

Full Length Research Paper

# Biochemical and histological studies on the effects of *Azadirachta indica* seeds kernel extract on albino rats<sup>ψ</sup>

Maha Babiker Dafalla<sup>1</sup>, Emadeldin. H. Konozy<sup>1\*</sup> and Haseeba Ahmed Saad<sup>2</sup>

<sup>1</sup>INRA - Unité de Génétique et Amélioration des Fruits et Légumes, 84143 Montfavet BP94, France.

<sup>2</sup>Department of Zoology, University of Khartoum, Khartoum, Sudan.

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The present study was carried out to investigate the effects of (*Azadirachta indica*) neem seed kernel aqueous extract on albino Wistar rats. Two experiments were conducted using 34 rats which received different doses of neem aqueous extract. In acute toxicity experiment, rats were treated orally with 0 mg/kg (control), 3 mg/kg (low), 80 mg/kg (medium), and 130 mg/kg (high) body weight for 14 days. In subacute toxicity experiment, rats were treated with 0.5 mg/kg body weight for 6 weeks. Rats that received medium and higher doses showed significant ( $P<0.05$ ) increase in activity of plasma glutamate pyruvate transaminase (GPT) enzyme. Necrosis of liver and kidney tissues was observed, while mild histopathological changes were seen in the testes. However, urea and glucose level did not show significant change. Testosterone hormone showed significant ( $P<0.05$ ) decrease in concentration at acute toxicity. In subacute toxicity no significant changes were observed except for significant ( $P<0.05$ ) increase in testosterone hormone concentration and significant ( $P<0.05$ ) increase of glucose concentration. Taken together, these results may indicate that neem seed kernel aqueous extract possesses dose-dependent hepatorenal toxicity and antifertility adverse effects.

**Key words:** *Azadirachta indica*, seeds kernel extract, toxicity, kidney, liver, testis.

## INTRODUCTION

Neem (*Azadirachta indica*), family Meliaceae, is a drought-tolerant plant, and thrives in many of the drier areas of the world in Asia, Africa and other tropical part of the globe. Different parts of neem have been traditionally used for treatment of human ailments and control of pests (Sidhu et al., 2003). Neem has been reported for its various medicinal properties such as antiseptic, healing of wound, curing skin diseases, antiulcer and anti-inflammatory activities (Subapriya and Nagini, 2005; Brahmachari, 2004). It is commonly used for the treatment of gastrointestinal and dental infection,

cardiovascular disorder, diabetes and cancer (Paul et al., 2011). Biological activities of neem extract have been documented for its strong antifeedant, insect growth regulator and reproductive effects (Schmahl et al., 2010). Azadirachtin from neem seed kernel has been reported as the most potent naturally-occurring insect control agent (Senthil et al., 2007; Sidhu et al., 2003). Neem extract has a powerful pesticidal activity and used to control wide varieties of pests such as bacteria, fungi, viruses and rodents (Parida et al., 2002; Tiwari et al., 2010). Neem is now considered as a valuable source of unique natural product of medicine against various diseases (Thakurta et al., 2007; Tripathi et al., 2009) and also for the development of industrial products (Abdel-Ghaffar et al., 2008; Heukelbach et al., 2006). In Sudan, the most common uses of neem were the treatment of fevers and dental infections. Neem was found to be toxic to malaria parasite (Udeinya et al., 2008) and can reduce

\*Corresponding author. E-mail: [ehkonozy@yahoo.com](mailto:ehkonozy@yahoo.com).

**Abbreviations:** EDTA; ethylenediaminetetracetic acid, GOT; glutamate oxaloacetate transaminase, GPT; glutamate pyruvate transaminase, U/L; unit per Litre

fever in malaria patients. Researches revealed molluscicidal properties of neem extracts, therefore, used to control schistosomiasis, the second most important parasitic disease in Sudan after malaria (Fayez, 2001). In Sudan, besides the use of neem in medical fields, neem seeds products are routinely used for vegetable pest control (Satti et al., 2010). Though neem has been subjected to many studies that dealt with its biological activities, its toxic effects were not well focused upon. Some toxicological manifestations of various parts of neem have been observed by a number of researchers (Akudugu et al., 2001; Chinnasamy et al., 1993; Goktepe et al., 2004; Senthil-Nathan et al., 2009). Therefore, in parallel to the extensive medical applications, little information is available on neem toxicity. The present study was conducted to investigate toxic effects associated with exposure to neem aqueous extract on rats, in attempt to prove the safety of neem for medicinal uses and pests' control.

## MATERIALS AND METHODS

### Preparation of neem seeds aqueous extract

Mature good quality neem seeds were washed with distilled water and left to dry for seven days under the shade.

The dried seeds were crushed in a mortar to remove the shell without damaging the kernel. The kernels were then ground by an electric blender into fine powder. The powder was passed through 925 mesh sieve, stored in tightly covered glass jars and left at room temperature till it was required for extraction. The aqueous extract of neem seeds was prepared by mixing 5 g of the powder with 100 ml of distilled water in a conical flask. The mixture was left for 4 h in the shaker and was then filtered through a light cloth and the filtrate was kept at -10°C till further use.

### Animals

Thirty four healthy Wistar Albino rats 11 to 12 weeks of age, weighing 195-200g were obtained from the Animal house of the Medicinal and Aromatic Plant Research Institute, Khartoum, and acclimatized for one week prior to the experiment with feed and water supplied *ad libitum*.

### Experimental design

#### Acute toxicity

The choice of acute and subacute doses were done according to Joshi et al. (1996). Twenty healthy rats were divided randomly into four groups 1, 2, 3 and 4, each group contains five rats. Rats of groups 1, 2 and 3 were treated daily by oral intubations with neem seed kernel

aqueous extract for 14 days as follows:- Group (1) Low dose 3 mg/kg body weight; Group (2) Medium dose 80 mg/kg body weight; Group (3) High Dose 130 mg/kg body weight. Rats of *group (4)* were kept as control 0 mg/kg body weight.

#### Sub-acute toxicity

Fourteen healthy rats were divided randomly into two groups. Group (1) consisted of 10 rats which received 0.5 mg/kg body weight of neem seed kernel aqueous extract daily by oral intubations for 6 weeks. Group (2) composed of 4 rats were kept as control.

#### Collections of blood samples

Blood samples were collected from cervical blood vessels of rats during slaughtering into clean dry bottles containing ethylenediaminetetracetic acid (EDTA). Plasma was separated by centrifugation at 3000 rpm for 5 min, and stored at -20°C until analyzed for biochemical parameters.

#### Tissue samples

Samples of liver, kidneys and testes were fixed in 10% neutral buffered formalin and processed for histopathological examinations (Presnel and Schreiber, 1997).

#### Biochemical investigations

Plasma was analyzed for glucose and urea. The activities of enzymes glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and creatinine concentration were determined. Plasma of male rats was used to measure level of testosterone hormone.

#### Determination of glucose

Plasma glucose level was measured by enzymatic spectrophotometric test as previously described by Miskiewicz and colleagues (Miskiewicz et al., 1973).

#### Determination of urea

Plasma urea level was measured by the enzymatic method as described earlier (Chaney and Marbach, 1962).

#### Determination of enzymes activities

#### Determination of glutamate oxaloacetate transaminase (GOT)

Plasma GOT activity was determined according to the method described by Reitman and Frankel (1957).

**Table 1.** Plasma constituents of rats given different oral doses of neem seed aqueous extract: Acute toxicity.

Treatment	Control group (4)	Low dose group (1)	Medium dose group (2)	High dose group (3)
Glucose (mg/dl)	68.0 ± 9.39 <sup>a</sup>	50.8 ± 2.04 <sup>a</sup>	50.4 ± 2.23 <sup>a</sup>	61.8 ± 5.91 <sup>a</sup>
Urea (mg/dl)	67.0 ± 4.07 <sup>a</sup>	74.5 ± 5.30 <sup>a</sup>	73.1 ± 6.90 <sup>a</sup>	82.7 ± 2.10 <sup>a</sup>
GOT (U/L)	29.0 ± 3.56 <sup>a</sup>	34.3 ± 3.71 <sup>a</sup>	15.8 ± 1.46 <sup>b</sup>	21.3 ± 1.44 <sup>b</sup>
GPT (U/L)	71.3 ± 8.03 <sup>a</sup>	87.0 ± 8.66 <sup>ab</sup>	86.0 ± 10.83 <sup>ab</sup>	101.8 ± 3.11 <sup>b</sup>
Creatinine (mg/dl)	1.10 ± 0.09 <sup>a</sup>	1.20 ± 0.03 <sup>a</sup>	1.12 ± 0.05 <sup>a</sup>	1.22 ± 0.03 <sup>a</sup>

Means values for the same row with alphabetic superscripts are significantly different at  $p < 0.05$ .

### **Determination of glutamate pyruvate transaminase (GPT)**

Plasma GPT activity was determined according to the method described by Reitman and Frankel (1957).

### **Determination of creatinine concentration**

Creatinine was determined by the method described by Husdan and Rapoport (1968).

### **Determination of testosterone hormone**

Testosterone hormone was quantitatively determined by using radioimmunoassay (RIA) Kit (IMK-457).

### **Statistical analysis**

Data were analyzed for significance according to the procedure described by Mendenhall (1971). Toxicological data were tested for significance by analysis of variance. Result was presented as mean ± standard error (SE) of mean. The difference between treated and control is considered significant at  $P < 0.05$ .

## **RESULTS**

### **Acute toxicity**

Rats that received 3, 80, and 130 mg/kg body weight of neem seeds aqueous extract did not show obvious clinical signs. The group that received the high dose of neem seed aqueous extract had dark liver, compared to that of the control group at post examination.

### **Biochemical changes**

Changes were presented in Table 1. GOT activity in group 4 was found to be 29.0 u/L, while that of groups 1, 2 and 3 are 31.3, 15.8 and 21.3 u/L respectively. Groups 2 and 3 showed significant ( $p \leq 0.05$ ) decrease when compared with control group (Figure 1). Activity of GPT in group 4 was found to be 71.3 u/L, while that of groups 1, 2 and 3 were found to be 87.0, 86.0 and 101.8 u/L, respectively. Group 3 showed significant ( $p \leq 0.05$ )

increase when compared with control group (Figure 2).

Glucose concentration in group 4 was found to be 68.0 mg/dl, while that of groups 1, 2 and 3 did not show significant change when compared with the control group 4.

The concentration of plasma urea in group 4 was found to be 67.0 mg/dl, while that of groups 1, 2 and 3 did not show significant change when compared with control group 4.

Plasma creatinine level in group 4 was found to be 1.10 mg/dl, while that of groups 1, 2 and 3 did not show significant change when compared with control group 4.

### **Hormonal changes**

Changes in testosterone concentration are presented in Table 2 and Figure 3. The concentration of plasma testosterone in group 4 was found to be 2.50 ng/ml, while those of groups 1, 2 and 3 were 1.80 ng/ml, 0.90 ng/ml and 0.97 ng/ml, respectively. Groups 2 and 3 showed significantly ( $p \leq 0.05$ ) decreased values when compared with the control group.

### **Histological changes**

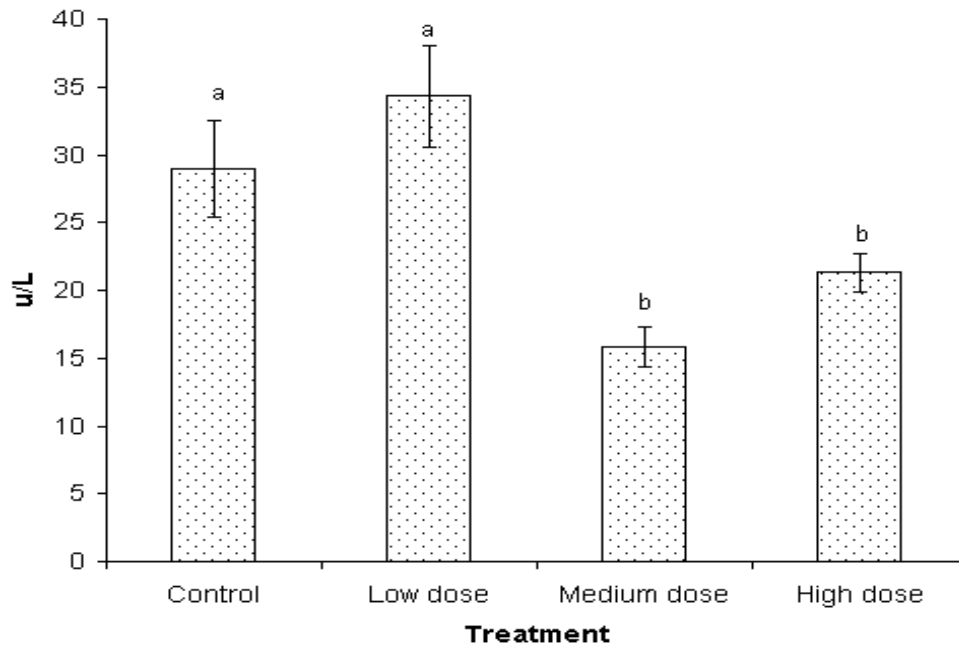
Lesions were observed in kidney, liver and testes. Liver of groups 2 and 3 showed marked mononuclear cell infiltration, nuclear degeneration, architectural disarray, hemorrhage and necrosis. Kidney in group 1 showed mild hyalinization, hydropic swelling and eosinophilia, while groups 2 and 3 showed nuclear loss, karyolysis, hyalinization, cyanophilia, glomerular destruction, complete swelling and marked hydropic changes to the point of necrosis. Testes showed mild architectural disarray and ductal swelling when compared with control group (Figures 4, 5 and 6).

### **Subacute toxicity**

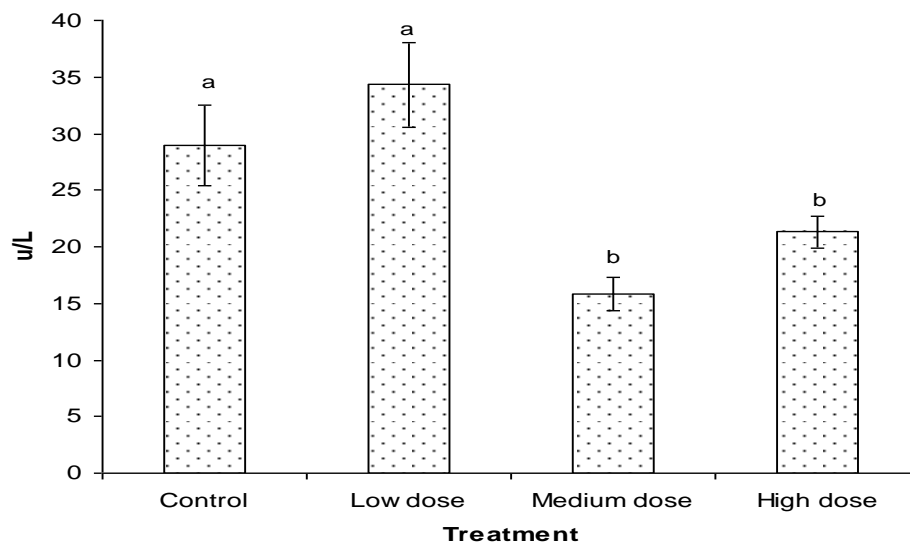
Rats that received 0.5 mg/kg body weight of neem seeds aqueous extract did not show obvious clinical signs or histopathological changes.

### **Biochemical changes**

Changes in different biochemical parameters such as



**Figure 1.** Acute toxicity effect of neem seed aqueous extract on plasma GOT activity in rats.

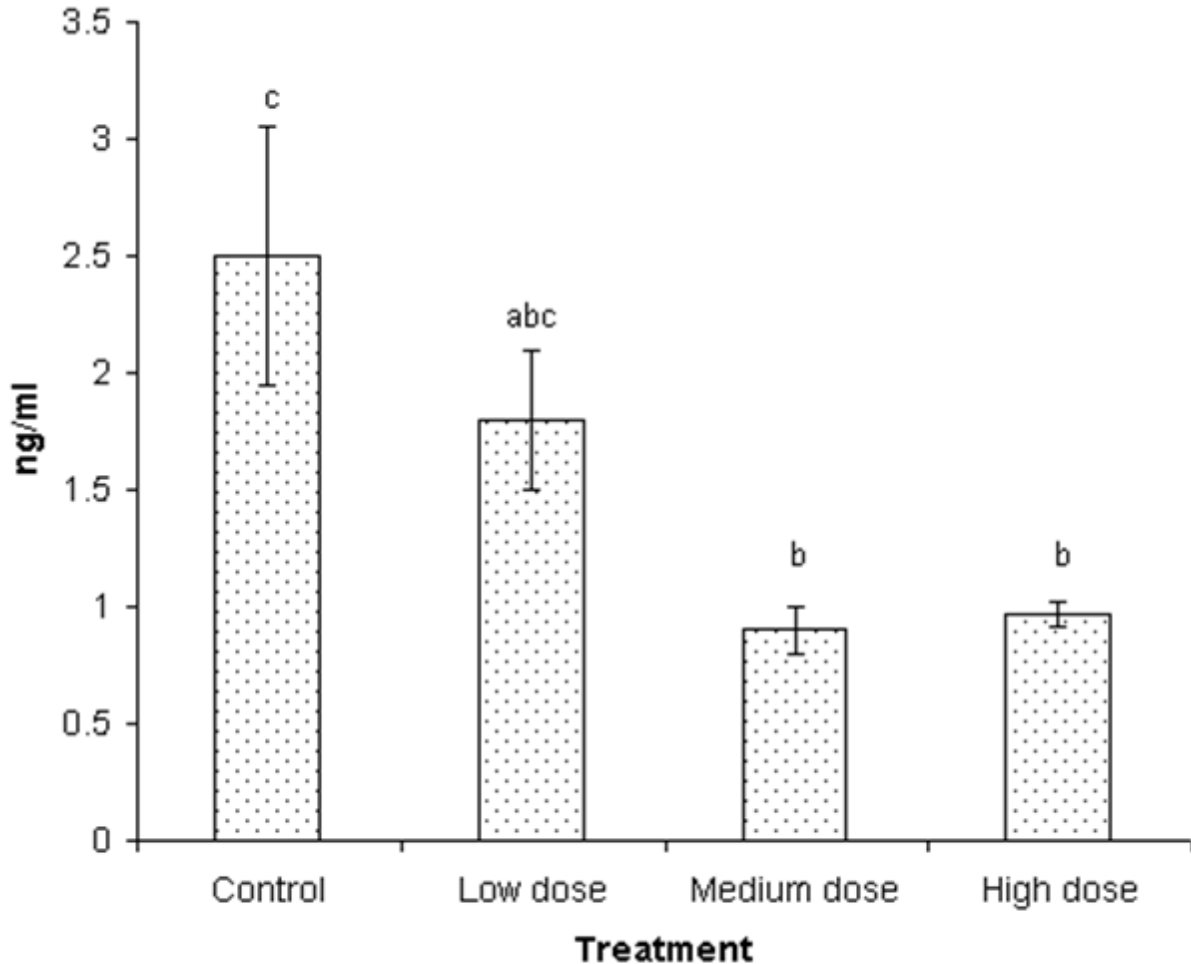


**Figure 2.** Acute toxicity effect of neem seed aqueous extract on plasma GPT activity in rats.

**Table 2.** Effect of neem seed aqueous extract on plasma testosterone concentration of rats: Acute toxicity (mean  $\pm$  SE).

Treatment	Hormone (ng/ml)
Control (4)	2.50 $\pm$ 0.55 <sup>a</sup>
Low dose (1)	1.80 $\pm$ 0.30 <sup>ab</sup>
Medium dose(2)	0.90 $\pm$ 0.10 <sup>b</sup>
High dose(3)	0.97 $\pm$ 0.05 <sup>b</sup>

Means values for the same column with different alphabetic superscripts are significantly different at  $p < 0.05$



**Figure 3.** Acute toxicity effect of neem seed aqueous extract on testosterone concentration in male rats.

glucose, urea, GPT and GOT were presented in Table 3. GOT activity was found to be 15.8 u/L in group 2 and 17.1 u/L in group 1. No significant change was observed in GOT activity. GPT activity was found to be 14.5 u/L in group 2 and 14.0 u/L in group 1. No significant change was observed in GPT activity. The concentration of plasma glucose was found to be 105.5 mg/dl in group 2 control, while that of group 1 significantly ( $p \leq 0.05$ ) increased to 127.1 mg/dl (Figure 7). The concentration of plasma urea was found to be 51.8 mg/dl in group 2 and 47.8 mg/dl in group 1. Plasma urea level did not show significant change between test group and the control group.

#### **Hormonal changes**

The effect of Azadirachta seed extract on the hormonal activities in the Wistar rats are presented in Table 4 and Figure 8. The concentration of plasma testosterone in group 2 control was found to be 0.53 ng/ml, while that of group 1 significantly ( $p \leq 0.05$ ) increased to 2.07 ng/ml.

#### **Histological changes**

Liver, kidney, and testes of rats treated with neem seed aqueous extract did not show any signs of histopathological changes when compared with control group 2 (data not shown).

#### **DISCUSSION**

Neem has traditionally been used as a medicine for various diseases. In fact each part of this plant has been reported to link with specific ailment treatment. Recently, a fractionated neem-leaf extract known as IRAB with reported activities against Malaria, acquired immune deficiency syndrome (AIDS) and cancer has been actively commercialized as a drug in Nigeria (Anyaehe, 2009). In recent years, due to the residual contamination of crops and water with chemical pesticides, the use of neem based-compound has significantly increased (Boeke et al., 2004). As little information on neem toxicity



**Figure 4.** Marked mononuclear cell infiltration in the liver of rats given neem seeds aqueous extract at 80 mg/Kg body weight for 14 days.

is available as compared to its application level, this has necessitated further studies to ascertain the safety of neem compounds and extract for various applications.

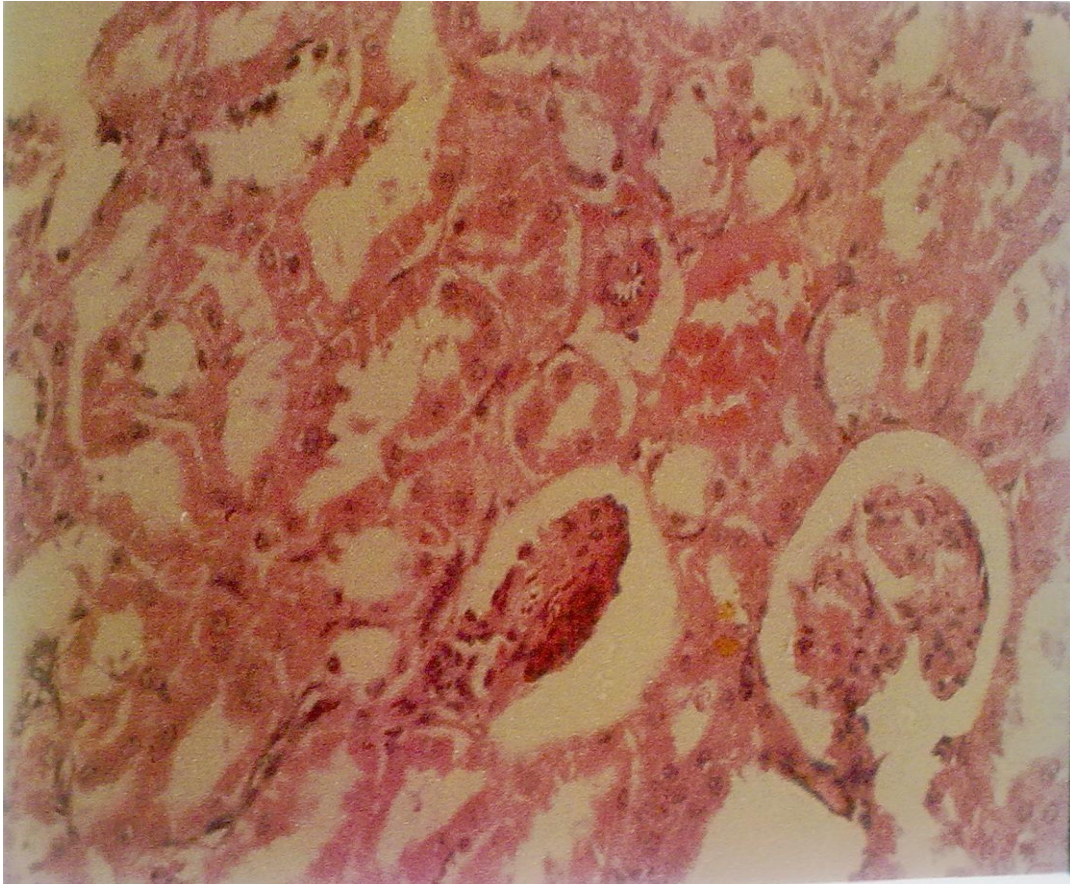
The present study was undertaken to investigate the effect of different doses of neem seed kernel aqueous extract on Albino rat. Acute toxicity study, along with subacute toxicity experiment are conducted, as both considered important for the assessment of risk posed by new chemicals and for better control of natural substances in the human environment.

From the results obtained, it appears that at low dose (3 mg/kg body weight) neem extract caused no significant change, while at a dose of (80 mg/kg body weight) of neem seed aqueous extract, significant increase in plasma GPT activity was observed when compared with control group. The elevated levels of GPT indicated liver and /or kidney damage caused by neem seed aqueous

extract. The plasma GOT level showed slight, however, non significant increase at 3 mg/Kg, while at 80 and 130 mg/Kg of the extract there was significant decrease.

Increases in serum levels of GOT and GPT indicate hepatocellular damage. GPT is the liver- specific enzyme in rats. GPT is more sensitive and specific than GOT in the detection of liver disease. Moreover, elevation of serum GPT persisted longer than that of GOT activity, and this may justify the decrease in GOT activity in rats dosed at 80 and 130 mg/Kg of neem seed aqueous extract (Tietz, 1982).

This result is not surprising since both liver and kidney tissues are known to possess high concentration of GPT. A rise in plasma transaminases (GOT, GPT) activities is a sensitive indicator of damage to cytoplasmic and/or mitochondrial membranes (Mayne, 1994). In the present study, Hepatorenal toxicity was further confirmed by



**Figure 5.** Packing of the renal glomerular destruction, vacuolation and necrosis of epithelial cells of the proximal convoluted tubules of rats given 130 mg/Kg body weight of Neem aqueous extract for 14 days.

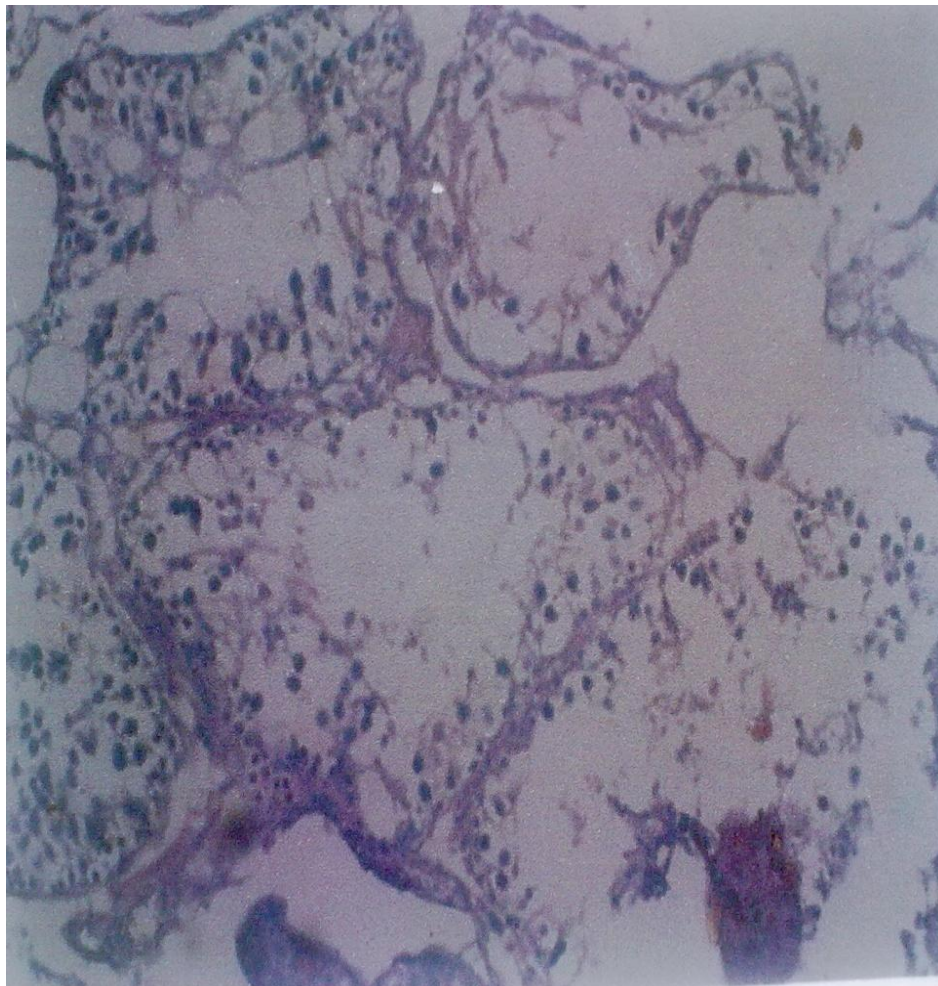
histopathological changes that revealed marked mononuclear cell infiltration, nuclear degeneration, architectural disarray, glomerular destruction and necrosis.

Hepatotoxicity was reported in the study that was carried out by Rahman et al. (2001) when rats had been treated with vepacide (isolated from neem oil). Rats treated with neem leaf aqueous extract showed adverse effects indicating liver damage (Bhanwra et al., 2000). Sadekar and colleagues reported significant increases in GOT concentration in calves (Sadekar et al., 1998). Similar result was obtained by Ibrahim et al. (1992) when Brown Hisex chicks had been fed with a diet containing 2 and 10% of neem leaf. However, this result is in contrast with that obtained by Raizada et al. (2001) who had reported no toxic effect or increase in GOT and GPT activities in rats treated with different doses of azadirachtin mixed with peanut oil for 90 days. This may be attributed to the vehicle used, peanut oil, which may interfere with the absorption of azadirachtin. Neither plasma urea concentration or plasma glucose concentration in the treated rats showed significant

changes when compared with the control group. Similar result was observed when rats exposed to different concentration of azadirachtin (Raizada et al., 2001). In contrast to these findings, Joshi et al. (1996) reported that glucose concentration increased in albino rats when treated with leaf powder of *A. indica*.

In subacute toxicity experiment no biochemical or histopathological changes were observed. However, the plasma glucose concentration showed significant increase when compared with the control group. This is in accord with results reported by Joshi and his colleagues (Joshi et al., 1996). This may be due to time factor and low dose used.

Antifertility adverse effect of neem seeds aqueous extract has been reported in both male and female of different animals. In the present study we considered the male reproductive system whereas few experiments were conducted compared to those performed on female. In addition very few experiments were conducted to investigate the effect of neem extract on sex hormone. The experiments revealed significant decrease in plasma testosterone hormone in rats that received 3, 80 and 130



**Figure 6.** Degeneration of the epithelial cells of the testicular tubules of rats orally given 130 mg/Kg body weight of neem seed aqueous extract for 14 days.

**Table 3.** Plasma constituents of rats given neem seed aqueous extract: Subacute (means  $\pm$  SE).

Treatment	Control (2)	Treated (1)
Glucose (mg/dl)	105.5 $\pm$ 5.7 <sup>a</sup>	127.1 $\pm$ 5.1 <sup>b</sup>
Urea (mg/dl)	51.8 $\pm$ 1.0 <sup>a</sup>	47.8 $\pm$ 1.6 <sup>a</sup>
GOT (U/L)	15.8 $\pm$ 1.9 <sup>a</sup>	17.1 $\pm$ 1.6 <sup>a</sup>
GPT (U/L)	14.5 $\pm$ 2.4 <sup>a</sup>	14.0 $\pm$ 1.4 <sup>a</sup>

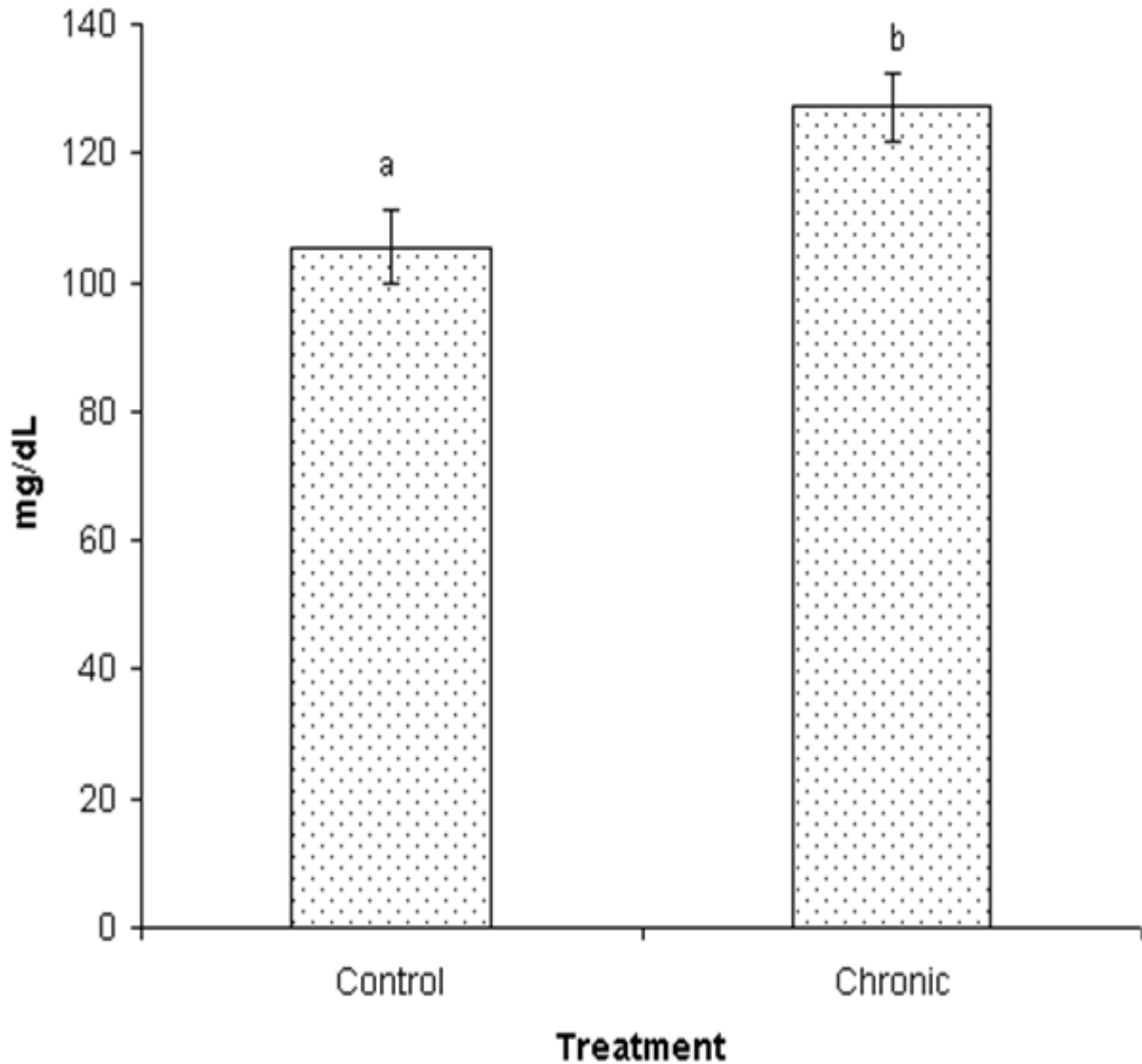
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mg/kg body weight) for 14 days. These changes were correlated with concomitant histopathological changes in the testes of treated rats.

Hormonal measurement has limited but important role in the assessment of gonadal dysfunction. The low plasma testosterone concentration indicates primary testicular dysfunction (Mayne 1994). Parshad et al.

(1994) reported significant decreases in testosterone level in rats receiving neem aqueous extract. *A. indica* produced dose dependant reduction in serum testosterone and LH with decreased weight of testes (Raji et al., 2003). Testosterone low level may be attributed to leydig cells failure. The study carried out by Kasturi and his colleagues showed clear leydig cells





**Figure 7.** Subacute toxicity effect of neem seed aqueous extract on plasma glucose concentration.

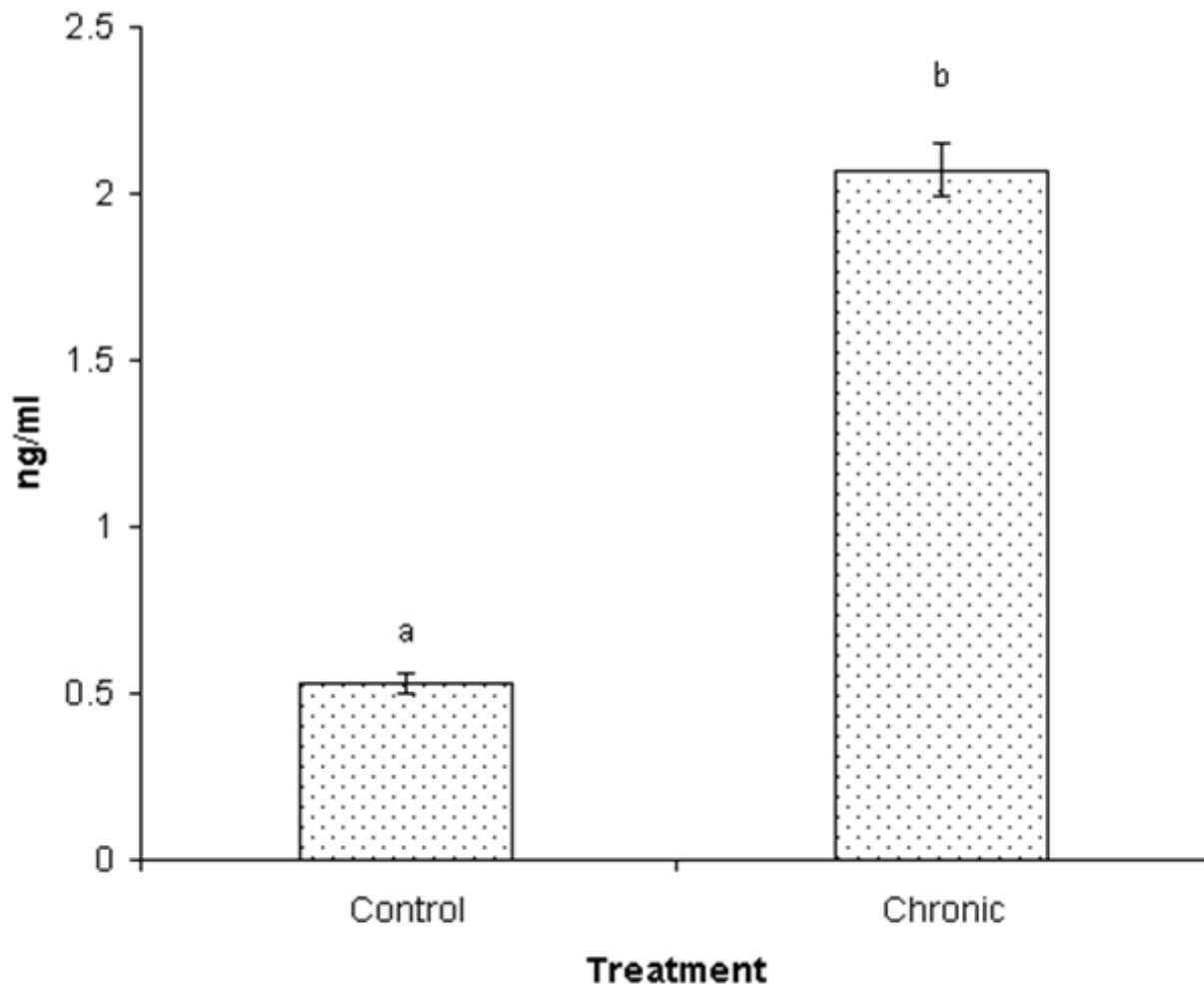
**Table 4.** Effects of neem seed aqueous extract on testosterone concentration of rats: Subacute toxicity (mean  $\pm$  SE).

Treatment	Hormone (ng/ml)
Control (2)	0.53 $\pm$ 0.03 <sup>a</sup>
Treated (1)	2.07 $\pm$ 0.08 <sup>b</sup>

Means values for the same column with different alphabetic superscripts are significantly different at  $p < 0.05$ .

damage in rats that received oral dose of neem leaves powder (Kasturi et al., 2002). Mohan et al. (1997) reported the antifertility effects of neem seed kernel on chickens, which included reduced semen volume and sperm concentration, increase the incidence of

morphological sperm abnormalities, higher number of degenerating cells and poor spermatogenesis. Similar result was observed in study that was carried out by Upadhyay et al. (1993) when male rats received neem oil in lumen of the vas deferens, the animal remained



**Figure 8.** Subacute toxicity effect of neem seed aqueous extract on plasma testosterone concentration.

infertile for 8 months without affecting testosterone production. Reduction in seminiferous tubules, nuclei of germinal element and mass atrophy of the spermatogenic elements were observed in the study that was carried out by Joshi et al. (1996) on rats treated with leaf powder of *A. indica*. In the previous studies it was clear that neem extract caused obvious changes in male reproductive organ. Another interesting investigation was carried out by Owolabi et al. (2008) whom demonstrated no histological impairment for rats dosed at 200 and 400 mg/Kg by methanolic leaves extract for a period up to 14 days of treatment. This group detected no effect on FSH level; however, serum levels of LH were significantly reduced when compared with the control, therefore they concluded a possible adverse effect of neem extract on female fertility.

### Conclusion

Taken these results together, we can come to the

conclusion that neem seed aqueous extract possesses dose-dependent hepatorenal toxicity in addition to its antifertility adverse effects. Therefore, the use of neem extract and its products for therapeutic purposes and control of pests may not be considered safe.

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