Trop J Nat Prod Res, November 2020; 4(11):1019-1024 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Effect of Methanol Extract of *Curcuma longa* L on some Biochemical and Haematological Parameters in Albino Rats

Chinelo C. Nkwocha[1], Raphael C. Ekeanyanwu[2]*, Kingsley I. Uka[1]

[1]*Department of Biochemistry, University of Nigeria Nsukka, Enugu State, Nigeria*
[2]*Department of Biochemistry, Imo State University Owerri, Imo State, Nigeria*

ARTICLE INFO

Article history:
Received 25 September 2020
Revised 16 October 2020
Accepted 18 November 2020
Published online 30 November 2020

ABSTRACT

C. longa (Turmeric) is a plant that is used both as spice and medicine. Despite all the documented importance of turmeric in both nutrition and medicine, there is little or no scientific evidence on the biochemical effect of the methanol extract in albino rats. This study was aimed at evaluating the effect of the methanol extract on some selected biochemical parameters in albino rats. In the assessment of the 15-day sub-acute toxicity profile of the methanol extract of *C. longa*, acute and sub-acute toxicity study was performed following standard laboratory procedures using twenty albino rats which were divided into four groups of 5 rats each. The rats were administered different concentrations of the methanol extracts, 100 mg/kg b.wt, 200 mg/kg b.wt and 500 mg/kg b.wt whereas control received distilled water only. At the end of each period of administration, some biochemical parameters and haematological parameters were analysed. There was a significant increase ($p<0.05$) in parameters such as High-Density Lipoprotein cholesterol (HDL-c), malondialdehyde, haemoglobin, Glutathione reductase (GHSR), red blood cell count, and packed cell volume in rats administered the methanol extract. In contrast, a significant decrease ($p < 0.05$) was also observed in parameters such as Catalase (CAT), Superoxide dismutase (SOD), and blood glucose level. The result has shown that the methanol extract of turmeric has blood glucose and blood lipid-lowering potentials, and as well possesses hematopoietic potentials.

***Keywords*:** *Curcuma longa*, Methanol extract, Toxicology, Plant extraction.

Introduction

Medicinal plants have provided a credible source for the preparation of new medicines as well as treating diseases, since the dawn of civilization. *Curcuma longa* (Turmeric) is a sterile plant and does not contain any seeds. The plant grows yellow flowers up to 3-5 ft high and slender. The rhizome, a thick and fleshy underground stem ringed with the bases of old leaves, is part of turmeric which possesses a potential medicinal property.[1] Rhizomes are fermented, then dried and ground to produce the signature bright yellow spice. *C. longa* powder has a bitter peppery flavour, and a slightly orange and ginger-like, mild aroma. Though *C. longa* powder is best known as one of the key ingredients used to make the curry spice, it also gives its bright yellow colour to the ballpark mustard.[2] In addition to its culinary uses *C. longa* was commonly used in traditional medicine in India, Pakistan and Bangladesh due to its various beneficial properties.[3] The *C. longa* plant is an excellent natural antiseptic, disinfectant, anti-inflammatory and analgesic for conventional Ayurveda though, at the same time, it is also used to support digestion, improve intestinal flora and treat skin irritations.[2] It was also used as a readily available antiseptic for wounds, burns, and bruises in South Asia. However, several other beneficial properties are reported in folk medicine.[4]
Curcumin the turmeric's yellow colour pigment is industrially derived from turmeric oleoresin. The comprehensive survey of literature finds that *C. longa* is widely regarded as a panacea in herbal medicine with a wide variety of pharmacological activities.[5] With antioxidant activities, it has many useful properties and is useful in conditions such as inflammation, ulcer, and cancer. The presence of various metabolites such as curcuminoids, oil content, flavonoids, phenolics, some essential amino acids, protein, and high alkaloid content show the co-relationship with its medicinal uses.[6] Despite the reported numerous pharmacological data on *C. longa*, no reported data on the biochemical and haematological effects from the methanol extract. Given this circumstance, the present study is a study aimed to assess the effect of *C. longa* methanol extract on some selected biochemical parameters in albino rats.

Materials and Methods

Plants
Fresh *C. longa* roots used were obtained from the botanical garden of the University of Nigeria Nsukka, Enugu State, Nigeria and authenticated by a Botanist at the Department of Botany by comparing with voucher specimen present in their herbarium (Voucher number: UNN/FBS/2019_049A).

Experimental animals
The animal used for the study were adult male albino rat of body weight 108.2g - 150.4g and Swiss albino mice (16.0g – 18.0g) obtained from the animal holding unit of the Department of Zoology and Environment Biology, University of Nigeria, Nsukka. Before the experiment, the animal was acclimatized under standard laboratory condition in the animal farm of the Veterinary Teaching Hospital University of Nigeria, Nsukka for 14 days with free access of water and feed *ad libitum*.

*Corresponding author. E mail: ekeanyanwu.chukwuma@imsu.edu.ng
Tel: +2348032744572

Citation: Nkwocha CC, Ekeanyanwu RC, Uka, KI. Effect of Methanol Extract of *Curcuma longa* L on some Biochemical and Haematological Parameters in Albino Rats.Trop J Nat Prod Res. 2020; 4(11):1019-1024. doi.org/10.26538/tjnpr/v4i11.30

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Ethics approval

The Department of Biochemistry, University of Nigeria's animal experimentation committee approved the protocol for all animal experiments performed in this report (Ethical Approval Number: 03/2019). Processes involving experimental animals were performed in compliance with the National Institute of Health Care Guide for the Treatment and Use of Laboratory Animals (NIH publication # 85 – 23, updated 1985). This research project was carried out from March 2019 through September 2019. Every effort was made to mitigate the suffering of the animal and to reduce the number of animals used in experiments.

Extraction of plant material

The fresh root of the turmeric plant harvested was washed with water to remove sand and unwanted materials. The harvested turmeric plant was then cut into smaller pieces to quicken the drying. The sliced turmeric was oven-dried, ground into powder form, and then 1kg of the powdered sample was soaked in 2.5L of methanol and allowed to stand for 48 h. The suspension was filtered using a muslin bag followed with Whatman no. 42 filter paper. The filtrate was evaporated under reduced pressure and dried using a rotary evaporator at 55ºC. The concentrated extract (24 g) was stored in a labelled sterile screw-capped bottle at 25ºC.

Animal experiment

Acute toxicity study (LD_{50})

The acute toxicity study of the crude methanol extract of *C. longa* was estimated in mice following the method described by Lorke[7] using 39 albino mice of both sexes of weight between 16.0g – 18.0g, which were dosed orally with different gradual doses (10 - 5 000 mg/kg body weight). In the first phase, mice were divided into three groups of nine mice each and treated orally with the extract using cannula at doses of 10, 100 and 1000 mg/kg body weight. Signs of toxicity, mortality and general behaviour were observed for 24 h. In the second phase, twelve mice were divided into four groups of three mice each and the extract wasalso given orally at doses of 1000, 1600, 2900 and 5000 mg/kg body weight. The symptoms of toxicity, mortality and general behaviour were observed for 24 h. The geometric mean of the highest non-lethal dose (without death) and the lowest lethal dose (where death occurred) was calculated as LD_{50}.

$LD_{50} = \sqrt{\text{minimum toxic dose} \times \text{maximum tolerated dose}}$.

14-day Sub-acute toxicity study

Animals used for the study were a total of twenty male albino rats of body weight 108.2g - 150.4g. Only male rats were selected for the study in other to eliminate hormonal effects on the experimental results. We housed the animals under standard colony and specific pathogen-free conditions and maintained with a 12 hour light/dark cycle (lights on at 6.00 am) at 25- 27ºC and relative humidity of 40 – 60% which was measured using a CEM hydrometer (DT-615, Shenzhen, China). They received humane care throughout the experimental period following the ethical rules and recommendations of the University of Nigeria committee on the care and use of laboratory animals. The treatment groups and protocol for the study is presented in table 1.

The doses were selected based on the outcome of our acute toxicity study on *C. longa*. The dosing of the extract started on day one (after acclimatization) and lasted 14 days (two weeks). Throughout treatment, the body weight of each rat was monitored on day 0 (before the experiment), and the end of the experiment (day 15).

Table 1: Treatment groups

Group	Description	Treatment administered
Group 1	Normal Control	Distilled water only
Group 2	Low Dose	100 mg/kg b.wt of extract
Group 3	Middle Dose	200 mg/kg b.wt of extract
Group 4	High Dose	500 mg/kg b.wt of extract

b.wt = bodyweight of the experimental albino rats.

Blood sample collection and preparation

On day 15, after overnight fasting, all rats were anaesthetized under light ether anaesthesia and blood samples were collected from all the rats by ocular puncture into plain tubes and were allowed to clot for 15 min. Briefly, for the analysis of haematological parameters, about 5 mL of the collected blood sample was immediately transferred into another sample bottle containing EDTA and shaken mildly to mix the blood and prevent clotting. For the analysis of biochemical parameters, about 5 mL of blood from each rat was transferred to a test tube and kept at ambient temperature for 30 min to clot. Afterwards, the test tubes containing the clotted blood sample were centrifuged at 4000 rpm/min for 10 min to enable complete separation of serum from the clotted blood. The clear serum supernatants were then carefully drawn out with a syringe and stored in clean sample containers for biochemical analysis.

Biochemical assay

The values for serum cholesterol, triglycerides, high-density lipoproteins as well as low-density lipoprotein-cholesterol (LDL-c) and lipid peroxidation were determined using standard laboratory procedures.[8-10]The blood glucose was determined with Accu-check glucometer using a pin to puncture the coccygeal plexus and dropping blood on the strips to be read in the glucometer.[11]

Haematological assay

The values for haematological parameters such as red blood cells (RBC) count, white blood cell count (WBC), packed cell volume (PCV) and haemoglobin (Hb) were determined by the method of Ochei and Kolhatkar.[12]

Quantitative phytochemical analysis of turmeric

The determination of tannin content was done using Folin Denis Colourimetric method described by Kirk and Sawyer.[13]The determination of flavonoids, terpenoid, phenol, steroid, alkaloid, saponins, glycosides and soluble carbohydrates were done using the method described by El-Olemyl.[14]

Determination of serum antioxidant enzymes activity

Catalase (CAT) activity was assayed via the method described by Aebi.[15] Superoxide dismutase (SOD) activity was assayed using the method described by Fridovich[16] as contained in the commercial kit. The concentrations of glutathione peroxidase (GPx) and glutathione reductase (GHSR) were determined according to the method of Habig*et al*.[17]

Statistical analysis

Data obtained from the laboratory were analysed using IBM Statistical Product and Service Solutions (SPSS), version 18. The results were expressed as mean ± standard deviation (SD) and presented in tables. One-way analysis of variance (ANOVA) was used to compare means across the groups. Mean values with $p < 0.05$ were considered statistically significant.

Results and Discussion

Phytochemical composition

The quantitative phytochemical analyses of the methanol extract showed steroid (4.21 ± 0.03%), terpenoid (3.66 ± 0.01%), anthocyanin (0.98 ± 0.01%), alkaloid (4.51±0.50%), phenols (4.55±0.06%), tannin (1.22±0.01%), flavonoid (4.26±0.20%), saponin (4.11±0.11%), glycosides (0.02±0.01%) were detected. Glycoside had the lowest amount and phenols had the highest amount when compared to others. Phytochemicals are found naturally in plants and are responsible for providing fruits and vegetables with colour, flavour, and aroma. Biologically, they function to protect plants from invasion, disease, and infection. Phenolic compounds are phytochemicals with at least one hydroxyl group, having one or more aromatic rings. They play a protective role in plants by minimizing the effect of predators, parasites on aggression, and also protecting plants from ultraviolet radiation. In fruits, cereals, legumes, and vegetables, phenolics and terpenoids are

all-time commons. Terpenoids possess antioxidant properties and interact with most regulatory proteins as well. In the treatment of cancer and inflammatory diseases plant extracts have been used both historically and in modern medicine. In modern medicine, terpenes are used as inhibitors of NF-kB.[18]

It is assumed that flavonoids have different therapeutic qualities. The antihyperglycemic effect has been identified in flavonoids.[19] Flavonoids are known to enhance heart function, reduce angina levels and lower cholesterol levels. These compounds function upon inflammation mediator control.[20]It has also been shown that flavonoids lower pathogenic thrombosis development in mice models.[21]

Anthocyanins are known to prevent the development of free radicals and thus to protect cardiomyocytes after ischemic episodes.[22]Anthocyanins have vasodilating and anti-aggregating tendencies, as well as lower oxidized LDL-c levels.[21] These compounds are also reported to decrease nitric oxide levels by inhibiting nitric oxide synthase activity.[22]Anthocyanins display anti-inflammatory behaviour as they inhibit enzyme cyclooxygenase. Clinically speaking, both types of tannins may be involved in regulating blood glucose levels. Tannin has been shown to promote carbohydrate use in the receptor cells. They are potent antioxidants and have been considered to be cardio-protective, anti-inflammatory, anti-carcinogenic and anti-mutagenic, among others.[23]

Also, terpenoids boost the skin tone, increase antioxidant concentration in wounds, and restore inflamed tissues by increasing blood supply.[24] Even terpenoids enhance lung function.[25]Terpenoids have been shown to minimize diastolic blood pressure and lower the sugar level in blood in hypertensive and diabetic patients, respectively.[26] Alkaloids are known to have blood-glucose-lowering activity. Alkaloids are known to possess antiarrhythmic effects, antihypertensive effects, anticancer and antimalarial activity.[27]Saponins are plant compounds that occur either as alkaloids, triterpenoid glycosides or as steroids. The hypocholesterolemic, immunostimulant, hypoglycemic activity, and anti-carcinogenic properties of these phytochemicals are known.[28] Cardiac glycosides are secondary plant metabolites that have a glycoside unit and function upon the cardiac muscle's contractile action. Traditionally, these compounds have been used to treat cardiac arrhythmias and congestive heart failure, as they improve contractile force.[29]Phytosterols is a type of steroids that have cholesterol-like structures and functions. In plants, phytosterols act as substrates for secondary metabolite synthesis to regulate the permeability and fluidity of cell membranes and also act as biogenic precursors of growth factors.[30]Several studies suggest the presence of these phytochemicals is the reason why *C. longa* is very effective in treating multiple ailments and overall body wellbeing.[6, 31]

Mean body weight gain

Table 3 shows the mean body weight of animals administered 100 mg/kg, 200 mg/kg and 500 mg/kg b.wt of methanol extract of *C. longa* and the control group. There was an increase in the bodyweight

Table 2: Quantitative Phytochemical analysis of *Curcuma longa* (Turmeric)

Phytochemicals	Amount (%)
Steroid	4.21 ± 0.03
Terpenoid	3.66 ± 0.01
Anthocyanin	0.98 ± 0.01
Phenols	4.51 ± 0.50
Tannin	1.22 ± 0.01
Flavonoid	4.55 ± 0.06
Saponin	4.11 ± 0.11
Glycosides	0.02 ± 0.01

Results are presented in mean ± SD.

Table 3: Mean body weight gain of the experimental rat

Group	Weight before (g)	Weight after (g)	%Weight difference
Normal control	140.60±35.17	156.82±44.59	11.54
Low dose (100mg/kg b.wt)	131.14±13.38	148.98±14.42	13.60
Mid dose (200mg/kg b.wt)	115.96±6.09	125.60±23.07	8.31
High dose (500 mg/kg b.wt)	122.86±16.61	134.88±19.35	9.78

Values are mean ± SD (n = 5).

of the animals at the end of the experimental period. However, no significant difference ($p > 0.05$) was observed across the group both before and after the experiment.

Results of assays

The HDL-c concentration as seen in Figure 1 showed a significant increase in test group-administered low dose, mid-dose and high dose compared with the control. However, a significant decrease ($p<0.05$) was observed in cholesterol, TAG and LDL-c levels of rats administered varying doses of the extract compared with the control. Several human studies have shown that *C. longa* has a significant lipid-reducing effect.[32-36]*C. longa* produces a protective impact on the cardiovascular system due to its reported antioxidant properties, including a reduction in cholesterol and triglyceride levels and a decrease in the susceptibility of low-density lipoprotein cholesterol (LDL-c) to lipid peroxidation. A study reports that turmeric extract administered to 18 low-dose atherosclerotic rabbits (1.6 – 3.2 mg/kg daily body weight) showed decreasing sensitivity of LDL-c to lipid peroxidation; besides, it decreases plasma cholesterol and triglyceride levels. The higher dose reduces levels of cholesterol and triglycerides but has not reduced LDL-c lipid peroxidation. The possible effect of turmeric extract on cholesterol levels may be due to reduced absorption of cholesterol in the intestines and enhanced conversion of cholesterol into liver bile acids.[37, 38]

Estimation of the extent of lipid peroxidation (malondialdehyde) of methanol extract of *C. longa* in albino rats shows a significant increase ($p<0.05$) in the test groups compared with the control group. The findings are consistent with several studies because it has been shown that Curcumin inhibits lipid peroxidation using linoleate, a polyunsaturated fatty acid that can oxidize and form a radical fatty acid.[39]Curcumin has also been shown to act as a chain-breaking antioxidant at the 3' position, resulting in a Diels-Alder intramolecular reaction and lipid radical neutralization.[39]Curcumin down-regulates the activity of iNOS in macrophages and thus reduces the number of reactive oxygen species (ROS) produced in response to oxidative stress.[40] It is implied from these results that *C. longa* has antioxidant activity because it inhibits free radical peroxidation and can also attenuate the lipid profile parameters.

From the result presented in Fig. 2, a significant decrease ($P<0.05$) was observed in the CAT and SOD levels of rats in the test group compared with the control group. However, an increase was observed in GSHR levels of rats in the test group compared with the control group.

The baseline glucose level of the experimental rats and blood glucose level of the experimental rats after the experiment is shown in Figure 3. A significant ($p < 0.05$) decrease in the blood glucose level of the rats treated with low and high doses of methanol extract of *C. longa* (turmeric), compared with the control was observed. However, there was non-significant ($p > 0.05$) decrease in the blood glucose levels of rats treated with mid-dose compared with the control.

A significant ($p < 0.05$) increase in the Hb concentration of the rats treated with different doses of the methanol extract of *C. longa* compared to the normal control (Figure 4). However, a non-significant decrease ($p > 0.05$) in the Hb concentration of rats treated with a high

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

dose of *C. longa* methanol extract was observed compared to the mid-dose treatment dose. This is in line with the results of Ukoha and Onunkwo.[41]

The result of the red blood cell (RBC) concentration (Figure 5) of the experimental rats was; normal control (9.01 ± 0.57×10^6mm), low dose (10.27 ± 0.40×10^6mm), mid-dose (10.61 ± 0.61×10^6mm), high dose (10.20 ± 0.14×10^6mm). Generally, there was a significant ($p < 0.05$) increase in the RBC concentration of the rats treated with methanol extract of *C. longa* compared to the normal control (Figure 4). This is in agreement with the previous investigation of Ukoha and Onunkwo[41] on the effect of *C. longa* on the haematology and blood chemistry of broiler chickens. There was, however, a non-significant difference in RBC concentration among the rats treated with *C. longa*methanolic extract at low, mid, and high doses. This shows that the *C. longa* methanol extract increases the concentration of RBC regardless of the dosage.

The white blood cell (WBC) concentration of the experimental rats was; normal control (10660.00 ± 194.94×10^3mm), low dose (10220.00 ± 438.18×10^3mm), mid-dose (10660.00 ± 680.44×10^3mm), high dose (10440.00 ± 230.22×10^3mm). A non-significant ($p > 0.05$) difference was observed in the WBC concentration of the rats treated with methanol extract of *C. longa* compared to the normal control. This showed that *C. longa* does not have a significant effect on the white blood cell concentration in male albino rat. This agrees with the results obtained by Ukoha and Onunkwo[41] on the effect of *C. longa* on the haematology and blood chemistry of broiler chickens.

The packed cell volume (PCV) concentrations of the experimental rats were; normal control (32.00 ± 2.00%), low doses (40.00 ± 1.58%), mid doses (42.60 ± 0.45%), high doses (42.80 ± 0.42%). Generally, there was a significant ($p < 0.05$) increase in the PCV concentration of the rats treated with *C. longa* compared to the normal control (Figure 4). This agrees with the results obtained by Ukoha and Onunkwo[41]. The increase is more significant as the doses increase. However, there was a non-significant ($p > 0.05$) increase in the PCV concentration of the rats treated with the high dose compared to those treated with mid-dose.

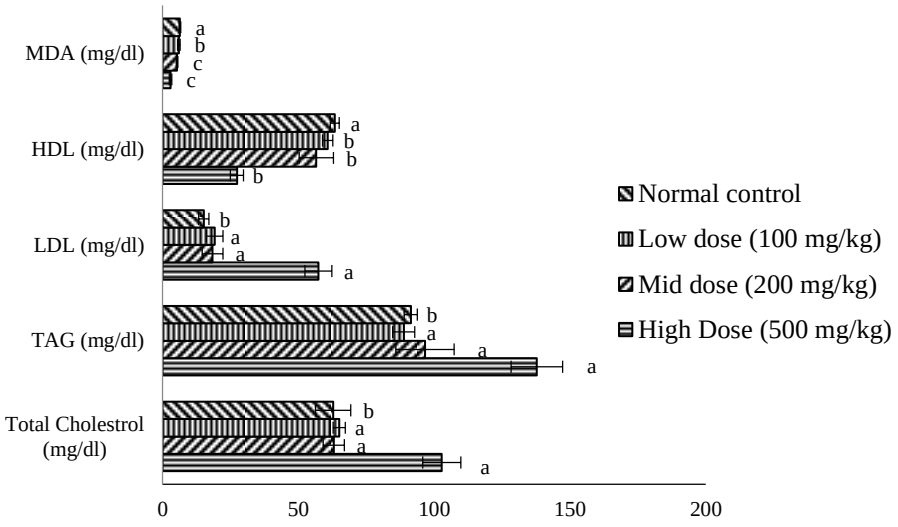

Figure 1: Some biochemical parameters in rats administered different doses of the methanol extract of *C. longa*.
Values are mean ± SD (n=5). [a] Values with the same superscript are significantly ($p < 0.05$), [b] Values with different superscript are significantly ($p > 0.05$).

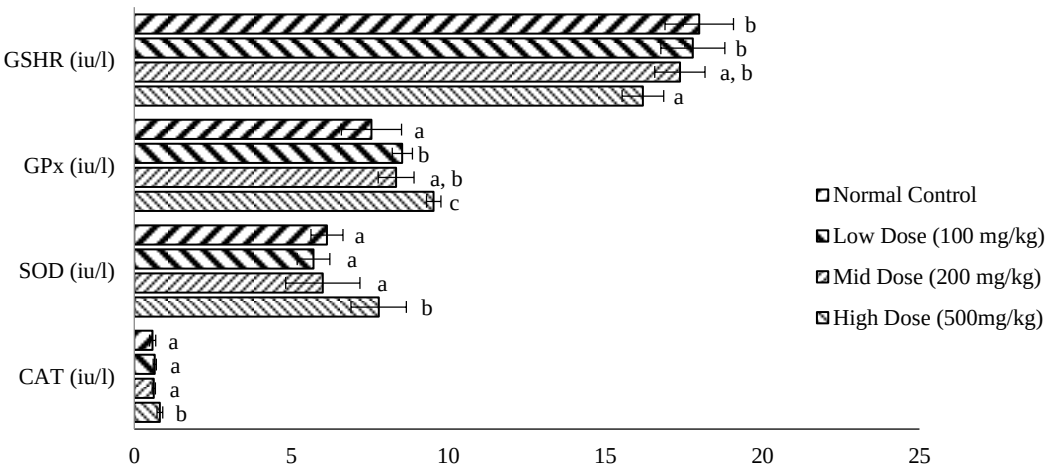

Figure 2: Antioxidant enzymes activity in rats administered different doses of the methanol extract of *C. longa*.
Values are Mean ± SD. [a] Values with the same superscript are significantly ($p < 0.05$), [b] Values with different superscript are significantly ($p > 0.05$).

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

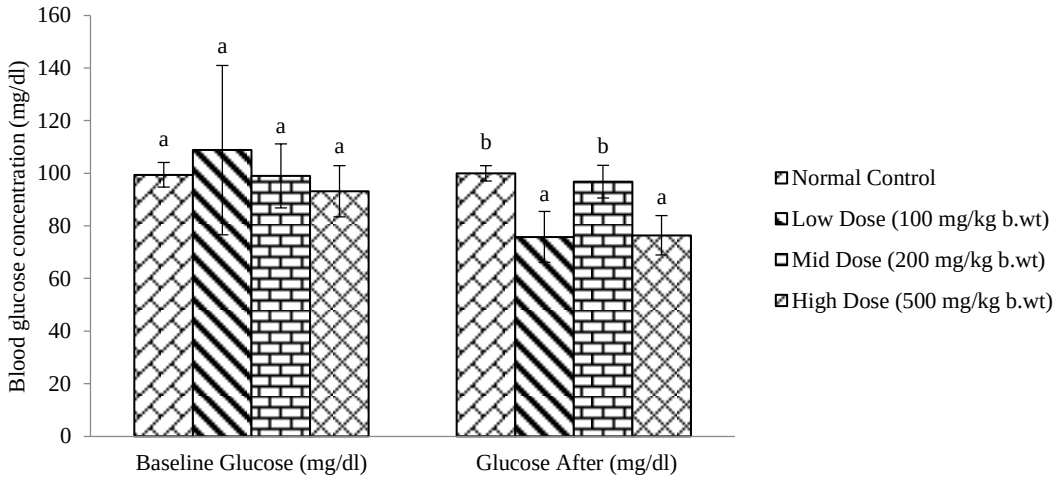

Figure 3: The blood glucose level of rats administered different doses of the methanol extract of *C. longa*.
Values are mean ± SD. [a] Values with the same superscript are significantly ($p < 0.05$), [b] Values with different superscript are significantly ($p > 0.05$).

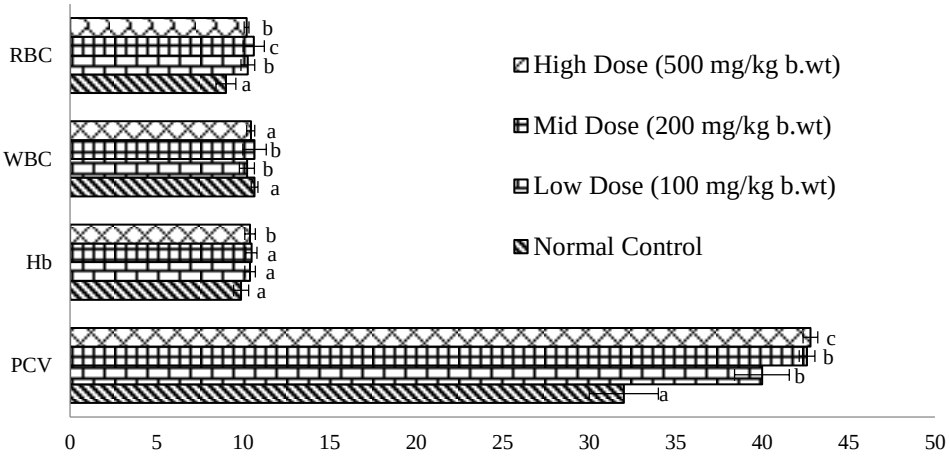

Figure 4: Some haematological analysis of rats administered different doses of the methanol extract of *C. longa*.
Values are mean ± SD (n=5). [a] Values with the same superscript are significantly ($p < 0.05$), [b] Values with different superscript are significantly ($p > 0.05$). Red Blood cell (RBC×10^6mm), White Blood Cell (WBC×10^6mm), Hb % (Haemoglobin), PCV g/dL (Packed Cell Volume).

Conclusion

This research work has shown that methanol extract of *C. longa* may be very effective in the management of high blood glucose level, lipid profile and also in boosting of haemoglobin, red blood cell and packed cell volume respectively.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the works presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors wish to acknowledge the Department of Biochemistry, University of Nigeria Nsukka, Enugu State for providing laboratory and infrastructural support.

References

1. Prabhakaran NKP. The Agronomy and Economy of Turmeric and Ginger: The Invaluable Medicinal Spice Crops. Elsevier USA. 2013. 544 p.
2. Odigie EB, Airiagbonbu BO, Airiagbonbu NT, Omorodion NT. Respiratory Tract Protective Herbs May Serve As Palliatives in the Battle against COVID-19 Pandemic. Int J Basic SciTech. 2020; 6(2):65-75.
3. Chattopadhyay I, Biswas K, Bandyopadhyay U, Banerjee RK. Turmeric and Curcumin: biological actions and medicinal applications. Curr Sci. 2014; 87(1):44-53.

4. Verma RK, Kumari P, Maurya RK, Kumar V, Verma RB, Singh RK. Medicinal properties of turmeric (*Curcuma longa* L.): A review. IntJ Chem Stud. 2018; 6(4):1354-1357.
5. Nasri H, Sahinfard N, Rafieian M,Rafieian S, Rafieian M, Shirzad F. Turmeric: A spice with multifunctional medicinal properties. JHerbmedPharmacol. 2014; 3(1):5-8.
6. Sarangthem K and Haokip MJ. The bioactive component in *Curcuma caesia*Roxb grown in Manipur. The Bioscan, 2010; 5:113-115.
7. Lorke D. A new approach to practical acute toxicity testing. ArchToxicol.1983;53:275-289.
8. Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total cholesterol. ClinChem. 1976;20(4):470-475.
9. Albers JJ, Cheung MC, Hazzard WR. High-density lipoprotein in myocardial infarction survivors. Metab. 1978; 27:479.
10. Wallin B, Rosengren B, Shertzer HG, Camejo G. Lipoprotein oxidation and measurement of thiobarbituric acid reacting substances formation in a single microtitre plate: its use for evaluation of antioxidants. AnalBiochem.1993;208:10-15.
11. Yogashi Y, Shirakawa J, Okuyama T, Yamazaki S, Kyohara M, Miyazawa A, Suzuki T, Hamada M, Terauchi Y. Evaluation of the appropriateness of using glucometers for measuring the blood glucose levels in mice. SciRep. 2016; 6:25465.
12. Ochei JO and Kolhatkar AA. Medical Laboratory Science: Theory and Practice, Tata McGraw-Hill Publication Company limited, New York. 2008. 637-745 p.
13. Kirk RS and Sawyer R. Pearson's composition and analysis of foods. Church hill, Livingstone, Edinburgh, 1998. 615-616.
14. El-Olemyl MM, Al-Muhtadi FJ, Afifi AA. Experimental Phytochemistry. A laboratory manual (King Saud University, KSA). 1994. 8-9 p.
15. Aebi HE. Catalase. In: Bergmeyer Hu (ed), Methods of enzymatic analysis, VerlaChemie, Weinnheim, Germany, 1983; 273-285.
16. Fridovich I. Superoxide dismutase. An adaptation to a paramagnetic gas. J Biol Chem. 1989; 264:7761-7764.
17. Habig WH, Pabst MJ, Jacoby WBC. Glutathione *S*-transferases: the first enzymatic step in mercapturic acid formation. J Biol Chem. 1974; 249:7130-7139.
18. Piero NM, Eliud NN, Susan KN, George OO, David NJMM. *In vivo*Antidiabetic Activity and Safety in rats of *cissampelospareira*traditionally used in the management of diabetes mellitus in Embu County, Kenya. J Drug MetabToxicol. 2015; 6:184.
19. Muriithi NJ, Maina GS, Maina MB, Klambi MJ, Kelvin JK. Determination of the haematological effect of methanolic leaf extract of *Vernonialasiopus*in normal mice. J Blood Lymph. 2015; 5:139.
20. Sanchez-Ramon S, Lopez-Longo FJ, Carreno L. Interleukins network in rheumatoid arthritis pathophysiology: beyond proinflammatory cytokines. RheumatolClin. 2011; 6:20-24.
21. Yang Y, Sterling J, Storici F, Resnick MA, Gordenin DA. Hypermutability of damaged single-strand DNA formed at double-strand breaks and uncapped telomeres in yeast Saccharomyces cerevisiae. PLoSGenetics. 2008; 4(11):e1000264.
22. Ngugi MP, Njue ME, Gathumbi K. P.Trace elements content of selected Kenyan antidiabetic Medicinal Plants.Int J Curr Pharm Res. 2012; 4(3):39-42.
23. Kumari M and Jain S. Tannins: An antinutrient with a positive effect to manage diabetes. Res J Recent Sci. 2012; 1(12):1-18.
24. Aggarwal BB, Ichikawa H, Garodia P. From Traditional Ayurvedic Medicine to Modern Medicine: Identification of Therapeutic Targets for Suppression of Inflammation and Cancer. ExpertOpinTher Targets. 2006; 10:87-118.
25. Mujoo K, Haridas V, Hoffmann JJ, Wächter GA, Hutter LK, Lu Y, Blake ME, Jayatilake GS, Baile D, Mills GB, Gutterman JU.Triterpenoid saponins from *Acacia victoriae* (Bentham) decrease tumour cell proliferation and induce apoptosis. Cancer Res. 2001; 61(14):5486-5490.
26. Grace G, Bomkazi MG, Collise N, Nomalungelo IG. The proximate composition of *S. Nigrum* plant leaves consumed in the Eastern Cape Province of South Africa.Int Pro ChemBiol Environ Eng. 2013; 53:103-106.
27. Dholi SK, Raparla R, Mankala SK, Nagappan K. *In vivo* antidiabetic evaluation of neem leaf in Alloxan-induced rats. J Appl Pharm Sci. 2011; 1(4):100-105.
28. Ros SJ, Cabiscol EC, Tamarit SJ. Oxidative stress in bacteria and protein damage by reactive oxygen species. Int. JMicrobiol. 2000; 3(1):3-8.
29. Liu Z, Solter P, Guzman R. Decreased Hepatic ALT Synthesis is an Outcome of Subchronic Microcystin-LR Toxicity. ToxicolApplPharmacol. 2000; 164:216-220.
30. Patel MD and Thompson PD. Phytosterols and vascular disease. Atheroscler. 2006, 186(1):12-19.
31. Ambrosio CLB, de Arruda CF, Siqueira C, de Faro ZP. Carotenoides Como Alternativa contra hipovitaminose A. Revista de Nutricao. 2016; 19(2):233-243.
32. Disilvestro RA, Joseph E, Zhao S, Bomser J. Diverse effect of low dose supplement of lapidated Curcumin in healthy middle-aged people. NutrJ. 2012; 11(1):79.
33. Madaric A, Kadrabova J, Krajcovicova-Kudlackova M, Valachovicova M, Spustova V, Mislanova C, Kajaba I, Blazicek P. The effect of a bioactive complex of quercetin, selenium, catechins, and curcumin on cardiovascular risk markers in healthy population after two months consumption. Bratislavskélekárskelisty - Bratislava Med J. 2013;114:84-87.
34. Mohammadi MT, Jahromi MG, Mirjalili MH, Binabaj M R, Jafari M, Salem F. Atorvastatin inhibits the brain oxidative stress of streptozotocin-induced diabetic rat. J ExpApplAnimSci. 2013; 1(1):35-43.
35. Panahi Y, Rahimnia A, Sharafi M, Alishiri G, Saburi A, Sahebkar A. Curcuminoid treatment for knee osteoarthritis: a randomized double-blind placebo-controlled trial. Phytother Res. 2014; 28:1625-1631.
36. Sanmukhani J, Satodia V, Trivedi J, Patel T, Tiwari D, Panchal B, Goel A, Tripathi CB. Efficacy and safety of curcumin in major depressive disorder: a randomized trial. Phytother Res. 2014; 23(4):579-585.
37. Botella-Pavia P and Rodriguez-Concepcion M. Carotenoid biotechnology in plants for nutritionally improved foods. Physiol Plant. 2006; 126(3):369-381.
38. Vance DE and Vance JE. Biochemistry of lipids, lipoproteins and membranes. Elsevier Amsterdam. 2012. 15-21 p.
39. Masuda T, Maekawa T, Hidaka K, Bando H, Takeda Y, Yamaguchi H. Chemical studies on antioxidant mechanisms of Curcumin: analysis of oxidative coupling products from curcumin and linoleate. J AgricFood Chem. 2011; 49:2539-2547.
40. Chan K, Shien J, Tiley L, Chen D, Chiou S, Wang S, Chang T, Lee Y, Hsu W. Curcumin inhibits influenza virus infection and haemagglutination activity. Food Chem. 2010; 119(4):1346-1351.
41. Ukoha OA and Onunkwo DN. The effect of turmeric (*Curcuma longa*) on the haematology and blood chemistry of broiler chickens. Sci-Afri J Sci Issues Res Essays. 2016; 4(6):964-968.