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Evaluation of the Systemic Serum Exposure and Acute Toxicity of the Aqueous Extract of *Curcuma longa* (Zingiberaceae) Rhizomes in Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AFN, TEF and FCN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EBB, TYO and NNB'BH, EBB managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Liver toxicity has become a public health concern as more people globally get exposed to xenobiotics with the potential to cause liver damage and consequent liver cirrhosis. The increase in liver toxicant abuse has necessitated the exploration of xenobiotic exposure levels when addressing therapeutic measures using alternative herbal remedies. The increasing use of herbal products as alternative therapy needs regulatory alignment through evidence-based support for the safety and efficacy of these natural products. To undertake preclinical discovery of new metabolites from medicinal products, the objective of this study was to investigate the systemic serum exposure and acute toxicity of the aqueous extract of *Curcuma longa* (Zingiberaceae) rhizomes on Wistar rat models.

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Methods: Phytochemical screening was carried out on the aqueous extract obtained by maceration of the dried plant rhizomes. Standard screening techniques for plant metabolites were used to screen blood serum after animal exposure with the extract. After a 500mg/Kg dose, systemic exposure was evaluated in blood samples collected at 30-minute intervals for one hour.
For acute toxicity, a single 2000mg/Kg by body weight dose of the plant extract and the reference (Silymarin 50mg/Kg) were administered to rats, and they were observed for 14 days. Biochemical markers of toxicity such as ALAT, ASAT, GGT, Bilirubin were quantified, and histological studies of the liver were carried out.
Results: No secondary metabolites were identified at 30 mins and 1hr in rat serum following a 500 mg/Kg oral dose. Administration of a 2000 mg/Kg oral dose to rats was well tolerated, and there were no deaths or significant target organ toxicity. The plant showed no lethality at the dose of 2000mg/kg body weight and decreased liver toxicity markers such as ASAT, ALAT, GGT, and Bilirubin. Histology revealed no significant damage to liver hepatocytes, no central vein occlusion, and no evidence of fibrosis.
Conclusion: There were no systemically available secondary metabolites at a dose of 500 mg/Kg after the qualitative screening; more sensitive and specific methods are required to test these secondary metabolites in serum. This study confirmed the safety margin of *Curcuma longa* with no lethality following a single oral dose of 2000mg/Kg and after observation for 14 days. There was a low expression of biochemical markers of toxicity ALAT, ASAT, and no histological indication of liver damage.

Keywords: Curcuma longa; acute toxicity; systemic serum exposure; lethality; ALAT; ASAT.

1. 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 an essential role in the development of cirrhosis, cutting across geographic, political, and economic boundaries [1-3].

The Global Burden of Disease (GBD) projection gave a global estimate of over two million liver disease-related deaths in 2020, including acute hepatitis, cirrhosis, and liver cancer [3-5]. 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 [4].

In 2016, the Centre for Disease Control (CDC) reported the number of adults in the United States of America (USA) with diagnosed liver disease as 4.9 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 the sub-Saharan region are sparse at best [7,8]. In sub-Saharan Africa, liver cirrhosis deaths doubled between 1980 and 2010, with western Africa having the highest cirrhosis mortality rates. The age-standardised 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, cirrhosis mortality was reported as 66 per 100,000 in 2016, with Alcohol Attributable deaths (ADD) given as 3639 (55%) [3,4].

Treatments for liver disease are usually costly for an average Cameroonian. There is, therefore, the need to explore herbal alternative treatment options for the management of liver diseases. Liver transplants remain the most effective treatment, but this treatment is inaccessible in most parts given the considerable shortage of management experts and the health technology platform [7,8].

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 plant. It belongs to the family Zingiberaceae and is also known as Indian Saffron or Curcuma. 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 [9].

Turmeric has been shown in preclinical models to have hepatoprotective effects against Carbon Tetrachloride (CCl_4) [10], Paracetamol [11], and Thioacetamide induced toxicity [12]. Clinical studies have shown its beneficial effects in peptic ulcer healing and irritable bowel syndrome [9]. Curcumin has a low oral bioavailability attributed to water insolubility and rapid Phase I and II metabolism in the liver into inactive metabolites. It is an oil-soluble compound, practically insoluble at room temperature in water at acidic and neutral pH. While it is soluble in alkali, it is very susceptible to auto-degradation [13].

The dried rhizome powder, as well as 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 [14,15].

Turmeric contains yellow matter called curcuminoids (5%) and essential oil (6%). The chief constituent of the yellow colouring is curcumin I (60%) in addition to small quantities of curcumin III, curcumin II and dihydrocurcumin. The volatile oil contains mono- and sesquiterpenes like zingiberene (25%), turmerone, and cineole. The volatile oil also contains camphene, limonene, terpinene, linalool, camphor, and eugenol [16,17].

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 the increased use of this plant in its management, the present study was undertaken to investigate the systemic serum exposure and acute toxicity of the aqueous extract of *Curcuma longa* (Zingiberaceae) rhizomes on Wistar rats.

2. METHODS

This was a preclinical *in vivo* experimental study carried out on Wistar rats. This work was carried out at the Laboratory for Preclinical Animal Studies and Pharmaco-Toxicology Research of the Department of Pharmacotoxicology and Pharmacokinetics from December 2018 to May 2019 in the Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, Cameroon. Ethical approval was sought from the institutional review board of the Faculty (N^0 008/UYI/FMSB/VDRC/CSD of 19th April 2019), and authorisation was obtained from the laboratory Head to work in the Animal House of this Faculty. The Organization for Economic community and Development (OECD) Guidelines for the use of animals in preclinical studies were applied [18].

2.1 Harvesting and Identification of Plant Material

The plant was harvested in Bafut, Mezam Division of the North West Region in December 2018. Fresh mature rhizomes were collected as well as other material for identification by the National Herbarium. A Botanist identified the plant samples at the National Herbarium by comparing the voucher specimen: Westphal botanic collection No 99674 registered at the National Herbarium as No 43153/HNC.

2.2 Preparation of Aqueous Plant Extract

The fresh rhizomes were washed, cut into small pieces, and air-dried 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 48hours, strained with a cloth and filtered with Whatman filter paper N^o2. 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 use when reconstituted with an appropriate amount of distilled water. The yield (%) is calculated from the formula:

% yield = mass of the extract obtained/mass of initial plant powder x 100

2.3 Animal Material

The experiments were carried out on adult Wistar rats from the Animal House of the Faculty. Animals were identified by cage card and corresponding bold marker body markings. The animals were subjected to a gross observation to ensure that selected rats were in good health. Rats were randomly selected for final allotment to the study. A total of 25 rats were required for the experiments: 20 rats for acute toxicity studies, two rats for preliminary studies and three rats for systemic exposure. The average mass of the rats used in the Acute Toxicity study was 62.1 g which is the value used in dose calculation.

2.4 Feeding and Accommodation

All rats were kept in cages at 25 ± 2°C, given tap 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 randomised assortment. Test substances were administered using intubation needles (adapted syringes). The dose administered to individual rats was calculated according to their average body weight.

2.4.1 Preparation of test aqueous extract solution

The doses of aqueous extract of *Curcuma longa* were: 500 mg/kg for systemic exposure and 2000 mg/kg of body weight per day for acute toxicity studies. 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 requires that for aqueous solvents, volumes for administration should not exceed 20 mL/Kg (2 mL/g) of body weight [18]. Hence, calculations were done according to guidelines by Oghenesuvwe [19].

2.5 Systemic Exposure

Plant metabolites were phytochemically screened from extracts using standard metabolite testing procedures [20,21]. The same screening techniques were used to determine the presence of key plant secondary metabolites in the serum of rats after oral administration of the extract by qualitative analysis, although systemic exposure generally is a quantitative process.

A satellite study with three rats was carried out. The animals were exposed to standard laboratory conditions. The first (T1) served as the control at 0min, the second as (T2) and the third as (T3). Rats were fed 500mg/Kg of the plant extract by oral gavage, and the rats were sacrificed by jugular incision at the start, 30minutes, and 1hour after, respectively. Their blood was collected and centrifuged at 3000 rpm and the serum collected. 1mL of serum of control was spiked with a few drops of diluted plant extract and screened after one hour for the presence of secondary metabolites that had been present in phytochemical screening. Serum from T1 andT2 were screened as well.

2.6 Acute Toxicity

This study was carried out according to the *OECD Guideline for Testing of Chemicals N° 420: Acute Oral Toxicity – Fixed Dose Procedure* [18]. 10 adult male and 10 female *Wistar* rats aged 7-12 weeks were divided and housed in four groups of five rats each for five days before the experiment. All animals had free access to water and food except for a 12-hour fasting period before oral administration of the extract (food was withheld, but not water). After fasting, the animals were weighed, and the extract was administered.

Animals in group A and B received distilled water (1 mL p.o) and served as control. The plant extract was administered by gavage at single doses of 2000 mg/kg respectively to groups C and D. After administration; the animals were starved for 3 to 4 hours. The general behaviour of rats was observed in cages continuously for one hour (1hr) after treatment and then intermittently for 4 hours and the next 24 hours for any signs of toxicity. The food and water consumption, signs of toxicity, appearance and behavioural patterns were observed daily.

The rats were euthanised on the fifteenth day. The jugular vein was cut; 1.5 ml of blood was collected in EDTA vacutainers, and 2.5 mL was collected into plain vacutainers without anticoagulant. The blood was centrifuged, aliquoted and analysed. Organs were immediately isolated, washed with cold saline and fixed in a neutral solution of 10% buffered formalin for histopathological evaluation.

2.6.1 Measurement of biochemical parameters

The following hepatic and renal biochemical parameters were quantified: ASAT, ALAT, GGT, ALP, Total Bilirubin, Total Proteins, Urea and Creatinine. ALAT, ASAT and Creatinine were measured using commercial CHRONOLAB® kits.

2.6.2 Evaluation of the hepatic activity /quantification of ALAT (CHRONOLAB® KIT)

Alanine aminotransferase (ALT) catalyses 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 and NADH. The rate of decrease in NADH concentration, measured photometrically, is proportional to the catalytic concentration of ALT present in the sample.

2.6.3 Quantification of ASAT (CHRONOLAB® KIT)

Aspartate aminotransferase (AST), or glutamate oxaloacetate (GOT), catalyses an amino group's reversible transfer from aspartate to α-ketoglutarate, forming glutamate and oxaloacetate. The oxaloacetate produced is reduced to malate by malate dehydrogenase and NADH. The rate of decrease in NADH concentration, measured photometrically, is proportional to the catalytic concentration of ASAT present in the sample.

2.6.4 Quantification of renal parameters: creatinine (CHRONOLAB® KIT)

The assay is based on the reaction of creatinine with alkaline sodium picrate to form a red complex, as described by JAFFÉ. The intensity of the colour formed is proportional to the creatinine concentration in the sample.

2.7 Urea Analysis

Urea is hydrolysed enzymatically into NH_4^+ and CO2. NH_4^+ reacts with Salicylate and Hypochlorite, NaClO in the presence of Nitroprusside to form a green-yellow indophenol. The colour intensity is proportional to the concentration.

2.7.1 Preparation of liver samples for histopathological analysis

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

2.8 Statistical Analysis

Raw data on weight, alimentation and biochemical parameters were collected and entered in Microsoft Excel 365. The GraphPad Instat version 5.1 software was used to compare the groups analysed 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 as statistically significant.

3. RESULTS

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

3.1 Systemic Exposure

Qualitative systemic exposure studies following a 500 mg/Kg dose of plant extract was carried out. Serum spiked with plant extract confirmed the presence of plant metabolites. However, pre-dose serum samples at 0mins and those at 30mins and 1hr after administration were negative for the presence of the main secondary metabolites present in phytochemical screening: Polyphenols, Flavonoids and Tannins (Table 1). Only the lead acetate test for polyphenols tested positive.

3.2 Acute Toxicity- Zootechnical Parameters

3.2.1 Weight

The average weight of all groups increased gradually from the first to the fourteenth day after a single dose administration of 2000 mg/Kg. Means appear to show a greater net weight gain in controls than test groups, but this difference is not statistically significant (Fig.1).

Table 1. Results of the qualitative test for secondary metabolites in serum after administration of 500mg/Kg dose

	Test	Spiked Serum	0mins	30mins	1hr
Polyphenols	FeCl3	+	-	-	-
	Lead Acetate	+		+	+
Flavonoids	H_2SO_4	+	-	-	-
Tannins	NH_3/ $CuSO_4$	+	-	-	-

Key: + Present - Absent

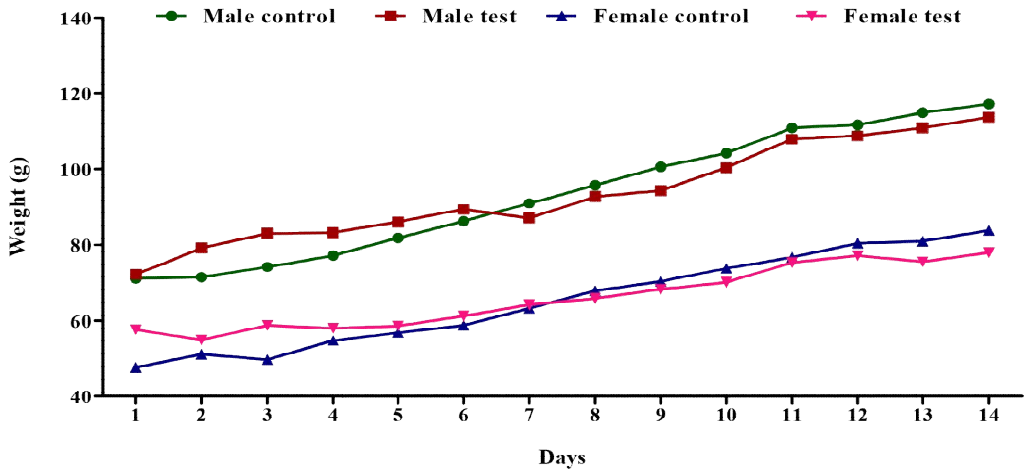

Fig. 1. Weight evolution of all groups in acute toxicity study

3.2.2 water and food intake

The weight for males and females following no treatment and a single 2000 mg/Kg oral dose of the aqueous extract showed that water and food intake values were greater for the control than the test groups and reflected weight changes (Table 2).

3.2.3 Relative mass of organs

The relative weight of organs was noted after sacrifice on the 15th day after administering a single 2000 mg/Kg oral dose of the plant extract in the test groups and no treatment in the controls. There was no significant variation in the weight of the organs between the test and control groups (Table 3).

3.3 Biochemical Parameters

3.3.1 Cytolysis parameters

There was a non-significant increase in ASAT in both test groups. The same is true of ALAT, except in the female test group with a slight decrease (Fig. 2).

Table 2. Food and water intake in acute toxicity study

Parameters	Male control	Male test	Female control	Test female
Weight gain (g)	46,20 ± 12,56	41,60 ± 11,59	36,40 ± 13,30	20,40 ± 16,02
Food intake (g)	88,31 ± 21,62	77,77 ± 16,40	76,31 ± 21,47	67,77 ± 22,76
Water intake (g)	90,31 ± 18,54	104,46 ± 13,73	77,54 ± 18,73	83,69 ± 16,49

The results are presented in terms of mean ± standard errors (n = 5)

Table 3. Effect of aqueous extract on organ weight in grams

Organs		Male control	Male test	Female control	Female test
Heart		0.39 ± 0.05	0.40 ± 0.11	0.40 ± 0.11	0.25 ± 0.17
Liver		3.67 ± 0.27	3.70 ± 1.00	4.06 ± 1.03	2.45 ± 1.65
Lungs		0.91 ± 0.24	0.85 ± 0.25	0.77 ± 0.24	0.53 ± 0.32
Brain		1.27 ± 0.22	1.40 ± 0.39	1.70 ± 0.49	1.00 ± 0.65
Rate		0.89 ± 0.22	0.88 ± 0.24	0.65 ± 0.27	0.51 ± 0.30
	Left	0.36 ± 0.04	0.37 ± 0.09	0.43 ± 0.10	0.25 ± 0.17
Kidney	Right	0.38 ± 0.04	0.39 ± 0.10	0.46 ± 0.11	0.30 ± 0.18
	Left	0.60 ± 0.15	0.48 ± 0.16	0.24 ± 0.06	0.24 ± 0.18
Testes/Ovaries	Right	0.62 ± 0.15	0.49 ± 0.16	0.25 ± 0.06	0.24 ± 0.18
	Left	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Adrenal glands	Right	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01

The values are expressed as means ± SEM, n = 5 animals in each group; the results are analysed for statistically significant difference using one-way analysis of variance (ANOVA) and Turkey's Kramer post hoc test. P-value<0,05 was considered significant

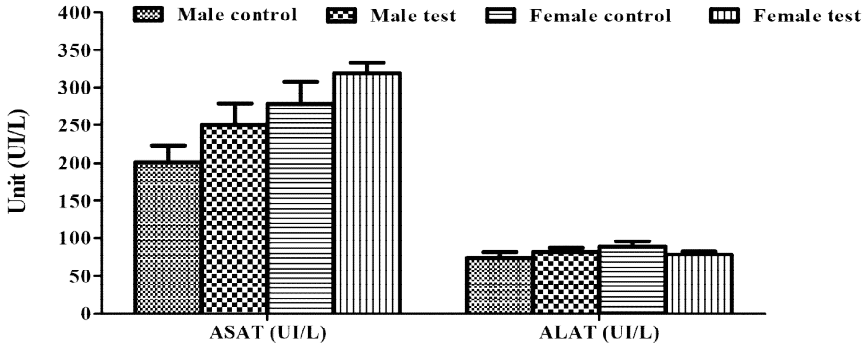

Fig. 2. Changes in the activities of serum aspartate transaminase (ASAT) and serum alanine transaminase (ALAT) in experimental groups

Data are expressed as the mean ± SEM of 5 rats in each group

3.3.2 Cholestasis parameters

Biochemical analysis of the liver results showed a non-significant decrease in ALPactivity in the test groups compared to control groups with a p-value> 0.05, indicating no hepatic cholestasis. Similarly, for GGT and total Bilirubin, there was a non-significant increase in the activity of these parameters in the test groups after administration of the plant compared with the control groups with a p-value> 0.05, as shown in Fig. 3.

Concerning renal function, there was no noticeable change in creatinine activity with a p-value> 0.05. However, in females, there was a very significant increase in Urea in the test group (1.11 ± 0.13 g/l) compared to the control group (0.59 ± 0.10 g/l) with a p-value< 0.001, indicating protein destruction in the liver, which was much more accentuated in females than in males (Table 4).

3.4 Histological Analysis of Liver and Kidney

There were no significant architectural changes in the histological analysis of the target organs; the liver and the kidneys. There was no visible effect of organ damage, with histological morphology showing no changes in control and treated organs, as indicated in Fig. 4.

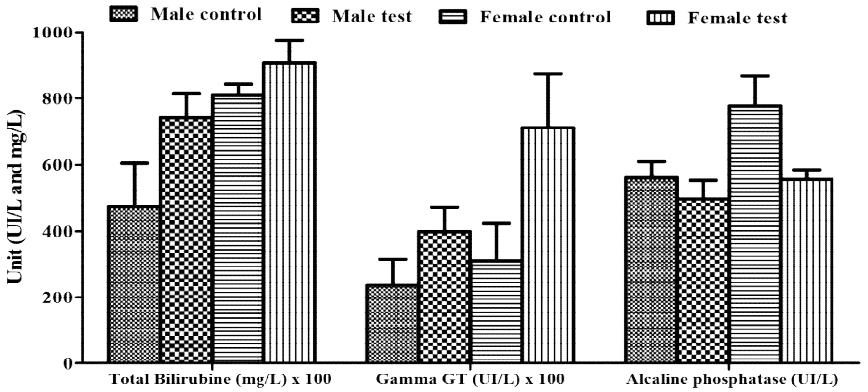

Fig. 3. Changes in the activities of serum alkaline phosphatase (ALP), Total Bilirubin and Gamma GT (GGT)

Data are expressed as the mean ± SEM of 5 rats in each group. Differ significantly at p < 0.05

Table 4. effect of aqueous extract of *C. longa* on renal biochemical parameters of study rats in acute toxicity

Parameters	Male control	Male test	Female control	Female test
Creatinine (mg/L)	4.32 ± 0.32	4.87 ± 0.68	4.84 ± 0.31	4.09 ± 0.368
Urea (g/L)	0.49 ± 0.06	0.58 ± 0.14	0.59 ± 0.10	1.11 ± 0.13***

3.4.1 Liver

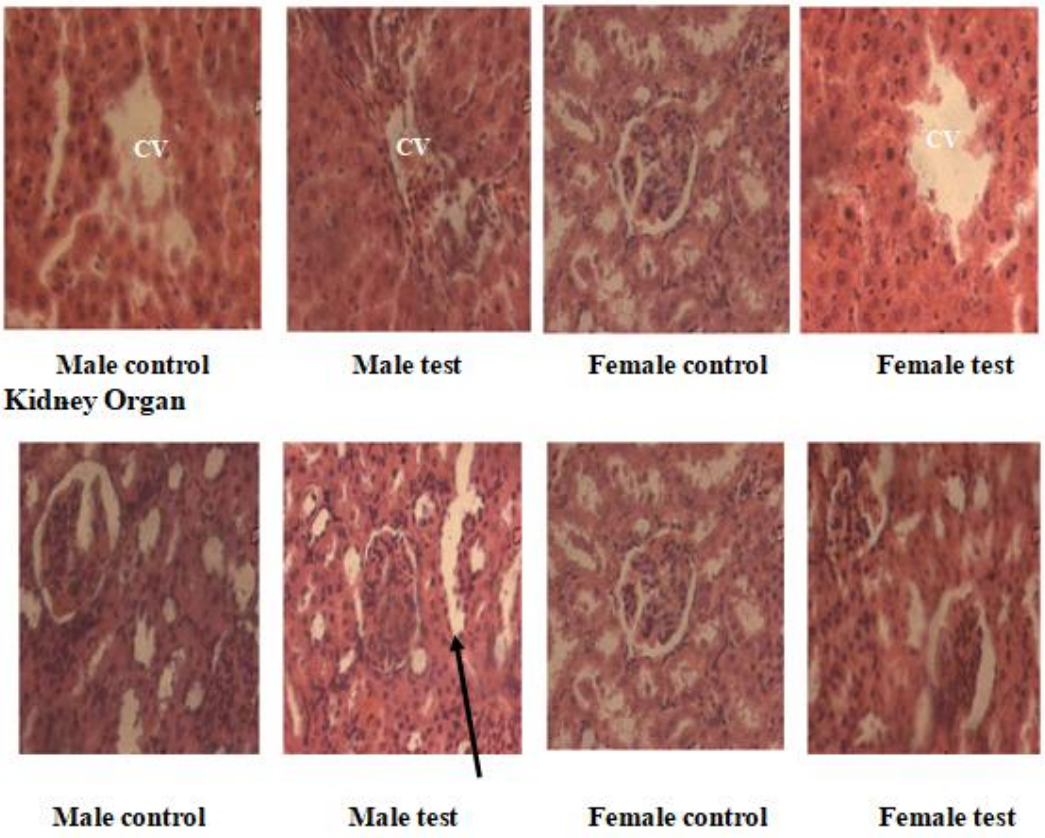

Fig. 4. Photomicrographs of the target organs (liver and kidney) in Acute Toxicity Study

Key: CV: Central vein white arrow: glomerulus Black arrow: tubule

4. DISCUSSION

Liver diseases, in general, are a serious global health problem. The unavailability of proper therapeutic drugs, poor access, and high cost of their management make the investigation of other management sources a necessity. Medicinal plants such as *Curcuma longa* are used. Hence, we decided to investigate the systemic exposure and acute toxicity of the aqueous extract of dried rhizomes of *Curcuma longa* on albino rats of the Wistar strain. To achieve this, we carried out a preclinical, experimental study that analysed biochemical and histological markers of liver function, determined systemic exposure of secondary metabolites and proved that our extract was safe by acute oral administration.

From earlier studies, phytochemical screening of the aqueous extract of dried *C. longa* rhizomes showed seven secondary metabolites, namely: Polyphenols, Flavonoids, Tannins, Phlobatanins, Mucilage, Saponins, and Quinones[17,21].

The preliminary qualitative screening for systemic exposure of secondary metabolites in serum yielded negative results due to difficulties in identifying metabolites in serum due to precipitation and coagulation. Hence, standard techniques used for plant extracts were not effective. Polyphenols regroup flavonoids and tannins, which explains why these two tests were also negative. The Lead Acetate test was inconclusive due to precipitation in all the tubes. This can be explained by the presence of serum proteins which interfered with the results. Curcumin, which is the primary polyphenol present in turmeric and generally conferred with its anti-inflammatory properties, has been long known to have low oral bioavailability (1%) even at very high oral doses (500mg to 2g) [22,23].

Studies in rats showed curcumin to have a $t_{1/2}$ of 28mins and very low systemic concentrations in the order of micro to nanograms per mL, hardly within the range of sensitivity of chemical tests [22]. Curcumin's low bioavailability is attributed to poor absorption, extensive first-pass metabolism and elimination [13,23]. While most of these studies have not been carried out on the whole rhizome, results may not be very different, although turmeric oil increases the bioavailability of curcumin. These in no way invalidate the pharmacological properties of turmeric plant. However, more work must be done on curcumin for consideration as a lead compound in drug development by testing more bioavailable formulations [24]. Also, the hepatoprotective activity of turmeric extract may not be significantly influenced by the low bioavailability of curcumin if the liver is sufficiently exposed during the first-pass effect [13,24]. Besides the properties of the plant metabolites, more sophisticated testing methods are required or a more refined simplistic model for serum screening that considers factors such as serum proteins, ions and low limits of detection.

Acute toxicity study based on the OECD 420 Guidelines on the aqueous extract of the dried rhizomes of *Curcuma longa* at a fixed dose of 2000 mg/Kg demonstrated that the extract did not show any signs of toxicity and mortality. The fixed-dose procedure differs from the classical method of acute toxicity testing because it uses fewer animals and causes less suffering. This approach avoids using the death of animals as an endpoint and relies instead on the observation of clear signs of toxicity. This shows that the LD50 is greater than 2000 mg/Kg [18]. This is like results obtained by Mohammed *et al.* in a study that found the LD50 of the aqueous, methanolic and hexane extracts of *Curcuma longa* to be >5000 mg/Kg in mice [25]. Another study by Aggarwal *et al.* obtained no toxicity at single doses of 5000 mg/Kg of a curcumin enhanced extract after 14 days of study [26]. Several clinical studies have found single doses of up to 12000 mg/Kg tolerable and minimal adverse effects [24, 27]. Turmeric has long been generally considered as safe (GRAS) by the FDA. There was a constant weight gain across all groups during the study period, with greater weight gain in control animals. The difference in weight between the test and control groups was not statistically significant. Similar observations were made in other studies [25,26]. However, *C. longa* extracts have been shown to cause dose-dependent weight loss. Food and water values reflect the weight changes. Determination of organ function and toxicity was carried out using biochemical and histological tests. Liver function was measured using ASAT, ALAT, ALP, Bilirubin etc [28,29]. Liver analysis showed a non-significant decrease in ALP in test groups as compared to controls. However, for ASAT, GGT and total Bilirubin, there were non-significant increases in test groups. These results indicate that the liver was not affected. Increases in Bilirubin could be due to partial haemolysis of blood samples. Since ASAT is nonspecific for the liver, its slight increase could be attributed to other sources. Other hepatic parameters remained unchanged, indicating maintained liver function. Administration of large doses of extracts could have adverse effects on the kidney responsible for excretion. Kidney function was evaluated using Urea and creatinine, which showed typical values for creatinine in both test and control groups, indicating that kidney function was not affected. However, there was a significant increase in Urea (p-value < 0,001) in the female test group showing high protein breakdown rates. This is different from results obtained by Mohammed *et al.* and Salama *et al.* at similar doses [12,25]. This can be explained by the fact that their results were not sex-segregated. Biochemical tests were further confirmed by histological sections of the kidney and the liver, showing no significant structural changes [30].

5. CONCLUSION

There were no systemically available secondary metabolites after a single 500 mg/Kg dose of the aqueous extract of dried rhizomes of *Curcuma longa* by qualitative screening. This study confirmed the safety of *Curcuma longa* with no lethality after a single oral dose of 2000mg/Kg and following observation for 14 days. There was a low expression of biochemical markers of toxicity such as ALAT, ASAT, and no histological indication of liver damage.

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CONSENT

Not applicable.

ETHICAL APPROVAL

Ethical approval was sought from the institutional review board of the Faculty (N^0 008/UYI/FMSB/VDRC/CSD of 19th April 2019), and authorisation was obtained from the laboratory Head to work in the Animal House of this Faculty

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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