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Original Article

Evaluating the Ameliorative Effect of Aqueous *Azadirachta indica* Leaf Extract on Alcohol-Induced Liver Damage in Adult Wistar Rats

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Abstract

BACKGROUND AND AIM: Alcoholism is a significant cause of disease with serious social and economic implications and consequent effect on the brain, liver and cardiovascular system. Neem (*Azadirachta indica*) has been studied for its potential hepatoprotective effects, and some studies have explored its relation to liver health. However, few literatures demonstrated the hepatoprotective activities of Neem on alcohol-induced liver toxicity, hence the aim of this study was to evaluate the ameliorative potential of Neem on alcohol-induced liver damage.
METHODOLOGY: Thirty Wistar rats weighing between 120 and 170 grams were divided into six groups,(I-VI) of 5 rats per group and received, 1 ml of distilled water for 28 days, 1 ml of 50% alcohol only, 250 mg/kg body weight of *Azadirachta indica* only, 500 mg/kg body weight of *Azadirachta indica* only, 1 ml of 50% alcohol and 250 mg/kg body weight of *Azadirachta indica*, 1 ml of 50% alcohol and 500 mg/kg body weight of *Azadirachta indica* respectively. Alcohol and *Azadirachta indica* were administered for 14 eacg. At the end of the 14 and 28 days of treatment, blood and liver tissue were collected for analysis of liver function test and histology of the liver respectively.
RESULTS: There was significant reduction in weight changes in rats treated with 1 ml of 50% alcohol and 250 mg/kg body weight of *Azadirachta indica* when compared to control. There was significant increase ($P<0.05$) AST in the alcohol only group when compared to control which was reversed with treatment of *Azadirachta indica*. There was a significant increase ($P<0.05$) in TP, ALB, TB levels of alcoholic groups treated with 500 mg/kg body weights of *Azadirachta indica* when compared to alcohol only group. Histopathological analysis showed improved architecture in rats liver treated with aqueous extract of *Azadirachta indica*.
CONCLUSION: *Azadirachta indica* has hepatoprotective and ameliorative activities against alcohol-induced liver toxicity by enhancing the activities of AST, AST, TB TP, ALB and normal liver architecture as observed in this study.

Keywords:
Alcohol, *Azadirachta indica*, Liver, Hepatoprotective, Wistar rats

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INTRODUCTION

Alcohol, a psychoactive drug with addictive traits, has a long history of use across civilizations, contributing to alcoholism—a major health concern with significant societal and economic impacts. It is linked to over 200 illnesses, injuries, and health issues, including mental disorders and severe non-communicable diseases (World Health Organization, 2022). The effects of alcohol on various tissues are influenced by its blood concentration, determined by absorption, distribution, digestion, and excretion. Understanding the balance between alcohol elimination and the accumulation of metabolic byproducts is crucial for comprehending its short- and long-term effects (World Health Organization, 2022).

The liver is the body's largest reticulo-endothelial cell network, as well as an important function in the host's ability to fight against infection. It is made up of parenchymal and non-parenchymal cells, each with a specific function. It primarily metabolizes alcohol and is involved in first part metabolism (FPM) of alcohol together with the gastric Alcohol dehydrogenase (ADH).

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The most prevalent types of alcoholic liver disease are fatty liver, alcoholic hepatitis, and cirrhosis. Often, as people continue to drink heavily, they progress from fatty liver to alcoholic hepatitis to cirrhosis (Mann *et al,* 2003). Alcohol is eliminated from the body by various metabolic mechanisms. The primary enzymes involved are Aldehyde dehydrogenase (ALDH), Alcohol dehydrogenase (ADH), Cytochrome P450 (CYP2EI) and Catalase Zakhan (2006); (Zimatkin and Deitrich, 1997).

Azadirachta indica, or neem, a widely cultivated pantropical plant, is recognized for its diverse applications, including religious, economic, medical, and decorative purposes. It serves as a 'wonder' tree with various valuable components (roots, trunk, bark, leaves, flowers, fruits, and seeds) utilized for wood, fuel, pharmaceuticals, insecticides, and oil (Siddiqui, 1992; Vietmeyer, 1992; Gupta, 1993).

The study evaluates the liver function activity of *Azadirachta indica* leaf extract on alcohol-induced damage in Wistar rats, using indicators such as Aspartate Transferase (AST), Alanine Transaminase (ALT), Total Protein (TP), Conjugated Bilirubin (CB), and Albumin (ALB) and also the histology of the liver of adult Wistar rats.

MATERIALS AND METHOD

Collection and identification of plant material and preparation of aqueous extracts

Azadirachta indica leaves was obtained from the wild in Benin City, Edo State, Nigeria's Egor Local Government Area. They were identified in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Nigeria, and then air-dried for seven (7) days before being ground into powder and weighed on an electrical weighing scale. Extraction was carried out utilizing proven methods (Eze and Akonoafua, 2020).

Preparation of aqueous leave extract of *Azadirachta indica* was conducted in the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City. Before being macerated in distilled water in a jar, the seeds were pulverized in a British milling machine. 500 g of powder was soaked in 2 litres of cold distilled water in a conical flask. After twenty-four (24) hours, the solution (a mixture of seed extract powder and distilled water) was filtered with a filter rag and funnel. Before decanting the supernatant, the filtrate was allowed to settle for a time. At 60^{o}C, the supernatant was steamed to dryness in an evaporating dish (Royal Worcester, England) using an H-H Digital Thermometer Water Bath (Mc Donald Scientific International – 22050Hz1.0A). The extracts were kept refrigerated at 4°C in plastic vials until needed.

Experimental Animals

Thirty Wistar rats weighing between 117g and 165g were used. The rats were allowed to acclimatize for two weeks before administration. They were given free access to conventional rat feed and water. The research ethics committee's guidelines for animal treatment at the University of Benin's College of Medicine were espoused and fully implemented and ethical approval obtained.

The animals were randomly assigned into six (I to VI) groups of five animals per group. Animals in group I served as the control group, given distilled water (1 ml) for 28 days; animals in group II were given 1 ml of 50 % alcohol only for 14 days; animals in group III were given 250 mg/kg body weight of *Azadirachta indica* only for 14 days; animals in group IV were given 500mg/kg body weight of *Azadirachta indica* only for 14 days; animals in group V were given 1ml of 50 % alcohol for 14 days and thereafter treated with 250mg/kg body weight of *Azadirachta indica* for 14 days; animals in group VI were given 1 ml of 50 % alcohol for 14 days and thereafter treated with 500 mg/kg body weight of *Azadirachta indica* for 14 days. All administrations were done by gavage and lasted for fourteen (14) and twenty-eight (28) days respectively.

Tissue collection, processing and staining, histopathology

The rats were sacrificed and the liver were taken at the end of the two weeks and four weeks study. Blood (5 mL) was collected in sterile bottles for analysis of liver function test. The liver tissues were preserved for 72 hours in 10% buffered formalin before being histologically processed and stained with Haematoxylin and Eosin using standard procedures (Drury *et al*., 1976). The sections obtained were examined and photomicrographs were taken using a Leica DM750 research microscope with an attached digital camera (Leica CC50). The tissues were photographed digitally at magnifications of 400x.

Biochemical assays

Reitman and Frankel (1957) technique was used to measure aspartate transferase activity, Englehardt (1970) method for alkaline phosphate activity, Bradford (1976) method for total protein activity, Doumas *et al* (1971) method for albumin activity and Jendrassik and Grof (1938) method for conjugated bilirubin activity.

Statistical analysis

Graph Pad Prism Version 9.0 for Windows (GraphPad Software Inc.) was used to analyze the data obtained in the study.Results obtained were expressed as Mean ± SEM (standard error of mean). Differences among the means were determined by one-way analysis of variance (ANOVA). Values were considered statistically significant if P value was less than 0.05 ($p < 0.05$).

RESULTS

There was a statistically significant decrease (P<0.05) in the Body Weight (g) for rats treated with 1ml of 50% alcohol for 14 days followed by 250mg/kg of *Azadirachta indica* for 14 days when compared with 1ml of 50% alcohol only (Fig 1).

There was a statistically significant increase (P<0.05) in aspartate transferase level in rats treated with 1ml of 50% alcohol only group for 14 days when compared with control. There was a statistically significant decrease(P<0.05) in aspartate transferase level in rats treated with 1ml of 50% of alcohol only for 14days followed by 250mg/kg and 500 mg/kg of *Azadirachta indica* when compared with 1ml of 50% of alcohol only group (Fig 2).

There was a significant decrease (P<0.05) in alanine transaminase level in rats treated with 1ml of 50% alcohol only for 14 days when compared with control. There was a significant decrease (P<0.05) in alanine transaminase level in rats treated with 1 ml of 50% alcohol only for 14 days followed by 500mg/kg of *Azadirachta indica* for 14 days when compared with 1ml of 50% alcohol only group (Fig 3)

There was a significant decrease (P<0.05) in total protein in rats treated with 1 ml of 50% alcohol only for 14 days group, and 250mg/kg of *Azadirachta indica* only group for 14 when compared with control group. There was a significant increase (P<0.05) in total protein in rats treated with 1 ml of 50% alcohol for 14 days followed by 500 mg/kg of *Azadirachta indica* for 14 days when compared with 1ml of 50% alcohol only for 14 days groups (Fig 4).

There was a statistically significant decrease (P<0.05) in serum albumin level in rats treated with 1ml of 50% alcohol for 14days and a statistically significant decrease (P<0.05) in Albumin in rats treated with 250mg/kg of *Azadirachta indica* only for 14days when compared with control (Fig 5).

There was a significant decrease (P<0.05) in Total Bilirubin in rats treated with 1ml of 50% of alcohol only group for 14days when compared with control. There was a significant increase (P<0.05) in Total Bilirubin in rats treated with 1ml of 50% alcohol for 14days followed by 500mg/kg of Azadirachta indica for 14days when compared with 1ml of 50% alcohol only for 14days (Fig 6).

Histological findings

Histological findings showed normal liver architecture in control. There was zonal necrosis, periportal infiltrates of inflammatory cells and vascular congestion in rats liver treated with 1 ml of 50% alcohol for 14 days. Rats liver treated with 250 and 500 mg/kg body weights of extract of *Azadirachta indica* showed normal liver architecture. However, the 500 mg/kg body weight dose of extract of *Azadirachta indica* also showed active vascular congestion. Rats liver given 1 ml of 50% alcohol for 14 days and then treated with 250 mg/kg body weight of extract of *Azadirachta indica* showed normal liver architecture in addition to Kupffer cell activation and periportal lymphocyte mobilization. Rats liver given 1 ml of 50% alcohol for 14 days and then treated with 500 mg/kg body weight of extract of *Azadirachta indica* showed zonal necrosis, vascular congestion and ulceration, heavy periportal infiltrates of inflammatory cells and ductal epitheliosis

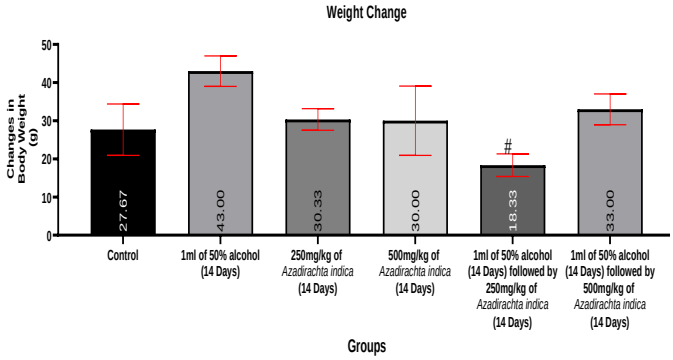

Fig 1. Changes Mean Body Weight.
Represents statistically significant difference (P<0.05) as compared to Alcohol only treated group.

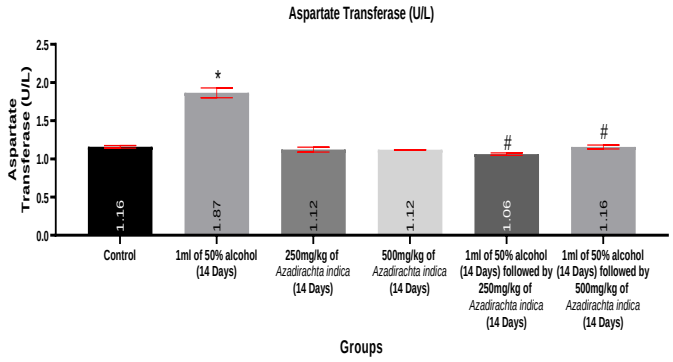

Fig 2. Aspartate Transferase.
*Represent comparison with control (P<0.05)
#Represent comparison with 1ml of 50% alcohol only group (P<0.05)

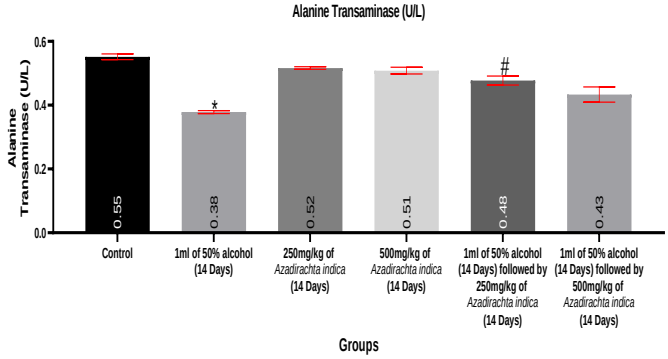

Fig 3. Alanine Transaminase.
*Represent comparison with control (P<0.05)
#Represent comparison with 1ml of 50% alcohol only for 14days group (P<0.04)

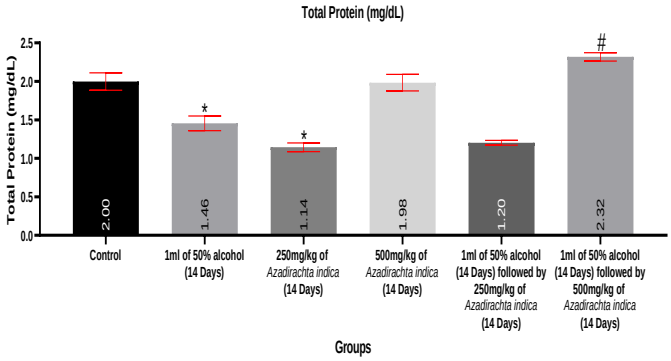

Fig 4. Total Protein.
*Represent comparison with control (P<0.05)
#Represent comparison (P<0.05) with 1ml of 50% alcohol only for 14days group

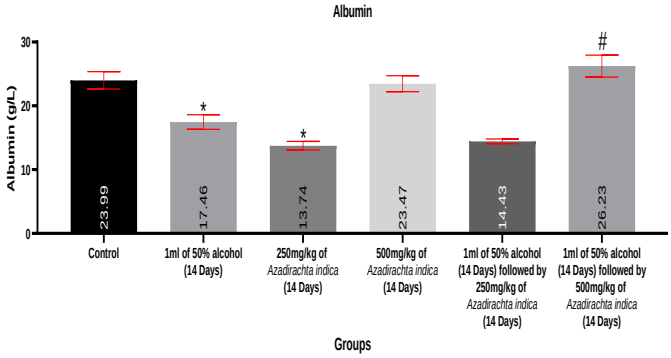

Fig 5. Albumin.
*Represent comparison with control (P<0.05)
#Represent comparison with 1ml of 50% alcohol only group for 14 days (P<0.05)

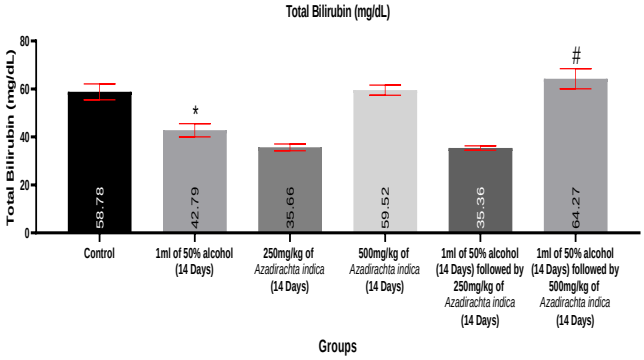

Fig 6. Total Bilirubin
*Represent comparison with control (P<0.05)
#Represent comparison with 1ml of alcohol only group (P<0.05)

Histological findings

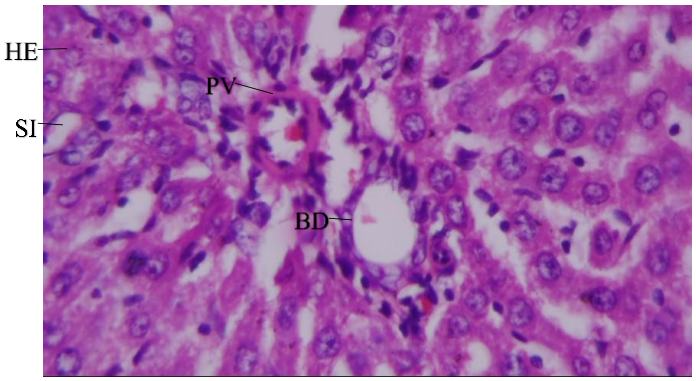

Plate 1: Rat liver. Control: Composed of normal architecture: hepatocytes (HE), sinusoids (SI), portal vein (PV), bile duct (BD) (H&E; 400x)

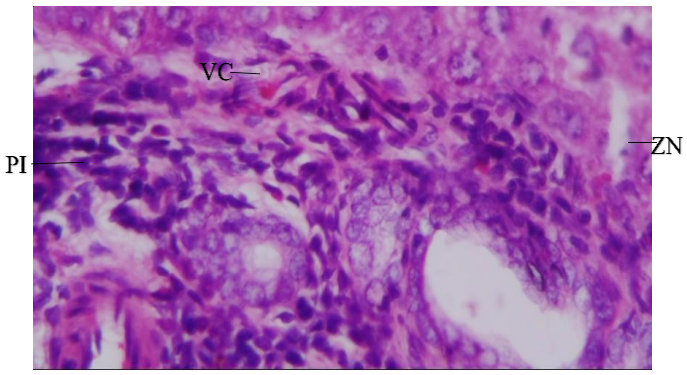

Plate 2. Rat liver given Alcohol only showing: zonal necrosis (ZN) periportal infiltrates of inflammatory cells (PI), vascular congestion (VC) (H&E; 400x)

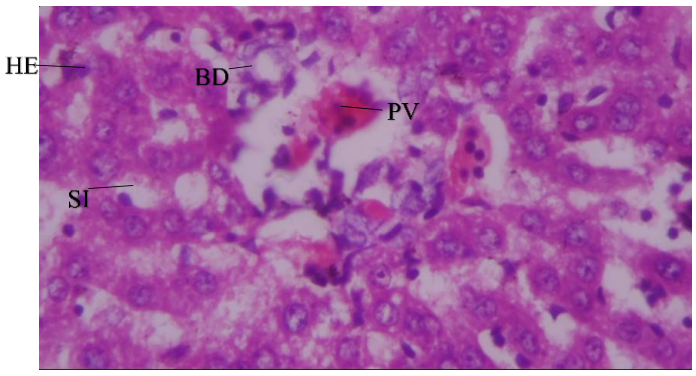

Plate 3. Rat liver given 250mg/kg of extract only showing normal architecture: hepatocytes (HE), sinusoids (SI), bile ducts (BD), portal vein (PV). (H&E; 400x)

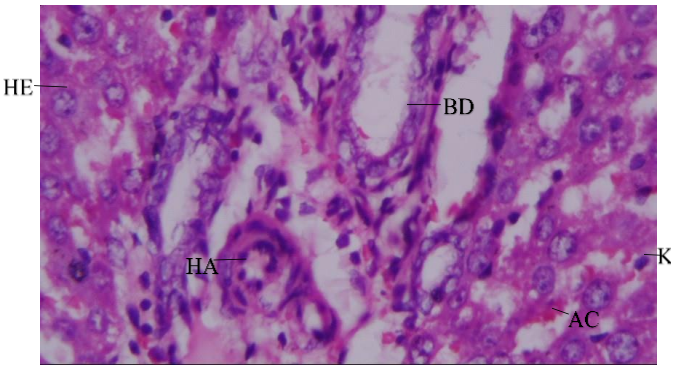

Plate 4. Rat liver given 500mg/kg of extract only showing normal architecture: hepatocytes (HE), sinusoidal Kupffer cell m(KA), active congestion (AC), bile duct (BD), hepatic artery (HA). (H&E; 400x)

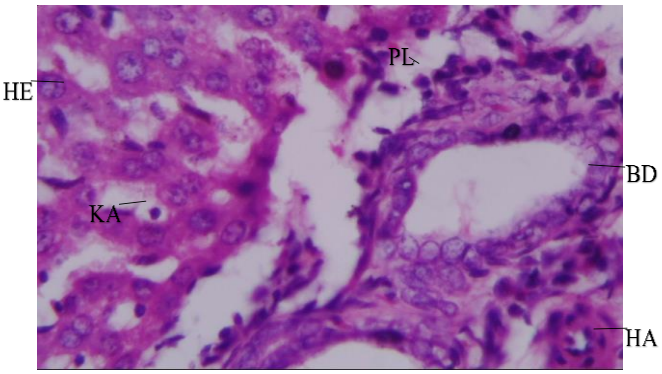

Plate 5. Rat liver given Alcohol then 250mg/kg of extract only showing normal architecture: hepatocytes (HE), Kupffer cell activation (KA), periportal lymphocyte mobilization (PL), bile ducts (BD), hepatic artery (HA). (H&E; 400x)

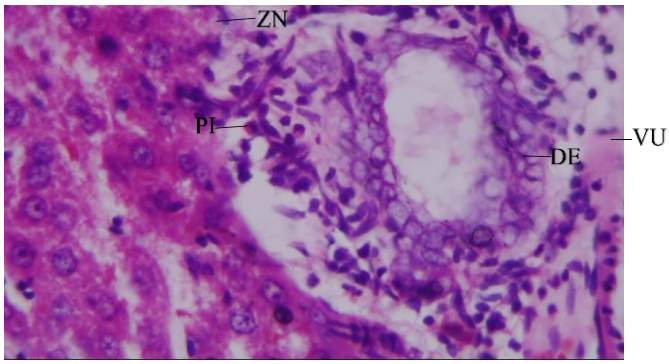

Plate 6. Rat liver given Alcohol then 500mg/kg of extract showing: zonal necrosis (ZN), vascular congestion and ulceration (VU), heavy periportal infiltrates of inflammatory cells (PI), ductal epitheliosis (DE): (H&E; 400x)

DISCUSSION

In this study, although there was an increase in the mean body weight of rats treated with 1 ml of 50% alcohol only for 14 days, there was no significant change in the mean body weight when compared to control Fig 1. Kolota *et al*. (2019) in a previous study also reported no significant change in the body weights of wine drinking rats when compared to control. The increase in mean body weight of rats observed in this study indicates some level of weight gain in the alcohol only group. Alcohol consumption has been associated with body weight gain in both animal and human studies. Suter *et al*. (1997) observed a body weight increase in rats treated with alcohol and attributed it to alcohol metabolism which tend to cause obesity. Wang *et al*. (2010) reported an increase in body weight with alcohol consumption in middle-aged and older women in their study. They observed that women who consume alcohol have a higher risk of becoming overweight.

This study also revealed significant reduction in the mean body weight of rats treated with 250 mg/kg body weight of *Azadirachta indica* following initial alcohol treatment. (Fig 1). *Azadirachta indica* has been reported in previous studies with potential for weight reduction. Jamshed *et al*. (2022), reported a significant reduction in body weight and body mass of experimental rats treated with *Azadirachta indica.* This finding also supports the anti-obesity claim of *Azadirachta indica* as reported in previous studies.

There was significant elevation of spartate transferase level in the alcohol-treated group when compared to control. Liver AST is an important marker of inflammation of the liver. This is evidenced by the presence of inflammatory cells in this group. A similar finding was observed by Nyblom *et al.* (2014) who observed a significant increase in AST level in alcohol treated rats.

Saravanan *et al.* (2006) reported a significant decrease in total bilirubin levels in rats that were administered with alcohol. Similar finding was recorded in this present study where 1ml of 50% alcohol caused a significant decrease in the total bilirubin of rats which was subsequently reversed upon treatment with 500mg/kg of *Azadirachta indica*.

Alcohol caused a significant decrease in the total protein in rats when compared with control and this correspond to finding reported by Saravanan *et al*. (2006). In their study they observed a significant decrease in the total protein of alcoholic treated rats.

There was significant reduction in the Alanine transaminase and total albumin levels in alcoholic treated rats in this present study. However, treatment with *Azadirachta indica* caused a reversal of this effect induced by alcohol. This is in agreement with findings by Nyblom *et al*. (2004). However, Eun *et al.* (2012) reported a significant increase in liver Alanine transaminase level of rats chronically treated with alcohol. Eun *et al*. (2012) also reported a significant reduction in the total albumin levels after treatment with alcohol. This could be due to the toxic effect of alcohol on the liver but for the extract induced significant increase in albumin, total protein and total bilirubin further studies may be required for better explanation.

Alcohol treatment caused infiltration of inflammatory cells in the liver tissue as shown in photomicrograph of alcohol only group indicating possible potential to induce hepatitis. It was also observed that treatment with extract of *Azadirachta indica* cause mobilization of lymphocytes and Kupffer cells .also indicating possible immunologic potentials of this extract. Studies by Ezz-Din *et al.* (2011) and Mossanen *et al*. (2019) reported similar findings of the immunologic potentials of extract of *Azadirachta indica*. However, the hepatic architecture seen in the 250 mg/kg body weight of extract of *Azadirachta indica* showed a better ameliorative potential when compared to the 500 mg/kg body weight dose

CONCLUSION

Azadirachta indica leave extract at 250mg/kg body weight has hepatoprotective potential by ameliorating the acute alcohol-induced liver injury.

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