British Journal of Pharmaceutical Research
14(2): 1-8, 2016; Article no.BJPR.27237
ISSN: 2231-2919, NLM ID: 101631759

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Acute and Subchronic Toxicity Studies of Aqueous, Methanolic and n-Hexane Root Extracts of *Curcuma longa L.* on Albino Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AM, AMW, AJA and IUM designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors AI and YA managed the literature searches and analyses of the study. Authors AM and IUM managed the experimental process. Author AM identified the species of plant. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2016/27237
Editor(s):
(1) Vasudevan Mani, Universiti Teknologi MARA (UiTM), Selangor, Malaysia.
Reviewers:
(1) B. Ravishankar, SDM Centre for Research in Ayurveda and Allied Sciences, Udupi, India.
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Complete Peer review History: http://www.sciencedomain.org/review-history/17345

Original Research Article

Received 25th May 2016
Accepted 26th November 2016
Published 24th December 2016

ABSTRACT

Introduction: The rapid increase in the use and consumption of herbal remedies worldwide has been stimulated by several factors, including the notion that most herbal products are safe and effective.
Aim: This work was designed to evaluate the acute and subchronic toxicity of oral administration of

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the aqueous, methanolic and n-Hexane root extracts in rats.
Methodology: The LD_{50} was conducted in two phases. In the first phase, oral doses of 10, 100 and 1000 mg/kg body weight of the extracts were administered and rats observed daily for two weeks. In the second phase, 1600, 2900 and 5000 mg/kg body weight of the extracts were administered respectively. Signs accompanying toxicity and possible death of animals were monitored for two weeks to ascertain the median lethal dose LD_{50} as well as effect on body weight. Subchronic toxicity were also determined using Lorke's method to assess the effect of the aqueous, methanolic and n-hexane extracts on kidney and liver parameters after 28 days of oral administration of 10, 100 and 1000 mg/kg body weight.
Results: The LD_{50} was found to be > 5000 mg/kg body weight, no casualty recorded in two weeks. There was no significant weight decrease (P>0.05) among dose groups up to 1000 mg/kg body weight. In subchronic toxicity study there was significant decreases (P>0.05) in AST, ALT and albumin levels while ALP and total protein showed significant increases (P>0.05). Also, there was significant (P>0.05) decreases in Urea, Creatinine, K^+ and HCO_3^- in treated groups III and IV while Na^+ and Cl^- shows no significant difference in all the treated groups.
Conclusion: The safety usage of extracts from *curcuma longa L.* at a dose of less than or equal to 5000 mg/kg body weight is considered to be safe.

Keywords: Acute toxicity; albino rats; Curcuma longa L.; subchronic toxicity.

1. INTRODUCTION

Lethal toxicity (acute toxicity) is the ability of chemical to cause ill effect "relatively soon" after one oral administration or a 4- hour exposure of chemical in air [1]. "Relative soon" is usually defined as a period of minutes, hours (24), or days (up to about 2 weeks) but rarely longer [1]. LD stands for "Lethal Dose" LD_{50} is the amount of materials given all at once, which causes the death of 50% of a group of test animals. The LD_{50} is one way to measure the short- term poisoning potential (acute toxicity) [2]. Toxicologist can use many kinds of animals but most often testing is done with rats and mice. It is usually expressed as the amount of chemical administered (e.g milligrams) per 100grams (for smaller animals) or per kilogram (for bigger subjects) of the body weight of the LD_{50} can be found for any route of entry or administration, but dermal and oral administration methods are the most common. The LD_{50} value obtained at the end of the experiment is identified as LD_{50} (oral), LD_{50} (skin) e.t.c as appropriate. The most frequently performed lethality study is the oral $LD_{50.}$ The results of oral studies are important for drugs, food and accidental domestic poisonings. In general, the smaller the LD_{50} value the more toxic the chemical. Also, the larger the LD_{50} value the lower the toxicity [2]. LD_{50} value can be compared to other values using a toxicity scale. Confusion sometimes occurs because several different toxicity scales are in use. The two most common scales used are the "Hodge and sterner scale" and the "Gosselin, smith and Hodge scale [3]." These tables/scales differ in both the numerical rating given each class and the terms used to describe each class. It is important to know that the actual LD_{50} value may be different for a given chemical depending on the route of exposure (oral, dermal, inhalation) [3].

The plant *Curcuma longa L.* also known as *Gangamau* in Hausa language is a rhizomatous herbaceous perennial plant of the ginger family (*Zingiberaceae*), *Zingiberaceae* grows 5 - 6 feet high in the tropical regions, with trumpet-shaped, dull yellow flowers. Its roots are bulbs that also produce rhizomes, which then produce stems and roots for new plants [4]. Studies suggest Turmeric originated in Southern India which continues to be the world's largest producer. It was used in treating indigestion, cough, arthritis, diabetes and purifying blood. Similarly, Chinese medicine uses Turmeric for the treatment of epigastric and abdominal pain, various menstrual irregularities, swellings and trauma [5].

A major and overriding criterion in the selection of herbal medicines for use in health services is safety. Plants extracts should not only be efficacious but safe for consumption. Therefore, this research work is aimed at finding out the acute and subchronic toxicity of the aqueous, methanol and n-hexane root extracts of *Curcuma longa* L.

2. MATERIALS AND METHODS

2.1 Sample Collection

The plant *Curcuma longa L.* was collected from Toro Local Government Area of Bauchi State,

Nigeria. The plant was identified and authenticated by a taxonomist from the Department of Plant biology, Faculty of Sciences, Bayero University Kano and was given a voucher number of (BUK/HAN/0188). The plant was air dried under a shade at room temperature.

2.2 Aqueous Extract Preparation

The dried plant was manually grounded using Mortar and pestle and the powered plant kept in air tide container until used. Exactly 500 g of the dried sample was soaked in 1 litre of distilled water in a conical flask. The suspension was shaken vigorously and left to stand at room temperature for 24 hours with intermittent vigorous shaking. The extract was there after filtered with muslin cloth and then was filtered by passing through Whattman's Filter No. 1. The filtrate thus obtained was concentrated by complete evaporation of solvent using rotary evaporator to yield the crude aqueous extract.

2.3 Methanolic and n-Hexane Extracts Preparation

Five hundred gram of the powder was weighed and soaked in two litres of 1:1 methanol and n-hexane, the solution was shaken vigorously and left to stand at room temperature for 24 hours. The suspension was transferred into a separatory funnel for separation and the extracts were concentrated and evaporated using rotary evaporator and dried under water bath to yield methanolic and n-hexane extracts.

2.4 Determination of Acute Toxicity (LD_{50})

The method of Lorke [6] was used in LD_{50} determination. Three groups of three rats each were orally administered aqueous, methanol and n-hexane root extracts of *curcuma longa L.* at doses of 10, 100 and 1000 mg per kg body weight. The animals were observed for signs of toxicity and possible deaths within 24 hours, 72 hours, and two weeks. In addition, a fourth group of three rats was set up as control and animals in this group were not given the extract. In the second phase which was deduced from the first phase, another three groups of three rats each were administered respective doses of 1500, 2900 and 5000 mg per kg body weight of the extract. The rats were equally observed for toxicity signs and possible deaths within 24 hours, 72 hours, and two weeks. Again, a fourth group of three rats was set up as control and animals in this group were not given the extract. Possible number of deaths was recorded and LD_{50} value was determined from Lorke's formula as follows:

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

Where a is the highest dose at which no death occurred and b is the least dosage at which death occurred. All animals were observed frequently on the day of treatment and surviving animals were monitored daily for 2 weeks for signs of acute toxicity. Recovery and weight gain were seen as indications of having survived the acute toxicity.

2.5 Determination of Subchronic Toxicity

Twenty rats were selected by stratified randomization and then divided into four groups of five each. Group I served as control, while the remaining groups II, III and IV were given 10, 100, and 1000 mg/kg of aqueous root extract orally for 28 days and was repeated for methanol and n-hexane root extracts. During the four-week dosing period, all the animals were observed on daily basis for likely clinical signs, behavioral and mortality patterns. All the rats were sacrificed after 28 days of administration, blood samples was collected and centrifuged for analysis of some liver functions indices Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline phosphatase (ALP), Direct Bilirubin, Total Bilirubin, Albumin, Total protein and some kidney functions indices Urea, Creatinine, Na^+, Cl^-, K^+, HCO_3^-.

2.6 Statistical Analysis

The statistical analyses were carried out using statistical package for social sciences (SPSS-computer package version 16.020). Body weights were expressed as mean ± SD. Values in all groups were compared using the analysis of variance (ANOVA). For all analyses the level of statistical significance was fixed at $P>0.05$ [7].

3. RESULTS

The acute lethal study of aqueous, methanol and n-hexane root extracts of *Curcuma longa Linn* on albino rats (Table 1) shows that no animal died within 24 hours after treatment with the extracts and the LD_{50} was greater than 5000 mg/kg body weight. Again, no death was recorded among all the dose groups throughout the two weeks

experimental period. It was observed that the LD_{50}> 5000 mg/kg body weight. Furthermore, a dose-dependent weight loss occurred in the second phase of the experiment as shown in (Table 2).

3.1 Hepatotoxicity

Effect of oral administration of aqueous, methanol, and n-hexane root extracts of *Curcuma longa L.* on liver enzymes Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Alkaline phosphatase (ALP), Albumin, Direct Bilirubin, Total bilirubin and Total protein in rats at doses of 10, 100 and 1000 mg/kg after 4 weeks is shown in Tables 3, 4 and 5. There was a significant decrease in the serum AST, ALT and Albumin level ($p<0.05$) in group III and IV when compared to the normal control group I. A significant increase ($p<0.05$) in ALP and Total protein was observed in group III and IV when compared with normal control group I, while Total and Direct bilirubin levels shows significant

Table 1. Acute toxicity effect of aqueous, methanolic and n-Hexane root extracts of *Curcuma longa L.* when administered orally to albino rats

Experiment	Dose (mg/kg b.w)	No of dead rats after 24 hrs		
		Aqueous	Methaolic	n-Hexane
Phase 1	10	0/3	0/3	0/3
	100	0/3	0/3	0/3
	1000	0/3	0/3	0/3
Control	0	0/3	0/3	0/3
Phase 2	1600	0/3	0/3	0/3
	2900	0/3	0/3	0/3
	5000	0/3	0/3	0/3
Control	0	0/3	0/3	0/3

Experiment was conducted in two phases; each dose group of phase one and two are made of 3 rats each

Table 2. Effect of oral administration of aqueous, methanolic and n-hexane root extracts of *Curcuma longa L.* on the body weights of rats during acute toxicity experiment

Experiment	Dose (mg/kg b.w)	Weight gain (g) (x±SD)		
		Aqueous	Methanol	n-Hexane
Phase-1	10	30.97 ± 0.13	28.90 ± 0.11	27.32 ± 0.17
	100	28.81 ± 0.62	27.99 ± 0.36	25.51 ± 0.44
	1000	27.07 ± 0.45	21.24 ± 0.21	23.71 ± 0.15
	0	31.92 ± 0.74	31.92 ± 0.74	31.92 ± 0.74
Phase-2	1600	18.12 ± 1.33^{a}	14.24 ± 0.88^{a}	9.92 ± 0.40^{a}
	2900	12.41 ± 0.98^{b}	7.80 ± 0.80^{b}	5.21 ± 0.10^{b}
	5000	9.30 ± 0.30^{c}	6.50 ± 0.20^{c}	3.50 ± 0.50^{c}
	0	$30.28 \pm 2.00^{a,b,c,}$	$30.28 \pm 2.00^{a,b,c,}$	$30.28 \pm 2.00^{a,b,c,}$

Test of significance was done in rows. Same superscripts indicate no significant difference (p > 0.05). Weight values in phase-2 (were n<3) were statistically significant (P> 0.05) different when compare with control

Table 3. Effect of oral administration of aqueous root extract of *Curcuma longa L.* On serum activity of AST, ALT and ALP in u/l, serum levels of albumin in g/dl, direct bilirubin and total bilirubin in mg/dl, total protein in g/dl

Liver parameters	Group I (Control)	Group II (10 mg/kg)	Group III (100 mg/kg)	Group IV (1000 mg/kg)
AST (u/l)	$32.33^{ab} \pm 2.33$	30.16 ± 1.00	$26.43^{a} \pm 0.43$	$20.34^{b} \pm 2.34$
ALT (u/l)	$29.46^{ab} \pm 0.46$	24.36 ± 0.06	$19.98^{a} \pm 1.50$	$14.68^{b} \pm 0.60$
ALP (u/l)	$54.72^{ab} \pm 1.44$	60.92 ± 1.92	$66.95^{a} \pm 0.45$	$69.27^{b} \pm 1.00$
Albumin (g/dl)	$6.42^{abc} \pm 0.42$	$5.10^{a} \pm 0.06$	$5.06^{b} \pm 0.06$	$4.84^{c} \pm 0.84$
D. Bilirubin (mg/dl)	$0.36^{abc} \pm 0.01$	$1.21^{a} \pm 0.21$	$1.32^{b} \pm 0.04$	$1.68^{c} \pm 0.08$
T. Bilirubin (mg/dl)	$1.25^{abc} \pm 0.10$	$1.31^{a} \pm 0.06$	$1.72^{b} \pm 0.02$	$2.44^{c} \pm 0.44$
T. Protein (g/dl)	$9.84^{ab} \pm 0.04$	10.37 ± 1.37	$14.15^{a} \pm 0.15$	$16.17^{b} \pm 0.12$

Values are presented as mean ± SD, n = 5. Values bearing same superscripts in the same row are significantly different (p< 0.05)

($p<0.05$) increase when compared to group I when administered with aqueous, methanol, and n-hexane root extracts respectively.

3.2 Nephrotoxicity

Effect of oral administration of aqueous, methanol, and n-hexane root extracts of *Curcuma longa L.* on serum Urea, serum Creatinine, Na^+, Cl^-, K^+, HCO_3^- in rats at doses of 10, 100 and 1000 mg/kg after 4 weeks is shown in Tables 6, 7 and 8. There was a significant decrease in the serum Urea, serum Creatinine, Sodium (Na^+), HCO_3^- and Chloride (Cl^-) levels ($p<0.05$) in group III and IV compared to the normal control group I when administered with aqueous, methanolic and n-hexane root extracts respectively while significant increase ($p<0.05$) in serum potassium (K^+) level was observed in group III and IV compared with normal control Group I when administered with aqueous and methanol root extract only.

4. DISCUSSION

The acute toxicity of Aqueous, Methanolic and n-Hexane root extracts of *Curcuma longa L.* on rats shows that no animal died within 24 hours after treatment with extracts (Table 1). The major signs of toxicity noticed within 24 hours included

Table 4. Effect of oral administration of methanolic root extract of *Curcuma longa L.* on serum activity of AST, ALT and ALP in u/l, serum levels of albumin in g/dl, direct bilirubin and total bilirubin in mg/dl, Total protein in g/dl

Liver parameters	Group I (Control)	Group II (10 mg/kg)	Group III (100 mg/kg)	Group IV (1000 mg/kg)
AST (u/l)	$32.33^{ab} \pm 2.33$	28.47 ± 3.00	$25.15^{a} \pm 3.00$	$18.77^{b} \pm 0.77$
ALT (u/l)	$29.46^{ab} \pm 0.07$	25.14 ± 0.14	$17.49^{a} \pm 0.49$	$11.34^{b} \pm 1.00$
ALP (u/l)	$56.4^{ab} 1 \pm 2.41$	62.72 ± 2.02	$67.88^{a} \pm 1.00$	$71.33^{b} \pm 2.33$
Albumin (g/dl)	$6.42^{ab} \pm 1.02$	5.97 ± 1.00	5.10 ± 0.10	4.72 ± 0.72
D. Bilirubin (mg/dl)	$0.36^{abc} \pm 0.08$	$1.34^{a} \pm 0.30$	$1.41^{b} \pm 0.41$	$1.77^{c} \pm 0.07$
T. Bilirubin (mg/dl)	$1.25^{abc} \pm 0.05$	$1.43^{a} \pm 0.43$	$1.69^{b} \pm 0.09$	$2.57^{c} \pm 0.57$
T. Protein (g/dl)	$9.84^{ab} \pm 2.00$	11.42 ± 2.42	$15.86^{a} \pm 1.00$	$18.22^{b} \pm 0.22$

Values are presented as mean ± SD, n = 5. Values bearing same superscripts in the same raw are significantly different (p< 0.05)

Table 5. Effect of oral administration of n-hexane root extract of *Curcuma longa L.* on serum activity of AST, ALT and ALP in u/l, serum levels of albumin in g/dl, direct bilirubin and total bilirubin in mg/dl, Total protein in g/dl

Liver parameters	Group I (Control)	Group II (10 mg/kg)	Group III (100 mg/kg)	Group IV (1000 mg/kg)
AST (u/l)	$32.33^{ab} \pm 2.17$	31.64 ± 1.04	$28.28^{a} \pm 0.28$	$24.65^{b} \pm 0.65$
ALT (u/l)	$29.46^{ab} \pm 4.23$	26.06 ± 0.06	$21.92^{a} \pm 1.92$	$18.11^{b} \pm 1.11$
ALP (u/l)	$56.41^{ab} \pm 3.20$	59.46 ± 4.23	$63.70^{a} \pm 3.00$	$65.14^{b} \pm 2.00$
Albumin (g/dl)	$6.42^{ab} \pm 1.22$	6.10 ± 0.10	$5.86^{a} \pm 0.86$	$5.04^{b} \pm 0.04$
D. Bilirubin (mg/dl)	$0.36^{abc} \pm 0.06$	$1.07^{a} \pm 0.02$	$1.22^{b} \pm 0.22$	$1.49^{c} \pm 0.40$
T. Bilirubin (mg/dl)	$1.25^{ab} \pm 1.00$	1.30 ± 0.68	$1.68^{a} \pm 1.00$	$2.04^{b} \pm 0.04$
T. Protein (g/dl)	$9.84^{ab} \pm 0.04$	10.10 ± 2.05	$13.17^{a} \pm 3.00$	$15.34^{b} \pm 1.00$

Values are presented as mean ± SD, n = 5. Values bearing same superscripts in the same raw are significantly different (p< 0.05)

Table 6. Effect of oral administration of aqueous root extract of *Curcuma longa L.* on serum Urea in mMol/l, serum creatinine in mMol/l, Na^+, Cl^-, K^+, HCO_3^- in Mol/l

Liver parameters	Group I (Control)	Group II (10 mg/kg)	Group III (100 mg/kg)	Group IV (1000 mg/kg)
Urea (mMol/l)	$8.48^{ab} \pm 1.48$	8.08 ± 0.08	$6.80^{a} \pm 0.80$	$6.28^{b} \pm 0.28$
Creatinine (mMol/l)	$86.60^{ab} \pm 4.26$	86.60 ± 1.00	$69.40^{a} \pm 1.80$	$67.40^{b} \pm 1.09$
Na^+ (Mol/l)	$135.20^{ab} \pm 3.22$	135.80 ± 2.44	$132.40^{a} \pm 0.40$	$128.60^{b} \pm 2.55$
Cl^- (Mol/l)	$100.90^{ab} \pm 3.33$	100.20 ± 1.98	$95.60^{a} \pm 0.90$	$87.40^{b} \pm 0.40$
K^+ (Mol/l)	$4.60^{ab} \pm 0.60$	4.70 ± 0.70	$4.80^{a} \pm 0.80$	$4.80^{b} \pm 1.05$
HCO_3^- (Mol/l)	$37.70^{abc} \pm 1.78$	$26.70^{a} \pm 2.00$	$26.80^{b} \pm 1.22$	$26.80^{c} \pm 0.80$

Values are expressed as mean ± SD for n= 5. Values bearing same superscripts in the same raw are significantly different (p< 0.05)

Table 7. Effect of oral administration of methanolic root extract of *Curcuma longa L.* On serum urea in mMol/l, serum creatinine in mMol/l, Na^+, Cl^-, K^+, HCO_3^- in Mol/l

Liver parameters	Group I (Control)	Group II (10 mg/kg)	Group III (100 mg/kg)	Group IV (1000 mg/kg)
Urea (mMol/l)	$8.48^{ab} \pm 1.6$	7.48 ± 0.48	$6.40^{a} \pm 0.40$	$6.03^{b} \pm 1.00$
Creatinine (mMol/l)	$86.60^{ab} \pm 1.00$	86.90 ± 2.56	$70.40^{a} \pm 1.22$	$72.60^{b} \pm 3.33$
Na^+ (Mol/l)	$135.20^{ab} \pm 4.32$	135.40 ± 2.69	$133.90^{a} \pm 4.01$	$130.90^{b} \pm 0.90$
Cl^- (Mol/l)	$100.90^{ab} \pm 2.99$	100.50 ± 3.41	$96.80^{a} \pm 0.80$	$89.60^{b} \pm 1.00$
K^+ (Mol/l)	$4.60^{ab} \pm 0.60$	4.60 ± 0.70	$4.70^{a} \pm 1.00$	$4.80^{b} \pm 0.60$
HCO_3^- (Mol/l)	$37.70^{abc} \pm 2.78$	$28.40^{a} \pm 1.00$	$27.80^{b} \pm 1.99$	$27.40^{c} \pm 2.60$

Values are expressed as mean ± SD for n= 5. Values bearing same superscripts in the same raw are significantly different (p< 0.05)

Table 8. Effect of oral administration of n-hexane root extract of *Curcuma longa L.* on serum urea in mMol/l, serum creatinine in mMol/l, Na^+, Cl^-, K^+, HCO_3^- in Mol/l

Liver parameters	Group I (Control)	Group II (10 mg/kg)	Group III (100 mg/kg)	Group IV (1000 mg/kg)
Urea (mMol/l)	$8.48^{ab} \pm 0.48$	6.18 ± 0.18	$5.30^{a} \pm 1.00$	$5.06^{b} \pm 1.00$
Creatinine (mMol/l)	$86.60^{ab} \pm 1.88$	87.8 ± 3.26	$71.90^{a} \pm 0.90$	$73.20^{b} \pm 1.20$
Na^+ (Mol/l)	$135.20^{ab} \pm 2.56$	134.60 ± 2.11	$132.50^{a} \pm 1.76$	$129.60^{b} \pm 1.66$
Cl^- (Mol/l)	$100.90^{ab} \pm 1.33$	99.50 ± 0.99	$94.40^{a} \pm 0.40$	$87.30^{b} \pm 1.60$
K^+ (Mol/l)	$4.60^{abc} \pm 1.00$	$4.80^{a} \pm 1.00$	$4.90^{b} \pm 1.00$	$4.90^{c} \pm 0.80$
HCO_3^- (Mol/l)	$37.7^{abc} \pm 1.33$	$27.50^{a} \pm 0.99$	$25.20^{b} \pm 3.00$	$25.40^{c} \pm 0.99$

Values are expressed as mean ± SD for n= 5. Values bearing same superscripts in the same raw are significantly different (p< 0.05)

loss of appetite, calmness, difficulty in breathing and general weakness. These signs were not seen in 10 and 100 mg/kg body weight dose groups of aqueous and methanol extracts, but in the case of n-hexane signs were not seen in 10 mg/kg body weight only. Progression became increasingly pronounced as the dose increased towards 5000 mg/kg body weight. The LD_{50}, being greater than 5000 mg/kg body weight is thought to be safe as suggested by Lorke [6]. Again, the absence of death among rats in all the dose groups throughout the two weeks of the experiment seems to support this claim. Furthermore, the dose-dependent weight loss observed, were not found to be statistically significant ($p>0.05$) when compared with the control group in phase one (Table 2).

A preliminary study has shown that this plant contains alkaloids and saponins. It has been shown that saponins enhance nutrient absorption and aid in animal digestion. Significant toxicity is usually as a result of suicide attempt or inappropriate self-administration for therapeutic purposes [8]. Also, alkaloids have some pharmacological effects and are used as medications, recreational drugs, or in entheogenic rituals for example the local anaesthetics and stimulant cocaine, the stimulant caffeine, the analgesic morphine or the antimalarial drug quinine [2].

Liver is an important organ of the body and is involved actively in different metabolic functions [9]. Hepatic damage caused by chemicals or infectious agents is associated with distortion of these metabolic functions and may lead to progressive liver fibrosis and ultimately cirrhosis and liver failure [10]. Liver damage is characterized by disturbances in the normal functions of the liver and is clinically diagnosed by determining the serum concentration of liver enzymes (ALT, AST and ALP), bilirubin, total protein and albumin [11]. ALT is an enzyme normally present in liver and heart cells once there is damaged to either organs the ALT level in blood will increased, and thus indicates liver or heart injury. During hepatocellular injury, enzymes are normally released into the blood flow particularly those located in the cytosol, their quantification in plasma is useful biomarkers to determine the extent and type of hepatocellular damage [9]. ALT and AST are used to assess the hepatocellular integrity of liver tissue. ALT is more predominantly found in liver while AST is normally found in equal amounts in the liver, heart, muscle, kidney and brain. Therefore, ALT is more liver-specific than AST [10]. ALP is also a non-plasma specific enzyme involved in the hydrolysis of a variety of phosphate esters at alkaline pH. These enzymes were reported to reach higher than normal level in the blood in events of impaired liver function [11]. Thus,

they are used as serum markers of hepatic damage.

Serum albumin is the main protein of human blood plasma [12]. It binds water, cations (such as Ca^{2+}, Na^{+} and K^{+}), fatty acids, hormones, bilirubin and thyroxine (T_4), its main function is to regulate the colloidal osmotic pressure of the blood, low albumin (hypoalbuminemia) may be caused by liver disease, nephrotic syndrome, burns, malabsorption, malnutrition e.t.c and high albumin (hyperalbuminemia) is almost always caused by dehydration. Serum protein estimation may be helpful in the assessment of liver function because impairment of protein metabolism will lead to elevated serum protein concentration. The liver also metabolises bilirubin, Impairment of normal conjugation and excretion of bilirubin would therefore results in its accumulation in the blood, thus estimation of serum bilirubin can be used to assess liver function [12].

From Tables 3, 4 and 5 shows a significant decreased ($p<0.05$) in serum activities of AST and ALT between groups III, and IV when administered with 100 mg/kg body weight and 1000 mg/kg body weight of Aqueous, Methanolic and n-Hexane root extracts of *Curcuma longa L.* in treated rats compared to control group. These results are similar to the work of Liju [13] on the oral administration of tumeric essential oil showed no significant changes in liver function parameters. While the significant increased ($p<0.05$) in total and direct bilirubin levels of groups II, III and IV when compared to the control group I may be as a result of haemolysis of the red blood cells whereas ALP and albumin shows significant increased $p<0.05$ in groups II, III and IV. This indicated that *Curcuma longa L.* aqueous, methanol and n-hexane root extracts at doses up to 1000 mg/kg body weight showed no sign of hepatocllular damage and did not cause any toxicity to the liver. Also this work is similar to the findings of Deshpande [14] on subcronic toxicity of *Euphorbia pulcherrima* methanol extract on wister rats.

Kidneys are the major organs in metabolizing toxic compound besides liver. It receives about 1200 ml of blood per minute [15] containing a lot of chemical compounds. Therefore, damage to the kidneys can be determined by measuring the level of urea, electrolyte and creatinine in blood as an indicator of kidney damage. Urea is a byproduct from protein breakdown. About 90% of urea produced is excreted through the kidney. While the creatinine is a waste product from a muscle creatinine, which is used during muscle contraction. Creatinine is commonly measured as an index of glomerular function [16], It is excreted exclusively through the kidney [17]. Therefore, damage to the kidney will make the kidney inefficient to excrete both urea and creatinine and causes their accumulation in the blood. Therefore, the high level of blood urea and creatinine will indicate kidney damage, also Electrolytes are substances that become ions in solution and acquire the capacity to conduct electricity. The balance of the electrolytes in human bodies is essential for normal function of cells and organs. The functions and normal range values for these electrolytes are important, and if an electrolyte is at an extreme low or high, it can be fatal.

Also from Tables 6, 7, and 8 there was a significant reduction ($p<0.05$) in levels of serum urea, creatinine Na^{+}, Cl^{-}, and HCO_3^{-} in groups III and IV when administered with 100 mg/kg body weight and 1000 mg/kg body weight of Aqueous, Methanol and n-Hexane root extracts of *Curcuma longa L.* in treated rats compared to control group I. The same observations have been made by [18] in normal rats treated with the petroleum ether extract of *N. sativa* for four weeks. While K^{+} shows significant increase in groups III and IV when compared with control group.

5. CONCLUSION

From the results of this study, it is hypothesized that extracts of *Curcuma longa L.* is safe for usage in traditional medicine. Higher doses should however be avoided and users should not rule out completely the possibility of chronic toxicity developing with the continual usage of these extracts. It is now left for the therapeutic dosage to be determined for clinical applications.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that Principle of laboratory animal care [19] and ethical guidelines for investigation of experimental pain in conscious animals [20] were observed during experimentation [21].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
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