



Morphological & Biochemical Effects of Aqueous Extract of Neem (*Azadirachta indica*) on Liver of Adult Albino Rats

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ABSTRACT

Introduction: Neem (*Azadirachta indica*) is an important medicinal plant which is traditionally used all over the world as household remedy in diabetic and hypertensive patients and also for the cure of various dermatological ailments. **Aims & Objectives:** To evaluate the gross and biochemical effects of aqueous extract of neem leaves on the liver of adult albino rats. **Place and duration of study:** The study was undertaken at the Anatomy department of Shaikh Zayed Postgraduate Medical Institute, Lahore. **Material & Methods:** 45 Albino rats of both genders were used and equally divided into group A (Control), group B (low dosage) and group C (high dosage), each containing 15 animals randomly. The rats of group A received distilled water, while group B and C received 40 mg/kg and 100mg/kg of aqueous extract of neem respectively for 20 days using an orogastric tube. At the end of complete dosing schedule, rats were sacrificed and the livers were dissected out for examination. Animals were evaluated for gross (appearance of rats, appearance of liver, body weight of animal, weight of liver, relative tissue body weight index) as well as for biochemical (Serum ALT levels) parameters. **Results:** The body weight and weights of livers of experimental groups were decreased as compared to control group and it was statistically significant with p-value < 0.001. Similarly biochemical parameters were markedly impaired in group B and group C. **Conclusion:** The present research work demarcates that the higher doses of neem extract induce remarkable gross and biochemical effects on liver.

Key words: *Azadirachta indica*, Albino rat, sluggish, hemorrhagic areas.

INTRODUCTION

Neem is related to the Mahogany tree of Meliaceae family. Its botanical name is *Azadirachta indica* A. Juss (Syn: *Melia Azadirachta*).¹ Neem is native of India and harvested in tropical and sub tropical countries with widespread distribution throughout the world.² *Azadirachta indica* is evergreen tall tree (up to 20-30 meters). It has white flowers that develops into bunches of small fruits which are swollen and look like olives. Its complex leaf pattern resembles to that of walnut with spreading branches like crown.³ It starts fruiting from 3-5 years and has a life expectancy up to 200

years. Neem shows an adaptation to wide range of temperature. It can survive in hot temperature as high as 44 °C and tolerates cold from 0°C – 4°C to an altitudes up to 500 m. Usually grows in those areas where annual rainfall is from 450 mm to 1200 mm.^{3,4} Literally each and every part of the plant is blessed with medicinal properties and is commercially available. It is mother of every therapeutically used plant that has been utilized extensively many decades back and still been utilizing for medicinal and ritual purposes. It's low cost and easy availability has enabled many individuals to pick up advantage from this dynamic plant.⁵

The neem tree contains about 140 bioactive ingredients and it is rich in proteins. *Azadirachta*

indica is bitter in taste because of the presence of complex compounds called "Triterpenes". Its tree contains about 40 different types of active agents known as Tetraterpenoids or Limonoids.^{6,7} Several chemical constituents have been isolated from neem leaves using different solvent systems like water (hot or cold), ethanol, methanol, acetone, petroleum, ether and hexane. The most active and well known chemical compounds found in neem are Azadirachtins.^{8, 9} It is highly oxidized triterpenoid. Azadirachtins rapidly dissociates in the presence of light and moisture. But Azadirachtin is stable when composed in oily medium together with neem natural compounds.^{10, 11, 12}

The accurate mechanism of neem is not well recognized but possible pharmacokinetics of neem leaves are involvement with mitochondrial bioenergetics which leads to inhibition of electrochemical proton gradient which is the main energy generated in mitochondria. This inability to utilize oxygen is presented as cytotoxic hypoxia and that ultimately leads to metabolic acidosis and hyperpnoea¹³. Neem also interacts with receptors and changes membrane permeability and integrity.^{13,14} It has been proven that *Azadirachta indica* leaf and bark extract inhibit prostaglandin synthetase as compared to acetyl salicylic acid.^{15,16} It has been documented that neem is an effective hypoglycaemic agent as it increases insulin secretion and promotes the utilization of glucose by peripheral receptors as it hinders the effects of epinephrine on metabolism of glucose.^{16, 17}

They have tremendous fungicidal and bactericidal properties along with the quality of regulating growth in insects. In addition one of the major ingredients of neem leaf aqueous extract is Nimbidin which has anti-inflammatory, anti-pyretic, anti-arthritis, hypoglycaemic, antiulcer and antitumor effects.^{17, 18}

Various researches have been carried out to evaluate the effects of neem on different animals. The *Azadirachta indica* leaf aqueous extract caused damage to seminiferous tubules of male mice at a dose of 200mg/kg destruction and also distortion of spermatogenesis.¹⁹ Similar effects are also observed in testis of monkeys.²⁰

A study was conducted to show the effects of Vepacide, a neem based pesticide on the biochemical enzymes like aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in serum and different tissues of rats of both

genders. Albino rats were given low (80mg/kg), medium (160mg/kg) and high (320 mg/kg) dosage of coconut oil containing vepacide orally for 90 days. Biochemical profile suggested that by increasing the dose of vepacide caused an increase in the ALT and AST enzymes of serum, kidneys and lung tissues whereas the levels of these enzymes were lowered in liver tissue.

As the neem is extensively used by people as self-medication without proper medical advice and its use is increasing day by day and the possible consequences lead to an evaluation of effects of chronic therapy with the drug.

Therefore, the present research is being conducted with an aim to observe the consequences of neem leaves aqueous extract on morphology of adult albino rats.

MATERIAL AND METHODS

45 adult albino rats of both strains (weighing about 180-200g) were obtained from Veterinary Research Institute, Lahore. They were divided in three groups; A (control), B (experimental low dose) and C (experimental high dose). Each group consisted of 15 rats. The weight of each rat was carefully recorded in a Performa. For identification, the rats were marked with permanent pointer and were placed in different cages for 21 days. A 12 hours light / dark cycle was maintained.²¹ The animals were allowed free access to food and water. .

ALT levels of all three groups were measured in 1cc of blood in each rat prior to the administration of the neem extract or the start of experiment. Fresh leaves of neem were collected locally and then its extract was obtained from the PCSIR, Laboratories Complex, Lahore. Water extraction was performed by refluxing the powdered leaves with distilled water.²²

Aqueous extract of neem leaves was given to the animals by orogastric intubation.

The control group A containing 15 rats and were not given any extract except for equivalent proportion of 0.1% distilled water, 20ml per kg of weight of body by orogastric intubation for 20 days. The experimental group B containing 15 rats, each of which received 40mg per kg body weight of neem extract by orogastric intubation for 20 days (for example: if the weight of rat was 180g, 7.2 mg dry neem powder dissolved in 2ml of distilled water was given. It means each ml contains 3.6 mg of powder.)

Then the experimental group C contained 15 rats. All the animals of this group were given 100 mg per kg body weight of neem extract by orogastric tube for 20 days (For example: if the weight of rat was 180g, 18 mg dry neem powder was dissolved in 2ml of distilled water. It means each ml contains 9 mg of powder). Hence the dose was adjusted according to weight of the rat.

At the end of study, the rats of all groups were weighed properly and recorded in performa. For ALT determination, blood samples were collected from each rat through tail and allowed to clot.

All the rats were euthanized by giving morphine 0.3–0.5mg/kg intraperitoneally, as an analgesic agent. The anaesthetic agent sodium pentobarbital was administered intraperitoneally with dose of 45mg/kg.¹³ The animals were put in a supine position with their belly facing up and limbs fixed to the dissection board. A midline incision was made with a pair of scissors from groin to chin and extended laterally. Liver was made free from surrounding structures and placed on a blotting paper to make it free of blood and fluids. After recording the weight of the liver, it was washed with normal saline to remove blood and fixed with 10% formalin for 48 hours in appropriately labelled tissue bottles.

Statistical analysis:

The overall data was calculated and compared with the help of computer software Social Package of Statistical Sciences (SPSS) version 24. Qualitative variables like gross appearance were described by using frequencies and percentage for each group. Comparison for these qualitative variables among groups was performed by using CHI-SQUARE test. Quantitative variables like weight of animal before sacrificing, weight of liver, relative tissue weight index (RTWI) & serum ALT levels were described by using mean ± S.D. Comparison for these quantitative variable was performed by using ANOVA, Tukey’s test for post-hoc analysis was used where required. P-value ≤ to 0.05 was considered statistically significant.

RESULTS

The animals of control group A were active and healthy looking throughout the experiment. Eating habits of this group was normal. Statistically this parameter is constant.

At the end of experiment, 3 animals in group B and 7 in group C looked apparently abnormal, as compared to control group A. These animals were lazy and sluggish in response. There was statistically significant difference in the gross appearance of rats among three groups with p-value 0.003. There was significant decrease in the weight of animals of group B & C as compared to control group A. When comparison was made for liver weight, the animals of group A had higher weight for liver than that of experimental groups. (Table-1, Table-2). Similarly the relative tissue body weight index was significantly reduced in group B and group. (Table-3)

The external surface of all 15 livers of control group A was smooth and their colour was reddish brown. But the gross appearance of livers of 3 rats of experimental group B and 7 rats of experimental group C showed haemorrhagic areas on their external surface, randomly affecting all lobes as compared to smooth surface of control group A. (Table-1, Fig-1,2,3)

The serum was rapidly separated by centrifugation of clotted blood. Sera were stored at -20°C until assayed for the biochemical parameter. Alanine amino transferase were determined on fully automatic chemistry auto analyzer, Dimension, RXL from Siemens, USA. There was slightly increase in ALT levels from experimental group B and group C with p-values 0.002 and <0.001 respectively.

Group	Gross appearance of rats						Gross appearance of liver					
	Normal		Any abnormal-ity		Total		Normal		Any abnormal-ity		Total	
	N	%	N	%	N	%	N	%	N	%	N	%
Group A	15	100	0	0	15	100	15	100	0	0	15	100
Group B	12	80	3	20	15	100	12	80	3	20	15	100
Group C	8	53.3	7	46.7	15	100	8	53.3	7	46.7	15	100
Total	35	77.8	10	22.2	45	100	35	77.8	10	22.2	45	100

Chi-square = 45.0 p-value < 0.001

Table-1: Comparison of gross appearance of rats and liver in control and experimental groups after administration of neem extract

Key

- N Number of animals in each group
- A Control Group
- B Experimental Group
- C Experimental Group

Body Weight of Rats						Liver Weights of Rats				
	Sum of Squares	df	Mean Square	F	p- value.	Sum of Squares	df	Mean Square	F	p- value
Between Groups	3729.7	2	1864.9	16.7	-	66.06	2	33.03	48.58	< 0.001**
					< 0.001**	28.55	42	0.68	-	-
Within Groups	4689.6	42	111.7	-	-	94.61	44	-	-	-
Total	8419.3	44	-	-	-	-	-	-	-	-

Table-2: Comparison of body weight and liver weights of rats in control and experimental groups after administration of neem extract

Based on ANOVA

df Degree of Freedom

F f-test (Ratio of Variances)

** Highly Significant Difference ($p < 0.001$)

Relative tissue weight index of Liver of Rats						ALT Levels of Rats				
	Sum of Squares	df	Mean Square	F	p-value	Sum of Squares	df	Mean Square	F	p- .value
Between Groups	10.5	2	5.2	39.9	< 0.001**	35.79	2	17.89	30.59	< 0.001**
Within Groups	5.5	42	0.1	-	-	24.57	42	0.59	-	-
Total	16	44	-	-	-	60.36	44	-	-	-

Table-3: Comparison of relative tissue weight index & ALT levels of rats in control and experimental groups after administration of neem extract

Based on ANOVA

df Degree of Freedom

F f-test (Ratio of Variances)

** Highly Significant Difference ($p < 0.001$)

Gross appearance



Fig-1: Liver of Control Group A Showing smooth external surface.

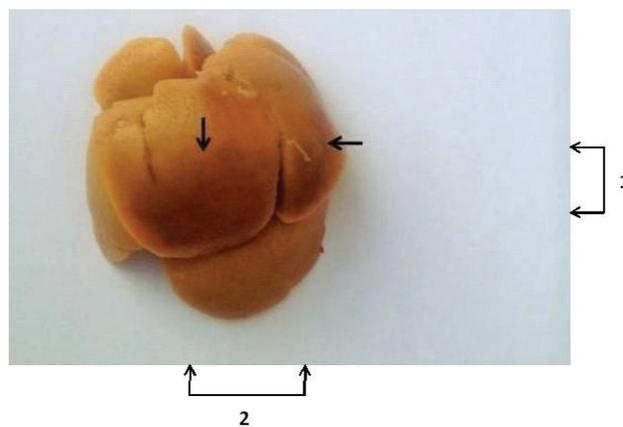


Fig-2: Liver of Experimental Group B, Black arrows shows gross hemorrhagic areas and extent of the lesions on the external surface.

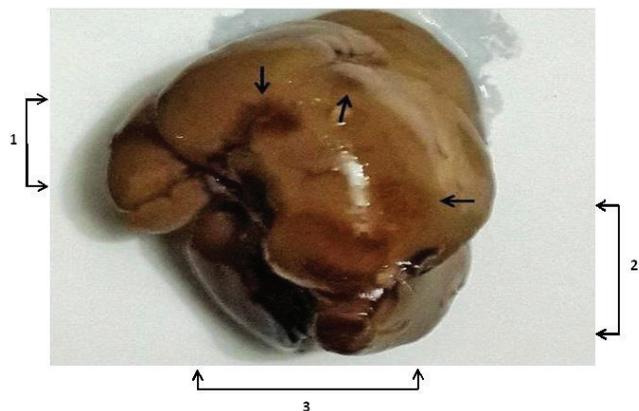


Fig-3: Liver of Experimental Group C, Black arrows show gross hemorrhagic areas and extent of the lesions on the external surface

DISCUSSION

Generally Neem has been utilized as a vital piece of our lives for quite a long time as an insecticidal,²³ disinfectant, prophylactic, antipyretic, antiparasitic, antiarthritic,^{21,22} antifungal and hypoglycaemic agent.^{23,24} People use it as an alternative treatment for a variety of health ailments and skin problems.²⁵ Neem leaf extract is common supplement which is easily available in drug stores and raw leaves are often consumed by many people in our society. It is usually considered as safer in wide range of doses. Now it has been proved that it is toxic at higher doses especially when used for longer duration. Its harmful effects on liver, kidneys, lungs and male and female reproductive organs have also been well documented. It is considered very dangerous to the children when used in any dose.^{25,26}

Azadirachtin is the most bioactive tetranortriterpenoid which is the bitter component of neem^{9,10,11} and has been proved lethal. Its unique quality is due to its anti-feedent properties against insects. It acts as systemic poison, moult inhibitor, causes delay in post embryonic development, antifertility effects, chitin and enzyme inhibition.^{16,26} The present work was designed to evaluate the harmful effects of neem on morphological and biochemical parameters of liver as it is the main metabolizing organ for neem and its constituents. It has been suggested that neem based formulations affect membrane alterations and influence the oxidant defence mechanism.²⁷ In the present study the gross appearance of rats of control group A were normal after the administration of neem extract but the 3 animals of group B and 7 animals of group C

were lazy, weak and not responding to the particular stimuli, at the end of experiment. The difference in appearance was significantly different among different groups with p-value 0.003.

In this study the average body weight of experimental groups B and C showed gradual weight loss and feed intake after neem consumption as compared to control group A. The difference was significant with p-value <0.001, suggesting toxic stress and growth retardation in treated rats. The result of this present research work showed overall weight loss which coincides with the findings of previous studies by Rahman and Siddique that showed decreased appetite and weight loss of albino rats at higher doses of neem extract.²⁸

The result of the present study revealed that the gross appearance of livers of 3 rats of experimental group B and 7 rats of experimental group C had haemorrhagic areas on external surface randomly affecting all lobes as compared to smooth surface of control group A. These results showed that by increasing the dose of neem, the haemorrhagic areas were also increased. It was possibly due to the toxic stress in treated rats especially on blood and blood forming elements.^{27,28} These findings correlate with the study performed by Samuel, Thomas and others, who observed the dose related prolongation of PT and APTT values by oral administration of crude neem leaf acetone-water extract on albino rats. They suggested that it may be due to impaired liver function which could influence directly or indirectly the synthesis of coagulation factors.²⁸

When the comparison of average liver weights was made among three groups, it was observed that the average liver weight was decreased in experimental group B which was low dose group and further reduced in high dosage that was group C. This clearly showed that the decrease in animal body weight and liver weights resulted in decreasing the RTWI values in experimental groups. The possible mechanism of toxicity due to neem based formulation is time, dose and tissue specific inhibition in glutathione-s-transferase, reduced glutathione and UDP-glucuronyl transferase activity in liver, lungs, kidneys and brain. Also there is decrease in cytochrome P-450 reductase activity in liver and brain.²⁸

In the present study, the average ALT level for group A was significantly lower than of group B and C with p-values 0.002 and <0.001, the level of group B was significant from group C with p-value

<0.001. This finding correlates with the studies of Rahman and Siddiqui, who proved that the exposure to neem based pesticides caused an increase in aspartate and alanine aminotransferase in serum, kidneys and lungs whereas these enzymes decreased in liver.^{26,27} This might be due to increased permeability of plasma membranes followed by the necrosis of cellular tissues caused by the neem.

CONCLUSION

This examination demonstrated that organization of aqueous neem leaf extract in high dosages for longer time caused noteworthy negative effect on the morphology and biochemical parameters of liver of adult albino rats. Although neem is commonly used as non-allopathic medicine but dosing still is not standardized. There is a need to evaluate safer dose and duration of usage of neem in general public.

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