



# Effect of Neem leaf extracts on testicular trace elements in albino mice

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## ABSTRACT

Alcoholic extract of neem leaves administered orally at the rate of 132, 200 and 300 mg/kg bw/day for 24 days caused variation in some of the testicular mineral & trace elements in male albino mice. A significant decrease in the diameter of seminiferous tubules and number of spermatogenic elements was also observed.

**Keywords** - Neem, testicular, trace elements, albino mice.

## INTRODUCTION

A mineral is said to be essential if it plays a direct role in structure and functioning of the body i.e. It can not be replaced by any other substitute and its deficiency affects the growth and reproduction (Smith and Akinbamijo, 2000; Kumar *et al.*, 2011). On the other hand excess of trace elements can cause direct toxic effects by causing secondary deficiency of other trace elements (Thompson *et al.*, 1991)

*The essential minerals may be divided into two groups :*

1. Major minerals which include Calcium (Ca), Phosphorous (P), Potassium (K), Sulphur (S), Sodium (Na), Chlorine (Cl) and Magnesium (Mg).
2. Micronutrients or trace elements which include Iodine (I), Iron (Fe), Zinc (Zn), Manganese (Mn), Copper (Cu), Cobalt (Co), Molybdenum (Mo), Selenium (Se), Vanadium (V), Chromium (Cr), Silicon (Si), Arsenic (As) etc.

The micronutrients are involved in functions such as intracellular detoxification of free radicals, synthesis of reproductive steroids and other hormones, carbohydrates, proteins and nucleic acid metabolisms, their deficiencies/excess may impair spermatogenesis and libido in male (Smith and Akinbamijo, 2000). Trace elements such as Zn, Cu, Fe and Se are essential for male fertility (Fayed, 2010).

Present study was carried out to establish the effect of leaf extracts on testicular minerals and trace elements such as Zn, Cu, Ca, Fe, Na and K and also on the diameter of seminiferous tubules and number of spermatogenic elements.

## MATERIALS AND METHODS

Leaves obtained from neem tree in botanical garden, Punjab Agricultural University, Ludhiana was dried and powdered. The extract was prepared according to the method of Chattopadhyay (1993). The powder was extracted by percolation at room temperature with 70 percent ethanol. The extract was then concentrated under reduced pressure and finally dried in a vacuum dessicator. The residue was dissolved in propylene glycol (vehicle) at the rate of 100 mg/ml and was used for present experimental study.

Male albino mice (8-10 weeks old with average 30 gm body weight) were maintained under controlled conditions. Standard rat feed and water were provided ad libitum. Acclimatized mice were divided into four groups having 8 animals each. Group I served as control and were administered propylene glycol (vehicle) only while that of group II, III and IV were administered leaf extract in vehicle at the dose level of 132, 200 and 300 mg/kg bw/day for 24 days. The mice of each group were sacrificed 24 hours after the administration of last dose. From the sacrificed mice, the testes were dissected out and blotted free of mucus. One of the testes from each mouse was used for biochemical study i.e. for estimation of trace elements and the other was

used for histological study for determining the diameter of seminiferous tubules and number of spermatogenic elements.

The concentration of minerals and trace elements was determined by Atomic absorption spectrophotometry (Ludmilla, 1976). A known weight of tissue was digested with triple acids (Conc. nitric acid; Perchloric acid; Sulphuric acid) in the ratio of 10:3:1 at 100°C in a conical flask. The residue was reconstituted in a known volume of triple distilled water. The data was statistically analyzed using students t-test.

## RESULTS AND DISCUSSION

Concentration of elements ( $\mu\text{g/g}$  tissue) such as Zn, Cu, Ca, Fe, Na, K was determined in the testes of vehicle treated / normal and neem extracts treated (Group II, III & IV) mice. Diameter of seminiferous tubules and number of spermatogenic elements were also determined.

**Zinc:** Group II and III mice showed insignificant change in the concentration of Zinc as compared to control while concentration of Zinc increased significantly in Group IV mice (Table 1). Zinc is one of the most important biological trace element and cofactor of several enzymes exerting variety of biochemical effects on cell membranes, hormones and is associated with carbohydrate metabolism, protein synthesis and nucleic acid metabolism (Smith and Akinbamijo, 2000). Zinc is an essential trace element for spermatogenesis (Yamaguchi *et al.*, 2009).

**Table 1 :** Effect of oral administration of leaf extracts (132, 200 and 300 mg/kg body weight/day for 24 days) on testicular trace elements ( $\mu\text{g/g}$  tissue)

	Group I (Control)	Group II (132 mg/kg bw)	Group III (200 mg/kg bw)	Group IV (300 mg/kg bw)
Zn	30.250 $\pm$ 2.380	30.120 $\pm$ 2.440	28.970 $\pm$ 5.110	81.450 $\pm$ 3.040** (269)
Cu	897.060 $\pm$ 17.880	900.220 $\pm$ 15.450	968.320 $\pm$ 12.300	1418.720 $\pm$ 15.600** (158)
Ca	35.160 $\pm$ 3.690	35.290 $\pm$ 4.050	34.970 $\pm$ 1.928	52.080 $\pm$ 2.240* (148)
Fe	154.150 $\pm$ 1.430	143.860 $\pm$ 5.360	220.660 $\pm$ 5.190** (143)	229.410 $\pm$ 8.900** (149)
Na	226.540 $\pm$ 22.530	216.760 $\pm$ 22.230	182.710 $\pm$ 7.440	179.330 $\pm$ 9.330
K	619.930 $\pm$ 16.360	402.360 $\pm$ 1.160** (65)	362.290 $\pm$ 25.550** (58)	324.540 $\pm$ 32.280 ** (52)

Values are mean  $\pm$  S.E., values in parenthesis are % of control

P  $\leq$  0.01 : \*\* indicates significant change as compared to control. P  $\leq$  0.05 : \* indicates significant change as compared to control.

**Table 2** : Effect of oral administration of neem leaf extracts (132, 200 and 300 mg/kg body weight for 24 days) on the diameter of seminiferous tubules ( $\mu\text{m}$ ) and number of spermatogenic elements.

	Group I (Control)	Group II (132mg/kg bw)	Group III (200 mg/kg bw)	Group III (300 mg/kg bw)
Seminiferous tubule diameter ( $\mu\text{m}$ )	190.970 $\pm$ 7.590	187.230 $\pm$ 5.588	156.260 $\pm$ 2.520** (82)	102.510 $\pm$ 2.383** (54)
Number of spermatogenic elements-				
Spermatogonia	37.000 $\pm$ 3.160	35.800 $\pm$ 4.090	33.330 $\pm$ 1.190	32.330 $\pm$ 1.540
Spermatocytes	42.170 $\pm$ 5.440	39.500 $\pm$ 6.740	26.670 $\pm$ 4.830* (63)	8.170 $\pm$ 3.380** (19)
Spermatids	95.330 $\pm$ 24.300	89.500 $\pm$ 24.780	80.330 $\pm$ 11.740	Nil

Values are mean  $\pm$  S.E., values in parenthesis are % of control

$P \leq 0.01$ : \*\*indicates significant change as compared to control.  $P \leq 0.05$ : \*indicates significant change as compared to control

It is also associated with testosterone production which is critical factor for growth, development and function of seminiferous tubules (Martin *et al* 1994). Zinc's function in regulating androgen metabolism may be at testicular level (Leathem, 1970). Deficiency of Zinc has pronounced effect on testosterone secretion. On the other hand higher levels of dietary Zinc caused significant decrease in testosterone secretion. A high concentration of Zinc has been observed after adrenalectomy (Nair *et al.*, 1995) and it is attributed to oedema probably formed by destruction of testicular tissue.

The rise in Zinc level can be related to (i) failure of regulatory mechanism that regulate the flow of element across the blood testes barrier and (ii) increased oedematous fluid formed by cellular death of germinal epithelium (Nair *et al.*, 1995). In present study, there was also decrease in the number of spermatogenic elements in higher dose groups (Table-2).

**Copper** - The testicular content of Copper increased significantly in Group III and IV mice (Table 1). Mehta *et al* (1989) reported that plasma testosterone levels decreased significantly when dietary Copper and Zinc levels were increased. Hydrocortisone when administered to adrenalectomized rats showed increased copper levels in the testes and this increased copper level in the testes of adrenalectomized rats showed increased Copper levels in the testes and this increased copper level in the testes of adrenalectomized rats can damage the testicular tissue (Nair *et al.*, 1995) Increased copper levels might have caused decrease in testosterone levels which in turn was responsible for degeneration of spermatogenic elements as revealed in the present study (Table 2).

**Calcium** - A significant increase in the Calcium content was observed in testes of Group IV mice while the lower doses revealed insignificant change (Table 2). Excess Calcium may impair reproductive function by causing a secondary deficiency of P, Mg, Zn and Cu and other micronutrients by inhibiting their absorption in the intestine (King,1971).

**Iron**- A significant increase was observed in testicular Iron level in mice after the administration of higher doses of neem extracts. Testicular Iron content increased significantly in Group III and IV mice. The change in testicular iron content may be due to metabolic change occurring with in tissue after the administration of leaf extracts (Puri, 2002).

**Sodium and Potassium**- Insignificant change in testicular sodium content was observed in mice administered with all doses of leaf extracts i.e. in Group II, III and IV mice (Table 1). Testicular content of potassium decreased significantly in mice of Group II, III and IV (Table 1). Sodium and Potassium are constituents of tissues as electrolytes and are concerned with maintenance of osmotic pressure, membrane permeability and tissue irritability (Underwood, 1977).

Diameter of seminiferous tubules reduced significantly in Group III and IV mice (Table2). There was no marked effect on the number of spermatogonia but number of spermatocytes reduced significantly in Group III and IV mice. Group IV mice showed no spermatids at all. Histological studies have also shown the degeneration of spermatogenic cells in mice fed with extracts of *Azadirachta indica* (Puri & Sangha, 2006). Mass atrophy of spermatogenic cells might be due to anti-androgenic (Shaikh *et al.*, 1993) and anti- spermatogenic (Lohiya *et*

al., 1999; Abu and Uchendu, 2010) of different plant extracts.

## CONCLUSION

Administration of neem leaf extracts resulted in the variation in the concentration of some of essential minerals & trace elements. A significant change was observed in higher dose groups. This varied concentration of mineral and trace elements might have become the cause of degeneration of spermatogenic elements.

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**Conflicts of interest:** author declare that there is no conflict of interest.

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