Phytochemical Characterization, Hepatoprotective Activity on Alcohol-Induced Toxicity of the Aqueous Extract of *Curcuma longa* (Zingiberaceae) in Wistar rats

Comment [u1]: Review title

ABSTRACT

Introduction

Liver or hepatic disease refers to different conditions that affect the liver. Chronic alcohol consumption is one of the most frequent causes of liver disease and accounts for about 55 % of liver cirrhosis deaths recorded in Cameroon in 2020. Standard accessible treatments focus on end-stage liver disease with safety and efficacy obstacles. We have a research gap in Cameroon to understand the alternative use of natural products as treatment with a long traditional history of safe use. *Curcuma longa* has long been a source of traditional and modern medicine. It is commonly used in Cameroon as a spice and herbal product with some level of activity against various forms of liver disease.

Objective: To phytochemically screen for bioactive metabolites and evaluate the hepatoprotective activity of the aqueous extract of *Curcuma longa* on alcohol-induced toxicity in Wistar rats.

Methods:

Phytochemical screening was carried out on the aqueous extract obtained from maceration of plant rhizomes. Three doses (125, 250 and 500 mg/Kg) of the plant extract and the reference (Silymarin 50mg/Kg) were administered *per os* to rats 30 mins before administration of 40% alcohol (2mL/100g p.o) for 21 days. Biochemical parameters such as ALAT, ASAT, GGT, Bilirubin and Lipid profile were quantified and histological studies of the liver using standard procedures.

Results

Phytochemical screening of the aqueous extract of *C. longa* revealed polyphenols such as flavonoids, tannins, quinones, saponins and phlobatanins. The plant showed hepatoprotective activity by decreasing liver toxicity markers such as ASAT, ALAT, GGT and Bilirubin. Histology revealed dose-dependent protection with 500 mg/Kg showing the most cellular integrity, no central vein occlusion and minimal fibrosis.

Conclusion

This study indicated the presence of polyphenols like flavonoids and tannins in the aqueous extract of *C. longa*. The presence of these secondary metabolites in the studied extract justifies its anti-oxidant and anti-inflammatory properties confirmed by its hepatoprotective effects on alcohol-induced toxicity. This was clearly shown by biochemical and histological parameters. More sensitive and specific methods are required to test for these secondary metabolites in serum.

Keywords: *Curcuma longa*, polyphenols, Alcohol-induced toxicity, Hepatoprotection.

INTRODUCTION

Liver, or hepatic, disease comprises a wide range of complex conditions that affect the liver. Alcohol consumption, viral hepatitis, metabolic syndromes and drug-induced toxicity are among the most frequent causes of liver disease. Alcohol consumption undoubtedly plays a vital role in the development of cirrhosis, cutting across geographic, political and economic boundaries [1, 2].

The Global Burden of Disease (GBD) project gave a global estimate of over two million liver disease-related deaths in 2020, including acute hepatitis, cirrhosis, and liver cancer [3]. This implies that liver disease represents a significant public health burden. Acute alcoholic hepatitis and liver cirrhosis are associated with high mortality (50% in acute alcohol hepatitis). WHO reported alcohol is responsible for 48% of liver cirrhosis cases [3, 4].

In 2020, the Centre for Disease Control (CDC) reported the number of adults in the United States of America (USA) with diagnosed liver disease as 7.5 million (2% of the population) with 40,545 deaths [5]. It also reported the number of alcoholic liver disease deaths at 21,815 [6].

Liver disease estimates for Africa and especially the sub-Saharan region is sparse at best. In sub-Saharan Africa, liver cirrhosis deaths doubled between 1980 and 2010. Western Africa had the highest cirrhosis mortality rate. The age-standardized alcohol-attributable burden of disease and injury is highest in the WHO African Region. Cameroon has an estimated value of 8.9 alcohol per capita consumption compared to the African average of 6.3 [4]. The GBD estimates suggest that alcohol misuse accounted for 18% of cirrhosis and 20% of liver cancer in Africa [3,7].

In Cameroon, the cirrhosis mortality was reported as 66 per 100,000 in 2016, with Alcohol Attributable deaths given as 3639 (55%) [3,4].

Comment [u2]: attributable

Treatments for liver disease are usually high-cost and nonspecific, hence the need for alternative means of management. Liver transplants remain the most effective treatment. Treatment is inaccessible in most parts of sub-Saharan Africa given the considerable shortage of management experts [8].

Medicinal plants have been proposed as an alternative means for the management of liver disease. One such plant is *Curcuma longa*, commonly known as turmeric. This plant has been used for thousands of years as a spice and natural remedy. It is used traditionally as a stomachic, antimicrobial, wound healing and anti-arthritic remedy. The dried rhizome powder and Curcumin, a polyphenol and one of its active metabolites, have been proven in several preclinical studies to have potent anti-inflammatory, antioxidant, antimicrobial, immunomodulatory and hepatoprotective properties [9-12]. Turmeric has been shown in preclinical models to have hepatoprotective effects against Carbon Tetrachloride ($CCl_{4)}$ [10], Paracetamol [2, 11], and Thioacetamide induced toxicity [13]. Clinical studies have shown its beneficial effects in peptic ulcer healing and irritable bowel syndrome [9]. This plant has been present in Africa for a long time, and in recent years, its use has increased exponentially as a liver remedy. However, there are very few studies on this plant in our setting.

Given the high prevalence of liver disease and high amounts of alcohol consumption in our context, and the activity of this plant against other liver toxicants, the present study

was undertaken to investigate the hepatoprotective effect of Turmeric rhizomes in alcohol-induced liver toxicity.

The objective of this study was to phytochemically screen for bioactive molecules and investigate the hepatoprotective activity on alcohol-induced liver toxicity of the aqueous extract of *Curcuma longa* rhizomes in albino Wistar rat models.

METHODS

This preclinical *in vivo* experimental study carried out on Wistar rats., conducted at the Laboratory for Preclinical animal and Pharmacotoxicology Research, Faculty of Medicine and Biomedical Sciences FMBS, University of Yaoundé I, was conducted between December 2018 and June 2019. Ethical approval was sought from the institutional review board of the institution. Authorization was obtained from the administration of FMBS through the Laboratory Head to work in the Animal House. The Organization for Economic Community and Development (OECD) Guidelines 420, for the use of animals in preclinical studies were applied.

Harvesting, identification and authentication of plant materials.

The plant was harvested in Bafut village in the Mezam Division of the North West Region, where the plant is of economic importance, in December 2018. Fresh mature rhizomes were identified by a Botanist, collected and pressed with other materials and transported to the National Herbarium in Yaoundé for identification and authentication. The National Herbarium authenticated the plant by comparing the voucher specimen: Westphal botanic collection No 99674 registered at the National Herbarium as **No 43153/HNC**.

Preparation of aqueous extract.

The fresh rhizomes were washed, cut into small pieces, and air-dried for two weeks before being finely grown to a powder. A litre of double distilled water was mixed with 100 g of powdered *C. longa* rhizome and allowed to macerate for 48 hours, strained with a cloth, filtered with Whatman filter paper (No2), and the extracted liquid was evaporated to dryness in a hot oven at 50^{o}C for two days. A dry, brown powder was obtained, refrigerated in an airtight container until when it was reconstituted with an appropriate

amount of distilled water. The dried extract obtained was weighed to determine the yield from the initial powder used. The yield (%) was calculated using the formula:

$$\text{percentage yield} = \frac{mass\ of\ the\ extract\ obtained}{mass\ of\ the\ initial\ plant\ powder} * 100$$

Animal Sample.

The experiments were carried out on adult albino Wistar rats, and the animals were identified by cage card and corresponding bold marker body markings.

Selection of animals

The animals were subjected to a gross observation to ensure that the selected rats were in good health. Rats were randomly selected for final allotment to the study, with a total of 65 rats required for the experiments: The rats were separated into treatment lots, 2 rats for preliminary studies and 30 rats for hepatoprotective activity. The average mass of rats used in the hepatoprotective activity study was 147.1 g (~150 g).

Feeding and accommodation

All rats were kept in cages at 25 ± 2°C, given portable water and a standard pellet diet. The diet consisted of a mixture of cornmeal (45 %), wheat flour (20 %), fish meal (20 %), soybean meal (10 %), palm kernel (5%), bone flour for calcium intake (0.98 %), cooking salt (0.5 %) and vitamin complex (0.5 %). They were exposed to a 12h:12 h light-dark cycle at 50–60% humidity in an animal room. Rats were grouped in stainless steel covered cages according to a randomized assortment for the different tests. Test substances were administered using intubation needles (adapted syringes). The dose administered to individual rats was calculated according to average body weight.

Reference Hepatoprotective Drug: Silymarin 140 mg Silybon® from Microlabs India was purchased from Pharmacie d'Emia in Yaoundé.

Toxicant: Alcohol used to induce toxicity was 90^{o} ethanol from *Cooper*, *France* purchased in 150 mL containers from *Pharmacie d'Emergence* in Yaoundé.

Comment [u3]: ml

Phytochemical screening

The plant extract was screened for the following secondary metabolites: alkaloids, phenols, flavonoids, terpenoids, tannins and saponins**.** This screening was carried out according to general methods proposed by Trease and Evans [15, 16].

Test for alkaloids: the extract was dissolved in dilute hydrochloric acid using a hot water bath and filtered. The following tests were carried out on the filtrate;

Mayer's test: To 1 mL of the extract, 2 mL of Mayer's reagent (Potassium Mercuric Iodide) is added, a dull white precipitate indicates the presence of alkaloids.

Wagner's test: To 1 mL of the extract, 2 mL of Wagner's reagent (Iodine/Potassium Iodide) were added. The appearance of a reddish-brown precipitate indicates the presence of alkaloids.

Hager's test: 1 mL of filtrate was treated with Hager's reagent (saturated picric acid solution). The presence of alkaloids was confirmed by the formation of a yellow precipitate.

Test for phenolic compounds

Lead acetate test: A few drops of 10% lead acetate solution was added to the test solution. The formation of a white precipitate indicates the presence of phenolic compounds.

Ferric chloride test: To the test solution, a few drops of ferric chloride solution are added. A dark green or bluish-black colour indicates the presence of phenolic compounds.

Test for flavonoids

Sulfuric acid test for flavonoids: 2mL of aqueous extract was placed in a test tube. To this was added a few drops of concentrated Sulphuric acid, being careful not to agitate. The presence of an orange colour indicates the presence of flavonoids.

Sodium hydroxide test for flavonoids: A small quantity of dried extract was dissolved in water and filtered; to 2 mL of this, 1 mL of 10 % aqueous Sodium Hydroxide was added to produce a yellow colouration. A change in colour from yellow to colourless upon addition of dilute hydrochloric acid indicates the presence of flavonoids.

Test for tannins

Reaction with ammoniacal Copper II Sulphate Solution: 1mL of 1% $CuSO_4$ solution was added to 1mL of the extract. To this mixture, two drops of Ammonia was added. The formation of a black, blue or green precipitate indicates the presence of tannins.

Characterization of catechin tannins: To 5 mL of extract was added 1 mL of concentrated HCl. The mixture was heated in a water bath for 15minutes and then filtered. The formation of a red precipitate indicates the presence of catechin tannins [16].

Comment [u4]: ml

Comment [u5]: ml

Comment [u6]: ml

Gallic tannins: To the filtrate was added pulverized Sodium Acetate and then 1 mL of $FeCl_3$. The presence of gallic tannins not precipitated by STAINSYS test is indicated by the presence of dark blue colouration.

Steroids

1 mL of extract was mixed with 2 mL of Acetic Anhydride and 2 mL of H_2SO_4 in a test tube. The presence of violet to blue to green colour indicates the presence of steroids [17].

Test for terpenoids

1 mL of the extract was mixed with 2 mL of chloroform, to which was carefully added 2 mL of Sulphuric Acid. The formation of a reddish-brown layer in the interface between the two liquids indicates terpenoids [18].

Resins

1 mL of the extract was placed in a test tube. To this was added a few drops of acetic anhydride and 1mL of Sulphuric acid. The apparition of a yellow colouration indicates the presence of resins.

Betacyanins

To 1 mL of extract in a test, tube was added 1 mL of 2N Sodium Hydroxide. The mixture was heated in a hot water bath for 5 minutes. The appearance of yellow colour indicates the presence of betacyanins [19].

Phlobatanins

To 1mL of extract in a test tube was added a few drops of HCl, and the mixture was heated in a hot water bath. The presence of a red precipitate indicates the presence of phlobatanins.

Anthocyanins

1 mL of the extract was placed in a test tube. To this was added 1 mL concentrated H2SO4 and then 1 mL of Ammonia. In the presence of anthocyanins, the colour increases in acidic medium then turns purplish-blue in basic medium, indicating the presence of anthocyanins [20].

Saponins

Foam test: Crude extract weas mixed with 5 mL of distilled water in a test tube and agitated. The presence of a stable foam after 15 minutes was considered an indication for saponins' presence.

Coumarins.

Comment [u7]: ml

Comment [u8]: ml

Comment [u9]: ml

In a test tube containing 1 mL of the plant and 1 mL of distilled water was added a few drops of 10% $FeCl_3$. Obtention of a green or blue colouration that turned yellow by the addition of Nitric acid (HNO_3) indicates coumarins [20].

Quinones

To 1 mL of extract was added 1 mL of conc. H_2SO_4. The presence of a red colouration indicates the presence of quinones.

Cardiac Glycosides.

0.5 mL of the extract was mixed with 2 mL of glacial acetic acid and a few drops of 5% ferric chloride solution ($FeCl_3$), followed by the addition of 1 mL of conc. H_2SO_4. The formation of a greenish or brown ring at the interface indicates the presence of cardiac glycosides [21].

Oxalates.

To 1 mL of extract was added a few drops of glacial ethanoic acid. The formation of a blue-black colouration indicates the presence of oxalates.

Mucilage

To 1 mL of extract was added 5 mL of absolute ethanol. The obtention of a fluffy precipitate upon agitation indicates the presence of mucilage [22].

***In-vivo* activity**

Preparation of test solutions

Extract Solution

Three doses of aqueous extract of *Curcuma longa* were used: 125 mg/kg, 250 mg/kg and 500 mg/kg of body weight per day. The concentrations of stock solutions were calculated by using the following formula:

$$\text{Va } (mL) = \frac{Dose \frac{mg}{Kg} \; x \; weight \; (Kg)}{concentration \; (mg/mL)}$$

OECD guidelines for preclinical animal testing states that for aqueous solvents, volumes for administration should not exceed 20 mL/Kg (2 mL/g) of body weight [23]. Hence, calculations were done following standard guidelines [24]. The extract was prepared at a

concentration of 37.5 mg/mL, i.e. 2.250 g of extract in 60 mL of distilled water. The 500 mg/Kg group received 2 mL, the 250 mg/Kg received 1 mL and the 125 mg/kg received 0.5 mL.

Comment [u10]: ml

Reference Drug Solution

Silymarin was used as a reference hepatoprotective drug at a dose of 50 mg/Kg of body weight per day. It was prepared at a concentration of 3.7 mg/Kg.

Induction of Toxicity

Alcohol (ethanol) was used at a dose of 40% ethanol v/v which is 2 mL/100 g of body weight per day, to induce liver toxicity. Substances were single dosed within 30-minute intervals. Silymarin and the various plant doses were administered 30minutes before the administration of alcohol daily for 21 days.

Study Groups

30 Wistar rats were used for this study. The rats were randomly divided into 6 groups of 5 and allocated as follows:

Group 1: Healthy control group (received water)

Group 2: Negative control group (received ethanol)

Group 3: Silymarin control group (received ethanol and Silymarin daily p.o for 21 days)

Group 4: Treatment group 1 (received ethanol and 125 mg/kg of extract daily p.o for 21 days)

Group 5: Treatment group 2 (received ethanol and 250 mg/kg of extract daily p.o for 21 days)

Group 6: Treatment group 3 (received ethanol and 500 mg/kg of extract daily p.o for 21 days)

5.2.5. Morphological parameters

Animal weights were measured daily. Liver weights were measured at the end of the study after sacrifice.

Measurement of biochemical parameters

The following biochemical parameters were measured: ALAT, ASAT, ALP, GGT, Total Bilirubin, Lipid profile, Albumin and Total serum proteins using different test methods.

Evaluation of the hepatic activity

Quantification of ALAT (CHRONOLAB KIT)

Principle: Alanine aminotransferase (ALT) or Glutamate pyruvate transaminase (GPT) catalyzes the reversible transfer of an amino group from alanine to αketoglutarate, forming glutamate and pyruvate. The pyruvate produced is reduced to lactate by lactate dehydrogenase (LDH) and NADH:

Alanine + α -Ketoglutarate → Glutamate + Pyruvate

Pyruvate + NADH + H^+ → Lactate + NAD^+

The rate of decrease in the concentration of NADH, measured photometrically, is proportional to the catalytic concentration of ALT present in the sample.

Reagents R1: TRIS at P^H 7.8 and L-Alanine R2: NADH, LDH and α-ketoglutarate

Procedure: The Assay conditions were wavelength at 340 nm, cuvette 1 cm light path and constant temperature of 25ºC/ 30ºC/ 37ºC. The instrument was adjusted to zero with distilled water or air. It was pipette into a cuvette: WR mixture of 1 part R1 to 4 parts R2- (ml) 1.0 and sample (µL) 100 The sample was mixed, incubated for 1 min. Initial absorbance (A) for the sample was read and started the stopwatch, and absorbance read at 1-minute intervals after that for 3 minutes. Calculation of the difference between absorbances and the average absorbance differences was reported per minute (ΔA/min).
Results: Δ A/min x 1746 = U/L of ALT
Units: One international unit (IU) is the amount of enzyme that transforms 1 µmol of substrate per minute under standard conditions. The concentration is expressed in units per litre of the sample (U/L).

Quantification of ASAT (CHRONOLAB KIT)

Aspartate aminotransferase (AST) or glutamate oxaloacetate (GOT), catalyzes the reversible transfer of an amino group from aspartate to α-ketoglutarate, forming glutamate and oxaloacetate. The oxaloacetate produced is reduced to malate by malate dehydrogenase (MDH) and NADH:

Aspartate + α -Ketoglutarate →Glutamate + Oxaloacetate

Oxaloacetate + NADH + H^+ → Malate + NAD^+

The rate of decrease in the concentration of NADH, measured photometrically, is proportional to the catalytic concentration of ASAT present in the sample.

Procedure: Assay conditions: 340 nm wavelength, 1 cm cuvette light path and constant temperature of 25ºC/ 30ºC/ 37ºC. The instrument was adjusted to zero with distilled water or air. Was pipette into a cuvette WR (mL) 1.0 and Sample (µL) 100. The sample was mixed and incubated for 1 minute. The initial was read absorbance (A) of the sample at 1-minute intervals after that for 3 minutes. The difference between absorbance and the average absorbance differences per minute (ΔA/min) was noted and the result presented as Δ A/min x 1746 = U/L of ALT

Units: One international unit (IU) is the amount of enzyme that transforms 1 µmol of substrate per minute under standard conditions. The concentration was expressed in units per litre of sample (U/L).

Cholesterol

Cholesterol present in the sample produces a coloured complex in the following set of reactions:

Cholesterol + O2 → Cholestene-4-one + H_2O_2

$2H_2O_2$ + Phenol + 4AP → Quinoimine + 4 H_2O

The intensity of the colour produced is directly proportional to the concentration

Reagents:

Buffer: Phosphate buffer, Sodium Cholate, Chloro-4-phenol

Enzymes: Cholesterol Oxidase, Peroxidase

Procedure: The description of the quantification process of creatinine is described in table 1.

Table 1: description of the quantification process of creatinine

	Control	Calibration	Dosage
Reagent (mL)	1	1	1
Distilled water (µL)	10	/	/
Calibrator (µL)	/	10	/
Sample (µL)	/	/	10

Samples were left for 10minutes at room temperature. Absorbances were read at 500nm against the control.

Triglycerides

Triglycerides incubated with Lipoprotein Lipase produce glycerol and free fatty acids. The glycerol is phosphorylated by GPO and ATP in the presence of Glycerol Kinase to produce Glycerol-3-Phosphate and ADP.

Triglycerides + H_2O → Glycerol + Free Fatty Acids

Glycerol-3-Phosphate + O2 → Dihydro oxyacetone Phosphate + H_2O

H_2O_2 + 4 AP + P-Chlorophenol → Quinone + H_2O

The Procedure is similar to that of Cholesterol.

Total proteins

The quantification of proteins was done following the procedure described in table 2:

Table 2: Description of the quantification process for total proteins.

Put in the test tubes	**Sample**	**White**
Sodium hydroxide 0.1N	190 µL	200 µL
Gastric juice	10 µL	/
Solution C	1000 µL	1000 µL
Incubate for 10 minutes		
Solution D	100 µL	100 µL
The tubes are then vortexed		

Incubate the tubes for 30 minutes at room temperature under shade, then read the optical density at 600 nm against the white.

Preparation of liver samples for histopathological analysis

Liver samples from all groups were washed with normal saline, observed for macroscopic signs of toxicity and fixed by complete immersion in 10% formol for further histopathological analysis.

STATISTICAL ANALYSIS

Raw data on weight, alimentation and biochemical parameters were collected and entered in Microsoft Excel 365[25]. The GraphPad Instat version 5.1 software was used to compare the groups analyzed using one-way analysis of variance, the ANOVA test followed by Turkey's Kramer post hoc test.

The results were expressed in terms of mean ± standard deviation. P-values ≤0.05 were considered statistically significant.

RESULTS

Extraction of the dried rhizomes of Curcuma longa by maceration gave a percentage yield of 10.76%.

Phytochemical screening of the aqueous extract of dried rhizomes of *Curcuma longa* indicated the following secondary metabolites: Polyphenols, flavonoids, catechin tannins, mucilage, phlobatanins and quinones. Polyphenols such as Flavonoids and catechin tannins were the most abundant metabolites given the intensity of the colour reactions. Quinones and phlobatanins were present in lesser amounts. Other important secondary metabolites such as alkaloids and terpenoids were absent (6). The result of phytochemical screening is shown in table 3.

Table 3: results of phytochemical screening of aqueous extract of *C. longa*

Secondary Metabolites	Reagents	Results
Alkaloids	Hager	-
	Mayer	-

	Wagner	-
Polyphenols	$FeCl_3$	+
	Lead Acetate	+++
Mucilage	Ethanol	++
Saponins	Foam Test	+
Flavonoids	H_2SO_4	+++
	NaOH	++
Tannins	$CuSO_4$	+++
Catechin Tannins	HCl	+++
Gallic Tannins	STIASNY	-
Cardiac Glycosides	Acetic Acid/ $FeCl_3$	-
Steroids	Acetic Anhydride/ H_2SO_4	-
Terpenoids	Chloroform/ H_2SO_4	-
Resins	Acetic acid/ H_2SO_4	-
Oxalates	Ethanoic acid	-
Quinones	Conc. HCl	+
Coumarins	Fe/ HNO_3	-
Anthocyanins	H_2SO_4/ NH_3	-
Phlobatanins	HCl	+
Betacyanin	NaOH	-

Key: + Mildly present; ++ present; +++ abundant; - absent

HEPATOPROTECTIVE ACTIVITY

Preliminary Tests

Preliminary tests were carried out with two rats to determine the appropriate dose of alcohol for the primary test that induced appropriate liver toxicity without death. 50% alcohol was administered at 2 mL/100 g daily for 14days.

A gradual weight decrease was observed in both rats, and they showed signs of severe toxicity such as ocular haemorrhage and trembling. Rat 1 died on the 12^{th} day of the test, and Rat 2 was sacrificed on the 15^{th} day. Examination of organs showed no visual signs of toxicity. It was concluded that a lower dose had to be chosen.

Main Test /Relative weight of organs

The results show no remarkable variation of organ weights in the various study groups. The Negative Control group that received alcohol only (3.71 ± 0.58) had a

greater relative liver weight than the Healthy Control (3.40 ± 0.64) and the other groups. However, this increase was not statistically significant with a *pvalue>0.05* (Table 4).

Table 4: relative weight of organs in all groups of hepatotoxicity study.

Organs (g)		Healthy Control	Negative Control	Silymarin (mg/ Kg)	Plant extract (mg/ Kg)		
				50	125	250	500
Liver		3.40 ± 0.64	3.71 ± 0.58	3.60 ± 0.53	3.21 ± 0.23	3.30 ± 0.33	3.24 ± 0.09
Spleen		0.75 ± 0.35	0.61 ± 0.07	0.54 ± 0.17	0.63 ± 0.10	0.25 ± 0.02	0.44 ± .06
Heart		0.37 ± 0.07	0.41 ± 0.04	0.39 ± 0.05	0.35 ± 0.003	0.37 ± 0.01	0.41 ± 0.02
Lungs		0.76 ± 0.14	1.00 ± 0.44	1.23 ± 0.30	1.07 ± 0.005	1.03 ± 0.16	0.93 ± 0.18
Brain		0.83 ± 0.16	0.86 ± 0.15	1.11 ± 0.17	0.98 ± 0.08	0.87 ± 0.13	0.94 ± 0.03
	L	0.33 ± 0.06	0.38 ± 0.09	0.36 ± 0.03	0.33 ± 0.02	0.32 ± 0.04	0.32 ± 0.02
Kidneys	**R**	0.34 ± 0.07	0.37 ± 0.06	0.33 ± 0.03	0.35 ± 0.01	0.32 ± 0.02	0.32 ± 0.01
Adrenal	**L**	0.01 ± 0.002	0.02 ± 0.006	0.02 ± 0.004	0.02 ± 0.005	0.02 ± 0.004	0.02 ± 0.009
Glands	**R**	0.01 ± 0.002	0.02 ± 0.004	0.02 ± 0.006	0.02 ± 0.005	0.02 ± 0.002	0.02 ± 0.008

Key: L left R Right.

Biochemical Parameters

Effect of plant extract and drug reference on lipid profile

The results obtained show an increase in Triglycerides, LDL and VLDL in the Negative Control (NC) group compared to the other study groups. Similarly, there was also a decrease in HDL in this group. In contrast, a non-significant decrease with a *p-value >0.05* in triglycerides, LDL and VLDL in the test groups compared to the controls and a non-significant increase in HDL with a *p-value >0.05* in the test groups compared to the controls (Figure 1).

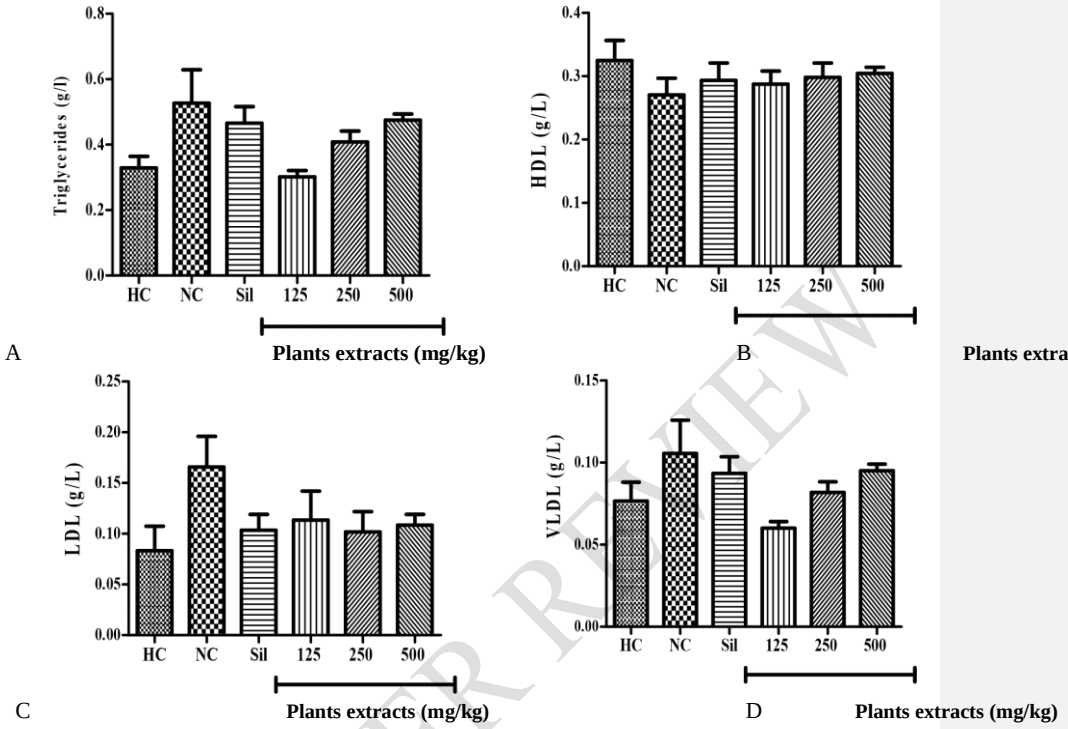


Figure 1: effect of plant extract on lipid profile of animals (Triglycerides, HDL, LDL and VLDL).

Effect of plant extract and drug reference on cytolysis induced by ethanol

Alcohol administration caused an increase in the cytolytic enzymes ASAT and ALAT in the Negative Control group compared to the Healthy Control. There was a decrease in ASAT in the Silymarin, 250, and 500 mg/Kg groups. As for ALAT, administration of the plant extract at different doses caused a decrease in enzyme levels. However, these values were not statistically significant (Figure 2).

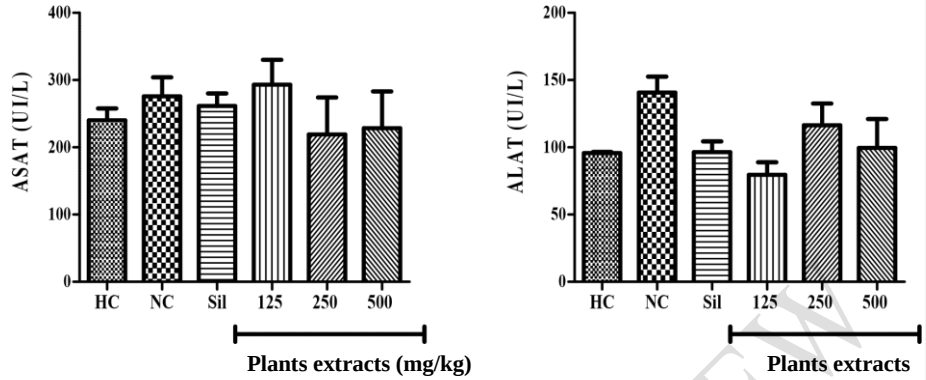
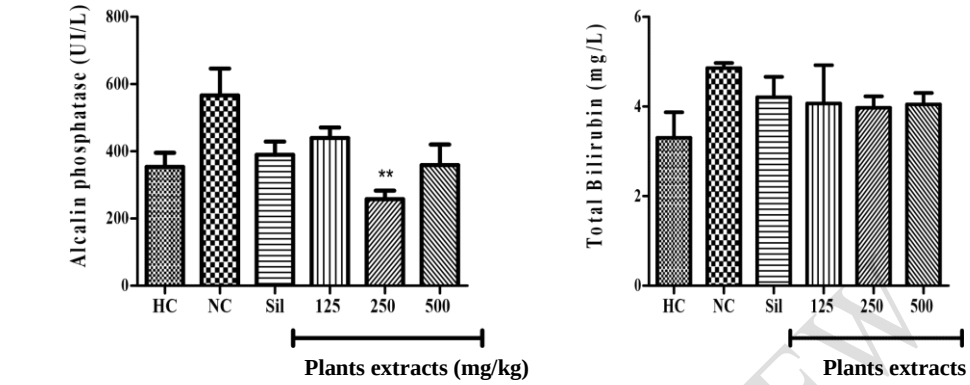


Figure 2: effect of plant extract on cytolysis induced by alcohol (ASAT and ALAT)

Effect of plant extract and drug reference on cholestasis induced by ethanol administration

Administration of alcohol led to an increase in ALP across the groups. The 250 mg/Kg plant extract caused a significant decrease in ALP activity compared to the other doses and the Silymarin group (Figure 2). For Bilirubin, there was a marked decrease across the different dose groups in comparison to the Negative Control, although the Silymarin group gave the lowest values (4.21 ± 0.91 mg/L)(Figure 2).

There was a decrease in GGT across all test groups in comparison with the Negative Control group. This decrease was non-significant with a p-value> 0.05 in the groups receiving 50 mg/Kg Silymarin and the plant extract at doses of 125 and 250 mg/Kg. However, there was a significant decrease with p-value < 0.01 in the 500 mg/Kg group (Figure 3).



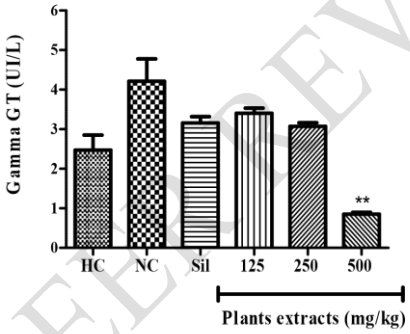
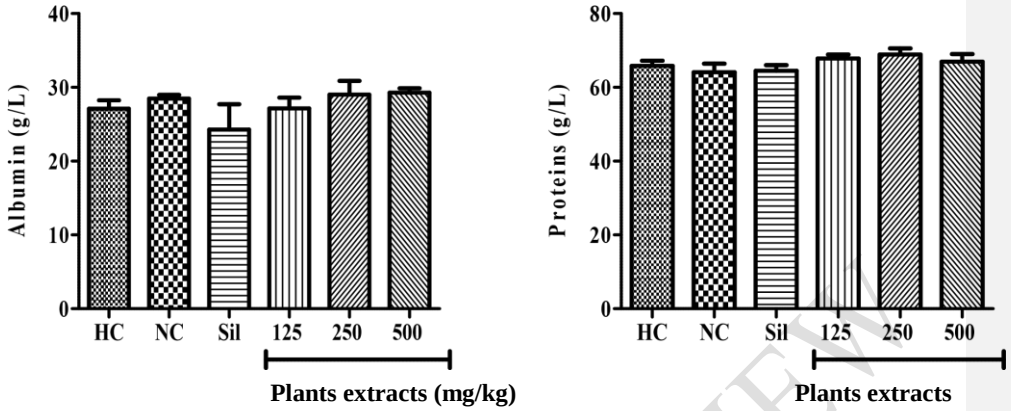


Figure 3: effect of plant extract on markers of cholestasis; A. Alkaline Phosphatase (ALP), B. Total Bilirubin and C. Gamma GT (GGT).

Effect of the plant extract on protein synthesis.

There was no significant variation in total proteins and albumin across all the test groups in the study. The Silymarin group, however, showed a decrease in albumin as compared to the other groups (4).



(mg/kg)

Figure 4: effect of plant extract on albumin and total proteins

Histological parameters

The gross appearances of the liver samples and microscopic assessment (H&E staining) of their sections in the experimental groups are shown in Figure 18. Healthy Control in Group 1 showed typical liver architecture (Figure5). Administration of alcohol-induced marked histological changes such as micro and macro-vesicular steatosis, central vein congestion, sinusoidal fibrosis and inflammatory cell infiltration in the Negative Control Group 2 (Figure 5). All these characteristics were reduced in the plant groups, with the 500 mg/Kg showing the most protection. The Silymarin (50 mg/kg) group, 125 mg/kg and 250 mg/kg group showed some central vein congestion and cell infiltration, but to a lesser extent than in the Negative Control (Figure 5C and D). Group 6 (500 mg/kg) treated group, however, showed the most conservation of liver histology with reduced micro-vesicular steatosis, sinusoidal fibrosis and inflammatory cell infiltration.

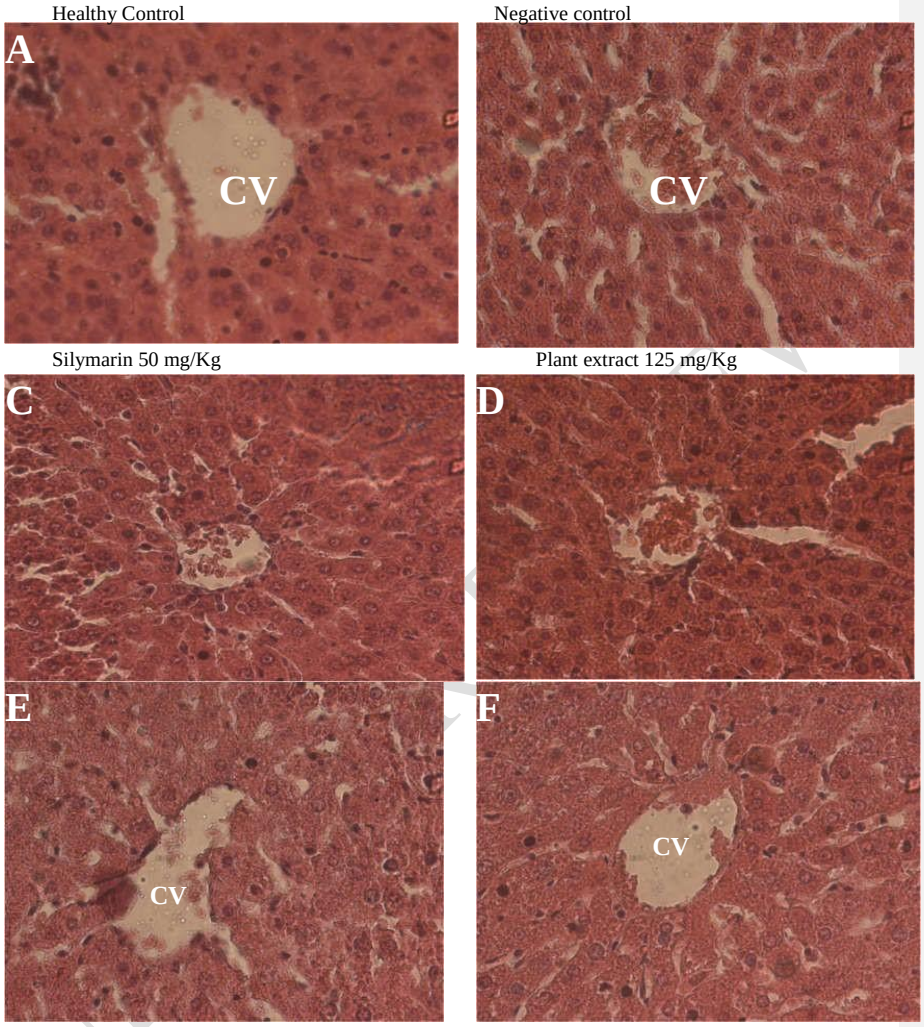


Figure 5: representative histopathological sections from livers sampled from rats in different experimental groups (H & E staining, 400 X).

Comment [u11]: Representative

A.Healthy control with typical liver architecture. B**.**Negative control group showing steatosis and central vein congestion, sinusoidal fibrosis and marked inflammatory cell infiltration. C.Sylimarin (50 mg/kg) group showing micro-and macro-vesicular steatosis with less central vein congestion. D.Plant (125 mg/kg) group with central congestion. E. Plant extract (250 mg/kg) group with diminution of central vein congestion. F. Plant

extract (500 mg/kg) treated group, showing reduced microvesicular steatosis, sinusoidal fibrosis and inflammatory cell infiltration.

DISCUSSION

Liver diseases in general and ALD are severe global health problems. The unavailability of proper therapeutic drugs, poor access, and high cost of their management make the investigation of other sources necessary. Hence, we decided to investigate the hepatoprotective activity of the aqueous extract of dried rhizomes of *Curcuma longa* on alcohol-induced liver toxicity in albino rats of Wistar strain. Phytochemical screening of the aqueous extract of dried *C. longa* rhizomes showed the presence of seven secondary metabolites, namely: Polyphenols, Flavonoids, Tannins, Phlobatanins, Mucilage, Saponins, and Quinones. These results are like those obtained by Dutta in India after screening an ethanolic extract by Soxhlet apparatus, which showed polyphenols, tannins, and saponins. However, they also found alkaloids and terpenoids [24]. Qualitative analysis by Sarangthem and Haokip also indicated significant amounts of polyphenols, flavonoids, alkaloids and tannins in the methanolic extract of *C. longa* [25-27]. However, our results are similar to those obtained in two other studies by Pawar *et al.* in India and Kodjio *et al.* in Cameroon, who did not find terpenoids in the aqueous extract of *C. longa* [28]. Differences in secondary metabolite content could be explained by geographical variation, different solvents and extraction methods. Polyphenols and flavonoids have anti-inflammatory and anti-oxidant properties. The plant extract contained polyphenols such as flavonoids and tannins, with reputed anti-oxidant and anti-inflammatory properties [29-31].

The hepatoprotective activity of the aqueous extract of *C. longa* on alcohol-induced liver toxicity by exposure to 40% alcohol at a dose of 3.76 g/Kg p.o daily was evaluated. The protocol induced ALD with similar pathology and etiology pattern to the human liver ALD including biochemical values for typical human ALD markers. The results were reconfirmed quantitatively by measuring the animals' liver index, the biochemical imbalances in the liver markers, lipid profile, and the altered total protein content. As previously described by other researchers, hepatic factors (ALP, GGT and ALAT) were increased in the negative control rats [32-35]. Histopathology also showed significant ant damage in the negative control group, which received 40% alcohol alone, and confirmed

these findings with noticeable changes such as micro-and macro-vesicular steatosis and central vein congestion (CV), sinusoidal fibrosis and marked inflammatory cell infiltration. The plant extracts exhibited hepatoprotective effects by causing recovery of these enzymatic activities and markers of alcohol-induced liver toxicity in a non-dose dependent matter, with the 250 mg/Kg showing the highest activity on parameters such as LDL, Albumin, Proteins, ASAT and ALP. This correlates with histological cuts showing marked conservation of liver structure in the plant-treated groups. However, this was in a dose-dependent manner with results suggesting that the 500 mg/Kg dose afforded the most protection. Parallel findings were also previously reported on other models of liver toxicity such as CCl_4 and paracetamol [10,12, 36]. Ethanol has been used to induce hepatotoxicity in experimental animals to produce various grades of liver damage, including steatosis, hepatitis and fibrosis. It is a potent hepatotoxic agent metabolized by CYP2E1 enzymes present in liver microsomes where it is converted to the toxicant, acetaldehyde [37, 38]. The development of ALD is reported to be multifaceted, involving multiple mechanisms. For instance, ethanol induces hepatocyte damage via its metabolite, acetaldehyde, which covalently binds to macromolecules of hepatocytes, causing DNA damage, protein oxidation and lipid peroxidation of the cell membrane biomolecules [39-40]. Alcohol metabolism also causes depletion of antioxidants such as glutathione and SAMe. In our study, we noticed an increase in triglycerides, LDL and VLDL in the alcohol-fed rats, and reduction in the treated groups. Histology also revealed steatosis, which is the earliest response of the liver to alcohol abuse and is characterized by the accumulation of fat (mainly triglycerides, phospholipids, and cholesterol esters) in hepatocytes. Alcohol increases fatty acid synthesis in the liver and inhibits its oxidation. Alcohol also increases fat mobilization from the gastrointestinal tract to the liver [25, 41]. Curcumin, the main polyphenol constituent of turmeric, has been shown to improve the lipid profile in similar studies by Rajakrishan *et al.* and Akila *et al.* in rats [42-44]. Alcohol administration caused increases in ALAT and ASAT. Another mechanism of alcohol toxicity is oxidative stress, which involves several pathways. It has been shown to activate phospholipases Al and A2 (PLA 1 and 2). The liberation of arachidonic acid by PLA2 sets in motion a range of oxidative catabolic processes. This compound is converted to prostaglandins, leukotrienes and thromboxanes

by cyclooxygenases and lipoxygenases, leading to considerable ROS generation and inflammation. Alcohol consumption also activates complement C3 and C5, which subsequently activate Kupffer cells via binding to their receptors on these cells; complement activation is followed by TNF production- and induction of hepatocyte injury [45 2, 46]. Hepatocyte injury by recruited neutrophils leads to the release of intracellular enzymes ALAT and ASAT, leading to their increase in the serum. It also explains the presence of cell infiltrates in histological cuts. Interestingly, Curcumin inhibits the production of arachidonic acid in the liver, kidney, and brain and inhibits PLA2 activity [26, 47]. Hepatocyte injury also leads to cholestasis, hence increased values of ALP and GGT. There is decreased conjugation of Bilirubin, leading to an increase in unconjugated Bilirubin. The protective effect of the plant extract on liver parameters wasn't clearing dose-dependent but was markedly greater than the effects achieved by the reference drug, Silymarin. Once liver cell integrity is compromised, the liver cannot ensure its regular metabolic functions of synthesis, and albumin is usually one of the first proteins to be affected, leading to hypoalbuminemia [6, 48]. Due to the increased production of antibodies and globulins in general to compensate, the overall protein profile might not reveal a change until a much later stage. This explains why the difference in total proteins wasn't significant across the groups. Histology revealed marked fibrosis. Activation of Stellate cells by mediators produced by Kupffer cells, acetaldehyde and Lipopolysaccharides leads to the secretion of extra collagen in the extracellular matrix, responsible for fibrosis [3, 17, 23, 49]. Turmeric by reducing inflammation reduces the possibility of necrosis and progression to fibrosis. Curcumin is reported to enhance apoptosis of damaged hepatocytes, which might be the protective mechanism by which Curcumin down-regulates the effects of inflammation and subsequent fibrogenesis of the liver. From histology, it can be concluded that the plant extract has antifibrotic properties [50]. We can deduce that the hepatoprotective effects of the aqueous extract of *C. longa* might be due to direct antioxidant and free radical scavenging mechanisms and the ability to indirectly increase glutathione levels, thereby aiding in hepatic detoxification. Turmeric extracts help maintain high levels of glutathione and SOD while maintaining low levels of MDA and other oxidative stress markers [11, 51].

CONCLUSION

According to this study, it was shown that the aqueous extract of dried rhizomes of *Curcuma longa:* contained polyphenols, flavonoids, catechin tannins, quinones and phlobatanins with anti-oxidant and anti-inflammatory effects. The extract showed dose-dependent hepatoprotective activity against alcohol-induced liver toxicity according to histological studies and decreased markers of hepatotoxicity such as ASAT, ALAT, GGT and ALP while preventing central vein congestion, inflammatory cell infiltration, steatosis and sinusoidal fibrosis.

Ethical Issue: Preclinical study not subject to ethical approval. However, the study approved by the scientific committee of the Faculty and OECD regulatory guidelines on animal studies strictly respected.

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