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Saponin Fractions of *Citrullus lanatus* (Watermelon) seed Ameliorates Ethanol-Induced Hepatotoxicity in Adult Male Wistar Rats

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ABSTRACT

Liver injury and disease caused by alcohol is a common complication to human health worldwide. Saponins derived from natural sources have long been known to offer several therapeutic effects in African herbal and traditional medicine. The study evaluated the therapeutic effects of the saponin fraction of Citrullus lanatus seed against ethanol induced hepatotoxicity. The rats were assigned into six groups: normal, ethanol (10 ml/kg of 50% ethanol), saponins (200 mg/kg/day), ethanol plus low and high dosages of saponins (ET + 50 and 100/200 mg/kg SCL), and ethanol plus Silymarin (ET + 100 mg/kg SLY). Plasma and serum enzymatic markers, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), Albumin, total protein and nonenzymatic markers (cholesterol triglycerides, low density and high density lipoprotein) were examine. Saponins (SCL) significantly ($p < 0.05$) reduced ethanol-induced elevations in ALP, AST, ALT while total protein and albumin were significantly increased ($p<0.05$). Non-enzymatic marker, when rats given SCL were compared to untreated ethanol rats, Triglyceride, Cholesterol, and low density lipoprotein levels dramatically decreased ($p < 0.05$), but high density lipoprotein levels increased. A histological examination significantly corroborated the biochemical assay result. According to the findings of this study, saponins diminish the rise in liver function enzymes and decreased in lipid profile parameters. The saponin-rich fraction of Citrullus lanatus seed exhibits anti-ethanol-induced liver damage potential. As a result, SCL may work as a plant-based natural therapy to prevent liver damage.

Keywords: *Citrullus lanatus, Ethanol, Antioxidant enzymes, liver, Inflammation*

INTRODUCTION

Alcohol is a popularly consumed beverage worldwide (Nowak and Relja, 2020). Consumption of excessive alcohol for a prolonged time can lead to a variety of sociomedical and public health issues around the world (Nowak and Relja, 2020) and can contribute to hepatic damage (Ghosh *et al.*, 2018). Alcohol-prompted hepatic damage causes steatosis, necrosis, and decreased liver cell regeneration, which eventually leads to cirrhosis and liver fibrosis (Sun *et al.*, 2021). Most population dies worldwide every year as a consequence of alcohol-producing hepatic damage (Chen *et al.*, 2020). Hence, alcohol-producing hepatic disease prevention and treatment are becoming more important public health concerns around the world (Chen *et al.*, 2020; Askgaard *et al.*, 2019; Rodriguez *et al.*, 2019).

Alcohol processed into acetaldehyde in the liver and high levels together can contribute to oxidative pressure and apoptosis in hepatocytes, which can result in tissue inflammation and fibrosis (Park *et al.*, 2017). Alcohol-evoked hepatic toxicity is largely influenced by oxidative pressure, lipid peroxidation, tenderness, and the creation of dangerous byproducts, according to an earlier study (Chen *et al.*, 2020; Askgaard *et al.*, 2019; Rodriguez *et al.*, 2019). The liver's cellular immune system can be stimulated by ethanol (EtOH) and acetaldehyde, which can lead to the generation of inflammatory markers like tumor necrosis factor-(TNF-α), (Ullah *et al.*, 2020). Early signs of alcoholic steatosis, also known as alcoholic fatty liver, can lead to alcoholic hepatitis and in the long run, end-stage liver illnesses such as liver cancer, cirrhosis, and liver failure (Hyun *et al.*, 2021). Clinically, full abstinence from alcohol and nutritional assistance are the antidotes for alcoholic liver damage (Fang *et al.*, 2022). However, there are no known particular medications for ALD. Disulfiram and corticosteroids are clinically prescribed drugs; nonetheless, they have drawbacks. To treat alcohol-induced liver damage, it is necessary to develop medications with high effectiveness and few adverse effects (Liu *et al.*, 2021).

Deregulated hepatic lipid metabolism is yet another serious health issue associated with ethanol use. For instance, it has been found that individuals who abuse alcohol have triglyceride build-up in their hepatocytes, which is a crucial step in the development of late-stage hepatitis and cirrhosis (Thomson *et al.,* 2008). Fat build-up in the liver brought on by an imbalance between fatty acid production and breakdown is an example of abnormal lipid metabolism. As a result, the negative consequences of excessive alcohol use are where the development of hepatic steatosis is first shown (Osna *et al.*, 2017). Additionally, patients with chronic alcohol use were found to have hypertriglyceridemia (increased blood triglyceride levels) brought on by increased blood levels of very low-density lipoprotein and chylomicrons (Cho *et al.*, 2014), as well as hypercholesterolemia brought on by increased cholesterol biosynthesis and decreased bile acid excretion (Dosumu *et al.,* 2012). The most often utilized animal models for alcohol-induced liver disease research are rodents (rats). Animal experiments with EtOH can imitate some symptoms of human illness and support identifying the process behind the growth of alcoholic liver disease (Ghosh *et al.*, 2018).

Antioxidants were reported to ameliorate the effect of oxidative stress and inflammation in liver-related ailments (Al-Harbi, 2019; Itoh *et al.,* 2010). Natural products are rich in bioactive compounds that are widely known to have pharmacological uses. Watermelon (*Citrullus lanatus*) is one of such medicinal plants that have attracted scientific interest due to its bioactivities (Erhirhie and Ekene, 2013).Water melon (*Citrullus lanatus*) is a tropical fruit which can be found in most parts of Africa, Asia, United States and Russia.

(FAOSTAT, 2016). It is an important horticultural crop, mostly grown in warm regions for its sweet and juicy taste (Acar *et al.,* 2012). It belongs to the cucumber family (Cucurbitacea) is commonly known as water melon and in local name Tarmuz (Hindi), Puchakaya (Telugu), Kankana (Hausa), Elegede(Yoruba), Anyu mmiri (Igbo).According to research, watermelon seeds (Citrullus lanatus) are a useful non-traditional food waste product having antibacterial, antihypertensive, antioxidative, anticancer, and cardioprotective properties (Shahein *et al.,* 2022). Despite the fact that they have strong functional and nutritional qualities due to their high content of numerous nutrients, these by-products of watermelon intake are underutilised (Otutu *et al.*, 2015).

Phytochemicals such as flavonoids, saponins, phenols, tannins, and alkaloids can be found in small amounts in the seeds of *C. lanatus* (Nwankwo *et al* 2014). Plants are a major source of Saponins, and their powerful antioxidant properties have attracted the interest of researchers (Farkhondeh *et al.,* 2021). The monomers of saponins and citrus extract have been the focus of studies on the effects of citrus on liver damage. The purpose of this study was to see if a saponin fractionate from Citrullus lanatus seed could protect rats from hepatotoxicity caused by ethanol.

Materials and Methods

Drugs and reagents

Silymarin (70 mg/tablet) is manufactured in India by Micrco Labs Ltd. An ethanol stock solution was made by dissolving 50 mL of ethanol in 100 mL of distilled water. Ketamine hydrochloride (PVT Ltd., India), sodium hydroxide (NaOH), isobutyl alcohol, magnesium carbonate ($MgCO_3$), and iron (III) chloride ($FeCl_3$) were the anaesthetic agents utilised in the investigation, along with hematoxylin and eosin. All products of the American company Sigma Chemical Co. in St. Louis, Missouri. Alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and biochemical kits (Randox test kits) were among the other high-analytical-grade chemicals and reagents used. The Lipid profile concentrations were determined using Agappe Diagnostic LTD.

Experimental Plant

Fresh Citrullus lanatus fruit was obtained at the Sabon Gari market in Zaria, Kaduna State, Nigeria, and authenticated there at the Department of Biological Sciences' Herbarium at Ahmadu Bello University, Zaria, Kaduna State, Nigeria, with the voucher specimen number 1266.

Extract Fraction

Before being ground into powder in an electric blender, the seeds were separated, cleaned, and allowed to air dry at room temperature. The active ingredients were extracted from 100 g of the powder by macerating it in 400 ml of distilled water at room temperature for 24 hours. The extractive solvent was then filtered, and the aqueous soluble extract was then concentrated to dryness in a hot air oven set at 45°C. To acquire the extract's saponin-rich sections, the oven-dried extract was first diluted in distilled water at room temperature, as described by Woo et al. (1980). The aqueous component of the extract was first defatted by adding N-hexane, and it was then let stand for a whole night. The two distinct layers that developed were separated, and the water residue was then mixed with a hydroalcoholic solution (water and methanol at a ratio of 70:30). By dissolving the hydroalcoholic extract in a diethyl ether solution and letting it sit for a whole night, polar molecules were further eliminated. Additionally, the two independent layers that developed were collected separately. The water residue was mixed with butanol, shaken, and then left to stand for the night. The butanolic fraction, which contains saponins were gotten. The fractions were next dried in an oven at 45°C. Thereafter, utilising an ethanol-induced paradigm, a hepatoprotective research on the obtained fractions was subsequently conducted.

Experimental Animals

Thirty (30) male Wistar rats were obtained from the Pharmacology Animal House, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. The animals were housed in the pharmacology department's animal housing and kept in typical lab settings, including a 12-hour cycle of darkness and light and room temperature (35°C). The animals were given an ordinary Grower Mash meal from Vital Feeds Nigeria Ltd., along with unlimited access to water. Before the administration began, the animals were given two weeks to acclimatize to their new surroundings. The Ahmadu Bello University Zaria Animal Use and Care Committee gave its approval for the experiment (ABUCAUC/2023/123), which was conducted in accordance with the ARRIVE recommendations.

Experimental design

The rats were randomly divided into six groups of five each. Group 1, the normal control group, receives distilled water (3 ml/kg) volume per body weight daily for 4 weeks; Group 2 receives 10 ml/kg of 50% ethanol per body weight daily for 4 weeks; Group 3 receives 3 ml/kg of distilled water for 3 weeks daily and 200 mg/kg of saponin for 1 week; Group 4; receives 10 ml/kg of 50% ethanol daily for 3 weeks, followed by 100mg/kg of saponin for 1 week; Group 5 receives 10 ml/kg of 50% ethanol daily for 3 weeks, followed by 200mg/kg of saponin for 1 week; Group 6 receives 10 ml/kg of 50% ethanol daily for 3 weeks, followed by 100 mg/kg of silymarin for 1 week. All treatments were given orally and carried out for 28 consecutive days. The dose of 10 ml/kg of 50% ethanol used in this study has been shown to induce hepatotoxicity and oxidative stress in rats (Elgendy *et al.*, 2022). The SCL dose in this study was based on prior research (Ejelonu *et al.,* 2021) and the silymarin dose was adopted from Popoola *et al.,* (2022).

Morphological and sampling procedure

Each animal's body weight was recorded using a digital weighted balance before, during, and after treatment. On day 29, the animals were sedated with a 50mg/kg intramuscular ketamine injection. Following sacrifice, the weight of each animal's liver was also recorded. The organ weights and relative organ weights were computed at the end of the experiment.

$$Relative\ Organ\ Weight = \frac{Absolute\ organ\ weight\ (g)}{Whole\ body\ weight\ (g)} \times 100$$

The cardiac puncture procedure was used to take blood from the heart, which was then placed into sterile centrifuge tubes and allowed to clot. Organs were removed, weighed. The bloods were centrifuged at 5000 g for 15 minutes for biochemical analysis. Serum were promptly frozen and kept at -20°C until needed.

Serum Biochemical Assay

Serum biomarkers of liver damage were assessed using commercially available diagnostic kits (Randox Laboratories Ltd., UK) and assay procedures given by the manufacturer. ALT and AST activities were measured using modified Reitman and Frankel (1957) procedures, and ALP activities were determined using modified Wright *et al.* (1972) methods. The serum total protein and albumin were estimated with the methods of Bjorsten *et al.* (2007) and George (2009). The techniques of Doumasa *et al.* (1971) and Rengarajan *et al.*, (1989) were used to measure the total protein content of serum albumin, respectively. The methods of Arntz (1979) were used to estimate total cholesterol (T. cholesterol), while the methods of Flegg (1972) were used to estimate HDL. The techniques of Schettler and Nusssel (1975) and Siedel *et al.* (1981), respectively, were used to measure triglycerides and LDL.

Histological Studies

The liver was fixed in neutral buffered formalin, then dehydrated in graded alcohol, embedded in paraffin wax, cleaned in xylene, sectioned at 5 microns, and stained with Haematoxylin and Eosin (H&E). A light microscope (Olympus CHNB107MVR, Tokyo,Japan) was used to view and photograph the tissue-processed slides.

Statistical analysis

The statistical analysis was done using GraphPad Prism for Windows (version 9.2, San Diego, CA). Data obtained from the study were presented as Mean ± SEM. One-way analysis of variance (ANOVA) was used to examine the mean difference between and within the groups. The significance level for each group was compared using Tukey's Post hoc test, and $p < 0.05$ was regarded as a statistically significant result.

Results

Effect of Saponins on Changes in Body Weight of the Wistar Rats.

Prior to the commencement of the experimental Wistar rat, there was no significant difference ($p > 0.05$) both between and within all the groups (Table 1). At the end of the experiment, rats given just ethanol exhibited a significant decrease in body weight gain ($p < 0.05$) when compared to rats in the normal control (NC) group and rats in groups given just saponins ($p < 0.05$). In comparison to the other treatment groups, the untreated ethanol control group had a significant increase in organ weight (liver). Saponin-treated groups (ET + 100/200 mg/kg SCL) showed a significant increase ($p < 0.05$) in their gain in body weight compared to the ethanol control group (ET). Similarly, the significant increase in weight gain was not dose-dependent, as there was no significant difference between rats treated with both saponins at high doses.

Table 1: Mean body weight changes of the experimental rats treated with saponin

Groups	In.wt (g)	Final wt. (g)	Body wt gain (g)	Liver Wt (g)
NC	139.00 ± 2.61	172.80 ± 2.59^{b}	33.80 ± 1.62^{e}	4.16 ± 0.10^{a}
Eth	136.60 ± 4.23	149.80 ± 6.02^{a}	13.20 ± 2.59^{b}	5.36 ± 0.07^{b}
200 mg/kg SCL	137.60 ± 5.93	171.00 ± 5.83^{b}	33.40 ± 2.56^{e}	4.30 ± 0.11^{a}
Eth + 100 mg/kg SCL	143.01 ± 3.27	162.20 ± 5.76^{ab}	19.20 ± 3.28^{ab}	4.45 ± 0.20^{a}
Eth + 200 mg/kg SCL	140.40 ± 5.69	164.60 ± 2.37^{ab}	22.60 ± 2.76^{bc}	4.50 ± 0.10^{a}
Eth + 100 mg/kg SLY	132.00 ±3.73	159.80 ± 3.78	27.80 ± 2.95^{cde}	4.32 ± 0.13^{a}
F	0.419	1.361	7.252	4.651
p-Value	0.902	0.246	0.000	0.001

n = 5; data analyzed using One–way ANOVA followed by Tukey's post hoc test. Values along the same column with different superscripts $^{a, b}$, ^{c} and ^{d} are significantly different ($p < 0.05$).

Effect of saponin rich extract on liver enzymes Parameters.

Hepatic serum liver enzymes (AST, ALT, and ALP) were significantly increased in the ethanol-control group ($p < 0.05$) compared to the treatment groups (Table 2). A significant reduction ($p < 0.05$) was observed in rats treated with ethanol plus saponins (ET + 100/200 mg/kg SCL), regardless of dosage, when compared to rats in the ethanol control (ET) group. For the ALT, it was observed that there was no significant difference observed in rats treated with 100 mg/kg saponin and silymarin compared with ethanol control (ET) rats. While the ALT decreased significantly ($p < 0.05$) at the high dose of the extracts when compared with the rats treated with just ethanol. Likewise, when compared to the rats in the ethanol group, the saponin extract-treated rats (ET + 200 mg/kg SCL) had a significant decrease in mean ALP. The effectiveness of the saponin-treated group in the case of ALP was dose-dependent; the high dose (200 mg/kg) had more efficacy than the low dose of the extracts. The albumin and total protein levels in the ethanol-control group were significantly lower ($p < 0.05$) than in the other treatment groups (ET + 100/200 mg/kg SCL; ET + 100 mg/kg SLY & 200 mg/kg SCL) and rats in the control group (NC).

Table 2: Mean Liver Enzymes of Ethanol-Induced Toxicity in Rats treated with saponin.

Groups	AST (IU/L) Mean ± SEM	ALT (IU/L) Mean ± SEM	ALP (IU/L) Mean ± SEM	TP (g/dL) Mean ± SEM	ALB (g/dL) Mean ± SEM
NC	48.00 ± 3.39^{a}	4.00 ± 0.55^{a}	11.00 ± 0.57^{a}	7.72 ± 0.75^{b}	3.18 ± 0.33^{c}
Eth	92.02 ± 6.63^{c}	9.80 ± 1.02^{d}	19.68 ± 1.90^{b}	3.96 ± 0.43^{a}	1.34 ± 0.22^{a}
200 mg/kg SCL	54.01 ± 3.39^{ab}	4.40 ± 0.81^{a}	10.96 ± 1.11^{a}	8.32 ± 0.24^{b}	3.24 ± 0.14^{c}
Eth + 100 mg/kg SCL	77.00 ± 8.60^{bc}	8.40 ± 0.51^{cd}	11.94 ± 0.92^{a}	6.18 ± 0.12^{ab}	2.58 ± 0.18^{bc}
Eth + 200 mg/kg SCL	55.23 ± 6.71^{ab}	7.00 ± 1.05^{abc}	14.28 ± 1.17^{a}	7.06 ± 0.34^{b}	3.02 ± 0.20^{bc}
Eth + 100 mg/kg SLY	60.00 ± 2.71^{ab}	8.00 ± 0.55^{cd}	13.92 ± 1.13^{a}	7.22 ± 0.73^{b}	2.94 ± 0.23^{bc}
F	**5.745**	**8.316**	**6.246**	**3.819**	**8.391**
p-Value	**0.000**	**0.000**	**0.000**	**0.002**	**0.000**

n = 5; data analyzed using One–way ANOVA followed by Tukey post hoc test. Values along the same column with different superscripts $^{a, b}$, ^{c} and ^{d} are significantly different ($p < 0.05$).

Effect of saponin-rich extract on the serum lipid profile

According to Fig. 1, there was a significant increase ($p < 0.05$) in the mean values of all lipid profile parameters (cholesterol, triglyceride and LDL) in ethanol-control treated rats compared to rats in the control and saponin treated groups, but a significant decrease ($p < 0.05$) in the HDL lipid profile parameter in ethanol-control (ET) treated rats compared to rats i n the saponin groups (ET + 100/200 mg/kg SCL). There is a significant reduction ($p < 0.05$) in the lipid profile parameters (cholesterol, triglyceride and LDL) of rats in ET + 100 mg/kg SCL, and Eth + 200 mg/kg SCL when compared with rats in the ethanol control group (ET). While for the HDL lipid profile parameters, a significant increase ($p < 0.05$) was observed in the ET + 100 mg/kg SCL and ET + 200 mg/kg SCL groups compared with the rats in the ethanol control group.

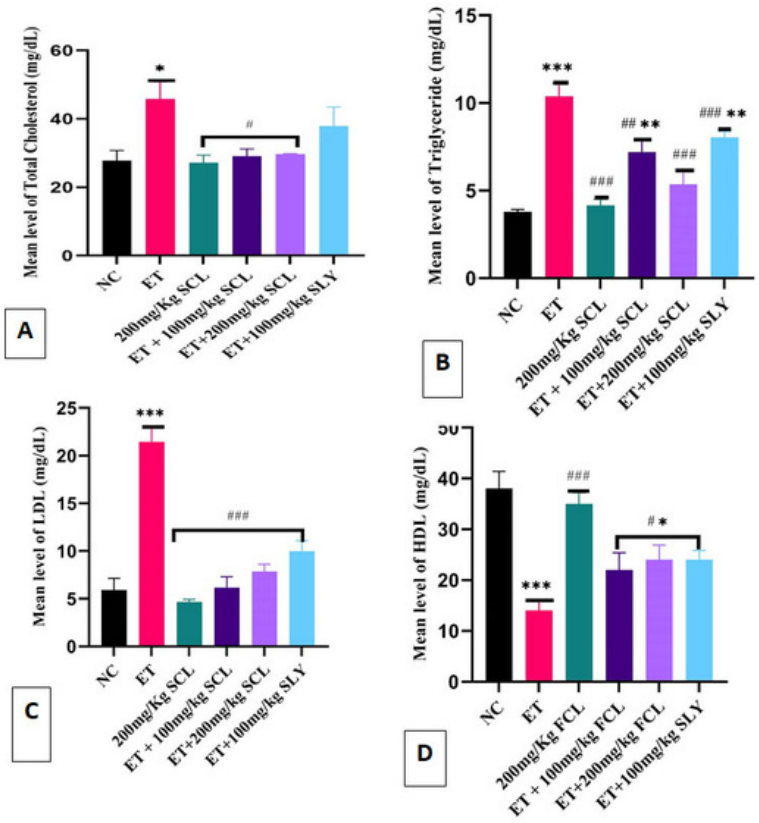

Figure 1: *Bar charts of serum lipid profile (a) Cholesterol (b) Triglyceride (c) Low density lipoprotein and (d) High density lipoprotein *P<0.033; ** P < 0.002; *** P < 0.0001 indicates a statistically significant difference as compared to the Normal control; #P<0.033; ## P < 0.002; ### P < 0.0001, indicates a statistically significant difference as compared to the ET Control group. n = 5, NC= Control, ET= Ethanol, SCL= Saponin Rich Fraction of Citrullus Lanatus seed, SLY= Silymarin.*

Photomicrographs of saponin-treated Wistar rat liver

Liver tissue sections from control and saponin-rich extract-treated rats showed normal hepatocytes with rounded nuclei, some bi-nucleated, sinusoids present between the cord and central vein, and Kupffer cells, which are inconspicuous but can be seen as flattened nuclei on the sinusoidal aspect of some hepatocytes (Fig. 2A,B,C). Liver tissue from only ethanol-treated rats (NC) showed dilated sinusoids and fat hepatocellular vacuoles of variable size with ballooning degeneration and necrosis. Rat liver sections treated with 100 mg/kg of saponin showed improved hepatocyte restoration, with few vesicular fat droplets and blood sinusoids, some necrotic and inflammatory hepatocytes, and a few vacuolated nuclei (Fig. 2D). Rat liver sections treated with 200 mg/kg of saponin showed remarkable restoration of the cytoarchitecture of the liver's histology, with scanty vesicular fat droplets and blood within the sinusoids (Fig 2E). Silymarin (100 mg/kg) gradually restored the liver's cytoarchitecture, with blood from hepatocellular vesicular fat droplets within the sinusoids, though some vacuolated hepatocytes were visible on the photomicrograph (Fig 2F).

Discussion

Alcohol-related liver disease (ALD) encompasses a range of liver conditions that are caused by excessive and prolonged alcohol consumption (Torruellas *et al.*,

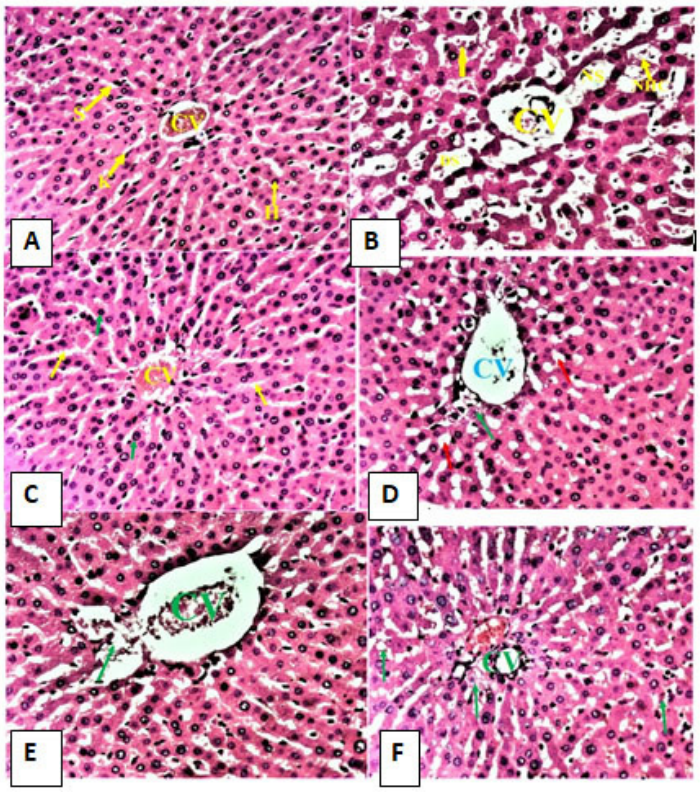

Figure 2: *Composite photomicrographs of liver of control (a) normal hepatocyte (H) with rounded nucleus with some binucleated (arrow), sinusoid (S), Kupffer cells (K) within the sinusoid, ET treated micrograph (b) dilated sinusoid (DS), fat hepatocellular vacuoles of variable size (arrow) with ballooning degeneration and necrosis (NHC) (c) showing normal hepatocyte (H) with rounded nucleus with some binucleated, sinusoid (S), within the sinusoid, with central vein ET + 100 mg/kg SCL (d)) showing normal gradual restoration of hepatocyte with some vacuolated and necrotic hepatocyte (green arrow), ET + 200 mg/kg SCL (e) showing normal central vein (CV), remarkable restoration of hepatocytes (green arrow), ET + 100mg/kg SLY treated group (f) showing gradual restoration hepatocyte and mild inflammation, ballooning degeneration and necrosis of the hepatocyte (green arrow), (H & E, X 200).*

2014). In the current study, saponin fractions of Citrullus lanatus seed were evaluated for hepaprotective activity against ethanol-induced hepatotoxicity in wistar rats. Liver function tests, lipid profile and histological studies were done to assess hepatoprotective. The hepatic microsomal ethanol-oxidizing system's major enzyme, CYP2E1, is crucial for the metabolism of ethanol (Liu *et al.*, 2019; Galicia-Moreno, and Gutiérrez-Reyes, 2014). Alcohol consumption promotes CYP2E1 activity, increasing the generation of ROS (Zeng *et al.*, 2018). C*itrullus lanatus* seed saponins extracts were chosen for this investigation to examine their hepatoprotective efficacy in the same gavage dosage and animal model under chronic alcohol use. Our data reveal that ethanol treatment causes typical clinical symptoms and pathological changes in rats, such as body weight loss, an increase in relative liver organ weight,

inflammatory infiltration, and necrosis. Furthermore, ethanol exposure damages the hepatic tissues and promotes vacuolation, resulting in a decline in liver function (Ding *et al.*, 2015; Zhao *et al.*, 2018). On the other hand, as compared to rats given only ethanol, the saponin extract was able to minimise liver weight gain; this may be due to the saponin's anti-oxidant properties, which were shown in the current study.

The liver enzyme such as AST, ALT, and ALP are the most sensitive indicators of the hepatocytes; when the liver is injured, ALT and AST are released from the hepatocytes (Dasgupta, 2015). It is reported that ALD elevates AST, ALT, and ALP (Torruellas *et al.*, 2015; Subramaniyan, 2021). However, saponin extract administered to ALD-induced rats significantly attenuated AST, ALT, and ALP toward normal. From the results, saponin extract may be a protective consequence against hepatic injury. Albumin, a critical component of plasma proteins that acts in transporting and binding proteins, lipids, medicines, and chemicals, is produced by the liver. While alcohol consumption can cause a decrease in albumin levels, it is worth mentioning that rats treated with saponin extract had higher amounts of this important protein (Kolankaya, 2002). Also, our results are also consistent with protective effects of different extracts with antioxidant ability against alcohol-induced hepatocyte cells of liver (Dahiru and Obidoa 2007; Sun *et al.*, 2001; Halliwell, and Gutteridge, 1986; Nordmann *et al.*, 1992; Ohkawa *et al.*, 1979). Ethanol administration decreased serum protein and albumin which caused the liver damage. This damage is attributed to the higher concentration of alcohol dehydrogenase enzyme which catalyses alcohol to aldehyde and accumulation of export type proteins due to inhibition of the secretion of the proteins from the liver of alcoholics (Yang *et al.*, 2001; Baraona and Lieber, 1982). Both doses of saponin extract restored the low level of protein in a dose dependent manner to normal level (Misra and Fridovich, 1972; Nordmann, 1994; Shaw *et al.*, 1981).

It is well known that adenosine monophosphate-activated protein kinase (AMPK) inhibits anabolic pathways while promoting catabolism (Tang *et al.,* 2014). Accordingly, drinking alcohol might lower AMPK expression, which in turn activates lipogenic enzymes by increasing the amount of the protein SREBP and decreasing the level of the receptor, peroxisome proliferator-activated receptor a (PPAR-a), leading to lipid accumulation (Louvet, and Mathurin, 2015). Triglyceride levels were significantly higher in the alcohol treatment group, indicating that alcohol does affect triglyceride levels. This result is consistent with the findings of Klop *et al.*, (2013) who reported that alcohol administration has a potent effect on raising triglyceride levels in the blood because alcohol is high in calories and sugar. Rats in groups treated with alcohol also showed a significant increase in serum cholesterol levels, as well as LDL levels, when compared with the control and saponin-treated rats. Al-Jameel *et al.,* (2018) who reported a significant increase in total serum cholesterol in rats treated with alcohol when compared with the control rats. In fact, the present results demonstrated that both saponins similarly alleviated the serum and hepatic lipid profile levels by mitigating the hepatic cholesterol and triglyceride levels. Additionally, the rats supplemented with saponin extracts gained a higher HDL-C level, which was in agreement with the previous results. Also, liver disease can affect hepatic lipid metabolism, causing changes in circulating lipid levels and contributing to dyslipidemia (Heeren and Scheja, 2021). Saponin extract reduced the elevated tryglycerides, total cholesterol and LDL in ethanol-treated rats with an increased in HDL implying that saponin extract may have a protective function against ethanol-induced irregularities in hepatic lipid breakdown in rats.

Histological studies also show enhanced permeability and damage and/or necrosis of hepatocytes. The histomorphology of the liver in ethanol-treated rats was improved to almost normal by the administration of saponin. Both the biochemical findings and the histological changes were linked. Rats were given saponin, which demonstrated a hepatoprotective effect and protected the liver's structural integrity against the harmful effects of alcohol.

Conclusion

Our study revealed that *Citrullus lanatus* seed's constituent saponin can protect the livers of rats from damage caused by alcohol consumption. This protective effect is likely due to the ability of saponin to restore liver function tests and lipid profile triggered by alcohol in the rat liver. These findings are encouraging and suggest that further research is necessary to determine the potential use of *Citrullus lanatus* seed's constituent saponin as a treatment option for ALD damage.

Conflicting interest: *The authors declare there are no conflicting interests.*

Abbreviations

ALD- Alcoholic liver disease
ALP - Alkaline phosphatase
ALT - Alanine aminotransferase
AMPK- adenosine monophosphate-activated protein kinase
AST- Aspartate aminotransferase
ET- Ethanol
SCL- Saponin Fraction of *Citrullus lanatus*
HDL- High density lipoprotein
LDL- Low density lipoprotein
NC-Normal Control
PPAR-a: peroxisome proliferator-activated receptor a
ROS- Reactive oxygen species
SLY-Silymarin

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