

# PHYTOCHEMICAL COMPOSITION AND TOXICOLOGICAL PROFILING OF *CURCUMA LONGA* (TURMERIC) ROOT EXTRACT IN RATS

## Abstract

**Background:** *Curcuma longa* Linn (turmeric) is a common medicinal plant used in Africa for the treatment of diseases. In this study, its extract was evaluated to ascertain its phytochemical composition and toxicity in wistar rats.

**Methods:** The prepared rhizome ethanol extract was first subjected to preliminary phytochemical evaluation and then lethal dose (LD<sub>50</sub>) test. In the sub-acute toxicity test, 20 adult wistar rats assigned to 4 groups of 5 rats each were administered oral graded doses of the extract. Group 1 served as control while groups 2, 3 and 4 received 250, 500 and 1000 mg/kg body weight of the extract over a period of 28 days, after which blood samples were collected for haematological and serum biochemical analyses.

**Results:** Results obtained from the phytochemical analysis revealed the presence of saponins, tannins, phenolics, flavonoids, steroids, terpenoids, cardiac glycosides and alkaloids in extract, with alkaloids being most abundant (41.20±0.53 mg/100g) and terpenoids, the least (3.98±0.03 mg/100g). Lethal dose value for the extract was found to be >5000mg/kg body weight. Results of sub-acute toxicity evaluation of the extract showed no deleterious effect on liver and kidney function parameters with these parameters being not significantly different from their control values (p<0.05). Treatment with the extract also caused significant hypolipidemia (p<0.05) but increased the values of high-density lipoprotein cholesterol. The activities of antioxidant enzymes and red blood cell counts were also significantly increased following treatment with the extract, especially at higher dose levels.

**Conclusion:** Therefore, we conclude that *C. longa* extract may be safe for use in the treatment of diseases when administered via the oral route.

**Key words:** Blood, *Curcuma longa*, Phytochemical, Toxicity, Wistar rats

## 1.0 INTRODUCTION

As the search for new potent, non-toxic and affordable medicines continues, global attention is currently beaming on medicinal plants. The recent development is not unconnected with the several toxic effect associated with the use of synthetic ones [1]. The use of extracts from plants leaves, stems, roots, backs, fruits, or seeds and their combinations as alternative treatment strategies in disease control has been accepted globally [2]. While many medicinal plants have

been explored for their healing potentials, it remains established that several others are yet to be screened, or even identified[3]. This gives credence to the current global acceptance of herbal medicine and supports the need for future sponsorship of medicinal plant research. *Curcuma longa* is one the medicinal plants that are currently being investigated.

“*Curcuma longa*(turmeric) is a plant that has an established history of medicinal use, dating back to nearly 4000 ago” [4].“The rhizome is used not only as spice but also during religious ceremonies. It is also known as Indian saffron because of its brilliant yellow coloration” [5]. “The plant is a perennial, rhizomatous, herbaceous plant native to the Indian subcontinent and Southeast Asia. Its temperature requirement is between 20<sup>0</sup> and 30<sup>0</sup>C (68<sup>0</sup> and 86<sup>0</sup>F) and a considerable amount of annual rainfall to thrive. The rhizome, from which the turmeric is derived, is tuberous, with a rough and segmented skin. The rhizome matures beneath the foliage in the ground. They are yellowish brown with a dull orange interior. The main rhizome is pointed or tapered at the distal end and measures 2.5-7.0cm (1-3 inches) in length and 2.5cm (1inch) in diameter, with smaller tubers branching off. More than 100 components have been isolated from turmeric, one of which is a volatile oil, containing tumerone, and another is a coloring agent called curcuminoids. Curcuminoids consist of curcumin, demethoxycurcumin, 5-methoxycurcumin and dihydrocurcumin, which are established natural antioxidants” [6,7].”The golden yellow colour of *Curcuma longa* is due to the presence of curcumin”[8].The agent has also been used traditionally for the treatment enlarged liver and other hepatic disorders, spleen, stomach ulcer, diabetes mellitus, pains, skin diseases, rheumatism and cough [9, 10]. Other pharmacological activities attributable to turmeric are anti-inflammatory, antimutagenic, and anticancer effects [4]. “Nutrition analysis of *Curcuma longa* powder showed the presence of

carbohydrate(60-70%), water(6-13%), protein (6-8%), fat(5-10%), dietary minerals (3-7%),essential oils (2-7%)and dietary fiber (1-6%)” [11].

Despite the widespread use of *Curcuma longa*, there is paucity of more data on its toxicity profiling. Hence this study is aimed at evaluating the phytochemical composition of turmeric and its effects on the haematology and biochemical activities in the body.

## **2.0 MATERIALS AND METHOD**

### **2.1 Collection and identification of plant material**

Fresh rhizomes of *Curcuma longa* were obtained from Apumirimarket in Umuahia South Local Government Area of Abia State and were authenticated at the Department of Forestry and Environmental Management by a forest scientist (Dr. E. Udoka), Michael Okpara University of Agriculture, Umudike.

### **2.2 Preparation of extract**

Soxhlet extraction technique described by Jensen,[12] was adopted. The *Curcuma longa* rhizomes were chopped into pieces and dried under shade on a laboratory bench at 25 °C within 14 days before being pulverized into fine powder. One hundred (100) grams of the powdered sample was introduced into the extraction chamber of the soxhlet extractor and extraction was done using ethanol as solvent. Temperature was maintained at 70 °C through-out the extraction period of 48 hours. At the end of the period, the collected extract in ethanol was dried in a laboratory oven at 40 °C to obtain a yellow oily extract which weighed 9.78 g and represented 9.78% extract yield. The *Curcuma longa* extracts had characteristic aromatic smell. The *Curcuma longa* extract was preserved in the refrigerator at low temperature until use.

### 2.3 Preliminary phytochemical screening

Qualitative evaluations of the phytochemicals in ethanol extract of *Curcuma longa* rhizome was carried out in accordance with the methods of Trease and Evans, [13] and Ukpai, *et al.* [14].

Quantitative analyses of the phytochemicals were determined using already established standard methods as indicated: Saponins (Obadoni, *et al.*), [15], alkaloids and cardiac glycosides (Harborne), [16], flavonoids (Miroslay *et al.*), [17], phenols (Wolfe, *et al.*), [18], steroids (Madhu *et al.*), [19], and terpenes and tannins (Mboso, *et al.*) [20].

### 2.4 Animals

Male wistar rats weighing 110-130 g obtained from the Animal production unit of the College of Veterinary Medicine Michael Okpara University of Agriculture, Umudike. Research animals were housed under standard housing conditions of room temperature, 24 to 27<sup>0</sup> C and humidity of 61 to 66 % with a 12 hours light and dark period. “They were provided with standard feed vital feed, Nigeria and water *al libitum* but were starved for 12 hours prior to commencement of experiments. All research animals’ experiments were carried out in compliance with NIH guidelines for care and use of laboratory animals OECD”, [21].

The study was carried out in the Biochemistry Laboratory of Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Nigeria. Ethical rules were strictly followed throughout the experimental procedures. All animal experiments were carried out in accordance with the internationally accepted guidelines for laboratory animal use and care as found in the US guidelines (NIH publication #85-23, revised in 1985).

## 2.5 Acute toxicity (LD<sub>50</sub>) evaluation of the extract

Acute toxicity studies were performed using the new Lorke's method used by Orieket *al.* [22], was adopted with little modification. Two stages were involved in the experiment using this method. In the first stage, 9 Wister rats were assigned to 3 groups (A, B and C) of 3 rats each and were treated with 10, 100 and 1000 mg/kg of the extract respectively. The animals were thereafter monitored for the manifestations of toxicity signs and deaths within 24 hours. With zero mortality recorded, the study proceeded to the second phase which also involved the use of 9 rats assigned to 3 groups (A-C). Single treatment doses assigned to the groups were 1600, 2900 and 5000 mg/kg respectively. The animals were again monitored for toxicity signs and deaths within 24 hours. When no mortality was observed at the end of the period, the highest dose used (5000 mg/kg) was repeated on another set of 3 rats to serve as a confirmatory test and was observed within 24 hours and a further one week.

Acute toxicity values calculated using Lorke's formula stated as:

$$LD_{50}(\text{mg/kg body weight}) = \sqrt{A \times B}$$

A= Maximum dose that produced no mortality

B= Minimum dose that killed all animals in a group

## 2.6 Sub-acute toxicity evaluation of *Curcuma longa*

Twenty (20) adult male Wister rats (110-130g) assigned to 4 groups of 5 rats each were treated via oral route according to the order below:

**Group 1:** Normal control

**Group 2:** 250 mg/kg *Curcuma longa*

**Group 3:** 500 mg/kg *Curcuma longa*

**Group 4:** 1000 mg/kg *Curcuma longa*

Treatment lasted for 28 days before animals were sacrificed for blood collection into EDTA and plain bottles for haematological and all serum biochemical studies respectively. Body weights of the rats were measured at the beginning and end of treatment.

### **2.7 Determination of haematological, biochemical and antioxidant values**

Determinations haematological values including red blood cells count (RBCC), packed cell volume (PCV), haemoglobin (Hb), white blood cells count (WBCC), platelets count (PLTC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined at once for each blood sample in an automated haematology analyser (BC-2300, Mindray Company, China). Determination biochemical parameters including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, bilirubin, urea, creatinine, sodium, potassium, chloride and bicarbonate were determined using commercial test kits with strict adherence to procedures prescribed by the producer, Randox Laboratories, UK. Antioxidant parameters including superoxide dismutase (SOD), catalase, reduced glutathione and Malondialdehyde (MDA) were all determined in serum according to the protocols adopted by Sun, *et al.* [23] and Kanu, *et al.* [24]

### **2.8 Statistical analysis**

Statistical Package for Social Sciences (SPSS, Version 23.0, IBM SPSS Inc, Chicago, IL) was used for data analysis. Level of significance was calculated by One Way Analysis of Variance

(ANOVA). Data were analyzed using Duncan Multiple Range Test and complemented with Student's t test for post-hoc test for comparisons of the means of the various doses and fractions. P- Values less than 0.05 were considered statistically significantly different between the test and control groups as well as among test groups for measured value.

### 3.0 Results

#### 3.1 Results of Phytochemical Analysis of *Curcuma Longa* (Turmeric)

Qualitative phytochemical analysis of turmeric extract showed the presence of phytochemical agents including saponins, tannins, phenolics, flavonoids, steroids, terpenoids, cardiac glycosides and alkaloids in different quantities. Quantitative tests showed that alkaloids were the most abundant ( $41.20 \pm 0.53$  mg/100g) while terpenoids were the least ( $3.98 \pm 0.03$  mg/100g). Table 1 shows results obtained for qualitative and quantitative analyses of *Curcuma longa* extract.

**Table 1: Results of qualitative and quantitative phytochemical analysis of *Curcuma longa***

Phytochemical agent	Inference	Quantitative (mg/100 g)
Saponins	++	$9.28 \pm 0.07$
Tanins	+	$4.81 \pm 0.08$
Phenolics	++	$20.13 \pm 0.44$
Flavonoids	+++	$32.22 \pm 0.51$
Steroids	+	$6.46 \pm 0.08$
Terpenoids	+	$3.98 \pm 0.03$
Cardiac glycosides	+	$7.37 \pm 0.08$
Alkaloids	+++	$41.20 \pm 0.53$

Negative (absent)

+ Present in small concentration

++ Present in moderately high concentrations.

+++ Present in very high concentrations.

### 3.2 Results of acute toxicity evaluation of *Curcuma longa* extract

No mortality was observed at all stages of the acute toxicity tests following the administration of doses ranging from 10 – 5000 mg/kg of the extract. Animals all appeared apparently healthy. Other signs of toxicity including agitations, roughness of hairs, depression, writhing reflexes, calmness, tremor and convulsions were also not observed throughout the period of the study. Hence the acute toxicity value for the extract was established to be >5000 mg/kg body weight (Tables 2 a&b).

**Table 2a: Result of acute toxicity evaluation of *Curcuma longa***

Group	Dose (mg/kg)	No. of death	Observation
1	10	0/3	Animals were active and physically stable.
2	100	0/3	Animals were active and physically stable.
3	1000	0/3	Animals were active and physically stable.

**Table 2b: Result of acute toxicity evaluation of *Curcuma longa***

Group	Dose (mg/kg)	No. of death	Observation
1	1600	0/3	Animals were active and physically stable.
2	2900	0/3	Animals were active and physically stable.
3	5000	0/3	Animals were active and physically stable.

### 3.3 Results of sub-acute toxicity evaluation of *Curcuma longa* extract

#### 3.3.1 Effects of *C. longa* extract on haematological parameters in rats

Treatment with *C. longa* extract at higher doses (500 and 1000mg/kg) significantly increased the values of RBC, PCV, Hb, WBC but not in PLT since the values remained the same when compared with the control ( $p < 0.05$ ). At the lowest dose used, the values of the haematological parameters did not differ from control ( $p > 0.05$ ). These results are presented in table 3.

**Table 3: Showing effects of turmeric extract treatment on haematological parameters in rats**

Groups	Dose	RBC ( $\times 10^6/\text{mm}^3$ )	PCV (%)	HB (g/dl)	WBC ( $\times 10^3/\text{mm}^3$ )	PLT ( $\times 10^3/\text{mm}^3$ )
1	Control	6.54±0.28 <sup>a</sup>	42.80±1.92 <sup>a</sup>	14.38±0.34 <sup>a</sup>	8.84±0.35 <sup>a</sup>	158.80±5.44 <sup>a</sup>
2	Tumeric 250mg/kg	6.51±0.28 <sup>a</sup>	42.80±1.64 <sup>a</sup>	14.44±0.37 <sup>a</sup>	9.42±0.46 <sup>a</sup>	156.20±6.22 <sup>a</sup>
3	Tumeric, 500 mg/kg	6.96±0.17 <sup>b</sup>	45.20±0.83 <sup>b</sup>	15.50±0.27 <sup>b</sup>	9.90±0.59 <sup>a</sup>	158.80±4.38 <sup>a</sup>
4	Tumeric, 1000 mg/kg	7.34±0.36 <sup>b</sup>	45.60±1.34 <sup>b</sup>	16.58±0.34 <sup>b</sup>	10.22±0.60 <sup>b</sup>	152.40±11.97 <sup>a</sup>

Values represent the mean  $\pm$  SD for N =5. Values in the same columns bearing the same letter of the alphabet are not significantly different from each other ( $p > 0.05$ ). PCV, Packed Cell Volume; Hb, Haemoglobin; RBC, Red Blood Cells; PLT, Platelets; WBC, White Blood Cell.

### 3.3.2 Effects of *C. longa* extract on liver function and urea and creatinine concentrations

Treatment of the animals with *C. longa* extract at all dose levels did not significantly alter liver function activities in the treated rats when compared with the control group ( $p > 0.05$ ), as values obtained for total protein, AST, ALT, ALP and total bilirubin in the treated rats did not differ significantly from those obtained in the control group (Table 4). Similar results were also obtained for renal function parameters, where both serum urea and creatinine concentrations were not significantly different from control values (Table 5).

**Table 4: Effect of *Curcuma longa* extract on liver function parameters**

GROUP	DOSE	TP	AST	ALT	ALP	BILIRUBIN
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	(mg/kg)	(g/dl)	(u/l)	(u/l)	(u/l)	(mg/dl)
<b>1</b>	Control	7.03±0.31 <sup>a</sup>	27.60±2.50 <sup>a</sup>	19.00±1.00 <sup>a</sup>	81.60±5.41 <sup>a</sup>	0.63±0.07 <sup>a</sup>
<b>2</b>	<i>C. longa</i> extract 250	7.10±0.18 <sup>a</sup>	28.40±2.50 <sup>a</sup>	16.80±2.94 <sup>a</sup>	77.00±4.63 <sup>a</sup>	0.58±0.03 <sup>a</sup>
<b>3</b>	<i>C. longa</i> extract, 500	7.35±0.20 <sup>a</sup>	28.20±1.78 <sup>a</sup>	15.60±3.28 <sup>b</sup>	73.20±5.63 <sup>a</sup>	0.59±0.01 <sup>a</sup>
<b>4</b>	<i>C. longa</i> extract 1000	7.65±0.23 <sup>b</sup>	28.00±1.41 <sup>a</sup>	16.40±2.30 <sup>a</sup>	72.80±3.11 <sup>b</sup>	0.61±0.05 <sup>a</sup>

*Values represent the mean ± SD for N =5. Values in the same columns bearing the same letter of the alphabet are not significantly different from each other (p > 0.05). TP, Total protein; AST, Aspartate amino transferase; ALT, Alanine amino transferase; ALP, Alkaline phosphatase; TB, Total bilirubin.*

**Table 5: Effects of *Curcuma longa* extract on renal function parameters**

GROUPS	DOSE (mg/kg)	UREA (mg/dl)	CREATININE (mg/dl)
<b>1</b>	Control	16.56±1.07 <sup>a</sup>	0.78±0.05 <sup>a</sup>
<b>2</b>	<i>C. longa</i> extract 250	15.88±0.78 <sup>b</sup>	0.68±0.04 <sup>b</sup>
<b>3</b>	<i>C. longa</i> extract 500	16.72±1.24 <sup>a</sup>	0.64±0.02 <sup>b</sup>
<b>4</b>	<i>C. longa</i> extract 1000	16.96±0.89 <sup>a</sup>	0.63±0.04 <sup>b</sup>

*Values represent the mean ± SD for N =5. Values in the same columns bearing the same letter of the alphabet are not significantly different from each other (p > 0.05).*

### 3.3.3 Effects of *C. longa* extract on serum electrolytes concentration in male rats

The results obtained showed no difference in Na<sup>+</sup> and K<sup>+</sup> concentrations between all groups treated with the extract and control (P > 0.05). However, lower values of Cl<sup>-</sup> and HCO<sub>3</sub><sup>-</sup> were obtained in

groups treated with 500 and 1000 mg/kg of the extract when compared with control ( $P < 0.05$ ).

Table 6 shows observed changes in values of serum electrolytes across the groups.

**Table 6: Effect of *C. longa* extract on serum electrolytes concentrations**

Groups	Dose (mg/kg)	Na <sup>+</sup> (mEq/L)	Cl <sup>-</sup> (mEq/L)	K <sup>+</sup> (mEq/L)	HCO <sub>3</sub> <sup>-</sup> (mmol/L)
<b>1</b>	Control	121.12±1.09 <sup>a</sup>	88.80±2.01 <sup>a</sup>	4.61±0.18 <sup>a</sup>	19.40±0.47 <sup>a</sup>
<b>2</b>	250	121.36±1.91 <sup>a</sup>	84.46±2.92 <sup>a</sup>	4.66±0.13 <sup>a</sup>	19.46±0.33 <sup>a</sup>
<b>3</b>	500	120.20±2.87 <sup>a</sup>	82.88±4.03 <sup>b</sup>	4.55±0.17 <sup>a</sup>	18.78±0.16 <sup>b</sup>
<b>4</b>	1000	122.58±1.99 <sup>a</sup>	79.82±1.80 <sup>c</sup>	4.77±0.10 <sup>a</sup>	18.90±0.46 <sup>b</sup>

*Values represent the mean ± SD for N =5. Values in the same columns bearing the same letter of the alphabet are not significantly different from each other (p > 0.05).*

### 3.3.4 Effects of *C. longa* extract on lipid profile parameters in male rats

Treatment with extract, especially at higher dose levels significantly lowered total cholesterol and low density lipoprotein cholesterol (LDL-c) concentrations, but increased the values obtained for high density lipoprotein cholesterol (HDL-c) in the treated rats when compared with control ( $P < 0.05$ ). Serum triglycerides concentration was significantly lower than control values only in the group administered 1000 mg/kg body weight of the extract ( $p < 0.05$ ), while very low density lipoprotein cholesterol (VLDL-c) concentrations did not significantly change across the groups following treatment (Table 7).

**Table 7: Effect of *C. longa* extract on lipid profile in rats**

GROUPS	DOSE (mg/kg)	Total Cholesterol (mg/dl)	HDL (mg/dl)	TAG (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)
1	Control	114.60±3.30 <sup>a</sup>	59.50±2.30 <sup>a</sup>	99.48±4.62 <sup>a</sup>	32.21±2.74 <sup>a</sup>	19.89±2.60 <sup>a</sup>
2	250	110.50±1.23 <sup>b</sup>	62.68±2.24 <sup>b</sup>	95.92±2.53 <sup>a</sup>	28.01±2.17 <sup>b</sup>	19.81±1.92 <sup>a</sup>
3	500	107.02±2.11 <sup>c</sup>	62.64±2.26 <sup>b</sup>	95.38±2.60 <sup>a</sup>	25.30±2.62 <sup>c</sup>	19.08±2.05 <sup>a</sup>
4	1000	105.60±2.19 <sup>d</sup>	64.22±1.50 <sup>c</sup>	91.60±3.58 <sup>b</sup>	23.06±2.40 <sup>d</sup>	18.32±1.77 <sup>a</sup>

Values represent the mean ± SD for N =5. Values in the same columns bearing the same letter of the alphabet are not significantly different from each other (p>0.05).

### 3.3.5 Effect of *C. longa* extract on Serum antioxidant parameters in male rats

While glutathione peroxidase activities were not significantly altered across the groups following treatment when compared with control (p>0.05), values obtained in the treatment groups for superoxide dismutase and catalase activities were significantly higher than control values following treatment with *C. longa* extract. Lipid peroxidation activity was also significantly lower than control value in group administered 1000 mg/kg body weight of the extract (Table 8).

**Table 8: Showing effect of *Curcuma longa* treatment on serum antioxidant parameters in wistar rats**

Groups	Dose (mg/kg)	GPx (U/L)	SOD (U/L)	CAT (U/L)	MDA (mmol/L)
1	CONTROL	46.40±1.82 <sup>a</sup>	27.80±3.70 <sup>a</sup>	21.40±3.05 <sup>a</sup>	0.63±0.07 <sup>a</sup>

2	250	46.40±1.58 <sup>a</sup>	31.00±2.74 <sup>b</sup>	22.00±1.58 <sup>b</sup>	0.60±0.05 <sup>a</sup>
3	500	46.40±1.82 <sup>a</sup>	31.00±2.74 <sup>b</sup>	23.20±1.30 <sup>c</sup>	0.58±0.04 <sup>a</sup>
4	1000	46.40±3.03 <sup>a</sup>	33.60±2.30 <sup>c</sup>	24.20±1.92 <sup>d</sup>	0.53±0.04 <sup>b</sup>

*Values represent the mean ± SD for N =5. Values in the same columns bearing the same letter of the alphabet are not significantly different from each other (p > 0.05). GSH, reduced Glutathione; SOD, Superoxide Dismutase; CAT, Catalase; and MDA, Malondialdehyde*

## Discussion

*Curcuma longa* is a plant that has a very long history of medicinal use, among other uses. The medicinal properties of *C.longa* have been known for many years. Results obtained following phytochemical evaluation of extract made from *C. longa* has shown that the plant is rich in saponins, phenols, flavonoids, steroids, terpenoids, cardiac glycosides and alkaloids, which may be responsible for its healing effects. Similar findings were made by Noor [25] following phytochemical evaluation of *C.longa*. The presence of alkaloids in leaves, barks, roots and seeds of plants have been reported and also associated with pharmacological activities like pain relief, anti-cholinergic, analgesic, antiprotozoal, antiarrhythmic, antimalarial, anti-tumor, anti-hypertensive and muscle relaxant effects [26]. Flavonoids and phenols have established antioxidant activity which usually achieve via free radical scavenging effects, and by that are of value in the prevention of a host of oxidative stress diseases [27, 28, 29]. Anti-inflammatory, anti-diabetic, anti-atherosclerotic effect, gastro-protective and hepatoprotective activities and hypolipidaemic activity have been attributed to saponins [30, 31]. The role of cardiac glycosides in the prevention and management of heart diseases have also been reported [32]. Steroids, as corticosteroids which regulate metabolism, inflammation and body sodium and water

levels[33].Tannins have been employed medically to treat diarrheal and control bleeding and also to treat bacterial and parasitic diseases [34, 35, 36].

Result of acute toxicity evaluation of *C. longa* (Turmeric) extract showed it was well tolerated, as such, was safe for consumption. No mortality was observed at all stages of the acute toxicity tests following the administration of doses ranging from 10-5000mg/kg of the root extract. As observed in the animals, they were healthy, did not show any sign of toxicity and moved about freely. Similar results were obtained by Ijioma et al., [37] following acute toxicity evaluation of plant extract.

Results of sub-acute toxicity evaluation of *C. longa* (turmeric) shows no negative effects on body. Haematological parameters were not negatively affected. A slight increase was observed in groups 3 and 4 for RBC and PCV and WBC for group 4 only when compared with the control group. The increase in white blood cell count observed in this work suggests that *C. longa* extract may have an immune-stimulatory effect. An immune-stimulatory extract would lead to an increase in white blood cells [38].This result is caused by the increase in the level of splenic proteins which may be due to an increase in immunoglobulin synthesis capable of defending the body against any, occurring foreign in vendor. Asoka, *et al.* [39]has shown previously that administration of immunostimulatory extracts in generally accompanied by an increase in the level of splenic proteins [40].

The increase in the rate of red blood cells observed shows that the plant extract does not affect erythropoiesis. This could mean also good functioning of kidney, since a slightest damage would have influenced the synthesis of the erythropoietin hormone that stimulates the bone marrow to

make red blood cells [41]. Again, this result could reveal that the extract could possibly induce hemoprotective effects since the extract could not induce haemolysis.

This is because according to Zohra and Fawzia, [42] a plant that does not cause haemolysis, protects the membranes of red blood cells against active agents that could get into the blood and therefore keep the red blood cell count constant.

In the case of liver function parameters an increase in the AST, ALT and ALP activities could indicate hepatic injury and when this hepatic damage increases the activities of this enzyme also increases [43]. However, the decrease in serum transminases and alkaline phosphate enzymes levels seen in this study when results are compared to the control could be an indication of hepatoprotective effect of the *C. longa* root extract in rats. These results are in agreement with those of Joshi, *et al.*, [44] who had shown that curcumin extracted from *C. longa* had a hepatoprotective effect. Curcumin is the major compound of this plant and could enhance the selective permeability of cell membranes and this will in turn result to lower serum AST, ALT and ALP [44]. In testing for total protein, low levels of total protein indicate a liver or kidney protein or it may be that the protein isn't being digested or absorbed properly. While a high total protein level could indicate dehydration or certain type of cancer, such as multiple myeloma, that causes protein to accumulate abnormally. Results of the total protein test in this study confirm the hepatoprotective effect of the rhizome extract of *C. longa* that limit the degradation of hepatocytes by adjusting the amount of serum ALT, AST and ALP that must be released into the blood, because excess increase or decrease in serum proteins could reflect liver cell damage [45]. The hepatoprotective functions of *C. longa* were further supported by the result obtained the serum bilirubin, which indicates a decrease in the serum bilirubin content when compared to control.

The kidney is the major system for excretion of waste metabolic products. Due to this function, the kidney may be regularly subjected to more or less high doses of toxic substances which can be harmful to it. Serum urea and creatinine levels are used to evaluate the functioning of the kidney [46]. The results from the study indicate good functioning of the kidneys since the values obtained for both urea and creatinine were not so significantly different from the control values. Malfunctioning of the kidney usually may lead to increased creatinine and urea values [47]. Hence, our finding here is that *C. longa* rhizome extract may have nephroprotective effects and agrees with the findings of Kamsuet *al.* [40] who evaluated the acute and sub-chronic toxicity of the ethanol extract of *C. longa* in wistar albino rats. This also corroborates the work of Rubina *et al.* [48] who showed that the aqueous extracts and the ethylene chloride/ methanol mixture of this *C. longa* remedied the renal damage caused by *Salmonella typhi* infection and Khorsandi and Orazizadeh, [49].

In this study, the decrease in the values of total Cholesterol, triglycerides, LDL cholesterol and the increase in the HDL cholesterol observed indicates that the root extract of *C. longa* could have a lipid lowering effect as confirmed by Kamsuet *al.* [40]. The extract has lipid lowering of serum levels of total cholesterol, triglycerides, LDL, VLDL and increase the levels of HDL [50, 51]. This also agrees with those of Sammani and Farrokhi, [51] who showed that curcumin extracted from *C. longa* had a lipid lowering effect. An increase in LDL cholesterol, triglycerides, VLDL and a decrease in HDL cholesterol increase the atherogenic index and therefore the risk of cardiovascular diseases [52, 53].

The root extract of *C. longa* could therefore prevent cardiovascular disease and in turn prevent hypertension since it lowers LDL cholesterol, VLDL cholesterol, triglycerides and increases the value of HDL cholesterol in the blood.

Electrolytes are important in many physiological processes, which include controlling of fluid levels, acid-base balance, nerve conduction, blood clotting, and muscle contraction, amongst other [54, 55]. Results obtained from its study showed an increase in serum sodium ion concentration ( $P < 0.05$ ) in rats that were given root extract of *C. longa* compared to the control. In the results also, values of chlorine ion decreased when compared with the control ( $P < 0.05$ ). Potassium ion concentrations increased slightly compared with the control ( $P < 0.05$ ). And the volume of bicarbonate ion decreased slightly. An imbalance in electrolytes level has been associated with hyperglycemia [55]. This may suggest that *C. longa* helps reverse diabetes-induced malabsorption syndrome, where potassium due to osmotic diuresis are the leading cause of hypokalemia in patients with diabetes [56]. The increase in sodium glucose co-transport system, suggesting an increased in renal glucose absorption *C. longa* root extract may have both renal and kidney protective effects. This result can be compared with that Gartuwa, *et al.* [57], who studies on the improvement properties of curcuma on serum electrolytes and lipid profiles of diabetes wister rats.

Results from serum antioxidant parameters in the Wister male rats show no significant difference for GSH which compared to the control. The same for MDA, except from group 4 administered with 1000mg/kg of the *C. longa* root extract. A significant increase was observed in SOD and CAT compared to the control. The presence of the endogenous antioxidant enzymatic defense is highly important for the amelioration of the free radical mediated tissue injuries [58]. MDA is one of the cellular redox states [59]. SOD and GSH are the primary free radical scavenging enzymes involved in the first-line cellular defense against oxidative injury before they form more reactive hydroxyl radicals. This study had showed that *C. longa* is a good anti-oxidant.

This was also observed also by Hossen, *et al.* [60]who studied the protective mechanism of turmeric (*C. longa*) on carbofuran-induced hematological and hepatic toxicities in a rat model.

### **Conclusion:**

The extract from *Curcuma longa* poses no deleterious threat, which is observed in the result gotten from both the oral administration of acute and sub-acute test. Again the extract is found to be rich in different phytochemical at various quantities and these are of medical importance. Therefore, the extract is safe for consumption and the use for treatment of different diseases at various doses used in this work.

### **Ethical Approval**

Animal Ethic committee approval has been collected and preserved by the author(s)

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