Sahel Journal of Life Sciences FUDMA (SAJOLS)
December 2024 Vol. 2(4): 149-155
ISSN: 3027-0456 (Print)
ISSN: 1595-5915 (Online)
DOI: https://doi.org/10.33003/sajols-2024-0204-20

Research Article

Acute Toxicity and Effects of Ethanolic Extract of Turmeric (*Curcuma longa*) on Some Serum Biochemical Parameters in Rats

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ABSTRACT

This study investigated the effects of ethanol extract of Turmeric (*Curcuma longa*) on serum biochemical parameters—total protein, albumin, cholesterol, and urea. Lorke's method was employed to determine the oral and intraperitoneal (I.P.) LD_{50} values using 24 rats. For oral LD_{50} determination, rats were divided into groups A–C (3 rats each) and D–F (1 rat each). Phase I involved administering 10, 100, and 1000 mg/kg body weight orally and intraperitoneally to groups A–C. In Phase II, groups D–F received 1600, 2900, and 5000 mg/kg orally, and 600, 1000, and 1600 mg/kg intraperitoneally. For biochemical analysis, 15 rats were randomly assigned to groups A, B, and C (5 rats each). Groups A and B received 400 mg/kg and 600 mg/kg of the ethanolic extract orally for 21 days, while Group C received distilled water as control. The LD_{50} was calculated to be 3807 mg/kg for the oral route and 774 mg/kg for the I.P. route. Oral administration of the extract significantly ($P<0.05$) reduced serum total protein and albumin levels after 21 days. Conversely, serum urea levels increased significantly ($P<0.05$) at both dosages, while cholesterol levels remained largely unchanged. These findings suggest that prolonged oral exposure to ethanolic extract of Turmeric affects serum protein and nitrogen metabolism, indicating potential impacts on liver and kidney function. The study underscores the importance of dosage considerations in the therapeutic use of Turmeric extracts.

Keywords: Biochemical parameters; Ethanolic extract; LD_{50}; Serum protein; Turmeric

Citation: Wakil, A.M., Stephen, J., Mohammed, A., Ekwem, P.C., Ngulde, S.I., Sandabe, U.K., Mbaya, A.W., Ojo, N.A. & Sodipo, O.A. (2024). Acute Toxicity and Effects of Ethanolic Extract of Turmeric (*Curcuma longa*) on Some Serum Biochemical Parameters in Rats. *Sahel Journal of Life Sciences FUDMA*, 2(4): 149-155. DOI: https://doi.org/10.33003/sajols-2024-0204-20

INTRODUCTION

Medicinal plants are natural sources of compounds that can be used for the treatment of many diseases today (Kubmarawa *et al*., 2007; Souad *et al.,* 2011). They play important role in individual and community's health which depends on some chemical compounds present in them that produces definite physiological and pharmacological actions in the human body (Zaku *et al.,* 2009). *Curcuma longa* is called Turmeric in English, Gangamau in Hausa, Ehiri in Igbo, Atalepupo in Yoruba and Gigir in Tiv. Curcuma longa (Linn) commonly known as turmeric; is a rhizomatous and erect perennial herb

with lanceolate (sword shaped) leaves and yellow flower (Blumenthal *et al.,* 2000; Ravindran *et al.,* 2007) and pulpy, orange, tuberous roots that grows to about 2 to 5 feet in length. It belongs to the ginger family *Zingiberaceae*, it is mostly cultivated in countries with tropical climate in some parts of Asia and Africa (Gopinathan *et al.* 2011; Abdelwahab and El-Bahr 2012; Sawant and Godghate, 2013; Olatunde *et al*. 2014). The plant is native to southeastern Asia (Dermardeosion 2001; Ravindra *et al.,* 2007). Many species are important ornamental genera and this includes the shell ginger (Alpinia), Siam or summer tulip (*Curcuma alisematifiolia*), Globba, ginger lily, Renealmia and ginger (zingiber). Some of the species of the family are; *Curcuma gracillima*, *Curcuma inodora, Curcuma petiola, Curcuma roscoeona, Curcuma zanthorrhiza, Curcuma zedoaria.*

Its rhizome is pungent, bitter and widely used in indigenous medicine and as household remedies (Aggarwal *et al*. 2004 and Gopinathan *et al.* 2011), studies have shown that this partof the plant contains volatile oil and curcuminoids which qualifies its medicinal uses. Curcuminoids is a combination of curcumin and two other related demethoxy compounds; demethoxycurcumin and bisdemethoxy curcumin (Eman *et al*. 2011; Gritsanapan, 2006). Curcumin is the active ingredient, which is a yellow coloured phenolic pigment derived from the powdered rhizome of the herb. It requires a temperature between 20^{O}C and 30^{O}C, a well-drained soil, frost free climate and 1000mm to 2000mm of rain fall annually or supplementary irrigation to grow. It cannot withstand water logging and heavy shade can reduce the yield of the plant but light shade is beneficial. Turmeric is widely used in the India system of medicine (AYurveda, Siddha and Unani) as well as in Eastern Asia system. In the Ayurvedia system depending on what it is combined with, its main therapeutic uses are for treatment of disorder due to poisoning, ulcers, skin diseases, urticaria, urinary disorders, anemia and chronic rhinitis and sinusitis (Ayurvedic pharmacopoeia committee 1989). It is also used for the treatment of anorexia, cough, diabetes, wounds, biliary and liver disorders and rheumatism (Ravindran *et al.*, 2007). Many ayurvedic healer integrate powder into paste or lotion for the treatment of dry and flaking skin, skin sores, inflammation of the skin, and painful arthritis (Blumenthal *et al.,* 2002; Ravindran *et al.,* 2007; Caleb *et al.,* 2002). The extract of *C. longa* has a diverse biological activity such as antioxidant, antimutagenic, antimicrobial, anticancer, radioprotective effect, antidiabetic, anticataract, wound healing, anti-ageing skin activities (Gupta *et al.* 2012; Panchatcharam *et al.,*2006) and anti-inflammatory (Ravindran *et al.* 2007; Teuscher *et al.* 2006). There is paucity of information on the use of ethanolic extract of *C. longa* and its effects on the serum biochemical parameters in rats. Therefore, this research is aimed at investigating the effect of acute toxicity of ethanolic extract of *C. longa* on some serum biochemical parameters in rats using standard methods.

MATERIALS AND METHODS

Sample Collection

Curcuma longa rhizomes were obtained from Oye market in Ekiti State, Nigeria in the month of October 2022. The sample was then submitted to Department of Biological Science University of Maiduguri and authenticated by a Botanist. A herbarium specimen was deposited in the Department of Veterinary Physiology and Biochemistry with a herbarium number VPBH/PG006A. The sample was air dried under shade for 10-14 days. It was then ground into powder using pestle and mortar, sieved to obtain fine powder and then stored in a plastic container at room temperature. The sample was submitted to the Department of Chemistry, University of Maiduguri, for quantitative phytochemical analysis.

Plant Extraction

Ethanolic extraction using reflux method was carried out. Four hundred gram (400g) of sample was transferred into two-litre round bottom flask and 95% ethanol was added until it covered the sample. The mixture was refluxed for about two hours. The solution was removed and decanted. The process was repeated twice using a new solution. After the sample has been extracted the solution was filtered and concentrated in hot air-oven at 40 – 50^{o}C after which a dark oily viscous substance was obtained and was then transferred into an air tight container.

Experimental Animals

A total of 39 adult albino rats of both sexes weighing between 70 – 280g was used for this study. They were maintained in clean plastic rat cages in the Physiology laboratory of the Department of Veterinary Physiology and Biochemistry, University of Maiduguri, Nigeria. They were fed with Pelleted commercial feeds (VITAL FEEDS, Nigeria Plc., Kaduna, Nigeria) and water was provided *ad libitum*. The animals were allowed to stay for 14 days to acclimatize to the laboratory environment before commencement of the experiment.

Acute Toxicity Study

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Acute toxicity study was carried out according to modified Lorke's method (Lorkes 1983). The study was conducted in two phases using a total of twenty-four (24) apparently healthy rats. The rats were fasted overnight prior administration of plant extract.

Phase I: Nine rats were weighed, and separated at random into three groups of three rats each (group A, B, and C). All rats in groups A, B, and C received orally 10mg/kg, 100mg/kg and 1000 mg/kg body weight of the extract respectively. The rats were observed for twenty-four hours for signs of mortality and the number of mortalities was recorded before proceeding to the next phase. The same process using the same dosage was performed for intraperitoneal route (I.P)

Phase II: In phase two of the study, three rats were grouped into Groups D, E and F, with each group containing one rat. All groups orally received 1,600mg/kg, 2,900mg/kg and 5,000mg/kg body weight respectively. The rats were observed for twenty-four hours for signs of mortality and the number of mortalities was recorded. The same process was repeated using intraperitoneal route (I.P) but the dosage was 600, 1000 and 1600 mg/kg respectively. The LD_{50} was calculated in mg/kg using the formula below;

$$\mathbf{LD_{50} = \sqrt{a \times b}}$$

Where: a = least dosage that killed the rat

b = the highest dosage that did not kill the rat

Serum Biochemical Analysis

Three groups of rats A, B, and C, containing five rats each, with a body weight ranging from 0.07kg – 0.28kg was used. Turmeric ethanolic extract was administered orally to groups B and C in dosages of 400 mg/kg and 600mg/kg respectively while group C is the control and received distilled water. The rats were sacrificed weekly and serum was collected and sent to the University of Maiduguri Teaching Hospital for biochemical analysis.

Determination of Serum Total Protein

The total protein in the serum sample was estimated using direct Biuret method (Peters *et al*., 1982). The peptide bonds of protein react with copper II ions in alkaline solution to form a coloured product whose absorbance can be measured spectrophotometrically at 540nm. Three test tubes labelled blank, standard and test were used. Five (5) millilitres of 3% NaOH solution was placed in the blank tube and 4.9ml of the same solution was added to each of the tubes labelled standard and test. Thereafter 0.10ml of protein standard was added into a standard tube while 0.1ml of serum sample was added into the tube labelled test. One millilitre of Biuret solution (5% $CuSO_4$) was added into each of the tubes. After 15 minutes, the absorbance of the standard and test were read against blank at 540nm using a spectrometer (Boeringer, 4010, West Germany). The protein concentration was calculated using the formula:

$$\text{Protein Concentration} = \frac{OD \times SA \times S\,T}{OD \times ST} = g/dl$$

Where: SA = Sample; ST = Standard; OD = Optical density

Determination of serum albumin: Serum Albumin level was determined by the dye Bromocresol green binding method introduced by Bartholomew and Delaney (1966) and modified by Doumas *et al*., (1971) and Spencer and Price (1977). In this method, Bromocresol green, an anionic dye, binds tightly to albumin when added to serum and the complex absorbs light much more intensely at pH 4.2 and 628nm, than does unbound to the dye. Colour formed was measured spectrophotometrically at 628 nm and the increase in light absorption is directly proportional to the albumin concentration (Doumas *et al*., 1971). Bromocresol green is the dye of choice because it binds so tightly to albumin and is not displaced by bilirubin and does not bind significantly to other serum proteins. In this experiment, 0.5ml of Bromocresol green (BCG) was pipetted into different tubes labelled unknown, standard and reagent blank. About 25µl (microliter) of the serum or working standard was added to the appropriate tubes and 25µl of water to the reagent blank tube. The contents of tubes were mixed and allowed to stand for 10 minutes at room temperature. The absorbance was read at 628nm against the reagent blank. The concentration of serum albumin was calculated using the formula:

$$\text{Serum albumin} = \frac{Au \times Cs}{As} = g/dl$$

Where Au = Absorbance of unknown, As = Absorbance of standard and

Cs = Concentration of standard

Determination of Serum Cholesterol

The cholesterol esters in serum are hydrolysed quantitatively into free cholesterol and fatty acid by cholesterolesterase. In the presence of oxygen, free cholesterol is then oxidized to choles – 4 – ene – 3 – one and hydrogen peroxide. The hydrogen peroxide reacts with phenol and 4- aminophenazone (POD) to form an O-quinoneimine dye. The intensity of the colour formed is proportional to the cholesterol concentration and can be measured photometrically between 480 – 520nm wavelengths.

Cholesterol was assayed by Trindar's reaction (Evan, 2014). Commercial Kits (Quinicaclinica Applicada, SA) were used for the assay. The mixtures were prepared using 3 test tubes, where 0.02ml serum was added to the first tube (test), 0.02ml Standard was added to the second tube (standard), and nothing was added to the third tube (blank). Then 2 mL of the reagent was added to each of the 3 test tubes.

Table 1. Determination of Serum Cholesterol concentration

	Test(ml)	Standard(ml)	Blank(ml)
Serum	0.02	-	-
Standard	-	0.02	-
Reagent	2.0	2.0	2.0

The contents of the tubes were thoroughly mixed and allowed to stand for 5 minutes at room temperature after which the absorbance was read at the wavelength of 510nm using a spectrometer. The cholesterol concentration was calculated using the formula:

Cholesterol concentration (mmol/L) = $\frac{\text{SA x OD}}{\text{ST x OD}} \boldsymbol{x\ 200}$

Where:

SA = sample, ST = standard; OD = Optical Density

Determination of Blood Urea

The diacetylmonoxime method of Natelson *et al*. (1951) as described by Randox Laboratories Ltd, United Kingdom, was used. When urea is heated in strongly acidic conditions with a substance such as diacetyl, containing two adjacent carbonyl groups or its monoxime, a yellow condensation compound is formed. The reaction is intensified by the presence of polyvalent ions such as ferric ions, then a red coloured complex is formed, which is linear with concentration than the yellow one. The intensity of the red complex, which is proportional to the concentration of urea in the sample, is measured calorimetrically.

Three test tubes, A, B, and C were used. Into tube A (test) was pipetted 10ml of distilled water and 0.1ml of serum. Into tube B (standard) was pipetted 10ml of distilled water and 0.1ml of 10mmol/L of standard reagent. The contents of the tubes were mixed thoroughly. Thereafter, 1ml each of distilled water was pipetted into test tubes A and B and 2ml into test tube C (blank). Two (2) ml each of working colour reagent and acid reagent were finally pipetted into the three tubes, mixed thoroughly and the tubes placed in an incubator at 100^0C for 20 minutes, cooled and optical density (OD) read at 520nm. The concentration of serum urea was calculated using the following formula:

Urea (mmol/L) = $\frac{\text{T - B}}{\text{S-B}} \boldsymbol{x\ 10}$

Where:

T = O.D of test sample S = O.D. of standard B = O.D. of blank

Data Analysis

Data obtained was presented as mean ± SD and analysed using Computer statistical software Graphpad Instat (2000), employing Analysis of Variance (ANOVA) and P value greater than or equal 0.05 was considered not significant.

RESULTS

Acute Toxicity

The administration of the extract at the dose of 2900mg/kg orally and 600mg/kg intraperitoneally did not produce death. Base on the results, the oral LD_{50} was calculated to be 3807mg/kg (Table 2) and intraperitoneal LD_{50} was 774mg/kg body weight (Table 3).

Effect of ethanolic extract of *Curcuma longa* rhizome on Serum albumin, protein, cholesterol and urea.

The effect of ethanolic extract of *Curcuma longa* rhizome on serum total protein, albumin, cholesterol and urea are presented in Table 3. Daily treatment with 400 mg/kg and 600 mg/kg body weight of the ethanolic extract of *C. longa* produced significant (P=0.010) decrease in the level of serum total protein from day 7, 14 and 21 as compared with the control. However, there was a slight decline in albumin level from day 7, 14 and 21 which was not significant (P=0.045) as compared with the control which remained constant throughout the experiment. There was no significant (P>0.05) increase in cholesterol levels as compared with the control. The administration of the extract also produced a significant (P<0.05) increase in urea levels from day 7, 14 and 21 as compared with the control. This shows that the extract has a prolonged effect on total protein and urea levels at both dosages (400 mg/kg and 600 mg/kg body weight) while prolonged effect on albumin was observed at 600 mg/kg body weight after three weeks of treatment.

Table 2 Oral LD_{50} ethanolic extract of *Curcuma longa* rhizomes in rats

Phase	Dosage	Death
1	10mg/kg	0/3
1	100mg/kg	0/3
1	1000mg/kg	0/3
2	1600mg/kg	0/1
2	2900mg/kg	0/1
2	5000mg/kg	1/1

Using the formula below

$LD_{50} = \sqrt{a \times b}$

Where a = least dosage that killed the rat

b = the highest dosage that did not kill the rat

$LD_{50} =$ $\sqrt{2900 \times 5000}$ = 3807mg/kg body weight

Table 3. Intraperitoneal LD_{50} ethanolic extract of *Curcuma longa* rhizomes in rats

Phase	Dosage	Death
1	10mg/kg	0/3
1	100mg/kg	0/3
1	1000mg/kg	1/3
2	600mg/kg	0/1
2	1000mg/kg	1/1
2	1600mg/kg	1/1

Using the formula below

$LD_{50} = \sqrt{a \times b}$

Where:

a = the least dosage that kill the rat

b = the highest dosage that did not kill the rat

= $\sqrt{1000 \times 600}$ = 774mg/kg body weight

Table 3: Effect of oral administration of ethanolic extract of *Curcuma longa* rhizomes on the mean of some serum biochemical levels in normal rats

Parameters	Groups (n=3)	Treatment days		Withdrawal period
		Day 7	Day 14	Day 21
		Concentration mean (Mean S.D) g/dl		
Total Protein	400 mg/kg	97.0±3.81[a]	70.40±6.01[b]	80.6±3.98[a]
	600 mg/kg	91.4±2.30[a]	67.80±3.89[a]	86.6±1.44[a]
	Control	70.4±1.14[a]	78.0±1.58[a]	91.6±1.14[b]
Albumin	400 mg/kg	36.0±3.20[b]	26.0±4.0[a]	24.2±11.6[a]
	600 mg/kg	31.0±4.74[b]	25.8±4.03[a]	15.4±5.32[a]
	Control	42.0±1.58[a]	42.0±1.58[a]	45.6±2.30[a]
Cholesterol	400 mg/kg	1.40±0.29[a]	1.36±0.31[a]	1.12±0.24[a]
	600 mg/kg	1.44±0.29[a]	1.38±0.29[a]	1.14±0.31[a]
	Control	1.60±0.16[a]	1.50±0.16[a]	1.36±0.11[a]
Urea	400 mg/kg	3.84±0.49[a]	5.02±0.19[a]	7.32±0.35[b]
	600 mg/kg	4.64±0.24[a]	5.24±0.59[a]	7.04±0.21[b]
	Control	6.10±0.16[b]	3.76±0.21[a]	3.72±0.30[a]

Column with same superscript not significant P>0.05; Column with different superscript significant P<0.05

DISCUSSION

The present study demonstrates that oral administration of ethanolic extract of *Curcuma longa* (C. longa) at doses of 400 mg/kg and 600 mg/kg body weight resulted in a significant decrease in serum total protein levels in normal rats. This outcome contrasts with findings by Olatunde *u* (2014), who reported a significant increase in albumin and total protein levels following the administration of an aqueous extract of *C. longa*. This discrepancy may be

attributed to differences in the extraction solvent used—ethanol versus water—as well as variations in the phytochemical profiles of the extracts, which could influence protein metabolism differently.

Furthermore, the study revealed that treatment with the ethanolic extract of *C. longa* at both doses significantly elevated serum urea levels. This observation aligns with the report of El-Ansary *et al.* (2007), who found that *C. longa* extract increased serum urea concentrations in *Schistosoma*-infected mice, with effects even surpassing those of praziquantel. Similarly, Oluwole (2001) reported a sustained increase in urea levels following the administration of garlic extract in rats. These findings collectively suggest that certain phytoconstituents in *C. longa* and related plant extracts may influence nitrogen metabolism, possibly through enhanced protein catabolism or impaired renal clearance of urea.

However, this result contradicts the findings of Amouoghli *et al.* (2011), who observed a reduction in serum urea, uric acid, and creatinine levels in diabetic rats treated with turmeric powder. This disparity may be explained by the physiological differences between diabetic and non-diabetic models, the form of turmeric used (powder vs. ethanolic extract), or the dosing regimen. Since urea is primarily synthesized in the liver from amino acids and excreted by the kidneys (Kaplan *et al.*, 1998; Murray *et al.*, 2006), an increase in serum urea could indicate an imbalance between production and excretion, possibly due to the extract's impact on protein metabolism or renal function.

In terms of lipid metabolism, the extract did not cause a significant reduction in serum total cholesterol levels across the treatment period. This result partially supports the work of Olatunde *et al.* (2014), who observed lipid-lowering effects following administration of an aqueous extract of *C. longa* rhizome in both normal and diabetic rats. The lack of hypocholesterolemic effect in the current study may again reflect differences in extract composition due to solvent choice or treatment duration. Despite the absence of a significant change, the potential of *C. longa* as a lipid-lowering agent remains promising given its previously reported effects on triglycerides and LDL levels.

The observed biochemical changes also have broader implications for understanding the therapeutic potentials and limitations of *C. longa*. While the increase in serum urea may raise concerns about renal or hepatic stress at higher doses of ethanolic extract, the unchanged cholesterol levels suggest a limited effect on lipid metabolism under normal physiological conditions. Given the known link between hypercholesterolemia, diabetes mellitus, and cardiovascular disease (Steiner, 1999; Cotran *et al.*, 1999; Iweala and Okeke, 2005), further studies are warranted to explore the extract's efficacy in disease models. Reducing serum cholesterol is a proven strategy for slowing the progression of atherosclerosis and decreasing cardiovascular risk (Evan, 2014), and thus, optimizing the extract formulation or dosage could enhance its clinical relevance.

CONCLUSION

In conclusion, the findings of this study indicate that prolonged administration of ethanolic extract of *Curcuma longa* may have notable effects on certain biochemical parameters in normal rats, particularly involving serum protein and renal function markers. The study also provides insight into the safety profile of the extract, suggesting a moderate level of toxicity depending on the route of administration. These results contribute to the understanding of the physiological impacts and relative safety of *Curcuma longa* extract, supporting further research into its therapeutic potential and toxicological evaluation.

Based on the findings, it is recommended that further toxicological studies be conducted on the ethanolic extract of *Curcuma longa* to evaluate its potential effects on various organ systems, including the cardiovascular, respiratory, nervous, reproductive, and digestive systems.

AKNOWLEDGEMENTS

The authors acknowledged the technical support of Mr Bitrus Wampana, Yusuf Zangoma, Abubakar Musa, and Ibrahim Oladimeji of the Physiology Laboratory, Department of Veterinary Physiology and Biochemistry,
University of Maiduguri, Borno State, Nigeria.

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