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Ethanolic Extract of *Allium cepa* mitigates the Effects of Ethanol-Induced Renal and Hepatic Damage and Exerts Antioxidant Activity in Wistar Rats: A Biochemical Study

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Abstract

Liver and renal injury caused by alcohol consumption is a common complication in many parts of the world with many deleterious effects to human health. *Alium cepa* (*A. cepa*) is a plant which is widely used in many parts of the world for its culinary and medicinal properties. The availability and affordability of this plant makes it a promising alternative to the medication used in the treatment of ethanol-induced renal and hepatic injury and complication. Thirty mice (30) were allocated to five groups (n=5). Each group receiving distilled water, 10 ml/kg of 20% ethanol, (50 and 100) mg/kg ethanol extract of *A. cepa* extract plus 10ml/kg of 20% ethanol, 100 mg/kg silymarin plus 20% ethanol orally, respectively for 18 days. The mice were euthanized a day after and blood was collected in a plain bottle for biochemical assay and determination of oxidative stress biomarkers. *A. cepa* was found to significantly ($p<0.05$) reduce serum ALT and AST concentration, increase serum albumin, reduce urea and increase creatinine levels, reduce MDA, elevate liver SOD, CAT and GSH concentration. The results obtained from the present study suggests that the ethanolic extract of *A. cepa* had hepato- and reno-protective properties, and effective in reducing the effects of ethanol-induced damage in a dose-dependent manner; however, further studies are required to determine the mechanisms involved.

Keywords: Allium cepa, Oxidative Stress, Biochemical Assay, Hepatic, Renal, Parameter

1. Introduction

Alcohol is a psychoactive substance with addictive and intoxicating properties. Its consumption is a public health problem globally affecting people of different age groups, sex, and race (Lai et al., 2019). The global alcohol use is estimated at over 2 billion with about 1 billion binge drinkers (Liu et al., 2021). In 2019, Europe was the leading consumer of alcohol in the world with Czech Republic leading having individuals consuming over 14 liters of pure alcohol per day. Latvia, Maldova, and Germany followed with a consumption rate of 13.19, 12.85, & 12.79 liters of pure alcohol respectively (WHO, 2021). In Asia, about 17% of men and 4% of women had a history of excessive alcohol use, while in Africa, Uganda is the leading alcohol consumer with individuals consuming more than 12 liters of pure alcohol (Bundy et al., 2018; WHO, 2021). Alcohol use is a risk factor for liver, kidney, pancreatitis, and cardiovascular diseases. In 2016, over 3 million global deaths (Park and Kim, 2020) and 130 million disability adjusted life years were attributed to alcohol use (Park and Kim, 2020; WHO, 2021). Oxidative stress is one of the main mechanisms of toxicity showing genotoxicity, renotoxicity, hepatotoxicity, teratogenicity and carcinogenicity (Al-Groom, 2022, Girgis et al. 2024).

Chronic kidney disease (CKD) is the loss of renal function for over 3 months with irreversible kidney damage. It is on the increase with about 120 million people affected worldwide (Pan et al., 2018; Fan et al., 2019). The role of alcoholism in kidney disease is contradictory. While other studies suggest that binge drinking is associated with CKD, others reported that it has to be in combination with smoking (Shankar et al., 2006). While alcohol consumption is a risk factor for liver disease, liver disease is still considered as a multifactorial disease process (Roerecke et al., 2019). About 35 % of binge drinkers are reported to develop chronic liver disease, while 24 million people are reported to have alcohol-related liver disease (Prince et al., 2023).

Onion (*Allium cepa/ A. cepa*) is the second most cultivated crop due to its uses in food, medicine, and cosmetics (Shabir et al., 2022). *A. cepa* has numerous nutritional and medicinal applications, used especially its antioxidant and inflammatory properties which has been used in the management and prevention of diseases like

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asthma, bronchitis and cough (Ishaya et al., 2024; William et al., 2025). The increased production has led to the generation of waste from its by-products (non-edible skin, dried and damage bulbs) which causes environmental pollution owing to the unpleasant odor after decomposition (Das and Mandal, 2015). Previous studies reported the use of onion waste to produce food ingredients/additives with antioxidant properties (Sharma et al., 2016; Santiago et al., 2020; Kumar et al., 2022). Onion waste has been attributed to have high antioxidant content and was used in the prevention of liver disease, various types of cancers, cardiovascular and neurodegenerative diseases (Compaore et al., 2017; Dagher et al., 2021; Dibal et al., 2022; Shabir et al., 2022; Dibal et al., 2023). In the light of this, the authors propose that onion waste can be channeled into products that can be used in managing and treating ethanol-induced liver and kidney disorders. The current study evaluated the kidney and liver functions as well as in-vivo antioxidant activity of ethanol extract of *A. cepa* peel on ethanol-induced kidney and liver damage in mice.

2. Materials and Methods

2.1. Chemicals

The chemicals used are of analytical grade and were either purchased from Sigma-Aldrich, USA, or Merck, Germany. They include ethanol which was used to induce kidney and renal damage, hematoxylin, Sodium chloride as a reagent and standard solution, trichloroacetic acid to remove contaminants, sodium carbonate, formic acid, potassium phosphate in catalysis of enzymatic reactions, hydrogen peroxide which was used as an antiseptic, acetic acid which was used as a solvent in the extraction process, and Ketamine injection which was used for anesthesia.

2.2. Onion Peel and Extraction

A. cepa peel was collected from a local market in Maiduguri and authenticated at Faculty of Pharmacy by a Botanist in Faculty of Biological Sciences, University of Maiduguri, and a voucher was deposited in the Faculty of Pharmacy with the voucher number (UMM/FPH/AMA/001). The peel was pulverized and onion powdered peel was diluted in 2.5 liters of ethanol (Sigma-Aldrich, Missouri). It was covered and agitated vigorously to mix, and allowed to steep for a period of 24 hours after which the resultant solution was filtered using filter paper to obtain the filtrate which was evaporated at a temperature of 47°C (Nafiu and Ogunsola, 2022).

2.3. Ethical Considerations

This study was conducted in strict accordance and adherence with the recommendations of the National Institutes of Health's Guide for the Care and Use of Laboratory Animals. The research protocol with animal experimentation was approved by the Scientific Ethics Committee of University of Maiduguri (Protocol Number: UM/UGP/22-23/080). All surgery was performed under ketamine hydrochloride, and great effort was made to minimize suffering.

2.4. Experiment design

Thirty mice were allotted to five groups (n=5). The groups received distilled water, 10 ml/kg of 20% ethanol, (50 and 100) mg/kg ethanol extract of *Allium cepa* peel plus 10ml/kg of 20% ethanol, 100 mg/kg silymarin plus 20% ethanol orally, respectively for 18 days. The mice were euthanized a day after using ketamine hydrochloride (Pfizer, Esentepe, Istanbul, Turkey) at a concentration of 100mg/kg was administered as an intramuscular injection on the left thigh of each mouse. A median incision was made on the abdominal wall of each mouse to expose the heart where a syringe was used to collect blood which was put in plain bottle for biochemical assay and for determination of oxidative stress biomarkers. The twenty percent ethanol intake for 18 days imitates two years of continuous alcohol consumption in humans (Dutta and Sengupta, 2016).

2.5. Biochemical analysis

The blood was centrifuged and serum levels of urea, creatinine, albumin, ALT, and AST were evaluated spectrophotometrically using their respective kits (Randox Laboratories Ltd, US) according to the manufacturer's instructions.

2.6. Oxidative stress markers

Part of the liver was homogenized in normal saline. The antioxidant and oxidant activities (catalase, superoxide dismutase, reduced glutathione, and malondialdehyde) were evaluated from the supernatant as described by Dibal et al. (2022), Attah et al. (2022).

2.7. Statistical analysis

One-way ANOVA followed by Tukey multiple comparisons was conducted using GraphPad Prism 10.0 (San Diego, USA). Statistical significance was considered at $p < .05$ and results were presented as mean ± standard error (SE).

3. Results

3.1. Effect of A. cepa Extract on Liver Enzymes

There was a significant ($p<0.05$) elevation in the serum concentrations of alanine aminotransferase (ALT) in all groups when compared to the control group, except in the group that was administered with silymarin, and this could be attributed to the alcohol that was ingested by the rats in these groups and protective effect of silymarin. Administration of ethanolic extract of *A. cepa* peel caused a non-significant decrease in ALT concentration in the groups that received the extract when compared to the group that was treated only with 20% ethanol (negative control). This decrease was more marked in the group that received a higher concentration (100mg/kg) of the extract, indicating that the higher dose was more effective in elevating serum ALT concentration. The reduction in serum ALT concentration in the silymarin group was also non-significant when compared to the negative control group. 100mg/kg of silymarin was more effective in reducing serum ALT concentration (Figure 1).

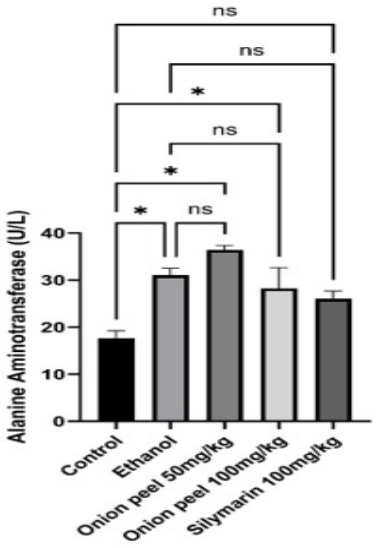

Figure 1. showing the effect of *A. cepa* peel extract on serum alanine aminotransferase in all groups (* - Statistical significance considered at p< .05. All value presented as mean ± standard error (SE), ns – not significant, U/L – units per liter).

Serum aspartate aminotransferase (AST) concentration was significantly ($p<0.05$) elevated in the negative control, demonstrating the deleterious effects of ethanol in elevating serum AST concentration. In the group that received 100mg/kg *A. cepa* peel extract, there was a significant ($p<0.05$) reduction in serum AST compared to the group treated with 50mg/kg *A. cepa* peel extract. In the silymarin group, administration of the extract caused a significant decrease ($p<0.05$) in serum AST compared to the negative control group. When comparing the groups that received 50mg/kg and 100mg/kg of extract to the positive control group, serum AST was significantly elevated. The negative control group that was administered with ethanol showed consistently elevated AST concentration when compared with all groups. Administration of 100mg/kg silymarin also caused a significant ($p<0.05$) decrease in serum AST concentration when compared to the positive control group (Figure 2).

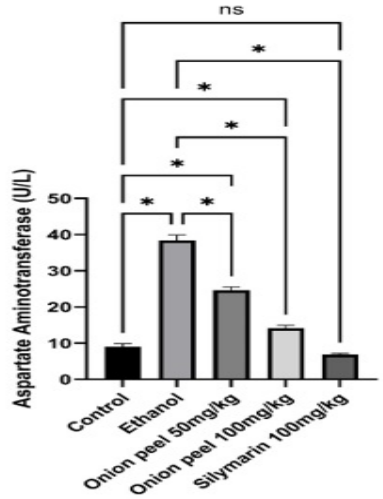

Figure 2 showing the effect of *A. cepa* peel extract on serum aspartate aminotransferase in all groups (* - Statistical significance considered at p< .05. All value presented as mean ± standard error (SE), ns – not significant, U/L – units per liter).

Albumin concentration was significantly ($p<0.05$) decreased in the negative control group which was administered with ethanol compared to all groups and was non-significantly decreased in the groups that received 50mg/kg and 100mg/kg. There was also a non-significant increase in the group administered silymarin when compared with the control group. Ethanolic extract of *A. cepa* peel caused a non-significant increase in the group administered 50mg/kg of extract when compared to the negative control. The increase was significant ($p<0.05$) in the group that received 100mg/kg of the extract and 100mg/kg of silymarin (Figure 3) demonstrating that the extract was effective in a dose-dependent manner.

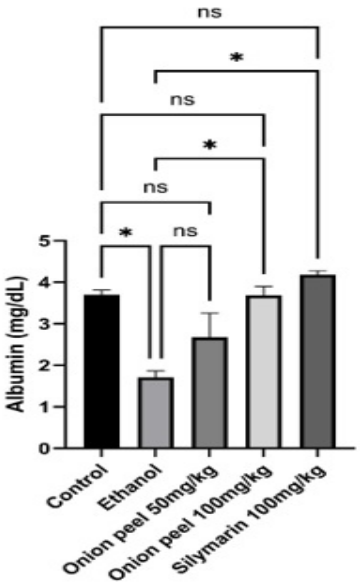

Figure 3. showing the effect of *A. cepa* peel extract on serum albumin in all groups (* - Statistical significance considered at p< .05. All value presented as mean ± standard error (SE), ns – not significant, mg/dl – milligram per deciliter).

3.2. Effect of A. cepa Peel Extract on Renal Biomarkers

There was a significant ($p<0.05$) increase in serum urea concentration in the negative control and silymarin groups when compared to the control group. Administration of *A. cepa* peel extract at a concentration of 50mg/kg and 100mg/kg significantly ($p<0.05$) decreased the serum urea concentration when compared to the control and negative control groups (Figure 4). The extract decreased serum urea concentration in the group administered a higher dose; however, the decrease was not significant when compared with the lower dose.

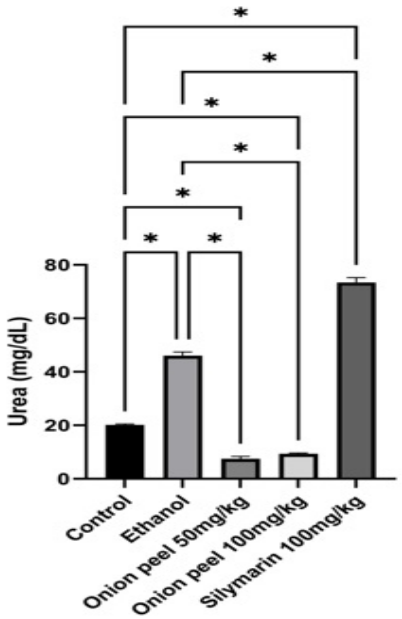

Figure 4. showing the effect of *A. cepa* peel extract on serum urea in all groups (* - Statistical significance considered at p< .05. All value presented as mean ± standard error (SE), ns – not significant, mg/dl – milligram per deciliter).

Serum creatinine concentration was significantly (p<0.05) increased in the negative control, 50mg/kg *A. cepa* peel, 100mg/kg of *A. cepa* peel and silymarin groups. Serum creatinine concentrations were also significantly (p<0.05) increased in the treatment groups (50mg/kg *A. cepa* peel extract, 100mg/kg *A. cepa* peel extract and 100mg/kg silymarin) when compared to the negative control group (Figure 5). The group administered with a higher dose showed a significant increase in serum creatinine concentration when compared to the group treated with a lower dose of the extract (Figure 5).

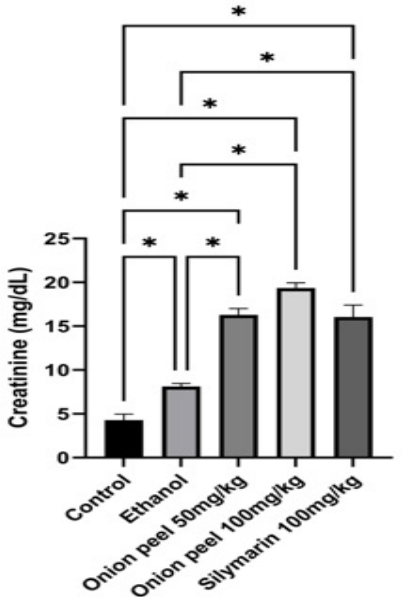

Figure 5 showing the effect of *A. cepa* peel extract on serum creatinine in all groups (* - Statistical significance considered at p< .05. All value presented as mean ± standard error (SE), ns – not significant, mg/dl – milligram per deciliter).

3.3. Effect of A. cepa Peel Extract on Oxidative Stress Biomarkers

Malondialdehyde (MDA) was significantly (p<0.05) elevated in the negative control and low dose groups when compared with the control group while in the high dose group, there was a non-significant increase compared to the control group. Silymarin administration reduced serum malonaldehyde level. There was a significant (p<0.05) decrease following the administration of the extract in both low and high dose groups when compared to the negative control group; however, the higher concentration non-significantly elevated serum MDA compared to the lower dose. Silymarin significantly reduced malonaldehyde concentration when also compared to the negative control group. The groups that were administered ethanol showed elevated MDA levels when compared to all groups (Figure 6).

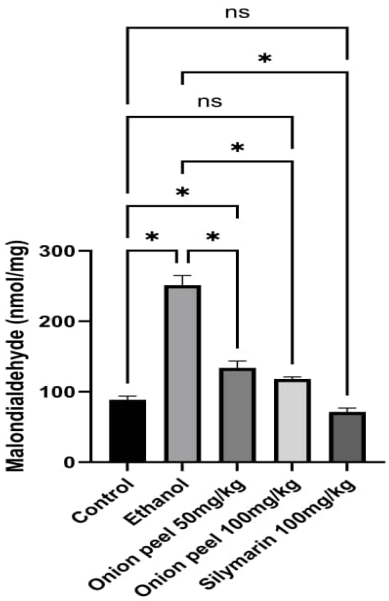

Figure 6. showing the effect of *A. cepa* peel extract on malondialdehyde concentration in all groups (* - Statistical significance considered at p< .05. All value presented as mean ± standard error (SE), ns – not significant, mg/dl – milligram per deciliter).

Catalase (CAT) was significantly (p<0.05) decreased in the negative control group when compared to all groups showing the deleterious effect of ethanol. There was a non-significant increase in catalase concentration in the low dose group and a non-significant decrease in catalase levels in the high dose group when compared to the control group. Catalase concentrations in the treatment groups were significantly (p<0.05) increased in the treatment groups (high and low dose) when compared to the negative control groups. In the group that was administered silymarin, there was a significant decrease when compared to the negative control group while catalase concentration appeared similar to the control group (Figure 7).

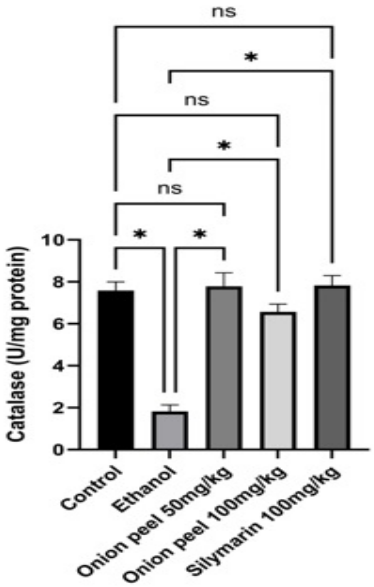

Figure 7. showing the effect of *A. cepa* peel extract on liver catalase concentration in all groups (* - Statistical significance considered at p< .05. All value presented as mean ± standard error (SE), ns – not significant, U/mg – units per milligram of protein).

In all groups, there was a significant (p<0.05) decrease in serum reduced glutathione (GSH) when compared to the control group. There was an increase in the treated groups when compared to the negative control group. Administration of the *A. cepa* peel extract increased reduced glutathione concentration in the low and high dose groups with no significant difference in the dosage of extract administered, yet there was a non-significant increase in the silymarin group when compared to the negative control group (Figure 8).

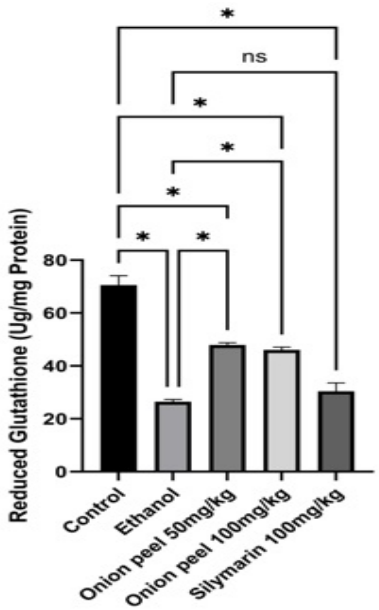

Figure 8. showing the effect of *A. cepa* peel extract on reduced glutathione concentration in all groups (* - Statistical significance considered at p< .05. All value presented as mean ± standard error (SE), ns – not significant, U/mg – units per milligram of protein).

Superoxide dismutase (SOD) concentration was significantly (p<0.05) reduced in the negative control group when compared to the control group and treated groups. Treatment with high and low doses of *A. cepa* non-significantly decreased SOD activity when compared with the positive control group and significantly increased SOD activity when compared with the negative control group in a dose-dependent manner. Silymarin administration caused a non-significant increase in serum SOD activity when compared to the positive control group. There was a significant (p<0.05) increase in the SOD activity in a dose dependent manner when compared to the negative control group with the group administered a higher dose having a significant increase in SOD level when compared to the low dose (Figure 9).

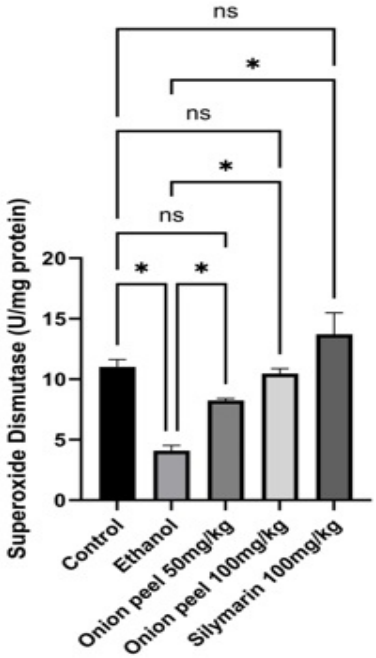

Figure 9. showing the effect of *A. cepa* peel extract on superoxide dismutase concentration in all groups (* - Statistical significance considered at p< .05. All value presented as mean ± standard error (SE), ns – not significant, U/mg – units per milligram of protein).

4. Discussion

Alcohol consumption has long been associated with a majority of liver diseases and has been found to influence both fetal and adult liver functions (Tian et al., 2016). In humans, alcohol intake can be acute (single occasion over several hours), short-term (for several days), or long-term/chronic (for years/decades). Binge drinking is the most common occurring alcohol intake and is a costly and deadly pattern of excessive alcohol use in the United States and many parts of the world (Arumugam et al., 2022). There are several detrimental consequences of binge drinking that affect the individual and society (Arumugam et al., 2022). There are immediate risks, including personal injury, driving accidents, unwanted pregnancies, and death due to alcohol overdose. The damage to the body mainly affects the liver, causing varying stages of fatty liver, liver fibrosis, liver cirrhosis, and hepatocellular carcinoma (Tian et al, 2016).

Many experimental studies have used the ethanol-induced model to simulate liver damage (Wang et al., 2020, Manu et al., 2022, Mahdi et al, 2023, Samia et al., 2023). Also, Silymarin which is a commercial drug was used for hepatoprotection and was administered as the positive control in the current study. Onion peel (*Allium cepa* L.) is a herb used for its culinary and medicinal properties. This plant belongs to the family of Amaryllidaceae and is found to contain the phytochemical flavonoids (quercetin and kaempferol), alk(en)yl cysteine sulfoxides (S-methyl cysteine sulfoxide and S-propyl cysteine sulfoxide, cycloalliin, thiosulfinates, and sulfides) as the main compounds (Galavi et al., 2021). The plant is used for its wide variety of activities including anti-oxidant, anti-inflammatory, lipid-modifying, anti-obesity, antihypertensive, and antidiabetic effects, and (in the present study) its effect on serum renal and hepatic biomarkers as well as oxidative stress parameters in ethanol induced hepatic and renal damage.

Administration of the ethanolic extract of *A. cepa* in the present research significantly elevated hepatic enzymes ALT, AST and there was a non-significant decrease in serum albumin concentration when compared with the normal control group. However, when compared with the negative control, *A. cepa* significantly decreased ALT in the higher dosage. AST concentration was also significantly reduced when compared with the normal control group and also, administration of the extract caused a significant increase in albumin concentration when compared to the negative control group. Sikder et al, 2016 and Emmamat et al., 2018 reported that *A. cepa* significantly reversed an elevation in plasma ALT, ALP and AST levels following the ingestion of a high fat, showing mechanistic role of quercetin and beta-sitosterol over dyslipidemia and subsequent hepatotoxicity. While Thomas et al. (2013) in an anti-diabetic study reported that AST and ALT concentration, Nishimura et al., (2019) reported that A. cepa had no significant effect on AST and ALP but decreased ALT in an antidiabetic study carried out.

Urea, commonly referred to as blood urea nitrogen (BUN) when measured in the blood, is a product of protein metabolism. BUN is considered a non-protein nitrogenous (NPN) waste product (Salazar, 2014). The concentration of urea is dependent on protein intake: the body's capacity to catabolize protein and adequate excretion of urea by the renal system. Creatinine, also a NPN waste product, is produced from the breakdown of creatine and phosphocreatine and can also serve as an indicator of renal function (Price and Finney, 2000). Creatine is synthesized in the liver, pancreas, and kidneys from the transamination of amino acids (arginine, glycine, and methionine) and then circulates throughout the body and is converted to phosphocreatine by the process of phosphorylation in the skeletal muscle and brain (Salazar, 2014). In the current study, *A. cepa* significantly reduced serum urea concentrations when compared to the normal, negative and positive control groups. The extract significantly increased serum creatinine concentration when compared with the normal, negative and positive control groups. In agreement to the current research, Babu and Srinivasan (1997) reported that *A. cepa* decreased urea and creatinine concentrations. Gomes et al. (2014) suggested that the nephroprotective effect of *A. cepa* was due to its antioxidant properties after reporting that the extract reduced uric acid, urea and creatinine concentrations in mice with in streptozotocin induced nephropathy. The elevation of serum creatinine in the present study could be attributed to initial prolonged renal damage in the treatment and silymarin groups.

Studies carried out to determine the antioxidant activity of different plant species contribute to revealing the value of these species as a source of new antioxidant compounds (Chaves et al., 2020). These include the use of wheat germ oil supplements to ameliorate cadmium chloride-induced oxidative stress in pregnant rats and fetuses (Abdou et al., 2017). Whole seed of durum wheat and barley is shown to have antioxidant activity (Hamil et al. 2017), as well as *Coix lacryma-jobi* (Diningrat et al. 2021), *Auricularia polytricha* (Agbor and Anyawu, 2021), *Leptadenia hastata* (Attah et al., 2022), *Hibiscus sabdariffa* (Al-Groom et al, 2022), Grape Seed Extract (Girgis et al., 2024), *Alium cepa* (Ishaya et al., 2024), Aloe vera (Abubakar et al., 2022, Manye et al., 2023, Ishaya et al., 2024)

In the present study, ethanolic extract of *A. cepa* revealed several antioxidant qualities as the extract elevated catalase (CAT), reduced glutathione (GSH) and superoxide dismutase (SOD) concentrations and reduced serum malondialdehyde (MDA) when compared with the negative control group. This result tallies with the result obtained by Henegan et al., 2015, who reported that *A. cepa* extract increased serum SOD, CAT and GSH in a hypolipidemic study. Vasquez-Prieto et al., 2011, El Soud and Khalil, 2010 reported that *A. cepa* reduced Thiobarbituric acid reactive substances (TBARS) in hypotensive rats. Gonzalez-Pera et al., 2014, Jung et al., 2011, Ogunmodede et al., 2012, Wu and Xu, 2014 stated that aqueous extract of *A. cepa* increased SOD, GSH, GPx, MDA concentration while reducing serum MDA concentration. *A. cepa* powder was reported to enhance the activity and levels of CAT, SOD, GSH, GPx and thiol, reducing MDA and effectively protecting against oxidative damage (Colina-Coca *et al.,* 2017, Marefati *et al*., 2018, Omer *et al.,* 2020, Nwonuma *et al.,* 2021).

In vitro and *in vivo* studies undertaken have explored the protective effect of *A. cepa* bulb against ethanol-induced hepatotoxicity and attributed its effect to the flavonoid content which quenched reactive oxygen species and regenerated membrane-bound antioxidants at both preventive and curative doses. as it displayed antioxidant potential against DPPH, hydroxyl and superoxide radicals (Kumar et al., 2013). In other studies, the antioxidant and anti-inflammatory effects of *A. cepa* were investigated and were shown to possess a high amount of polyphenol which displayed high DPPH and ABTS radical scavenging activities, and increased SOD, and CAT activities. It was shown to decrease the secretions of NO and inflammatory cytokines (IL-1β, IL-6, and TNF-α), and mRNA expressions of IL-1β and IL-12 (Kim et al., 2022, Williams et al., 2025). The effects were comparable to quercetin, another component of *A. cepa* which is well known for its antioxidant and anti-inflammatory activities. Therefore, *A. cepa* is proposed for use as an antioxidant and anti-inflammatory material to prevent related diseases, such as cardiovascular disease, asthma, atopic dermatitis, and arthritis as it has been shown to be as effective as silymarin at a concentration of 600mg/kg (Kumar et al., 2013).

5. Limitations of the Research and Recommendations for Further Studies

The current study was limited to investigating the protective effect of A. cepa on oxidative and biochemical parameters that measure liver and kidney function. The histology of these organs was not investigated. Further studies are recommended to determine the enzymatic pathways that *A. cepa* employs to exert its antioxidant and hepatoprotective effect and determine the effect of the extract on these organs at cellular level via histopathological investigations, consider the reason for elevated creatinine levels despite the protective effect of the extract, determine if a larger sample size would affect the outcomes, and explore the long-term effect of administration of *A. cepa*.

6. Conclusion

In the present study, the ethanolic extract of *A. cepa* was administered to rats to determine its effect on ethanol-induced renal and hepatic injury and to determine the antioxidant effect of the extract. Administration of *A. cepa* significantly reduced the serum concentration of hepatic and renal biomarkers of ethanol-induced damage and was found to have an antioxidant effect by reducing MDA concentration, increasing concentrations oxidative stress biomarkers. The extract was effective in a dose-dependent manner, with the 200mg/kg dose having a better effect compared to the 100mg/kg dose. Ethanolic extract of *A. cepa* can be considered an effective and affordable alternative to the treatment of ethanol-induced renal and hepatic damage.

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Conflict of Interest

The authors do not have any conflicting interests to declare.

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