EFFECT OF NEEM LEAF EXTRACTS ON EPIDIDYMAL SPERM PARAMETERS IN ALBINO MICE

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ABSTRACT

The effect of oral administration of neem (*Azadirachta indica*) leaf extract was studied on various sperm parameters in male albino mice. Oral administration of neem leaf extracts at a dose level of 132, 200 and 300 mg/kg body weight/day for 24 days caused alterations in various sperm parameters of cauda epididymis in albino mice. The change in cauda epididymal sperm parameters suggests that neem leaf extract has the potential to alter the fertilizing ability of mature sperms.

Keywords: Neem, Sperm, Fertilizing ability

INTRODUCTION

Neem is a versatile gift of nature (Tripathi, 1998). Earlier work has also revealed the antifertility effect of neem bark in male albino mice (Chakravarty et al., 2003). The antifertility properties of different neem extracts have also been reported by various workers (Mukherjee et al., 1999; Aladakatti and Ahmed, 1999; Puri et al., 2003). The present study was undertaken to know the effect of oral administration of different doses of neem leaf extracts on fertilizing ability of cauda epididymal sperm parameters by studying various sperm parameters like sperm count, per cent sperm motility, live sperm, dead sperms, normal sperms and abnormal sperms.

MATERIALS AND METHODS

Preparation of extracts: Leaves of neem trees (*Azadirachta indica)* were collected, shade dried and powdered. The leaf extract was prepared according to the method suggested by Chattopadhyay (1993). The powder was extracted by percolation at room temperature with 70 % ethanol. The extract was then concentrated in boiling water bath and finally dried in a desiccator. The residue was dissolved in propylene glycol at the rate of 100 mg/ml and was then used.

Grouping of mice: Adult male albino mice of 8-10 weeks age and average 30 gm body weight were procured from breeding house at Small Animal Colony of Department of Zoology and Fisheries, Punjab Agricultural University, Ludhiana. The mice were kept under standard conditions and fed with standard rat feed and water *ad libitum*. The adult mice were divided into different groups (8 in each group) for the administration of neem extracts.

Treatments: All the doses of neem extracts were administered orally for 24 days to the adult male mice. Three different doses (treatments) given to adult male mice are given below.

Dose 1. 132 mg/kg body weight (bw)/day for 24 days.
Dose 2. 200 mg/kg bw/day for 24 days.
Dose 3. 300 mg/kg bw/day for 24 days.

Each treatment was compared with control that received propylene glycol (vehicle) only for 24 days. The mice of each group were sacrificed 24 hours after the administration of last dose

Study of different sperm parameters: From the sacrificed mice, the epididymides were dissected out, blotted free of mucus and weighed accurately. Cauda epididymal fluid was obtained by incising cauda epididymis in 0.5 % glucose-saline for the study of various sperm parameters such as

(i) Sperm count (millions/ml)
(ii) Sperm motility (%)
(iii) Live sperms (%)
(iv) Dead sperms (%)
(v) Normal sperms (%)
(vi) Abnormal sperms (%)

Sperm count was determined by haemocytometric method (Salisbury et al., 1978). The motile sperms were calculated per unit area and expressed as per cent motility (Prasad et al., 1972). Cauda epididymal fluid were smeared with 0.1 % eosin stain to count live and dead sperms. Air dried smears of cauda epididymal fluid were stained with 2 % Giemsa stain to count normal and abnormal sperms.

RESULTS

The cauda epididymides of all the groups were accurately weighed. Various sperm parameters such as sperm count and per cent sperm motility, live and dead sperms and normal and abnormal sperms were studied by mincing the cauda epididymides of vehicle administered

group and of neem leaf extract administered groups in saline solution.

Weight of cauda epididymis: The change in the weight of cauda epididymides was observed after the administration of different doses of leaf extracts as compared to their control groups. A non-significant reduction in the cauda epididymal weight was observed in mice treated with leaf extract of 132 mg/kg bw in comparison to their control (Table 1).

Table 1. Effect of oral administration of leaf extract (132 mg/kg bw/day) for 24 days on cauda epididymal weight and various sperm parameters in albino mice

Parameters	Control	Treated (Dose 1)
Weight of cauda epididymis (g/100g bw)	0.044 ± 0.001	0.041 ± 0.001
Sperm count (millions/ml)	48.00 ± 1.247	43.33 ± 1.666
Sperm motility (%)	71.333 ± 1.515	39.33 ± 1.440**
Live sperms (%)	74.401 ± 0.722	57.00 ± 1.700**
Dead sperms (%)	25.567 ± 0.722	43.00 ± 1.700**
Normal sperms (%)	88.667 ± 0.272	82.00 ± 2.055*
Abnormal sperms (%)	11.333 ± 0.272	18.00 ± 2.055*

Values are mean ± standard error; **indicates significant change as compared to control at P ≤ 0.01; *indicates significant change as compared to control at P ≤ 0.05

Moreover, a significant reduction in the weight of cauda epididymides was observed in mice treated with 200 mg/kg bw (Table 2) and 300 mg/kg bw (Table 3) in comparison to their vehicle treated groups.

able 2. Effect of oral administration of leaf extract (200 mg/kg bw/day) for 24 days on cauda epididymal weight and various sperm parameters in albino mice

Parameters	Control	Treated (Dose 2)
Weight of cauda epididymis (g/100 g bw)	0.036 ± 0.005	0.025 ± 0.001*
Sperm count (millions/ml)	44.667 ± 1.186	19.000 ± 0.471**
Sperm motility (%)	66.667 ± 1.963	9.667 ± 0.720**
Live sperms (%)	75.333 ± 1.186	24.573 ± 1.490**
Dead sperms (%)	24.667 ± 1.186	75.427 ± 1.490**
Normal sperms (%)	85.000 ± 2.055	31.593 ± 1.392**
Abnormal sperms (%)	15.000 ± 2.055	68.403 ± 1.392**

Values are mean ± standard error; **indicates significant change as compared to control at P ≤ 0.01; *indicates significant change as compared to control at P ≤ 0.05

able 3. Effect of Oral administration of leaf extract (300 mg/kg bw/day) for 24 days on cauda epididymal weight and various sperm parameters in albino mice

Parameters	Control	Treated (Dose 3)
Weight of cauda epididymis (g/100g bw)	0.038 ± 0.004	0.026 ± 0.002*
Sperm count (millions/ml)	44.333 ± 1.785	< 1 million **

Sperm motility (%)	66.667 ± 1.963	3.00 ± 0.47**
Live sperms (%)	77.210 ± 1.276	9.10 ± 1.791**
Dead sperms (%)	22.790 ± 1.276	90.90 ± 1.791**
Normal sperms (%)	84.000 ± 1.700	10.83 ± 0.36**
Abnormal sperms (%)	16.000 ± 1.700	89.167 ± 0.36**

Values are mean ± standard error; **indicates significant change as compared to control at P ≤ 0.01; *indicates significant change as compared to control at P ≤ 0.05

Sperm count: There was dose dependent reduction in sperm count of groups administered with different doses of leaf extract. In the mice administered with 132 mg/kg body weight of leaf extract., the number of sperms reduced non-significantly (43.33 ± 1.666) (Table 1) as compared to control mice (48.00 ± 1.247). The sperm count reduced significantly in groups administered with leaf extract at the dose level of 200 mg/kg body weight (19.00 ± 0.471) and 300 mg/kg body weight (< 1 million) as compared to their vehicle controls in which sperm count was 44.667 ± 1.186 and 46.333 ± 1.785, respectively (Tables 2 and 3).

Per cent sperm motility: The sperm motility was affected significantly after the administration of all three doses of leaf extract. In vehicle control mice for different doses, it was 71.333 ± 1.515, 66.667 ± 1.963 and 66.667 ± 1.963 as compared to 39.333 ± 1.44, 9.667 ± 0.72 and 3.00 ± 0.471, respectively after the administration of 132, 200 and 300 mg/kg bw/day of leaf extract, respectively.

Per cent live sperms: There was significant reduction in the per cent live sperms of cauda epididymides of mice of groups administered with different doses of leaf extract. Per cent live sperms decreased from 74.401 ± 0.722, 75.333 ± 1.186 and 77.21 ± 1.276 in vehicle treated mice to 57.000 ± 1.70 (Table 1); 24.573 ± 1.490 (Table 2) and 9.100 ± 1.791 (Table 3) in 132, 200 and 300 mg/kg body weight leaf extract treated mice, respectively. Reduction in per cent live sperms in different groups was dose dependent.

Per cent dead sperms: A significant increase in per cent dead sperms of cauda epididymides was observed in mice administered with all the three doses of leaf extract. Per cent dead sperms in vehicle treated mice was 25.567 ± 0.722, 24.667 ± 1.186 and 22.790 ± 1.276 while, it was 43.00 ± 1.70, 75.427 ± 1.490 and 90.90 ± 1.791 in 132, 200 and 300 mg/kg bw in treated mice, respectively (Table 1, 2 and 3).

Per cent normal sperms: There was significant reduction in per cent normal sperms of groups administered with different doses of leaf extract. In vehicle control group per cent normal sperms were 88.667 ± 0.272, 85.00 ± 2.055 and 84.000 ± 1.700 while, it reduced to 82.00 ± 2.055, 31.593 ± 1.392 and 10.83 ± 0.36 in 132 (Table 1), 200 (Table 2) and 300 mg/kg bw of treated mice (Table 3), respectively.

Per cent abnormal sperms: A number of sperm abnormalities were observed in mice after the administration of various doses of neem extracts. Per cent abnormal sperms increased from 11.333 ± 0.272, 15.00 ± 2.055 and 16.000 ± 1.70 in vehicle treated mice serving as control to 18.00 ± 2.055, 68.403 ± 1.392 and 89.167 ± 0.360 in 132 (Table 1), 200 (Table 2) and

300 mg/kg bw of treated mice (Table 3), respectively.

DISCUSSION

The reduction in weight of cauda epididymis can be attributed to androgen deficiency. Earlier studies have also revealed that androgen deficiency was the cause of reduced epididymal weight in animal species treated with plant extract (Khanna et al., 1986; Akbarsha et al., 1990). Treated groups revealed significant change in all sperm parameters (Tables 1, 2 and 3). Decrease in cauda epididymal sperm count was also observed after administration of various plants extracts such as *Solanum xanthocarpum* in rats (Seth et al., 1981; Sarkar et al., 2000). Khanna et al. (1986) reported decreased sperm count in vas deferens of rats treated with Tulsi pellets. Administration of different neem extracts at all dose levels caused significant reduction in the number of motile sperms of cauda epididymides. Reduced motility has also been reported in rodents administered with different plants extracts such as *Ocimum sanctum* leaves (Seth et al., 1981), *Vinca rosea* leaves (Murugavel et al., 1989), and *Carica papaya* seeds (Chinoy et al., 1995). Reduction in protein concentration of spermatozoa can be one of the causative factors for reduction of sperm motility (Hoskins et al., 1978). Significant reduction in live and normal sperms was also reported by Murugavel et al. (1989) in mice administered with *Vinca rosea* leaf extract. Increase in per cent dead sperms indicated that *Azadirachta indica* is deleterious at the level of cauda epididymis and has the potential to decrease the fertilizing ability of epididymal sperms. Androgen deficiency was attributed to reduce the number of normal sperms as androgens are essential for maturation and survival of spermatozoa (Shaikh et al., 1993).

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