



Therapeutic Effect of Aqueous *Allium cepa* Extract on Ethanol-Induced Hepatotoxicity in Albino Rats

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Abstract:

In recent years, alcohol consumption has seen a significant rise, particularly driven by social activities. However, chronic alcohol intake has been widely recognized as a major risk factor for the development of numerous diseases, particularly liver disease. This study aimed to evaluate the therapeutic effect of aqueous extract of Allium cepa (onion) on ethanol-induced hepatotoxicity in albino rats. A total of twenty-five rats, weighing between 130-180g, were randomly assigned into five groups, each containing five rats. The rats were given a 14-day acclimatization period with free access to standard feed and water before the experiment. Group I served as the negative control and received only standard feed and water. Group II, the positive control, was administered 50% ethanol orally at a dose of 0.5 ml/100g body weight. Groups III, IV, and V received the same ethanol treatment, followed by oral administration of 200mg/kg, 400mg/kg, and 600mg/kg of aqueous Allium cepa extract, respectively, once daily for 30 days. At the end of the experiment, the rats were anesthetized using chloroform, and blood samples were collected for the analysis of liver enzymes, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) using enzymatic methods. The livers were also excised for histological analysis using hematoxylin and eosin (H&E) staining. Statistical analysis was performed using SPSS version 24, with significance set at p<0.05. The results revealed a significant reduction in the levels of AST (p=0.000028), ALT (p=0.00003), and GGT (p=0.000050) in the groups treated with Allium cepa extract compared to the positive control group. However, no significant differences were observed in ALP levels (p=0.610) across all groups. These findings suggest that aqueous Allium cepa extract may have ameliorative potential against ethanol-induced liver toxicity, possibly due to its antioxidant properties.

Keywords: *Allium cepa*, Ethanol-induced hepatotoxicity, Liver enzymes, Antioxidant properties, Albino rats, Hepatoprotective effects

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1. Introduction

In recent years, alcohol consumption has surged significantly, largely influenced by social, recreational, and emotional factors such as stress relief, celebration, and relaxation (1). Alcohol is commonly consumed in social settings like parties, festivals, and gatherings, often as a way to enhance social bonding and relieve stress (2). Additionally, some individuals consume alcohol to cope with anxiety, depression, or emotional challenges (3). Despite its shortterm effects of euphoria and relaxation, chronic alcohol use can lead to various health complications, including cardiovascular diseases, liver disorders, neurodegeneration, and immune dysfunction (4).

Among these health concerns, alcohol-induced hepatotoxicity is a prevalent and serious condition, characterized by liver damage due to excessive alcohol intake (5). Hepatotoxicity refers to the injury or damage to the liver caused by chemical substances, in this case, ethanol, the active compound in alcoholic beverages. The liver, being the primary organ responsible





for metabolizing alcohol, is highly susceptible to damage when overwhelmed by chronic alcohol consumption (6). Ethanol metabolism generates harmful byproducts such as acetaldehyde, which leads to oxidative stress, lipid peroxidation, and inflammation in liver tissues (7). This process disrupts normal liver function, causing conditions such as steatosis (fatty liver), alcoholic hepatitis, fibrosis, and eventually cirrhosis or liver cancer (8).

However, there is a growing interest in herbal medicines due to their affordability, minimal side effects, and long history of use in traditional systems of medicine (9). Herbal medicines have shown potential in ameliorating various disease conditions, including liver disorders, due to their bioactive compounds that possess antioxidant, anti-inflammatory, and hepatoprotective properties (10). One such promising herbal remedy is *Allium cepa*, commonly known as onion.

Allium cepa belongs to the Allium genus and the Amaryllidaceae family. It is a widely cultivated vegetable species known for its strong flavor and numerous health benefits (11). Onions are rich in flavonoids, sulfur-containing compounds (such as allicin), and phenolic acids, which contribute to their potent antioxidant, antimicrobial, and anti-inflammatory properties (7). These bioactive compounds have been shown to counteract oxidative stress and inflammation, making onions an excellent candidate for preventing and mitigating liver damage. Moreover, this study was aimed at evaluating the effect of aqueous extract of Allium cepa on ethanol-induced hepatotoxicity in albino rats.



Figure 1: Bulbs of Allium cepa (Onion)

2. Materials and Methods

2.1 Experimental Animals

In this study, twenty-five (25) albino rats, each weighing between 150 and 200 grams, were randomly selected. These rats were obtained from the Department of Anatomy, College of Medical Sciences, Rivers State University, and transported in ventilated wire cages to the Animal House in the Department of Animal and Environmental Sciences at Rivers State University, Port Harcourt. The animals were housed under a 12-hour light/dark cycle and provided with unlimited access to solid poultry chow and water. A two-week acclimatization period was observed prior to the initiation of the experiment.

2.2 Preparation of 50% percent Ethanol

A stock solution of absolute ethanol was purchased from a chemical store located on Hospital Road in Port Harcourt, Nigeria. To create a 50% ethanol solution, 50 milliliters (50 mL) of absolute ethanol was measured into a mixing beaker, followed by the addition of 50 milliliters (50 mL) of distilled water, and the mixture was gently stirred.





2.3 Preparation of Onions Extract

Fresh onion bulbs were sourced from the Mile 3 market in Port Harcourt, Nigeria. Among the three local onion varieties recognized by the National Institute of Horticultural Research (NIHORT) in Ibadan, the Kano Red variety was selected for its high antioxidant content and strong pungency (12). Sixty grams (60g) of the bulbs were thoroughly cleaned, blended, and homogenized in 100 mL of distilled water for one hour. The homogenate was then filtered three times using Whatman filter paper No. 1, resulting in a crude extract with a concentration of 60g/100mL or 6000mg/mL; this process was repeated daily. Furthermore, extract concentrations of 400mg/mL and 200mg/mL were prepared from the crude extract using the appropriate dilution formula. Additionally, the qualitative phytochemical analysis of the *Allium cepa* was also conducted.

2.4 Dose Determination

The 50% ethanol solution was given at a dose of 0.5 mL per 100 g of body weight, according to the method described by Lodh et al. (13). Each rat was weighed individually, and 0.5 mL of the 50% ethanol solution was administered for every 100 g of its body weight. For example, a rat weighing 168.9 g received 0.84 mL of the ethanol solution.

2.5 Acute Toxicity Study (Ethanol)

The Fixed Dose procedure, as specified by OECD (14), was followed, in which three rats were orally administered 50% ethanol at a dosage of 0.5 mL per 100 g of body weight for a duration of two weeks.

2.6 Acute Toxicity Study (Allium Cepa)

The acute toxicity study of the Allium cepa extract was performed following Locke's method, as detailed by Ibama et al. (15). Nine rats were divided into three groups, with three rats in each group. The groups were orally administered the aqueous extract of Allium cepa at doses of 400 mg/kg, 800 mg/kg, and 1600 mg/kg, respectively. The animals were then monitored for 48 hours to observe any signs of toxicity or mortality.

2.7 Experimental Design

After a 14-day acclimatization period, the rats were allocated into five groups, each containing five rats. Group 1 served as the negative control and was given only rat pellets and water ad libitum for 30 days. Group 2 received oral administration of 50% ethanol at a dose of 0.5 mL per 100 g once daily for 30 days, acting as the positive control. Groups 3, 4, and 5 were also administered 50% ethanol orally at a dose of 0.5 mL per 100 g, followed by 1.0 mL of Allium cepa aqueous extract (onion) at doses of 200 mg/kg, 400 mg/kg, and 600 mg/kg, respectively, administered one hour later, once daily for 30 days.

2.8 Blood Specimen Collection

At the conclusion of the 30th day of the experimental study, the animals in each group were fasted overnight. They were then anesthetized using cotton wool soaked in chloroform placed in a jar, and blood samples were obtained via cardiac puncture. Blood samples of 4 mL each were aseptically collected into plain bottles using sterile 5 mL syringes. The samples were centrifuged at 3000 rpm for 5 minutes to separate the serum, which was subsequently transferred to another plain bottle. Additionally, the liver tissues were excised for histological analysis.

2.9 Blood Sample Analysis

The serum samples were analyzed for hepatic enzymes, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT). The absorbances for each parameter in the samples were measured using the UV1720 UV-Vis Spectrophotometer (Shanghai Yoke Instrument Co., Ltd., China) along with a Randox test kit.

2.10 Histological Analysis





After euthanizing the animals, the liver tissues were carefully excised for histological analysis. Using a sterile scalpel, an incision was made in the abdominal cavity to access and detach the liver while preserving its integrity. The excised liver was immediately placed in a fixative solution, such as formalin, to preserve cellular structure. Once fixed, the liver samples were processed, embedded in paraffin, and sectioned into thin slices. The tissue sections were subsequently stained using histological dyes, such as hematoxylin and eosin (H&E), following the procedure outlined by Ibama and Konne (16). They were then examined under a microscope to assess any pathological changes associated with the experimental treatment.

2.11 Statistical Analysis

The analysis results were expressed as Mean \pm Standard Deviation and processed using Statistical Package for the Social Sciences (SPSS) version 26 (IBM Corp., Armonk, NY, USA). The mean and standard deviation values for various parameters between the test and control groups were compared using one-way ANOVA and Tukey tests. A statistical significance level of 95% confidence interval (P < 0.05) was established.

3. Results

3.1 Qualitative Phytochemical Analysis

The findings of the qualitative phytochemical analysis of the aqueous extract of *Allium cepa*, is presented in Table 3.1. It indicates that the extract contains alkaloids, flavonoids, cardiac glycosides, phenols, saponins, terpenes, and steroids.

Phytochemical compounds	Status
Alkaloids	+
Flavonoids	+
Saponin	+
Cardiac glycosides	+
Terpenes	+
Steroids	+
Phenols	+

Table 3.1: Result of the Qualitative Phytochemical Analysis

3.2 Toxicity Study of Allium cepa Extract

The results of the toxicity study for *Allium cepa* extract, is displayed in Table 2a. It indicates that administration of the aqueous extract at doses of 400 mg/kg, 800 mg/kg, and 1600 mg/kg did not produce any signs of toxicity or cause mortality after 48 hours of observation.

Table 3.2a: Results of Toxicity Study of Allium cepa Extract

Dose (mg/kg)	Observation
400	No mortality
800	No mortality
1600	No mortality





Toxicity Study of 50% Ethanol

The results of the toxicity study on 50% ethanol, as presented in Table 4.2b, show that oral administration of 0.5 ml/100g once daily for two weeks did not result in any signs of toxicity or mortality.

Table 3.2b: Results of Toxicity Study of 50% Ethanol

Dose	Observation
0.5ml/100g	No mortality

3.3 Comparison of the Levels of Liver Enzymes of the Control and Test Groups

The comparison of liver enzyme levels in groups I, II, III, IV, and V is summarized in Table 3.3 AST levels were significantly higher in group II (22.24 ± 4.39 U/L) compared to group I (10.98 ± 1.9 U/L, p=0.000028), while groups III, IV, and V had significantly lower levels than group II, with no significant difference among groups I, III, IV, and V. ALT levels followed a similar trend, with group II (56.16 ± 3.27 U/L) being significantly higher than group I (28.08 ± 1.8 U/L, p=0.00003), and groups III, IV, and V significantly lower than group II (28.08 ± 1.8 U/L, p=0.00003), and groups III, IV, and V significantly lower than group II, but not different from each other. ALP levels showed no significant difference among the groups (p=0.610). GGT levels were significantly higher in group II (209.54 ± 21.05 U/L) compared to group I (128.37 ± 29.52 U/L, p=0.000050), while groups III, IV, and V had significantly lower levels than group II, with no difference among groups I, IV, and V.

Table 3.3: Mean Levels of AST, ALT, ALP, and GGT of Group I, II, III, IV, and V Compared

GROUPS	AST (U/L)	ALT (U/L)	ALP (U/L)	GGT (U/L)
Group I (NC)	10.98 ± 1.19^{b}	28.08 ± 1.82^{b}	126.14±19.25	128.37±29.52 ^b
Group II (PC)	22.24±4.39ª	56.16±3.27ª	144.06±28.49	209.54 ± 21.05^{d}
Group III (200mg/Kg)	14.88±3.95 ^b	29.36±5.28 ^b	137.33±25.29	174.58±18.21ª
Group IV (400mg/Kg	15.90±2.43 ^b	37.12±12.46 ^b	124.37±17.28	145.87±21.94 ^b
Group V (600mg/Kg)	10.72±1.22 ^b	29.26±2.11 ^b	129.66±18.78	137.94±14.91 ^b
F-value	12.565	17.351	0.686	11.569
P-value	0.000028	0.00003	0.610	0.000050
Remark	S	S	NS	S

Key: AST = Aspartate amino transferase, ALT = Alanine amino transferase, ALP = Alkaline phosphatase,GGT = Glutamyl transferase. Values with different superscripts are significantly different (p<0.05), S = Significant,NS = Not Significant





3.4 The Results of the Histological Analysis of the Liver of the Various Groups of the Rats



Figure 2: Photomicrograph of the liver tissue of Group I rats showing distinct hepatocytes in radiating core, sinusoids and central vein containing blood deposit. Tissue shows no distortion of cytoarchitecture. H & E, X400



Figure 3: Photomicrograph of the liver tissue of Group II rats showing hypertrophy, degeneration and pockets of necrosis of hepatocytes in severe distorted radiating hepatic core. Several degenerating nuclei and edematous cells as well as dilated and congested sinusoids are observed. Tissue also contains central vein with no blood deposit and diffused vacuolations. Severe distortion of tissue cytoarchitecture indicated. H & E, X400







Figure 4: Photomicrograph of the liver tissue of Group III rats showing mildly distorted hepatocytes in radiating core, sinusoids containing blood/lymphoid cells and severely congested central vein. Pockets of vacuolations observed. Tissue shows distortion of cytoarchitecture. H & E, X400



Figure 5: Photomicrograph of the liver tissue of Group IV rats showing distinct and proliferation of hepatocytes in radiating core, sinusoids containing blood deposit and lymphoid cells spread within the tissue. Focal cellular degeneration is also noted. Mild tissue distortion of cytoarchitecture. H & E, X400







Figure 6: Photomicrograph of the liver tissue of Group V rats showing distinct and proliferation of hepatocytes in radiating core, dilated sinusoids containing blood deposit. There no deposit in central vein but perivascular distortion observed. Pockets of cellular degeneration (with nuclei content distortion) are also noted. Mild tissue distortion of cytoarchitecture indicated. H & E, X400.

4. Discussion

This study aimed to evaluate the therapeutic potential of *Allium cepa* (onion) aqueous extract on ethanol-induced hepatotoxicity in albino rats. Phytochemical analysis revealed that the extract contained bioactive compounds such as alkaloids, flavonoids, cardiac glycosides, phenol, saponin, terpenes, and steroids, aligning with earlier findings by Formica et al. (17). These phytochemicals are known for their antioxidant properties, which are crucial in combating oxidative stress, a primary factor in liver injury.

Ethanol administration significantly increased the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT) in Group II, which served as the positive control. This rise in enzyme levels is indicative of liver damage, as these enzymes, typically contained within hepatocytes, are released into circulation when the liver cells are damaged. Hassan and El-Gendy (18) explain that oxidative stress induced by ethanol can cause hepatocellular damage, leading to elevated serum enzyme levels.

The results of the study demonstrated that treatment with *Allium cepa* extract led to a significant decrease in AST and ALT levels in the treatment groups (III, IV, and V), with the extract administered at doses of 200 mg/kg, 400 mg/kg, and 600 mg/kg, respectively. These findings align with previous research by Dibal et al. (19), Ogunlade et al. (20), and Ozougwu et al. (21), who also observed reductions in liver enzyme levels following *Allium cepa* treatment in animal models with liver damage. The decrease in AST and ALT levels in the treated groups can be attributed to the antioxidant and free radical-scavenging properties of the *Allium cepa* extract, which help mitigate oxidative damage to liver cells (18).

Similarly, the administration of *Allium cepa* extract significantly lowered GGT levels in the treatment groups. GGT is another marker of liver damage, and its reduction further supports the hepatoprotective role of *Allium cepa*. The presence of phytochemicals such as flavonoids and phenols, known for their antioxidant capabilities, may explain the observed effects





(17). These results are consistent with those of Dibal et al. (19), who reported similar hepatoprotective effects using dry onion peels in ethanol-induced liver injury models.

However, no significant difference in alkaline phosphatase (ALP) levels was observed among the groups. This result contrasts with the findings of Ozougwu et al. (21), who noted a decrease in ALP levels with *Allium cepa* treatment, but is consistent with Dibal et al. (19), who found no significant changes in ALP levels following treatment. ALP is predominantly produced in the bile ducts, and since ethanol primarily affects hepatocytes rather than bile ducts, it is possible that AST and ALT serve as more sensitive markers of ethanol-induced liver damage (22).

The histological analysis further confirmed the protective effects of Allium cepa. The liver tissues of rats in Group II (positive control) showed severe damage due to ethanol administration, while Groups III, IV, and V exhibited significant improvements in liver architecture after treatment with *Allium cepa* extract. This aligns with the findings of Ogunlade et al. (20) and Dibal et al. (19), who also reported improved liver histology in rats treated with *Allium cepa* following ethanol-induced liver injury.

5. Conclusion

The hepatotoxicity induced by ethanol, characterized by elevated levels of AST, ALT, and GGT, signifies the oxidative stress and cellular damage inflicted upon liver tissues. Ethanol is known to disrupt cellular membranes and promote the release of hepatic enzymes into the bloodstream, thereby indicating liver injury. However, the aqueous extract of *Allium cepa* proved effective in counteracting these effects by significantly reducing the enzyme levels in the treatment groups, regardless of the dosage administered. The antioxidant properties of *Allium cepa*, attributed to its rich content of flavonoids, phenols, and other phytochemicals, likely played a critical role in scavenging free radicals and neutralizing the oxidative stress induced by ethanol. These bioactive compounds not only protected hepatocytes from damage but also facilitated the restoration of normal liver enzyme levels, suggesting that the extract promotes liver cell repair and regeneration.

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