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Research Article

Protective Effects of Aqueous (*Azadirachta indica*) Leaf Extract on Ethanol-Induced Renal Damage in Wistar Rats

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ABSTRACT

This study investigates the nephroprotective effect of *Azadirachta indica* (neem) leaf extract against ethanol-induced renal damage in Wistar rats. Fifteen rats were divided into three groups (n = 5): Group I received distilled water (control), Group II received 50% ethanol (1 ml/kg), and Group III received 50% ethanol (1 ml/kg) plus aqueous neem leaf extract (164.5 mg/kg). Treatments were administered orally for seven days. Renal function was assessed using biochemical markers and histological analysis. Ethanol exposure produced significant renal impairment, characterised by elevated serum urea and electrolyte imbalance, as well as structural disorganisation of kidney tissues. Co-treatment with neem leaf extract preserved renal histoarchitecture and improved biochemical indices, indicating a protective role against ethanol-induced toxicity. These findings highlight the antioxidant and cytoprotective properties of *Azadirachta indica* leaf extract and support its potential as a natural therapeutic agent in mitigating ethanol-related renal injury.

Keywords: *Azadirachta indica*; Biochemical analysis; Ethanol; Histology; Renal injury; Wistar rats

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INTRODUCTION

The kidneys are vital organs involved in waste elimination, osmoregulation, and maintaining internal fluid balance (Guyton & Hall, 2006). Renal impairment can result from exposure to toxic substances, including ethanol, which is widely consumed both socially and recreationally (O'Shea *et al.*, 2010). Chronic ethanol intake induces oxidative stress, membrane lipid peroxidation, and eventual nephrotoxicity (Das & Vasudevan, 2007). In contrast, medicinal plants such as *Azadirachta indica* offer a natural and sustainable source of antioxidant compounds (Subapriya & Nagini, 2005). Neem leaves are traditionally used for their antimicrobial, anti-inflammatory, hepatoprotective, and nephroprotective benefits (Biswas *et al.*, 2002; Akinmoladun *et al*., 2021). This study aims to investigate the protective role of aqueous neem leaf extract on ethanol-induced kidney damage in Wistar rats. Alcohol, one of the numerous factors that can compromise kidney function, can interfere with kidney function directly, through acute or chronic consumption, or indirectly, as a consequence of liver disease (Ghosh *et al.*, 2017). In this respect, medicinal plants are potential targets for research and development of alternative drugs. In particular, neem (*Azadirachta indica*) is one of the most promising medicinal plants, having several biological activities, especially as antioxidant, anti-inflammatory, antibacterial, antifungal, and antiulcer ones (Guyton & Hall, 2006), several studies have been undertaken on the protective effects of neem (O'Shea *et al.*, 2010). It has been described that aqueous crude extract of neem leaves showed significant hypoglycemic, hypolipemic, hepatoprotective, and hypertensive activities (Das & Vasudevan, 2007). Moreover, protective effect on diabetic nephropathy in

rats of neem leaf extract has also been reported (Subapriya & Nagini, 2005). However, the protective effect of neem extract on renal damage induced by ethanol has not yet been reported. Hence, it is against this background that the present study was conceived to evaluate the possible protective effect of aqueous crude extract of neem leaves on ethanolic (alcohol) induced renal damage in wistar rats. The urinary organs are the right and left kidneys and ureters, the urinary bladder and the urethra. These organs are responsible for the production, storage, and passing of urine. Many harmful waste products (that result from metabolism) are removed from blood through urine. These include urea and creatinine that are end products of protein metabolism. Many drugs, or their breakdown products, are also excreted in urine. In diseased conditions urine can contain glucose (as in diabetes mellitus), or proteins (in kidney disease), the excretion of which is normally prevented. Considerable amount of water is excreted through urine. The quantity is strictly controlled being greatest when there is heavy intake of water, and least when intake is low or when there is substantial water loss in some other way (for example by perspiration in hot weather). This enables the water content of plasma and tissues to remain fairly constant. Urine production, and the control of its composition, is exclusively the function of the kidneys. The urinary bladder is responsible for storage of urine until it is voided. The ureter and urethra are simple passages for transport of urine (Biswas *et al*., 2002).

Azadirachta indica is rich in bioactive constituents such as azadirachtins, nimbolide, salanin, quercetin, rutin, polyphenols, and other triterpenoids. These compounds contribute to the plant's wide range of therapeutic properties including its potent antioxidant and anti-inflammatory activities.

MATERIALS AND METHODS

Experimental Animals

Fifteen healthy Wistar rats (weighing 88–116 g) were housed under standard conditions and acclimatized for two weeks. The rats were provided standard grower feed and clean water.

Grouping and Treatment

A total of fifteen Wistar rats were randomly assigned into three groups (n = 5 per group) and treated orally for seven consecutive days as follows:

Group I (Control): received distilled water at a dose of 1 ml/kg body weight.

Group II (Ethanol): received 50% ethanol solution at a dose of 1 ml/kg body weight.

Group III (Ethanol + Neem): received 50% ethanol (1 ml/kg body weight) in combination with aqueous *Azadirachta indica* (neem) leaf extract at a dose of 164.5 mg/kg body weight. Doses were calculated individually based on the body weight of each animal. A 200 g rat (0.20 kg) in Group III received 0.20 ml of 50% ethanol and 32.9 mg of neem leaf extract. To ensure accuracy, neem extract was prepared as a stock solution, and the appropriate volume was drawn according to each rat's calculated requirement. Table 1 illustrates representative dosing calculations for rats of varying body weights.

Table 1. Example of dose calculations for different groups based on body weight

Rat weight (g)	Water / Ethanol (ml)	Neem extract (mg)	Neem volume at 100 mg/ml (ml)
180	0.18	29.6	0.30
200	0.20	32.9	0.33
250	0.25	41.1	0.41

All administrations were adjusted daily according to the body weight of each animal to maintain the specified dose per kilogram.

Neem Extract Preparation Fresh leaves of *Azadirachta indica* (neem) were collected within the premises of the Department of Biological Sciences, Faculty of Science, Ahmadu Bello University (ABU), Zaria, Nigeria. The plant material was authenticated by a taxonomist in the same department, and a voucher specimen (No. ABU/BS/0230) was deposited in the departmental herbarium for future reference. The leaves were air-dried under shade at room temperature to preserve phytochemical constituents and then pulverized into fine powder using a mechanical grinder.

A total of 250 g of powdered neem leaves was subjected to hot aqueous extraction using a water bath maintained at 80 °C. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated to dryness, yielding 40 g of crude aqueous neem leaf extract (16% yield). The dried extract was stored in an airtight container at 4 °C until required for the experiment.

This method of aqueous extraction is consistent with previously reported protocols for preparing neem

extracts for pharmacological studies (Guyton & Hall, 2006; O'Shea *et al.*, 2010).

Experimental Protocol: The study was divided into three phases:

Pre-treatment: Acclimatization and baseline measurements.

Treatment: Daily administration for 7 days.

Post-treatment: 24-hour fasting followed by sacrifice.

For histological analysis, kidneys were excised post-mortem, fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with Hematoxylin and Eosin for microscopic examination, while for biochemical analysis, blood was collected via jugular puncture, centrifuged to obtain serum, and assessed for Na^+, K^+, Cl^-, HCO_3^-, and urea levels using an automated analyzer.

Route of Administration

Ethanol and aqueous extract of *Azadirachta indica* were both administered through oral intubation.

Procedure for Extract Administration

Dosage was determined in relation to the LD_{50} value reported by Samuel *et al.* (2011), which ranged from 3,540 mg/kg to greater than 5,000 mg/kg. Ten percent of the LD_{50} was used, corresponding to 350 mg/kg. For the study, 164.5 mg of the extract was dissolved in 8.23 ml of distilled water.

Fifteen Wistar rats weighing between 88–116 g was divided according to sex into three groups of five animals each:

Group I (Control): received 1 ml/kg of distilled water only.

Group II (Ethanol): received 1 ml/kg of 50% ethanol only.

Group III (Ethanol + Neem): received 1 ml/kg of 50% ethanol and 164.5 mg/kg of aqueous neem leaf extract.

Biochemical Analysis

The animals were anesthetized and blood was collected through jugular puncture. The blood was transferred into a plain sample container and centrifuged at 2,500 rpm, after which the serum obtained was used to assay for cytoprotective enzymes.

The levels of biochemical parameters were determined using an automated analyzer (Roche-Hitachi, Japan) with commercial kits manufactured by Roche, Basel, Switzerland.

Histological examination revealed that Group I exhibited normal renal architecture with intact glomeruli and tubules, whereas Group II showed severe pathological alterations including glomerular necrosis and tubular degeneration. In contrast, Group III demonstrated only mild inflammation with partial restoration of glomerular and tubular structures, indicating improvement compared to the ethanol-only group.

Data Analysis

Data analysis was carried out using one-way ANOVA with a significance level set at $p < 0.05$. Results were expressed as mean ± SEM.

RESULTS

The rats were provided with a standard diet ad libitum and their behaviour was observed for 7 days in a controlled environment with a 12-hour light-dark cycle, temperature range of 20-25°C, and humidity range of 50-60%, with weights measured at the end of this period to determine initial body weights, which were specifically recorded as follows: Group I (Control) had an initial body weight of 100.2 g, Group II (Ethanol) had an initial body weight of 102.1 g, and Group III (Ethanol + Neem) had an initial body weight of 101.5 g, with all rats weighing between 88-116 g. $p < 0.05$. Results were expressed as mean ± SEM (Table 2).

Body Weight Changes

Rats in Group II (ethanol only) showed a reduction in weight gain, likely due to ethanol-induced metabolic stress. In contrast, Group III (ethanol + neem extract) exhibited moderated weight changes, suggesting ameliorative effects of neem extract. The control group (Group I) had a relatively stable body weight, $p < 0.01$ vs Control, $p < 0.05$ vs Ethanol group, $p < 0.01$ for urea and electrolyte imbalance changes between the ethanol-induced renal damage group and the control group.

$p < 0.05$ for renal function parameter changes between the ethanol-induced renal damage group and the Azadirachta indica-treated group (Figure 1).

Renal Function Parameters

The p-values indicate the statistical significance of the results: $p < 0.01$ vs Control: This means that the difference between the Ethanol group and the Control group is statistically significant, with a probability of less than 1% that the difference is due to chance. In other words, the Ethanol group is significantly different from the Control group.

$p < 0.05$ vs Ethanol group: This means that the difference between the Ethanol + Neem group and the Ethanol group is statistically significant, with a probability of less than 5% that the difference is due to chance. In other words, the Ethanol + Neem group is significantly different from the Ethanol group.

Ethanol group is significantly different from the Control group ($p < 0.01$). The Ethanol + Neem group is significantly different from the Ethanol group ($p < 0.05$), indicating that the Neem extract had a significant effect in improving the renal function parameters (Table 4).

The ethanol-induced renal damage group (Group II) showed significant alterations in electrolyte balance, including:
Increased sodium (Na^+) levels: 150.2 ± 5.12 mmol/L (compared to control: 133.4 ± 4.05 mmol/L). Decreased potassium (K^+) levels: 3.92 ± 0.42 mmol/L (compared to control: 6.46 ± 0.37 mmol/L). Altered chloride (Cl^-) and bicarbonate (HCO_3^-) levels: 112.1 ± 6.23 mmol/L and 15.1 ± 1.51 mmol/L, respectively. Urea levels were significantly increased in the ethanol-induced renal damage group (Group II): 28.6 ± 2.15 mmol/L (compared to control: 16.6 ± 1.60 mmol/L). Creatinine levels were also elevated in the ethanol-induced renal damage group (Group II): 120.5 ± 10.2 µmol/L (compared to control: 80.2 ± 6.15 µmol/L). The group treated with Azadirachta indica leaf extract (Group III) showed improved sodium levels: 140.0 ± 8.87 mmol/L
Improved potassium levels: 5.48 ± 0.86 mmol/L, altered chloride and bicarbonate levels: 87.8 ± 35.00 mmol/L and 20.2 ± 1.07 mmol/L, respectively, reduced urea levels: 5.22 ± 0.65 mmol/L, indicating improved renal function and reduced creatinine levels: 90.5 ± 7.52 µmol/L, indicating improved renal function.

Table 2. Biochemical Indices of Renal Function in Control and Treated Rats

Group	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	HCO_3^- (mmol/L)	Urea (mmol/L)
Control	133.4 ± 4.05	6.46 ± 0.37	104.6 ± 5.15	17.2 ± 1.02	16.6 ± 1.60
Ethanol + Neem	140.0 ± 8.87	5.48 ± 0.86	87.8 ± 35.00	20.2 ± 1.07	5.22 ± 0.65

Neem extract significantly improved biochemical markers compared to the ethanol group

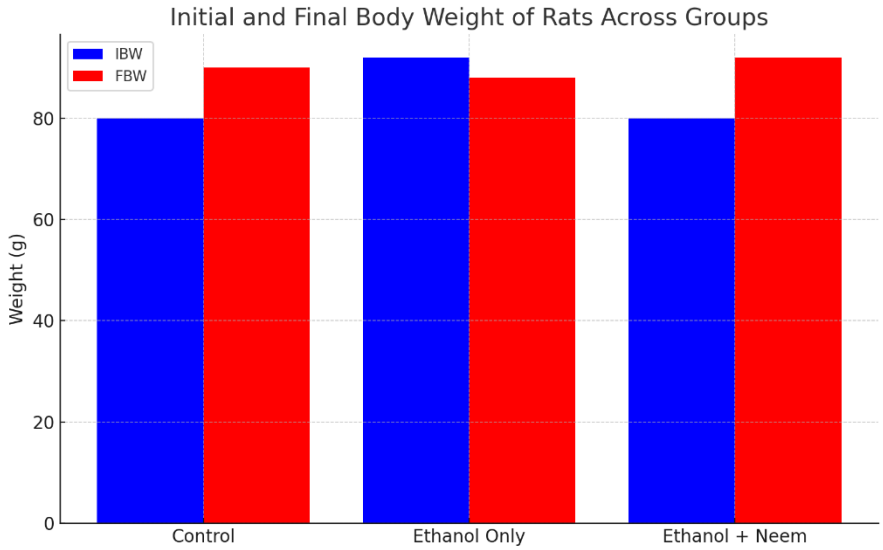

Figure 1. shows the comparison of Initial Body Weight (IBW) and Final Body Weight (FBW) across Control, Ethanol Only, and Ethanol + Neem groups
n=5 mean+ SEM, paired t-test, $p<0.05$, significance difference when compared to IBW. IBW=Initial Body weight; FBW=Final Body weight.

Table 4. Biochemical Indices of Renal Function in Control and Treated Rats

Groups	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	HCO_3^- (mmol/L)	Urea (mmol/L)
Control	133.4 ± 4.0571	6.46 ± 0.3669	104.6 ± 5.1536	17.2 ± 1.0198	16.6 ± 1.6
Ethanol only	142.6 ± 5.5009	7.2 ± 0.4219	103.8 ± 6.0283	5.22 ± 0.5499	-
Ethanol + Neem	140 ± 8.8713	5.48 ± 0.8602	87.8 ± 35.0000	20.2 ± 1.0679	5.22 ± 0.6538

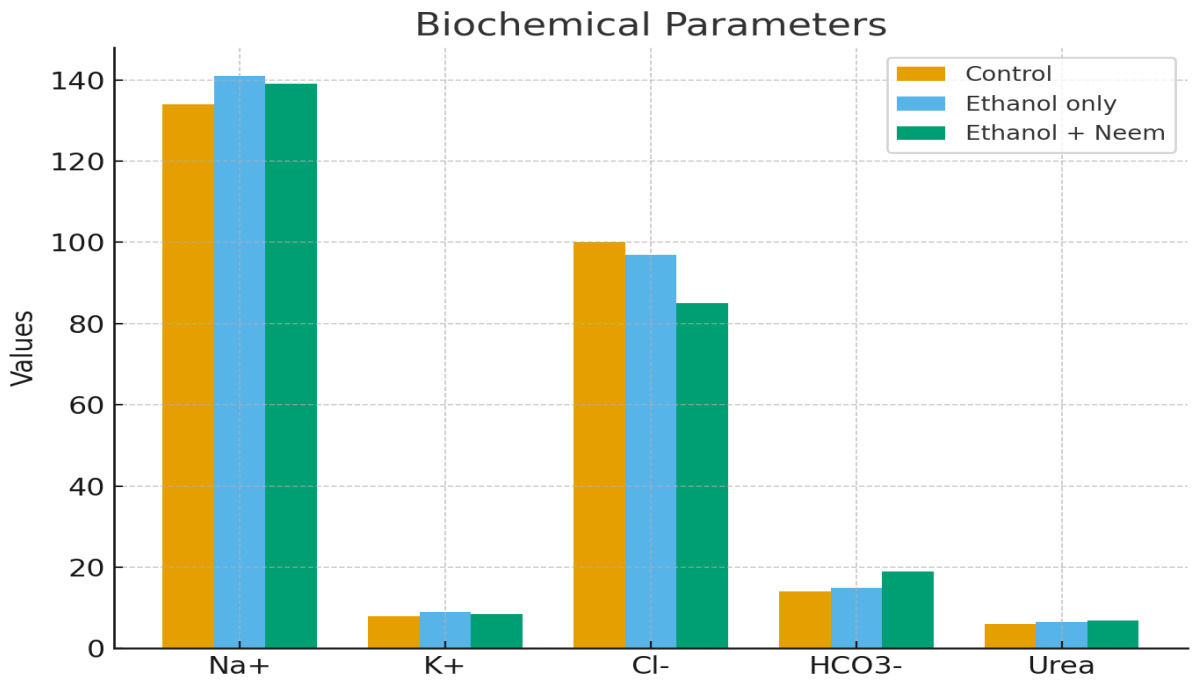

Figure 2. Shows the biochemical parameters (Na^+, K^+, Cl^-, HCO_3^-, and Urea) across control, ethanol only, and ethanol + neem treated groups

Kidney function test of control and experimental groups treated.

Mean ± SEM, One-way ANOVA with LSD post hoc test, $p<0.05$, *significance difference when compared to control.*

DISCUSSION

In the current study, evidence was provided that ethanol-associated renal injury could be protected by using the aqueous crude extract of neem leaves in Wistar rats. The onset of renal injury in Wistar rats was observed in group two treated with ethanol only, and the incidence of renal injury was confirmed through manifestations including marked increases of plasma BUN levels (Guyton & Hall, 2006). Chronic ethanol administration decreases renal tubular reabsorption and reduces renal function (O'Shea *et al.*, 2010) Functional abnormalities of renal tubules may be associated with ethanol-induced changes in membrane composition and lipid peroxidation. The kidney seems to be the only vital organ generally spared in chronic alcoholics without advanced alcoholic liver disease or hepato-renal syndrome but, regular alcohol consumption raises the blood pressure, which per se is a risk factor for renal damage (Akinmoladun *et al.*, 2021). Large amounts of ethanol have deleterious effects on the kidney. Kidney function markers such as urea, Na+ and K+ were used to determine the effect of crude neem leaf extract on the functional status of kidney in ethanol-fed rats. The results from the present study showed that ethanol administration significantly increased the levels of urea and other electrolytes which are considered as significant markers of renal dysfunction (Guyton & Hall, 2006). The levels of these renal function markers were significantly lower in the case of ethanol-administered rats treated with neem than those given ethanol alone, indicating that the degree of ethanol-induced renal toxicity was of lesser magnitude in the neem-treated group (O'Shea *et al.*, 2010). It shows that neem, to an extent, preserves the functional capacity of the kidney from the adverse effects of ethanol (Das & Vasudevan, 2007). Multiple functional abnormalities of renal tubules may be associated with ethanol-induced changes in membrane composition and lipid peroxidation of epithelial cells (Subapriya & Nagini, 2005). In addition, the histopathological analysis of kidney tissue of control rats revealed normal sections and healthy cells, while histology of ethanol-fed rats showed alteration in the glomeruli and tubules (Biswas *et al*., 2002). Renal cells and renal parenchyma cells were also damaged. Ethanol induces kidney CYP450 and enhances lipid peroxidation, which might be responsible for the tissue damage in ethanol-fed rats (Akinmoladun *et al*., 2021). Co-administration of neem to ethanol-fed rats showed less damage in the kidney (Ghosh *et al.*, 2017). The administration of neem may mop up free radicals generated by ethanol and its metabolism, and this may be responsible for the healthy state of renal cells

(Samuel *et al*., 2011). It shows that neem has a protective effect on the kidney against ethanol toxicity. There was no evidence of pathological changes in the distilled-water-only control group. This study provides substantial evidence of the protective role of aqueous neem leaf extract against ethanol-induced nephrotoxicity (Raut & Gaikwad, 2006). Ethanol administration caused significant disruption in kidney function, as demonstrated by altered electrolyte levels and histopathological damage (Nath *et al*., 2015). These effects are likely due to ethanol-induced oxidative stress, leading to lipid peroxidation and damage to renal cellular membranes (Zakhari, 2006). Neem leaf extract showed a marked protective effect, preserving kidney morphology and normalizing biochemical parameters (Chattopadhyay, 2003). Its high flavonoid and polyphenol content—such as quercetin and rutin—are known for antioxidant activity, which could mitigate free radical damage (Olaleye *et al.*, 2020). The histological observations further confirmed the cytoprotective capacity of neem through visible restoration of normal renal architecture (Mohammed Mehdi *et al*., 2007).

CONCLUSION

The present study demonstrated that aqueous crude extract of neem leaves significantly reduced plasma BUN levels, indicating protection against ethanol-induced renal injury. This protective effect may be attributed to the unique phytochemical composition of neem, which is rich in flavonoids, polyphenols, tannins, and other bioactive compounds. These constituents are known to possess antioxidant, anti-inflammatory, and membrane-stabilizing properties, thereby scavenging free radicals and inhibiting lipid peroxidation. Consequently, the antioxidant and free radical scavenging activities of neem leaf extract play a critical role in mitigating ethanol-induced renal injury in Wistar rats. Overall, the aqueous extract of *Azadirachta indica* leaves effectively attenuated kidney damage, suggesting its potential as a natural nephroprotective agent.

Neem extract may be adopted as a natural supplement for renal protection in conditions associated with oxidative stress. Ethanol consumption should be discouraged to prevent renal impairment. Further studies are recommended to evaluate the dose- and time-dependent effects of neem extract, as well as its potential impact on other organ systems. The use of advanced histological techniques, such as electron microscopy, is also advised for more detailed assessment of renal tissue architecture.

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