Trop J Nat Prod Res, September 2019; 3(10):302 – 306 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

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

Original Research Article

Acute and Sub-Chronic Toxicity Studies on the Methanol Leaf Extract of *Leptadenia hastata* in Wistar Rats

Ibrahim H. Sani*, Abdullahi R. Abubakar, Abdullahi H. Yaro, Sani Malami

Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical sciences, Bayero University Kano, Nigeria.

ARTICLE INFO

Article history:
Received 20 September 2019
Revised 01 November 2019
Accepted 14 November 2019
Published online 10 December 2019

ABSTRACT

Leptadenia hastata (Pers.) Decne is a wild plant used as vegetable and medicine in Africa due to its nutritive and therapeutic properties in the treatment of pain, hypertension, psychiatric disorders and stomach upset in children. The present study evaluated the acute and sub-chronic toxicity effects of the methanol leaf extract of *Leptadenia hastata* (LHME) in rats. For the acute toxicity study, a single oral dose (5000 mg/kg) of LHME was administered to three rats and observed for 14 days for signs of acute toxicity. The sub-chronic toxicity study involved daily oral administration of LHME at 250 mg/kg, 500 mg/kg and 1000 mg/kg body weight for 28 days. Phytochemical screening of the extract revealed the presence of steroids, glycoside, tannins, flavonoids, alkaloids and saponins. The oral median lethal dose (LD_{50}) was estimated to be > 5000 mg/kg. There were no mortalities or signs of toxicity observed in the rats following acute and sub-chronic administration of LHME. Sub-chronic administration of LHME also did not cause any changes in body weight of the rats. There was no significant difference ($p>0.05$) observed in the relative organ weights, body weights, haematological indices, biochemical parameters. Therefore, it is concluded that the oral administration of the LHME for 28 days does not cause acute or sub-chronic toxicity effects.

Keywords: *Leptadenia hastata*, Acute toxicity, Sub-chronic toxicity, OECD; Hematological indices, Ethnomedicine.

Introduction

Traditional medicine is recognized as the most common form of alternative medicine. World Health Organization (WHO) estimates that 80 % of the world's population relies on these plant-based medicines as their primary health care intervention in developing countries.[1] Over the decades, the use of herbs as natural remedies in the treating health problems has been very successful and its historic usage has been useful in drug discovery and development.[2]

Leptadenia hastata belongs to the family Asclepiadaceae widely used in Tropical Africa as food and medicinal plants.[3] The major chemical compounds found in *L. hastata* are: triterpenes, fatty acids, amino acids, lutein and β-carotene.[4] The plant is medicinally important in the treatment of many ailments, used to ease labour, back pain, scorpion bite, scabies, sexual impotency, hypertension and skin diseases. [5,6,7] This plant appears to be benign with lack of toxicity and used in the treatment of evil spirit, psychiatric disorders, loss of consciousness, hallucination and urinary ailments.[3,5,8] Among the low income earners, leafy vegetables contribute much in alleviating the protein deficiency symptoms associated with starchy diets and so play a vital role in providing the health and well-being of the people.[9]Vegetables not only provide important components such as ascorbic acids, β-carotene and folic acid that play important role in pregnancy, but are also sources of bioactive compounds and high fiber diets that are directly associated with the prevention of disorders such as diabetes, cardiovascular disease and gastrointestinal carcinoma.[10] However, with the increase in the use of medicinal plants globally, either for primary treatment or as complementary and alternative medicine, safety and efficacy of these medicinal plants have become a public health concern.[11] Hence, the toxicity assessment of plants with proven therapeutic use is of ultimate importance. Thus, the present study aimed to evaluate the safety of methanol leaf extract of *Leptadenia hastata* (LHME) through acute and sub-chronic toxicity studies.

*Corresponding author. E mail: harunaibrahim81@yahoo.com, ihsan0193@nwu.edu.ng
Tel: +2348036858682

Citation: Sani IH, Abubakar AR, Yaro AH, Malami S. Acute and Sub-Chronic Toxicity Studies on the Methanol Leaf Extract of *Leptadenia hastata* in Wistar Rats. Trop J Nat Prod Res. 2019; 3(10): 302-306. doi.org/10.26538/tjnpr/v3i10.1

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

Materials and Methods

Collection of plant material

The leaf of *Leptadenia hastata* was collected from Kumbotso town, Kano State, Nigeria in November 2017. It was authenticated by a botanist at the Department of Biological Sciences Herbarium, Bayero University, Kano with a voucher specimen (BUKHAN 0248).

Preparation of plant extract

Fresh leaves of *Leptadenia hastata* were dried under shade for three weeks after which they were powdered using a mortar and pestle and sieved until a fine powder that weighed 435g was produced. The powdered plant material was cold macerated with 2.5 L 70% v/v methanol with constant shaking for 5 days and then filtered using Whatman filter paper No 1. The filtrate was then concentrated to dryness in an oven at 45°C. The resulting extract (methanol leaf extract of *Leptadenia hastata* – LHME) was then kept in a desiccator until further use.

Experimental animals
Female Wistar rats weighing 108–120g were used for the study. They were obtained and maintained in the Animal House Facility of the Department of Pharmacology and Therapeutics, Bayero University Kano (BUK). The animals were randomized into experimental and control groups and housed six (6) per group in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellets as basal diet and water *ad libitum*. Ethical clearance was obtained from Bayero University, ethics committee on Animal research (BUK/CHS/REC/VII/53). All experimental protocols were in compliance with the guidelines provided by the committee.

Phytochemical screening
The methanol leaf extract of *Leptadenia hastata* (LHME) was subjected to phytochemical tests for the detection of various phytochemical constituents.[12]

Acute Toxicity Study
The oral median lethal dose (LD_{50}) was determined using the Organization for Economic Co-operation and Development (OECD) guidelines in rats.[13] Three rats fasted 3 h prior to dosing in the experiment and doses calculated according to the fasted body weight. Food was further withheld for 1-2 h after which the extract was administered orally. The limit test was conducted in two stages. In the first stage, 5000 mg/kg was used for one rat and observed for 48 h. On survival, the second stage was carried out with two additional rats. Animals were observed during the first 30 minutes of treatment and then occasionally within 24 h, and finally daily for 14 days. Animals were monitored for tremors, convulsions, salivation, diarrhoea, sleep, behavioural changes and coma.

Sub-chronic Toxicity Study
This was performed according to the Organization of Economic Co-operation and Development guideline (OECD) for testing of chemicals.[14] Twenty-four (24) rats were randomly assigned into 4 groups of 6 rats each. Group 1, the control group was given distilled water (dose) while groups 2, 3 and 4 were orally administered with 250, 500 and 1000 mg/kg of the plant extract (LHME) respectively daily for 28 days. The weight of the rats in each group was determined and documented weekly. Also, signs of toxicity such as body weight, mortality, food and water and intake were monitored. After 28 days, all surviving animals have fasted overnight and on 29th day, the animals were euthanized with chloroform and blood was collected through cardiac puncture. An aliquot (2 mL) of the blood was collected into ethylene diamine tetra-acetic acid (EDTA) bottle and was used for the analysis of haematological parameters. Another 5 mL of the blood was collected in non-heparinized bottles, centrifuged at 1000 r/min for 10 minutes and the resulting serum was aspirated and used for biochemical analysis. The animals were quickly dissected and the brain, heart, kidney, liver and lungs were excised and weighed to determine the relative organ weights.

Weekly body weight
The body weight of each rat was assessed at different times during the study including; during the acclimatization period, and before the commencement of each dosing, once weekly and finally on the day of sacrifice.

Mortality and clinical signs
During the four-week dosing period, animals were observed daily for clinical signs and mortality patterns once before dosing, immediately after dosing and up to 4 h after dosing.

Relative organ weight
On the 29th day, all the animals were euthanized with chloroform. Organs namely; the brain, heart, kidney, liver and lungs were carefully dissected out and weighed in grams. The relative organ weight of each animal was then calculated as follows:

$$Relative\ organ\ weight\ = \frac{Absolute\ organ\ weight\ (g)}{Body\ weight\ (g)} \times 100$$

Determination of haematological parameters
Blood sample was collected through cardiac puncture into EDTA containing tubes and analyzed using Sysmex SF-XE-21N Automated Haematological Analyzer. The haematological parameters analyzed include: Red Blood Cell (RBC), Packed Cell Volume (PCV), White Blood Cell (WBC), Haemoglobin (Hb), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC).[15]

Biochemical estimations
Blood collected in non-heparinized tubes were centrifuged at 1000 r/min for 10 min. The serum separated was analyzed for various parameters such as creatinine, urea was done using BS-200 biochemistry autoanalyzer.

Statistical analysis
Results were expressed as Mean ± Standard Error of the Mean (SEM) in the form of tables. Statistical analysis for the difference between means were carried out using One-Way and Repeated Measures Analysis of Variance (ANOVA) followed by Bonferroni's post hoc test. Values of $p< 0.05$ were considered statistically significant.

Results and Discussion

Medicinal plants have maintained high popularity worldwide, especially in developing nations and this is evidenced by the rapid increase in their use.[16,17] Thus, there are many medicinal plant products available, but only a few have been scientifically evaluated and found to be non-toxic to man while for a vast majority their efficacy and safety are still questioned.[18] Hence, the toxicity assessment of plants with proven therapeutic use is of ultimate importance. The present study aimed to evaluate the safety of the methanol leaf extract of *Leptadenia hastata* (LHME) through acute and sub-chronic toxicity studies.
Therapeutic benefits of traditional remedies depend upon one or a combination of phytochemical constituents found present in the plants. However, the phytochemical constituents of the methanol leaf extract of *Leptadenia hastata* revealed the presence of alkaloids, flavonoids, tannins, glycosides, saponins and steroids.

Acute toxicity study
Toxicity data are required to predict the safety of a medicinal product before use.[11]The oral median lethal dose of LHME in mice was estimated to be >5000 mg/kg body weight. The administration of 5000 mg/kg body weight to rats did not result in the death of any of the animals after the 14-days experimental period. Therefore, the oral median lethal dose (LD_{50}) of LHME in the experimental rats was estimated to be higher than 5000 mg/kg. There were no clinical signs of acute toxicity salivation, piloerection, urinary incontinence and defecation) and central nervous system toxicity (ptosis, drowsiness, locomotor, tremors and convulsion) among rats administered 5000 mg/kg body weight of LHME.

Table 1: Preliminary phytochemical constituents of the methanol leaf extract of *Leptadenia hastata*

Phytoconstituents	Inference
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	+
Glycosides	+
Steroids	+
Anthraquinones	-

Key: + = Present, - = absent

Sub-chronic toxicity studies

Following repeated administration of toxicants (ingestion, inhalation or parenteral), toxicants are transported by the blood to various organs including the brain, heart, liver, lung and kidney where they may eventually cause harmful effects to these organ systems.[11] However, after repeated administration of LHME for 28 days no deaths were recorded, no significant clinically relevant changes were observed in general behaviour and other physiological activities in this study.

Effect of oral administration of Leptadenia hastata on body and organ weight

The effects of 28 days oral administration of LHME on body and organ weight, the percentage change in mean body weight with a period of treatment, and organ/body weight ratio at the termination of treatment in control and treated groups are shown in (Table 2). The results show no significant difference (p>0.05) in body weight changes and organ weights between control and treated groups with time.

Body and relative organ weights provide information on the effect of a compound on an animal's sensitivity to toxicity, physiologic dysfunction and organ damage.[19] However, the assessment of haematological and biochemical parameters provides an insight into possible damage brought about by the LHME in the renal and liver functions. In toxicity studies, assessment of renal and liver functions is vital because both organs are essential for the survival of the animals.[20]

Effect of oral administration of Leptadenia hastata on Haematological indices

The effects of 28 days oral administration of LHME on haematological indices at the termination of treatment are shown in (Table 3). The results show that there was no significant difference in all parameters measured between control and treated groups.

Blood can act as a pathological and physiological indicator of animal health.[21]In this study, there were no significant increase or decrease in all the haematological parameters evaluated that can indicate pathological conditions which may be caused due to exposure to toxicants. Red blood cell indices measured includes MCV, MCH and MCHC. These aid in the diagnosis of the cause of anaemia in the exposed animals. The values obtained for MCV were indicative of normocytic red blood cells. Similarly, the MCH and MCHC readings also reflected a normochromic red blood cell. Thus, no significant difference between the control and study groups in terms of size and haemoglobin level of red blood cells.

Normocytic hypochromic anaemia can occur which could be attributed to the increased destruction of the red blood cells by the toxicants beyond the production capacity of the bone marrow and the fall in the levels of the body iron content.[22]

Biochemical studies

The effects of 28 days oral administration of LHME on biochemical parameters at the termination of treatment are shown in Tables 3, 4 and 5. The results of serum biochemical indices indicate that there were no significant differences (p>0.05) in all parameters measured between control and treated groups.

Effect of oral administration of Leptadenia hastata on renal function indices

The effects of 28 days oral administration of LHMEon Renal function parameters at the termination of treatment as shown in (Table 4). The results show that there was no significant difference in all parameters measured between control and treated groups.

Effect of oral administration of Leptadenia hastata on Liver function indices

The effect of 28 days of oral administration of LHMEon liver function parameters at the termination of treatment is shown in (Table 5). The results show that there was no significant difference (p>0.05) in the levels of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in the treated rats when compared with the control. Similarly, no significant difference (p>0.05) in total protein (TP), Albumin (ALB), total bilirubin (TB) and direct bilirubin (DB) in the treated rats when compared with the control.

Effect of oral administration of Leptadenia hastata on Blood glucose levels

The effects of 28 days of oral administration of LHME on blood glucose level on a weekly basis is shown in (Table 6). The results show that there was no significant difference in weekly fasting blood sugar (FBS) levels measured over the period of treatment between control and treated groups.

Oral administration of LHME for 28 days did not produce any clinical signs of toxicity or mortality in the animals used in this study. There was no significant reduction in food and water intake of the treated rats in the study; suggests that LHME is well tolerated by the rats, and thus did not in any way alter the normal physiology of the rats.

Table 2: Effect of 28 days oral administration of methanol leaf extract of *Leptadenia hastata* on body weight, organ weight and a relative organ-to-body weight ratio of Wistar rats.

Parameters	**Treatment (mg/kg)**			
	Control	**LHME (250)**	**LHME (500)**	**LHME (1000)**
Initial Weight (g)	102.50 ± 1.31	106.00 ± 3.93	104.00 ± 2.65	100.33 ± 0.33
Final Weight (g)	156.67 ± 3.06	147.00 ± 7.76	148.50 ± 2.06	158.83 ± 5.88
Weight Change (g)	54.17 ± 4.03	41.00 ± 5.08	44.50 ± 3.23	58.60 ± 7.54
Weight Change (%)	53.09 ± 4.44	38.56 ± 4.43	43.21 ± 3.83	58.47 ± 7.65
Brain (g)	1.42 ± 0.04	1.39 ± 0.08	1.55 ± 0.07	1.51 ± 0.11
Heart (g)	0.65 ± 0.02	0.61 ± 0.04	0.75 ± 0.17	0.62 ± 0.40
Kidneys (g)	0.97 ± 0.06	0.94 ± 0.04	0.92 ± 0.03	1.09 ± 0.08
Liver (g)	6.47 ± 0.18	6.92 ± 0.23	6.16 ± 0.25	6.08 ± 0.23
Lungs (g)	1.63 ± 0.12	1.58 ± 0.20	1.48 ± 0.18	1.56 ± 0.11
Brain (%)	0.91 ± 0.02	0.95 ± 0.06	1.04 ± 0.05	0.95 ± 0.07
Heart (%)	0.41 ± 0.01	0.41 ± 0.02	0.51 ± 0.12	0.39 ± 0.03
Kidneys (%)	0.62 ± 0.04	0.64 ± 0.03	0.62 ± 0.02	0.69 ± 0.05
Liver (%)	4.13 ± 0.12	4.71 ± 0.16	4.15 ± 0.17	3.83 ± 0.14
Lungs (%)	1.04 ± 0.08	1.07 ± 0.14	0.99 ± 0.12	0.98 ± 0.07

Data represented as Mean ± SEM, analyzed using one-way ANOVA followed by Bonferroni test as the post hoc: with no significant difference between control and treated groups. Control = distilled water (10 mL/kg), n = 6, LHME = *Leptadenia hastata* methanol extract.

Table 3: Effect of 28 days of oral administration of methanol leaf extract of *Leptadenia hastata* on some Haematological indices of Wistar rats.

Parameters	**Treatment (mg/kg)**			
	Control	**LHME (250)**	**LHME (500)**	**LHME (1000)**
WBC (10^3/uL)	5.40 ± 0.23	4.13 ± 0.31	4.45 ± 0.17	4.85 ± 0.19
LYMPH#	6.45 ± 0.29	6.23 ± 0.28	6.15 ± 0.18	5.98 ± 0.18
MID#	0.58 ± 0.025	0.68 ± 0.25	0.58 ± 0.10	0.80 ± 0.15
GRAN#	1.58 ± 0.10	1.78 ± 0.13	1.60 ± 0.04	1.23 ± 0.11
LYMPH%	62.08 ± 2.42	60.43 ± 0.92	63.43 ± 1.61	65.15 ± 1.83
MID%	5.53 ± 0.73	5.63 ± 0.65	6.75 ± 0.88	3.98 ± 2.15
GRAN%	32.70 ± 2.41	33.98 ± 0.88	29.65 ± 1.03	27.30 ± 2.82
RBC (10^6/uL)	4.21 ± 0.11	4.65 ± 0.13	4.58 ± 0.22	4.70 ± 0.27
HGB (g/dL)	13.85 ± 0.93	13.03 ± 1.08	15.18 ± 0.59	14.70 ± 0.92
HCT (10^3/uL)	41.50 ± 3.01	37.75 ± 3.35	44.75 ± 1.79	43.15 ± 2.79
MCV (fL)	90.75 ± 2.66	88.25 ± 0.59	88.53 ± 0.44	88.73 ± 0.47
MCH (pg)	28.55 ± 1.99	30.75 ± 0.11	32.35 ± 1.89	30.80 ± 0.73
MCHC (g/dL)	34.03 ± 1.30	33.55 ± 0.58	32.38 ± 1.02	33.75 ± 0.89
PLT (10^3/uL)	243.33 ± 23.97	322.43 ± 34.87	251.00 ± 27.97	263.75 ± 36.59

Data represented as Mean ± SEM, analyzed using one-way ANOVA followed by Bonferroni post hoc test with no significant difference between control and treated groups. Control = distilled water (10 mL/kg), n = 6, LHME = *Leptadenia hastata* methanol extract. WBC = White Blood Cell, LYMPH# = Lymphocyte Count, MID# = Monocyte Count, GRAN# = Granulocyte Count, LYMPH% = Percentage Lymphocytes, MID% = Monocyte Percentage, GRAN% = Percentage Granulocyte, RBC = Red Blood Cells, HGB = Hemoglobin, HCT = Hematocript, MCV = Mean Corpuscular Volume, MCH = Mean Corpus Hemoglobin, MCHC = Mean Corpuscular Hemoglobin Concentration, and PLT = Platelets Count.

Table 4: Effect of 28 days of oral administration of methanol leaf extract of *Leptadenia hastata* on renal function test of Wistar rats

Parameters	**Treatment (mg/kg)**			
	Control	**LHME (250)**	**LHME (500)**	**LHME (1000)**
Urea (mg/dL)	31.25 ± 2.29	32.25 ± 2.66	33.75 ± 5.23	22.25 ± 3.17
Sodium (mmol/L)	119.42 ± 12.55	129.093 ± 12.73	120.37 ± 9.52	134.33 ± 6.21
Potassium (mmol/L)	8.96 ± 1.05	9.64 ± 0.65	11.49 ± 1.34	11.00 ± 0.55
Creatinine (meq/L)	0.75 ± 0.09	0.88 ± 0.08	0.88 ± 0.07	0.78 ± 0.08
Chloride (mg/dL)	24.50 ± 2.63	21.50 ± 2.39	21.25 ± 2.25	22.75 ± 2.46
Bicarbonate (mg/dL)	83.50 ± 5.33	80.00 ± 3.94	85.75 ± 2.29	85.50 ± 2.72

Data represented as Mean ± SEM, using one-way ANOVA followed by Bonferroni post hoc test with no significant difference between control and treated groups. Control = distilled water (10 mL/kg), n = 6, LHME = *Leptadenia hastata* methanol extract.

Table 5: Effect of 28 days of oral administration of methanol leaf extract of *Leptadenia hastata* on liver function indices of Wistar rats

Parameters	**Treatment (mg/kg)**			
	Control	**LHME (250)**	**LHME (500)**	**LHME (1000)**
ALT (iu/L)	20.75 ± 1.03	25.50 ± 2.59	22.75 ± 2.17	26.50 ± 4.01
AST (iu/L)	27.25 ± 5.31	40.25 ± 3.25	40.00 ± 0.70	35.25 ± 6.26
ALP (iu/L)	25.06 ± 3.57	17.79 ± 1.38	29.37 ± 6.87	32.54 ± 6.68
TP (g/dL)	6.19 ± 0.64	6.47 ± 0.39	5.68 ± 0.38	6.16 ± 0.72
ALB (g/dL)	3.64 ± 0.64	2.53 ± 0.11	3.19 ± 0.57	2.88 ± 0.11
TB (mmol/L)	7.47 ± 0.55	7.46 ± 0.25	7.78 ± 0.60	6.99 ± 0.53
DB (mmol/L)	5.39 ± 0.25	4.99 ± 0.66	5.28 ± 0.39	5.29 ± 0.18

Data represented Mean ± SEM, no significant difference in liver function parameters between the control and treated groups comparisons were made by using a one-way ANOVA followed by Bonferroni test as the post hoc: with no significant difference between control and treated groups. Control = distilled water, n = 6, LHME = *Leptadenia hastata* methanol extract, ALT = Alanine Aminotransferase, AST = Aspatate Aminotransferase, ALP = Alkaline Phosphatase, TP = Total Protein, ALB = Albumin, TB = Total Bilirubin, DB = Direct Bilirubin

Table 6: Effect of 28 days of oral administration of methanol leaf extract of *Leptadenia hastata* on blood glucose levels (mg/dL) of Wistar rats

Treatment (mg/kg)	**Day** 0	**Day** 7	**Day** 14	**Day** 21	**Day** 28
Control	94.33 ± 22.90	108.17 ± 8.26	114.33 ± 11.02	77.83 ± 7.55	72.67 ± 7.53
LHME (250)	93.17 ± 7.88	99.17 ± 6.79	110.67 ± 10.39	79.00 ± 6.66	79.33 ± 6.02
LHME (500)	88.17 ± 10.03	102.50 ± 9.07	102.67 ± 6.83	86.33 ± 11.52	73.33 ± 12.98
LHME (1000)	97.00 ± 16.42	111.00 ± 15.62	100.83 ± 5.78	82.50. ± 5.68	76.50 ± 9.02

Data represent as Mean ± SD (mg/dL), Following repeated measures ANOVA, no significant interaction was observed between the treatment and number of weeks in the blood glucose level of the rats. Control = distilled water, n = 6, LHME = *Leptadenia hastata* methanol extract.

Conclusion

The results from this study showed that acute and sub-chronic oral administration of LHME at doses of 250, 500 and 1000 mg/kg did not cause signs of toxicity or death in the animals. LHME can thus be considered to be safe and non-toxic at the doses used in the present study. Further investigation into its chronic toxicity may further elucidate the toxicity profile of *Leptadenia hastata* plant.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

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

Acknowledgements

The authors acknowledge the assistance of the laboratory staff, Mr Aliyu Ahmed of the Department of Pharmacology and Therapeutics, Bayero University, Kano.

References

1. World Health Organization. Epilepsy: aetiology, epidemiology. 2012. Retrieved from http://www.whoint/media centre/Fact sheet No 999. Accessed 21st July 2017.
2. Patrick-Iwuanyanwu KC, Amadi U, Charles A, Ayalogu EO. Evaluation of acute and sub-chronic oral toxicity study of baker cleansers bitters -a Polyherbal drug on experimental rats. EXCLI Journal. 2012; 11:632-640.
3. Burkill HM. The Useful Plants of West Tropical Africa. (2nd ed.). Royal Botanic Gardens. Kew, UK. 1985. 228-232 p.
4. Aliero AA and Wara SH. Validating the medicinal potential of *Leptadenia hastata*. Afr J Pharm Pharmacol. 2009; 3:335-338.
5. Hussain HSN, Karatela YY. Traditional Medicinal Plants used by Hausa Tribe of Kano State of Nigeria. Int J Crude Drug Res. 1989; 27(7):211-216.
6. Salamatu D. Medicinal Plants of Nigeria: Nigeria Natural Medicine Development Agency. Federal Ministry of Science and Technology. 2009: 21 p.
7. Dambatta SH and Aliyu BS. A survey of major ethnomedicinal plants of Kano North Nigeria, their knowledge and uses by traditional healers. Bayero JPure Appl Sci. 2011; 4:28-34.
8. Kinda PT, Zerbo P, Guenné S, Compaoré M, Ciobica A, Kiendrebeogo M. Medicinal Plants Used for Neuropsychiatric Disorders Treatment in the Hauts Bassins region of Burkina Faso. Med. 2017; (4)32:1-21.
9. Badi SH, Dabeli N, Jibung GG. Medicinal value of vegetable consumption among Berom pregnant women. Asian J Med Sci. 2012; 4(3):86-88.
10. Flavia MVF, Elen LG, Sandra MB, Maricelma SS, Patricia CSB, Claudemir GM, *et al*. Effect of *Psidium quajava* on the metabolic profile of Wistar rats. J Med Plants Res. 2012; 6(18):3450-3454.
11. Chanda S, Parekh J, Vaghasiya Y, Dave R, Baravalia Y, Nair R. A Review: Medicinal Plants - From Traditional Use to Toxicity Assessment. Int J Pharm Sci Res 2015; 6(7):2652-2670.
12. Evans WC. Trease and Evans Pharmacognosy London Villiere Tindal UK; 1996. 57 p.
13. OECD, Organisation for Economic Cooperation and Development. Guidelines for the Testing of Chemicals/Section 4: Health Effects Test No. 420: Acute oral toxicity - Fixed Dose Procedure: Organization for Economic Cooperation and Development; 2001. 1-14 p.
14. OECD. OECD guidelines for the testing of chemicals. Test No. 407: repeated dose 28-day oral toxicity study in rodents. Paris: OECD Publishing; 2008. 10 p.
15. Stiene-Martin EA, Lotspeich-Steininger CA, Koepe JA. Clinical Haematology (2nd ed.) Lippincott. New York.SF-XE-21N Operation Manual; 1999. 45 p.
16. Ogbonnia SO, Mbaka GO, Anyika EN, Osegbo OM, Igbokwe NH. Evaluation of acute toxicity of hydro-ethanolic extract of *chromolaena odorata* (L.) king and Robinson (*Fam. Asteraceae*) in rats. Agric Biol J North Am. 2010; 1:859-865.
17. Sani IH, Sulaiman I, Iliyasu Z, Abdussalam US, Adzim, MKR. Antioxidant Potential of *Phoenix dactylifera Linn* Extract and its Effects on Calcium Channel Antagonist in the Treatment of Withdrawal Syndrome in Morphine Dependent Rats. Trop J Nat Prod Res. 2018; 2(7):309-313.
18. Cheng CW, Bian ZX and Wu TX. A systematic review of Chinese herbal medicine for functional constipation.World J Gastroenterol. 2009; 15:4886–4895.
19. Michael B, Yano B, Sellers RS, Morton D, Roome N, Johnson JK, Schafer K, Pitsch S. "Evaluation of organ weights for rodent and non-rodent toxicity studies: a review of regulatory guidelines and a survey of current practices," Toxicol Pathol. 2007; 35(5):742–750.
20. Olorunnisola OS, Bradley G, Afolayan AJ, "Acute and sub-chronic toxicity studies of methanolic extract of *Tulbaghiaviolacea* rhizomes in Wistar rats," Afr J Biotechnol. 2012; 11:14934–14940.
21. Jorum OH, Piero NM, Machocho AK. Haematological Effects of Dichloromethane-Methanolic Leaf Extracts of *Carissa edulis* (Forssk.) Vahl in Normal Rat Models. J Hematol Thromboembolic Dis. 2016; 5:2.
22. Holy B, Kenanagha B, Onwuli DO. Haemato-pathological effects of dichlorovos on blood picture and liver cells of albino rats. J Toxicol Environ Health Sci. 2015; 7:18–23.
23. Kanu KC, Ijioma SN, Atiata O. Haematological, Biochemical and Antioxidant Changes in Wistar Rats Exposed to Dichlorvos Based Insecticide Formulation Used in Southeast Nigeria. J Toxics. 2016; 4(28):1-8.