Effect Of Aqueous Extract of *Citrullus Lanatus* (Watermelon) Seed on Lipid Profile And Electrolyte Function In Alcohol-Induced Toxicity In Male Rats

Abstract

Alcohol abuse and alcoholism are serious health and socioeconomic problems globally, and chronic alcohol intake can spawn abundant reactive oxygen species (ROS), including superoxide anion radical, hydroxyl radical, and hydrogen peroxide. The study aims to ascertain the impact of an aqueous extract of *Citrullus lanatus* seed on lipid profile and electrolyte parameters following alcohol-induced toxicity in male Wistar rats. The animals were divided into five groups of six animals each, as follows: Group A served as a positive control and received feed and water only; Group B served as a negative control and received alcohol only; Group C received 500 mg/kg of ASCL for 6 weeks; Group D received 35% conc of alcohol for 3 weeks and was treated with 500 mg/kg of ASCL for 6 weeks; and Group E received 35% conc of alcohol for 3 weeks and was treated with 1000 mg/kg of ASCL for 6 weeks. The experiment lasted for 9 weeks, and administration was done through oral gavage. The alcohol-only treated group had a significant difference (*P≤0.05*) when compared to the normal control, and a significant difference (*P≤0.05*) was seen in the alcohol-only treated group in comparison with ASCL-treated groups for lipid profile, electrolyte parameters. Further, the alcohol report revealed hyperlipidemia, which resulted in increased levels of TC, TG, LDL, and VLDL and decreased levels of HDL, and treatment with ASCL indicated hypolipidemia. It was indicated that alcohol intake resulted in hyponatremia and hypokalemia, which were attenuated by ASCL.

Key words: Citrullus lanatus, Alcohol-induced toxicity, lipid profile, electrolyte function, hematological parameters

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INTRODUCTION

Alcohol abuse and alcoholism is a serious health and socioeconomic problems globally, and chronic alcohol intake can spawn abundant reactive oxygen species (ROS), including superoxide anion radical, hydroxyl radical, and hydrogen peroxide *(Capurso, 2016)*. Alcoholism is a serious socioeconomic health issue globally, and it is one of the leading causes of preventable mortality, second only to cigarette smoking.

Electrolytes play critical roles in body fluid regulation and a variety of other biological processes *(Maeda et al., 2016).* However, the principal electrolytes of significance are sodium, potassium, and chloride, which go along with magnesium, calcium, phosphate, and bicarbonate. Thus, an imbalance in the aforementioned electrolytes above either a low or high levels could disrupt normal physiological processes of life, thus leads to complex situations that threatens life *(Fawwad, 2016)*. Electrolyte disturbances play a role in a variety of illnesses as underlying disorders, pathophysiological changes caused by diseases and traumas, and changes or complications following insults *(Maeda et al., 2016).*

Lipids are described as the significant building blocks of life and are the major substances, which mammalian cells functions *(Dasgupta and Wahed, 2021)*. Cholesterol is essential in the progression of cardiovascular disease., which is linked to a high level of lipids in the serum, including cholesterol and triglycerides, known as hyperlipidemia and increases the risk of developing atherosclerotic cardiovascular disease (CVD) Lee *(Fawwad, 2016).* Lipid profile consists majorly of total cholesterol, triglyceride, LDL-cholesterol (LDL-C) and HDL-cholesterol (HDL-C), which are classified as diagnostic tool for cardiovascular risk factors *(Dasgupta and Wahed, 2021)*. Alcohol increases HDL cholesterol and decreases LDL cholesterol, potentially preventing cardiovascular disease. However, excessive alcohol consumption can cause lipid metabolism imbalances, hypertriglyceridemia, and impair liver function, contributing to fatty liver *disease (Li et al., 2019).* Thus, chronic alcohol abuse also hinders triglyceride breakdown, further affecting cardiovascular health. *Li et al.,* (2019) revealed that alcohol intake could result in lowered levels of high-density lipoproteins, and increased levels of low-density lipoproteins, total cholesterol, and triglycerides.

Medicinal plants are significant in natural remedies, health, diet, and folk healing. They are used as spices and in herbal medicine for various ailments *(Izunwanne et al., 2020).* Plants are important in drug discovery due to their bioactive molecules (Neglo et al., 2021). Citrullus lanatus, a member of the Cucurbitaceae family, has been traditionally used for treating various illnesses globally *(*Odo et al., 2021*).* Its various parts have pharmacological actions, including hepatoprotective, anti-ulcergenic, anti-diabetic, laxative, antisecretory, anti-prostatic hyperplasia, antioxidant, analgesic, antifungal, and anti-inflammatory properties. Consumption of *Citrullus lanatus* has been linked with changes in the levels of lipids as well as some electrolytes levels (Nwoye *et al*., 2013).

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MATERIALS AND METHOD

Location of the Study

This study was conducted in the Animal House of the Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medicine and Health Sciences, Nnamdi Azikiwe University, Awka.

Ethical Approval

Ethical approval was obtained from the Animal ethics committee, Faculty of Basic Medical Sciences, College of Medicine and Health Sciences, Nnamdi Azikiwe University, Awka.

Extract Collection

The seeds of Watermelon (*C. lanatus*) were collected from the Nkwo market Nnewi, Nnewi North Local Government Area, Anambra State and processed to prepare a Citrullus lanatus Seed Extract. The seeds were washed, air-dried, milled into coarse powder, macerated in Luke-warm water, filtered, concentrated, and dried in a laboratory oven. The extract was preserved in a refrigerator for future use, The extraction method was done with modifications as described according to the method employed by Quek, (2012).

Experimental Wistar rat and Design

30 male Wistar rats were used in the experimental study. The rats were divided into five groups:

Group A as a positive control and received feed and water only,

Group B as a negative control and received Alcohol only,

Group C received 500mg/kg of ASCL for 6-weeks,

Group D received 35% conc. of Alcohol for three-weeks and treated with 500mg/kg of ASCL for 6-week,

Group E received 35% conc. of Alcohol for three-weeks and treated with 1000mg/kg of ASCL for 6-weeks.

The experiment lasted for 9 weeks and involved oral gavage.

Acute toxicity of aqueous seed extract of *Citrullus lanatus* and Alcohol

The median lethal dose (LD_{50}) of the Alcohol and aqueous seed extract of *C. lanatus* (ASCL) was determined using the Lorkes method (1983) and divided into two phases. The study aimed to determine the acute toxicity of the extract and alcohol in a study conducted at Nnamdi Azikiwe University.

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Sample Collection and Termination of the Experiment

At the end of the experiment, Wistar rats in the different groups were anesthetized using chloroform in an enclosed container after 24-hours of the last administered dose of the Alcohol and aqueous seed extract of *C. lanatus* (ASCL). Blood samples were collected using a heparinized capillary tube through ocular puncture as described by Parasuraman, Raveendran, and Kesavan (2010). Blood obtained was stored in a plain bottle and EDTA bottle and was allowed to cloth, and centrifuged for 10-minutes at 3000rpm, after which the serum was retrieved using a micropipette from the plain bottle. The serum retrieved was used for serum electrolyte (sodium, potassium) and lipid profile (total cholesterol, low-density lipoprotein, high-density lipoprotein, triglyceride, and very low-density lipoprotein). The heart was harvested through intra-abdominal incision and then weighed and washed using normal saline. The heart was fixed in 10% formo-saline as a primary fixative for histopathological studies.

Statistical Analysis

The values were expressed as mean ± SEM. Hypothesis testing method included one way analysis of variance (ANOVA) followed by post hoc performed with Least Significant Difference (LSD) dunnett. P value of less than 0.05 was considered to indicate statistical significance and 0.001 as highly significant respectively.

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RESULTS

Table 4.1 effect of aqueous seed extract of *Citrullus lanatus* on total cholesterol and triglyceride level following alcohol toxicity

	Total cholesterol (mmol/L)	**Triglyceride (mmol/L)**
	MEAN±SEM	**MEAN±SEM**
Group A (Positive control)	72.34±5.10*	67.95±8.26*
Group B (Alcohol only)	154.06±11.64	112.00±7.27
Group C (500 mg/kg of ASCL)	75.78±8.41*	77.87±7.78*
Group D (35% Alcohol + 500 mg/kg of ASCL)	135.98±9.01*	69.66±9.22*
Group E (35% Alcohol + 1000 mg/kg of ASCL)	115.67±10.13*	59.90±7.75*
F-ratio	**7.70**	**5.05**

Data was analysed using ANOVA followed by post Hoc LSD multiple comparison and values were considered significant at *p<0.05*. ASCL: aqueous seed extract of *Citrullus lanatus*, * (significant), a (not-significant).

Table 4.1 result showed a significant increase in the total cholesterol levels in group B compared to A (*p=0.01*), while groups C, D and E (*p=0.02, p=0.03, p=0.00*) had a significant decrease compared to group B. The triglyceride result revealed a significant increase in group B compared to A (*p=0.00*), while groups C, D and E (*p=0.01, p=0.00, p=0.00*) had a significant decrease compared to group B.

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Table 4.2 effect of aqueous seed extract of *Citrullus lanatus* on HDL, LDL following alcohol toxicity

	High-Density-Lipoprotein (mmol/L) MEAN±SEM	Low-Density-Lipoprotein (mmol/L) MEAN±SEM
Group A (Positive control)	52.62±4.20 *	77.67±10.26 *
Group B (Alcohol only)	42.52±6.38	117.27±7.76
Group C (500 mg/kg of ASCL)	69.16±7.93 *	87.47±9.49 *
Group D (3ml of Alcohol + 500 mg/kg of ASCL)	79.06±9.61 *	78.93±10.32 *
Group E (3ml of Alcohol + 1000 mg/kg of ASCL)	75.76±8.25 *	67.13±8.68 *
F-ratio	**8.81**	**7.98**

Data was analysed using ANOVA followed by post Hoc LSD multiple comparison and values were considered significant at *p<0.05*. ASCL: aqueous seed extract of *Citrullus lanatus*, * (significant), a (not-significant).

Table 4.2 result revealed a significant decline in the levels of HDL in group B compared to A (*p=0.02*), groups C, D, and E (*p=0.01, p=0.04, p=0.03*) revealed a significant raised levels compared to B. The LDL result showed a significant increased level in group B compared to group A (*p=0.01*), while groups C, D, and E (*p=0.00, p=0.02, p=0.03*) revealed a significant decline compared to B.

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Table 4.3 effect of aqueous seed extract of *Citrullus lanatus* on sodium and potassium ion level following alcohol toxicity

	Sodium ion (mmol/L) MEAN±SEM	**Potassium ion(mmol/L) MEAN±SEM**
Group A (Positive control)	139.50±2.59*	6.60±0.30*
Group B (Alcohol only)	112.25±7.94	4.30±0.26
Group C (500 mg/kg of ASCL)	131.75±1.31 *	5.60±0.52 *
Group D (3ml of Alcohol + 500 mg/kg of ASCL)	144.25±2.46 *	6.57±0.39 *
Group E (3ml of Alcohol + 1000 mg/kg of ASCL)	134.25±2.46 *	7.87±0.39 *
F-ratio	**6.60**	**9.27**

Data were analyzed using ANOVA followed by post Hoc LSD multiple comparison and values were considered significant at $p<0.05$. ASCL: aqueous seed extract of *Citrullus lanatus*, * (significant), a (not-significant).

Table 4.3 result showed a significant decrease in the sodium ion level in group B compared to A ($p=0.01$), while groups C, D, and E ($p=0.00$, $p=0.02$, $p=0.01$) had a significant increase compared to group B. The potassium result revealed a significant decrease in group B compared to group A ($p=0.02$), groups C, D, and E ($p=0.01$, $p=0.01$, $p=0.01$) had a significant increase compared to group B.

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Discussion

The determined concentrations of total cholesterol, triglycerides, low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol in both the positive control, negative control and the different treatment groups were presented in table 1 and 2 respectively of the results. There was significant ($P < 0.05$) decrease in total cholesterol, triglyceride and LDL cholesterol in groups treated *C. lonatus* extract. This observation is based on the comparative measures of the positive control, negative control and final concentrations (after 6weeks of administration of the extract) of the assessed indices in the treated groups. while HDL result on the other hand, increased as the dose level of the extract increases at ($P < 0.05$). Since the extract result in the increase in concentration of HDL and a decrease in the concentration of TG, LDL and cholesterol, then the extract could be used for the treatment of cardio vascular diseases. High concentration of cholesterol leads to formation of plaque in the arterial wall, which serves as a cardio vascular risks factor.

Dennis et al., 2001 Reported that; HDL carries cholesterol from the arteries to the liver for excretion and this serves to protect the body's cardiovascular well-being. LDL oxidation leads to fat accumulation in the arteries, which cause atherosclerosis and other cardio vascular diseases (Butler, 1964; Steinberg, 1997). The increase in HDL suggests that the crude extract can be used to treat heart failure due to coronary arteries, which is a leading cause of death in industrialized societies. There is a negative correlation between the HDL and LDL. Atherosclerosis is due to high level of cholesterol and LDL in the blood. The plant extract has the ability to lower the cholesterol and the LDL levels and increase in the HDL level. The HDL is responsible for the clearance of cholesterol from the blood.

Lipids are generally characterized by insolubility in aqueous or polar solvents but highly soluble in nonpolar or organic solvents. Biochemical reactions and transportations of molecules generally occur in aqueous medium. Hence, lipids are normally combined with specific proteins to form structures called lipoproteins which possess substantial degree of hydrophilicity. Low density lipoproteins, high-density lipoproteins, and chylomicrons which are basically composed of triglycerides are integral parts of the serum lipoproteins (Rang et al., 1995). Except for the HDL cholesterol, high level of all lipids in the blood is arguably a high-risk factor in the onset of cardiovascular disorders. High serum concentrations of triglycerides and LDLs have been reported to cause atherosclerosis and coronary heart diseases (Eisenhaver et al., 1998). Cholesterol is the principal sterol in animal tissues and occurs mainly in the cell membrane due to its amphipatic nature (Lehninger et al., 2000). It is also found in the adrenal gland, liver, brain, and nervous system (Osmund, 2001). The molecule is synthesized basically from acetyl CoA in the liver from where it is distributed through the blood to extrahepatic tissues where it is utilized for the synthesis of bile acids and steroid hormones as well as regulation of membrane fluidity. However, high level of cholesterol in the blood has adverse effects on human health. It is reportedly a major cause of cardiovascular derangements such as atherosclerosis, myocardial infarction and coronary heart diseases.

In this case, *C. lonatus* extract at the administered doses (500 and 1000 mg/kg) has been observed to cause significant reduction in the serum levels of total cholesterol, triglycerides, and LDL

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cholesterol in rats. The extract also simultaneously produced elevated level of HDL cholesterol. There is a possibility that the extract possesses the ability to facilitate the transport of cholesterol and triglycerides from the blood into tissues. This may have probably occurred through the induction or suppression of certain enzymes critical to the metabolism of these lipids. This finding is consistent with the report of Adebayo et al., 2006 in which Commiphora africanana extract showed antilipidaemic and anticholesterolaemic activities in rats and the work of Ighodaro *et al* (2012) titled: Effects of Nigerian *Piliostigma thonningii* Species Leaf Extract on Lipid Profile in Wistar Rats.

Conclusion

The study found that alcohol ingestion report revealed hyperlipidemia, which resulted in increased levels of TC, TG and LDL and decreased levels of HDL, and treatment with ASCL indicated hypolipidemia. It was indicated that alcohol intake resulted in hyponatremia and hypokalemia which were attenuated by ASCL.

CONSENT It is not applicable.

ETHICAL APPROVAL All experimental protocols were subjected to the scrutiny and approval of Institutional Animal Ethics Committee.

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