CIBTech Journal of Zoology ISSN: 2319–3883
An Online International Journal, Available at http://www.cibtech.org/cjz.htm
2025 Vol.14, pp.278-285/Anju
Research Article

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

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ABSTRACT
Oral Administration of Neem (*Azadirachta indica*) bark extracts at a dose level of 200 and 300 mg/kg body weight per day for 24 days caused reduction in the weight of epididymis and remarkable alterations in various sperm parameters of cauda epididymis in albino mice. The changes in sperm parameters of cauda epididymis suggest that neem bark possesses incredible potential in altering the fertilizing ability of the matured sperms.

Keywords: *Neem bark, Fertilizing Ability, Sperm*

INTRODUCTION
Nature has provided us with several resources. One of these resources are plants. Man has depended upon plants for his survival like food, clothes, shelter and medicines. (Brahmachari, 2004). Medicinal plants used to be the most important part of human society. In Ayurvedic medicine neem has been used for more than 4000 years due to its medicinal properties. In Sanskrit, Neem is called "Arishta" which means perfect, complete and imperishable (Girish and Bhat, 2008). Neem possesses anti-bacterial, anti-fungal, anti- parasitic, anti- inflammatory and many other medicinal properties. Neem also possesses a remarkable property and that is its antifertility effect. The antifertility property of neem extracts has also been reported by various workers (Mukherjee *et al.,* 1999; Aladakatti and Ahamad, 1999; Puri *et al.,*2003; Puri, 2015). Neem extracts have been tested for its contraceptive efficiency in both males and females (Shweta *et al.,* 2011; Azamthulla *et al*.,2015). A large number of chemical agents have been known to induce contraceptive effect in males but these chemical agents have enormous side effects and may lead to total spermatogenic arrest and induce irreversible sterility (El-Kashoury *et al.,* 2009). Therefore, the present study was carried out to know the effect of neem bark extract as an anti- fertility agent in male rodents.

MATERIALS AND METHODS
Grouping of mice: Adult male albino mice of 8-10 weeks age and average 30 grams (g) body weight (bw) 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 bark extracts.
Preparation of extracts: Bark collected from neem plant was powdered. The powder was extracted by percolation at room temperature with 70 percent ethyl alcohol. The extract was finally concentrated under reduced pressure and dried in vacuum desiccator. The bark residue was dissolved in propylene glycol (vehicle) at the rate of 100 mg/ml and was used in present experimental study.
Treatments: Acclimatized mice were divided into different groups. All the doses of neem extracts were administered orally for 24 days to adult male mice. Two different doses (treatments) given to adult male albino mice are given below:
Dose 1: 200mg /kg bw/day for 24 days.
Dose 2: 300mg /kg bw/dayfor 24 days.

Each treatment was compared to its own control that received vehicle for the same period of treatment. The mice of each treatment were sacrificed 24 hours after the administration of last dose.

Study of different sperm parameters: The epididymis were dissected out from the sacrificed mice, mucus was removed and weighed accurately. Cauda epididymal fluid was obtained by incising the cauda epididymis in 0.5 per cent glucose-saline solution for the study of various sperm parameters such as:

- Sperm count (millions/ml)
- Sperm motility (%)
- Live sperms (%)
- Dead sperms (%)
- Normal sperms (%)
- 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 was 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. Epithelial height of cauda epididymides was determined by Stage-ocular meter.

RESULTS AND DISCUSSION

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

Effect on Cauda epididymides: The change in the weight of cauda epididymides was observed after the administration of different doses of bark extracts as compared to their control groups. A non- significant reduction in cauda epididymal weight was observed in mice treated with dose 1 (200 mg/kg bw) (Table 1) in comparision to their control while weight of cauda epididymides reduced significantly in mice treated with dose 2 i.e. mice treated with 300 mg/kg bw (Table 2).A remarkable reduction in mean epithelial height of cauda epididymis was observed in mice treated with bark extract at the dose level of 300 mg/kg bw/day for 24 days.(Figure I). In both the treated groups, the lumen showed less number of sperms as compared to control (Figure I).

Sperm count: There was dose dependent reduction in sperm count of groups administered with two different doses of bark extracts. The sperm count decreased significantly in both groups treated with dose1 and dose2 of bark extracts (Table 1 & 2).

Percent sperm motility: The sperm motility was affected significantly after the administration of both the doses of bark extracts (Table 1 & 2).

Percent live and dead sperms: There was significant reduction in the percent live sperms of cauda epididymides of mice of groups administered with two different doses of bark extracts (200 and 300 mg/kg bw/day). On the other hand, a significant increase in per cent dead sperms of cauda epididymides was observed in mice treated with both the doses of bark extracts (Table 1 &2).

Percent normal and abnormal sperms: There was significant reduction in per cent normal sperms of groups administered with different doses of bark extracts. A number of sperm abnormalities were observed in mice administered with different doses of neem bark extracts. Percent abnormal sperms increased significantly in groups administered with dose 1 and dose 2 of bark extracts (Table 1& 2).

A number of sperm abnormalities were observed in mice after the administration of neem bark extracts. The various characteristics of abnormal sperms were coiled tails, abnormal position and shape of head, bending of middle piece, headless spermatozoa and agglutination of sperms etc (Figure. II & III).

The reduction in the weight of cauda epididymis may be due to androgen deficiency. Earlier studies have also revealed that androgen deficiency was the cause of reduced epididymal weight in animal species treated with plant extracts (Khanna *et al.,* 1986; Akbarsha *et al*., 1990).
Groups treated with dose 1 and dose 2 of neem bark extracts revealed significant changes in all sperm parameters (Table 1& 2). Administration of various plant extracts also resulted in decrease in cauda epididymal sperm count in rats (Seth *et al.,* 1981; Sarkar *et al.,* 2000). Sheikh *et al* (1993) suggested that anti androgenic effect of plant product to be responsible for decreased epididymal sperm count. FSH dprivation may also be the cause of decreased sperm count (Lohiya *et al.,* 1999). Khanna *et al.* (1986) reported decreased sperm count in vas deferens of rats treated with tulsi pellets. Administration of different doses of bark extracts caused significant reduction in the number of motile sperms of cauda epididymides. Reduced motility has also been reported in rodents administered with different plant extracts (Chinoy *et al*., 1995; Sarkar *et al.,* 2000).

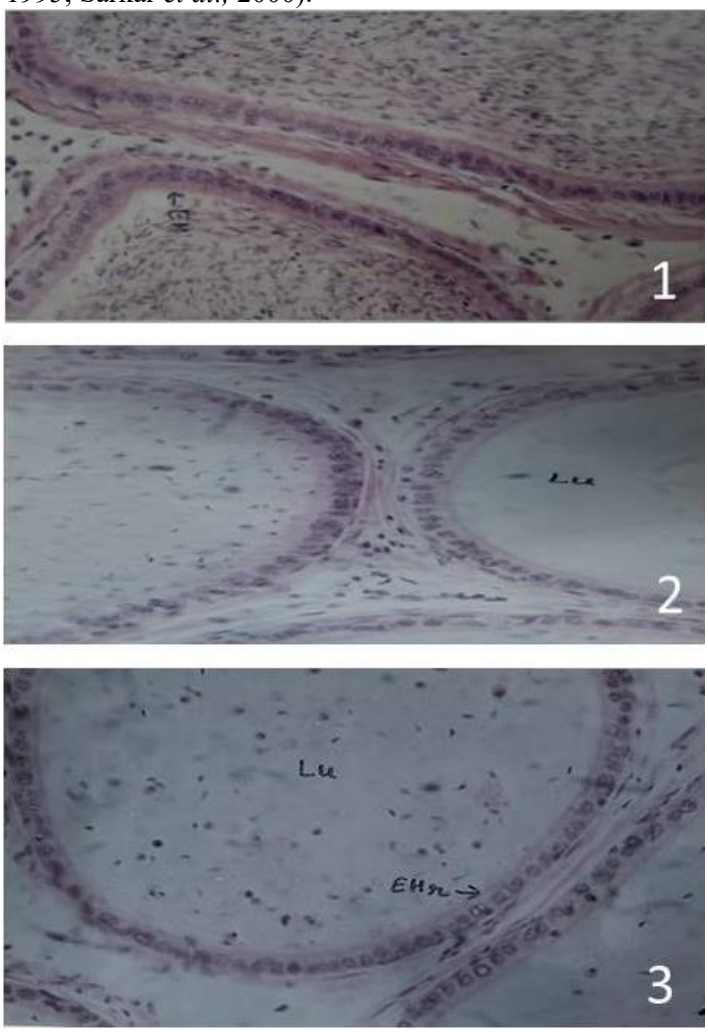

FIGURE I: *Mpg 1: Cauda epididymal tubules of control mice showing epithelial height and lumen full of sperms(10 × 40)*
Mpgs 2&3: Epididymal tubules of mice showing reduced epithelial height (mpg3) and lumen devoid of sperms in mice treated with bark extract at the dose level of 200 and 300 mg/kg bw/day for 24 days respectively(10 × 40).

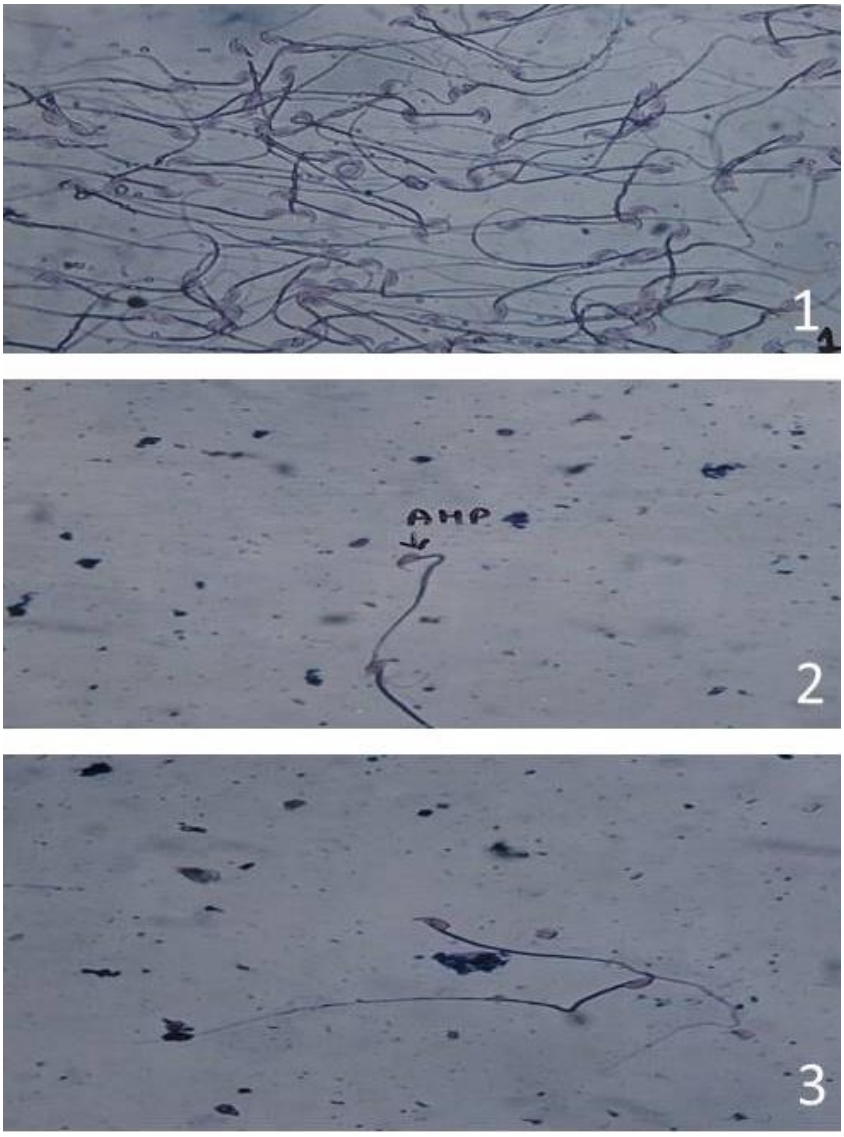

FIGURE II: *Mpgs 1: Showing normal sperms (10×40) in untreated normal mice.*
Mpgs 2&3: Showing sperms with abnormal head shape, bending of mid piece and coiled tail in mice treated with bark extract at the dose level of 200 mg/kg bw/day for 24 days(10×40).

Motility and fertilizing ability are two main characteristics of sperms. Motility is an important pre-requisite for fertilization in case of organisms with internal fertilization. Fertilizing ability is seriously impacted with reduced sperm motility (Murugavel et al 1989). Gupta *et al* (2001) reported that androgen deprivation resulted in decreased sperm motility in male rats treated with *Colebrookia oppostifoli**a***. In the present study reduction in sperm motility might have resulted due to alterations in epididymal milieu as it has been reported earlier after the administration of plant extracts (Chinoy *et al*., 1997).
Significant reduction in live and normal sperms was also reported by Murugavel *et al* (1989) in mice administered with *Vinca rosea* leaf extract. Shaikh *et al* (1993) suggested that reduction in the percentage of live sperms might be due to androgen deficiency as androgens are essential for maturation and survival of spermatozoa.

The percentage of abnormal sperms increased significantly in mice after the administration of neem bark extracts. Sperm abnormalities like coiled tails, detached head, irregular head and mid piece bending were observed in mice after the administration of different doses of
bark extracts. Aladakatti and Ahamed (1999) observed morphological changes in spermatozoa in rats treated with *Azadirachta indica* leaf extract.
The present study has clearly revealed that neem bark extract is deleterious to diiferent sperm parameters and hence has a remarkable potential in affecting the fertililizing ability of matured epididymal sperms and thus can be exploited to great extent as an antifertility agent.

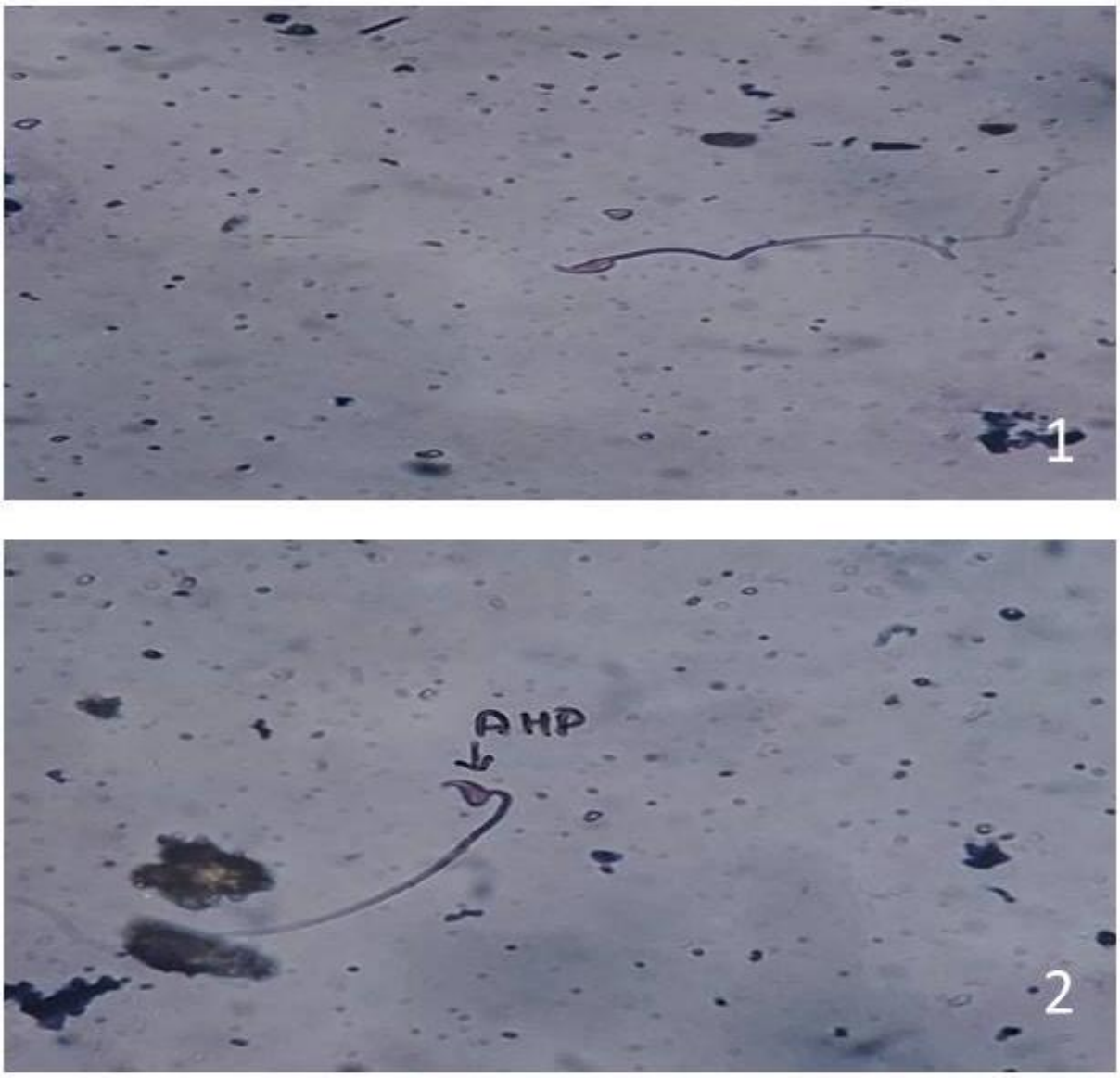

FIGURE III: *Mpgs 1 & 2 : Showing sperms with coiled mid piece and tail, abnormal position of head in mice treated with bark extract at the dose level of 300 mg/kg bw/day for 24 days(10×40).*
***Abbreviations**: EH: Epithelial height; Lu: Lumen; EHr: Reduced epithelial height; AHP: Abnormal head position.*

Table 1: Effect of oral administration of bark extract (200 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)	0.038 ± 0.004	0.034 ± 0.008
Sperm Count(Millions/ml)	43.323 ± 1.785	20.667 ± 1.440* (48)
Sperm motility (%)	70.00 ± 0.471	12.333 ± 3.538*(18)
Live sperms (%)	77.210 ± 1.276	26.333 ± 5.894* (34)
Dead sperms (%)	22.790 ± 1.276	73.667 ± 5.894* (323)
Normal sperms (%)	82.333 ± 1.515	52.667 ± 1.440* (64)
Abnormal sperms (%)	17.667 ± 1.515	47.330 ± 1.440* (268)

Values are Mean ± S.E.,values in parenthesis are % of the control.
*P ≤ 0.01 : * indicates significant change as compared to control.*

Table 2: Effect of oral administration of bark extract (300 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)	0.038 ± 0.005	0.031 ± 0.002* (82)
Sperm count (millions/ml)	42.333± 1.186	23.333 ± 1.440*(55)
Sperm motility(%)	66.660 ± 1.970	12.667 ± 2.681* (19)
Live Sperms (%)	71.797 ± 0.763	34.167 ± 1.879* (48)
Dead Sperms (%)	28.202 ± 0.763	65.883 ± 1.879* (233)
Normal sperms(%)	80.333 ± 0.720	42.667 ± 2.177* (53)
Abnormal sperms(%)	19.667 ± 0.720	57.333 ± 2.177* (292)

Values are Mean ± S.E., values in parenthesis are % of the control.
*P ≤ 0.01 : * indicates significant change as compared to control*

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