	80.2 ± 7.9	80.2 ± 7.9	80.2 ± 7.9	80.2 ± 7.9	80.2 ± 7.9	78.3 ± 2.9	78.3 ± 2.9	74.2 ± 7.1
1:1	-	1	69.1 ± 5.7	55.1 ± 6.3	35.2 ± 4.0	26.2 ±2.4	16.1 ± 3.2	35.2 ± 3.8	8.2 ± 1.9	All dead
1:1	-	5	35.2 ± 3.8	22.2 ± 3.8	15.1 ± 3.8	9.0 ± 2.4	All dead			
1:1	-	10	13.5 ± 3.1	6.9 ± 2.0	All dead					

pages 77 - 81