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***Research Article** (Open Access)*

ANTI-SPERMATOGENIC EFFECT OF NEEM OIL IN MALE ALBINO MICE

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ABSTRACT
Feeding neem oil at the dose level of 2.0, 3.3 and 4.6 ml/kg body weight/day for 24 days resulted in reduction in testicular weight, reduction in the diameter of seminiferous tubules and shrinkage of seminiferous tubules with increased inter-tubular space.

Keywords: *Neem oil, Seminiferous Tubules*

INTRODUCTION

Since the dawn of civilisation, plants have always been used as a safe and natural source of medicine. Man relies on plants to meet the basic needs of life such as food, shelter and health (Sharma *et al* 2013). Plants are important sources of pharmacologically active compounds and these have been proved to have anti-microbial (Arora and Kaur, 1999), anti-fungal, anti-inflammatory and their anti-fertility effects in both male and female. Neem is a plant well known for its outstanding properties (Tripathi, 1998). The anti-fertility properties of different neem extracts have been reported earlier by various workers (Shaikh *et al.,* 1993; Mukherjee *et al* .,1999; Aladakatti and Ahamed,1999; Kasturi *et al.,* 2002) in male animal species. Neem oil is derived from seeds of neem (*Azadirachta indica*) plant. Previous studies show that neem oil induces spermicidal activity in vitro and in vivo (Riar *et al.,* 1990; Bardhan *et al.,* 1991). Neem oil has contraceptive efficacy after intrauterine administration in female rats.

Different neem extracts have already been investigated for their anti-spermatogenic and antifertility effect in male animal species when administered orally (Chakravarty and Hans, 2004 ; Prasad *et al*., 1997).

The present investigation was carried out to study the effect of oral administration of neem oil on histophysiology of testes of mature albino mice.

MATERIALS AND METHODS

Adult male albino mice of 8 weeks old and average 30 grams (g) body weight (bw) were procured from the breeding house at Small Animal Colony, Department of Zoology and Fisheries, Punjab Agricultural University, Ludhiana. The mice were allowed to acclimatize for few days and were fed with standard rat feed and water ad libitum. For oral administration, neem oil was purchased from local market of Ludhiana. Acclimatized mice were divided into four different groups (8 in each group). Group I served as control and did not receive any treatment. Group II, III and IV received neem oil orally at the dose level of 2.0, 3.3 and 4.6 ml/kg bw/day for 24 days. The mice of all the groups were sacrificed 24 hours after the administration of last dose. Their testes were dissected out, mucus was removed and then weighed accurately. After that the testes of sacrificed mice were processed for histophysiological studies. The diameter of seminiferous tubules was recorded with stage-occular meter. To record the mean seminiferous tubule diameter, about 25 seminiferous tubules were studied. Identification of different stages of spermatogenic cells was done with criterion used earlier (Belleve *et al.,* 1977).

RESULTS AND DISCUSSION

A significant reduction in testicular weight was observed after the administration of all the three doses of neem oil with respect to control group (Table 1).

The oral administration of neem oil caused significant reduction in the diameter of seminiferous tubules in treated mice as compared to control mice. Seminiferous tubule diameter of Control group

mice was 187.600±8.500 while that of Groups II, III and IV mice was 147.810± 3.406,133.770 ±2.345 and 133.770 ±2.578 respectively (Table 1; Fig. 1&2).

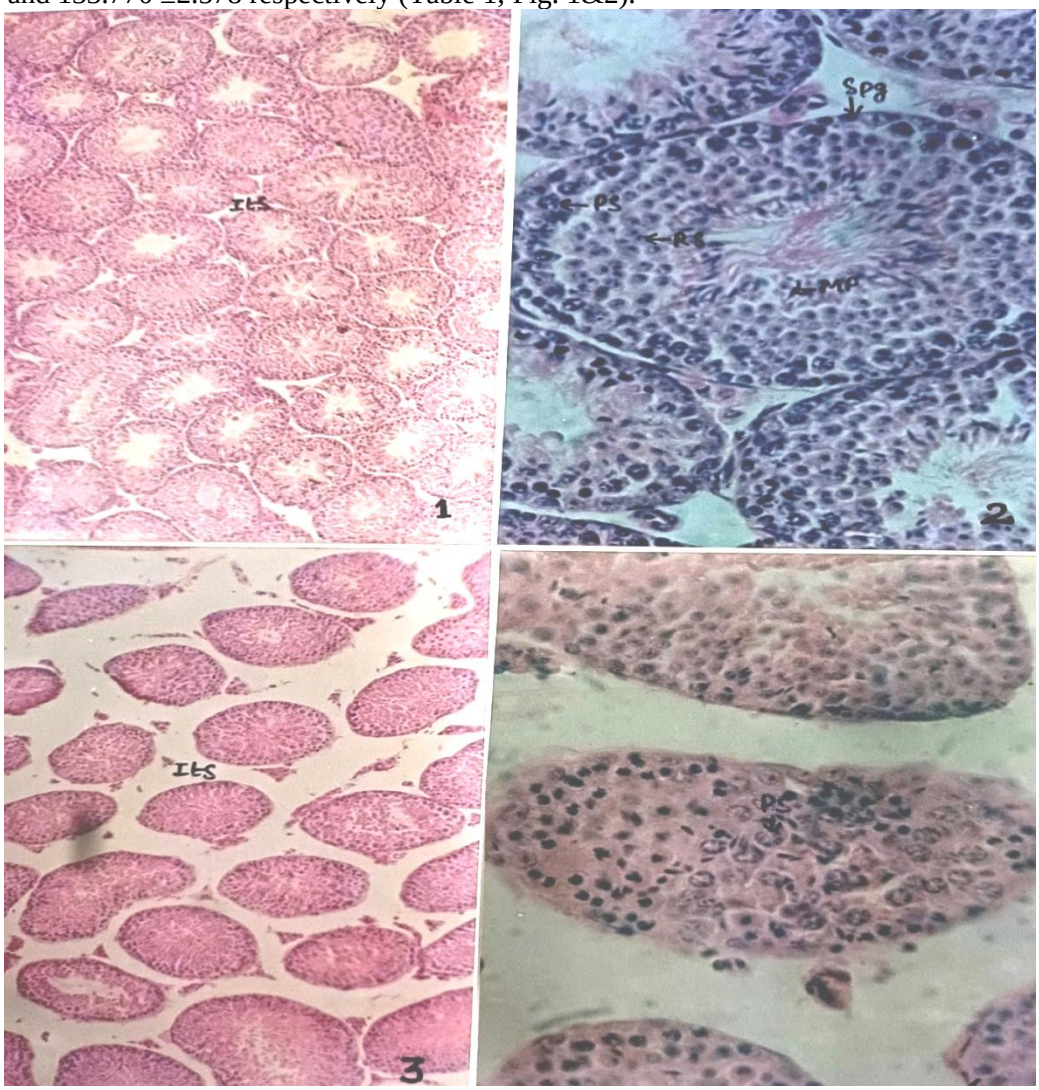

Figure 1: Hematoxylin-eosin stained sections of testis of control and neem oil treated mice at the dose level of2.0 ml/kg bw/day for 24 days.
Mpg 1 Seminiferous tubules of control mice showing compactly arranged seminiferous tubules with little inter-tubular space (10X10)
Mpg 2 Magnified view of seminiferous tubule of control mice showing spermatogenic activity in full swing (10X40)
Mpg3 Seminiferous tubules of mice treated with neem oil showing shrinkage with increased inter-tubular space (10X10)
Mpg4 Magnified view of seminiferous tubules of mice treated with neem oil showing loose arrangement with reduced number of spermatogenic cells (10X40).

Spermatogenic activity in mice was studied after the administration of neem oil and compared with control mice. The Control mice showed normal spermatogenesis. There was little inter-tubular space in between seminiferous tubules. The various spermatogenic cells of seminiferous epithelium observed were spermatogonia, spermatocytes, spermatids and spermatozoa in maturation phase.
In the treated groups, the seminiferous tubules showed shrinkage with increased inter-tubular space. In spermatocytes, the degenerative changes were observed as revealed by the displacement from their

original position. In most of the tubules the mean number of spermatogenic cells were reduced but the reduction was non-significant.

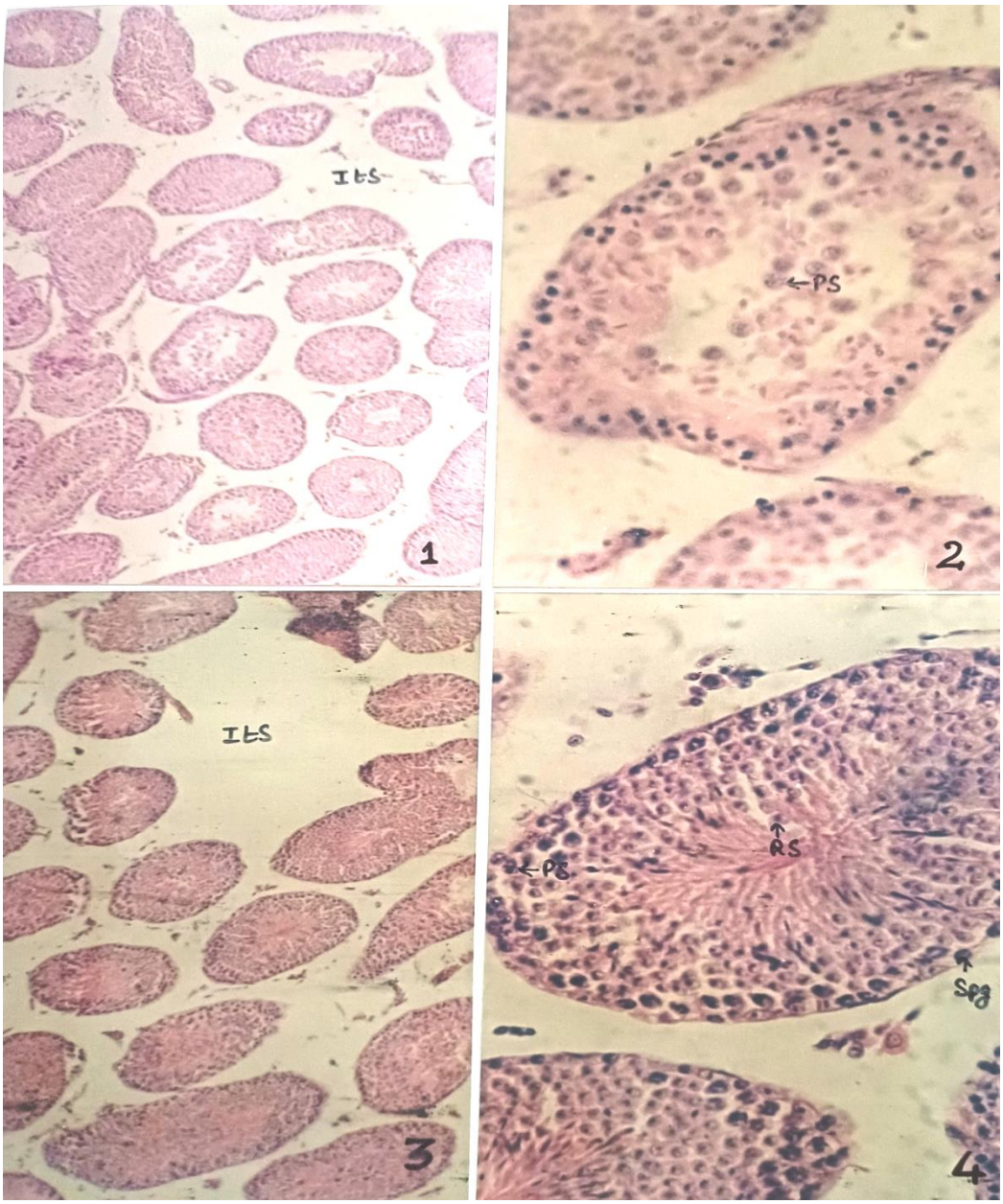

Figure 2: Hematoxylin-eosin stained sections of testis of treated mice at the dose level of 3.3 and 4.6 ml/kg bw/day for 24 days.
Mpg1 Seminiferous tubules of testis of treated mice with neem oil(3.3 ml/kg bw/day) showing shrinkage(10X10)
Mpg2 Magnified view of seminiferous tubules of mice treated with neem oil at the dose level of 3.3 ml/kg bw/day(10X40)
Mpg3 Seminiferous tubules of testis of mice treated with neem oil at the dose level of 4.6 ml /kg bw/day showing shrinkage of seminiferous tubules (10X10)
Mpg4 Magnified view of seminiferous tubules of mice treated with neem oil at the dose level of 4.6 ml showing reduced number of spermatogenic cells particularly mature sperms(10X40).
Its Inter-tubular space, Spg Spermatogonia, PS Primary Spermatocyte, RS Round Spermatid.

Degeneration/reduction in the number of spermatogenic cells might be responsible for decrease in testicular weight. Upadhyay *et al.*, (1993) also recorded similar observation in neem oil treated rats. Anti-androgenic property of neem seed oil has been reported earlier in rats. Earlier studies have also been reported to have anti-spermatogenic and anti-androgenic effect in male animal species after the administration of different plant extracts (Gupta *et al.*, 1985; Das RP, 1990; Gupta *et al.*, 1990; Hammami *et al.*, 2008). Androgen deficiency may the reason for decreased testicular weight. The administration of different plant extracts have also revealed similar result in male rodents (Pakrashi and Pakrasi, 1977; Adhikary *et al.*, 1989; Choudhary *et al.*, 1990; Sarkar., 2000). Administration of different leaf extracts have also caused significant reduction in the diameter of seminiferous tubules. Saini and Prasad (1991) highlighted the fact that a certain level of testicular necrosis may have occurred in treated mice. Administration of different plant extracts have also brought similar results in male albino mice (Choudhary *et al.*, 1990*;* Joshi *et al.*, 1996*;* Verma *et al.*, 1980; Adhikary *et al*., 1989; Murugavel and Akbarsha, 1991; Reddy *et al*., 1997).

Table 1: Effect of oral administration of Neem oil (2.0,3.3 and 4.6 ml/kg bw/day for 24 days) on Testicular weight, Diameter of seminiferous tubules and the Number of Spermatogenic cells

	Control	Group I (2.0 ml/kg bw)	Group II (3.3 ml/kg bw)	Group III (4.6 ml/kg bw)
Testes weight (g/100g body weight)	0.392±0.006	0.332±0.016** (85)	0.295±0.008** (75)	0.289±0.011** (74)
Seminiferous tubule diameter(µm)	187.600± 8.500	147.810± 3.406** (79)	133.770± 2.345** (71)	133.770± 2.578** (71)
Number of Spermatogenic cells				
Spermatogonia	31.670±1.680	31.000±3.290	30.330±3.470	29.500±3.760
Spermatocytes	41.830±5.210	39.830±3.320	30.000±3.170	28.000±3.110* (67)
Spermatids	78.330±21.450	66.330±9.650	49.830±11.290	49.830±11.120

Values are Mean ±S.E. values in parenthesis are % of Control.
*P≤0.01 **indicates significant change as compared to Control.*
*P≤0.05 *indicates significant change as compared to Control.*

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