EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES

http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 1
130-140
Year: 2018

ASSESSMENT OF *IN VITRO* ANTIOXIDANT ACTIVITY, ACUTE AND SUB-ACUTE TOXICITY OF AQUEOUS EXTRACT (DECOCTION) OF *TRICLISIA GILLETII* (DE WILD.) STANER (MENISPERMACEAE) STEM BARK IN WISTAR RATS

Dr. Cimanga Kanyanga R.*[1,3], Kikweta Munduku C.[1], Mbamu Maya B.[2], Nsaka Lumpu S.[1], Tshodi Ehata M.[1], Bakana Phongi D.[1], Opota Onya D.[2], Kambu Kabangu, O.[1], Vlietinck A. J.[3], Pieters L.[3]

[1]Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Laboratory of Pharmacognosy and Phytochemistry, University of Kinshasa, P.O. BOX 212, Kinshasa XI, Democratic Republic of Congo.
[2]Department of Galenic Pharmacy and Medecines Analysis, Faculty of Pharmaceutical Sciences, Laboratory of Galenic Pharmacy, University of Kinshasa, P.O. BOX 212, Kinshasa XI, Democratic Republic of Congo.
[3]Department of Pharmaceutical Sciences, Natural Products & Food Research and Analysis (NaturA), University of Antwerp, Universiteistplein 1, B-2610, Antwerp, Belgium.

***Corresponding Author: Dr. Cimanga Kanyanga R.**
Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Laboratory of Pharmacognosy and Phytochemistry, University of Kinshasa, P.O. BOX 212, Kinshasa XI, Democratic Republic of Congo.

Article Received on 15/11/2017 **Article Revised on 06/12/2017** **Article Accepted on 27/12/2017**

ABSTRACT

The present study was undertaken to evaluate the antioxidative activity, the acute and sub-acute toxicity of aqueous extract of *Triclisia gilletii* stem bark in experimental animals. Wistar rats in acute toxicity were used and given single oral dose of 1000, 2000 and 5000 mg/kg body weight respectively and were observed for 28 days for toxic effects. Oral administration of aqueous extract at all oral doses did not induced any mortality or other toxic symptoms. Therefore the lethal dose 50 (LD50) of the extract was estimated to be more than 5000 g/kg body weight. The body weight of treated animals with all oral doses showed significant increase ($p <0.05$) compared to untreated animals as also observed in sub-acute toxicity via oral route in animals orally received daily doses of 200, 400 and 800 mg/kg body weight of the extract. The concentrations of hematocrit, RBC, RWC, hemoglobin in treated rats receiving 5000 mg/kg of aqueous extract of *T. gilletii* stem bark did not show significant statistically differences compared to untreated animals. Only the platelets level in treated rats showed significant increase in treated rats compared to untreated rats, but its value was within normal physiological ranges.[8,9,40,47] The increase of platelets number by the administration of the aqueous extract AE-1 of *T. gilletii* stem bark showed that the extract has no anticoagulant properties. Significant reduction of glucose in treated animals compared to untreated animal was observed and this effect is due to the hypoglycemic **properties** of the extract. The administration of aqueous extract at the highest oral dose of 5000 mg/kg body weight revealed slight increase or decrease of haematological and biochemical parameters with no significant difference. From these results, it was concluded aqueous extract of ***T. gilletii*** stem bark by oral route was considered to be practically non-toxic since the extract was found to be safe and well tolerated by animals.

KEYWORDS: *Triclisia gilletii*, Menispermaceae, stem bark, aqueous extract, antioxidative activity, acute and subacute toxicity.

INTRODUCTION

The use of different medicinal plants prepared and dispensed by herbalists for the treatment of diseases is very common in some rural and urban communities in many developing countries. Their pharmacological effects are proven in different laboratories and are due to the presence of various secondary metabolites (active organic compounds) belonging to different phytochemical groups. Thus medicinal plants remain the principal source of active natural metabolites which contribute in traditional medicine for treating a number of various ailments.

Herbal medicines has received greater attention as an alternative to clinical therapy and the demand for these remedies has currently increased.[1,2] It well known that 80% of the world's population relies on traditional medicine for health care delivery[3,4] since the access to traditional medicine is very easy and shows better compatibility for economic and social reasons.[1,5]

Nowadays, several studies on many medicinal plants need to be evaluated for their toxicity. The data of the acute, sub-acute and sub-chronic toxicity studies should

be obtained in order to increase the confidence in their safety and tolerability to animals and humans.[1,6-17]

The organization of Economic and Cooperation Development (OECD) defines acute toxicity as the adverse effects occurring within a short time of oral administration of a single dose of a substance or multiple doses given within 24 h. It defines the sub-acute toxicity as the adverse effects occurring as result of the repeated daily oral dosing of a chemical to experimental animal for part (not exceeding 10%) of the life span. It gives valuable information on the cumulative toxicity of a substance on forget and physiological organs at low dose, prolonged exposure and wide variety of adverse effects can be detected.[15]

Hence, systematic scientific studies of medicinal plants should include a trough toxicity study with the hope that the obtained results would provide information on their safety and tolerability prior to the evaluation of their therapeutic efficacy in humans.

Triclisia gilletii is a medicinal plant largely used in traditional medicine to treat various ailments.[18,19] Taking account of the current use of aqueous extract of the stem bark (decoction) of this medicinal plant in traditional medicine to treat various **ailments**, the present study deals with the assessment of antioxidative activity, acute and sub-acute toxicity of aqueous extract (decoction) of ***T. gilletii*** stem bark as the typical traditional preparation used by people.

2. MATERIALS AND METHODS

2.1. Plant material

Stem barks of *Triclisia gilletii* (De Wild) Staner also called *Triclisia dictyophylla* Diels were collected in Kinshasa (Democratic Republic of Congo) in May 2011, the plant was identified by Mr. Nlandu Lukebiako, B. of the Institut National d'Etudes et des Recherches en Agronomie (INERA), Faculty of Sciences, Department of Biology, University of Kinshasa. A voucher specimen (NL29052011TGSB) has been deposited in the herbarium of this institute.

The plant part was dried at room temperature and reduced to powder by pulverization using an electronic blender.

Triclisia gilletii **(De Wils) Staner, Menispermaceae.**

2.2. Preparation of the aqueous extract

A decoction of *T. gilletii* stem barks (20 g) was prepared by mixing with 150 ml distilled water and heated on a hoteplate for 15 min. After cooling and filtration, the filtrate was evaporated *in vacuo* yielding corresponding dried aqueous extract denoted as Extract AE-1 (13.32 g).

2.2. Qualitative Phytochemical screening

The phytochemical screening was carried out by using TLC on precoated silica gel plates (thickness layer 0.25 mm, Merck) using with different mobile phases and chemical reagents described in the literature for the identification of major phytochemical groups such as alkaloids, flavonoids, anthraquinones, terpenes, steroids, coumarins and proanthocyanidins.

The Froth test, hydrochloric acid/*iso*-amyilic alcohol, Stiasny's reagent (formol + conc. HCl) were used for the identification of saponins, anthocyanins and tannins, respectively.[20]

2.3. DPPH radical scavenging assay

The antioxidant activity of the aqueous extract and its fractions from *T.* ***gilletii*** stem bark was evaluated on the basis of the radical scavenging effect of the stable DPPH free radical according to method previously described by[21] with minor modifications. Ascorbic acid (Vitamin C) was used as reference product. Briefly, 1 ml of the test sample (5 mg of extract or fractions dissolved in 5 ml water/ ethanol 9:1) or standard diluted twofold to have test concentrations of 1,85 - 500 µg/ml was mixed with 1 ml of DPPH methanolic solution (4 mg DPPH in 100 ml methanol) in test tube. After incubation at 37°C for 30 minutes in a dark room, the absorbance of each test solution was determined at 571 nm. The percentage of free radical scavenging inhibitory effect of DPPH by test samples was calculated according to the following formula:

% Inhibition = Ac - Ats/Ac X 100

Where Ac was the absorbance of control (DDPH) and Ats the absorbance of the test sample.

The IC50 value of each test sample was graphically determined (n=3).

2.4. ABTS radical cation scavening assay

The antioxidant activity of samples from *T. gilletii* stem was evaluated by the improved ATBS+ radical cation scavenging ability as previously described by[22] with slight modifications. ABTS+ radical cation was produced by mixing 7 mM 2,2'-azido-bis (3- ethylbenzothiozoline-6 sulfonic acid) diamonium salt (ABTS) and 2.45 mM potassium persulfate (K2S2O3) incubated at room temperature in dark for 24 h. To determine the ABTS radical scavenging activity, 4 ml of ATBS solution was mixed **thoroughly** with 1 ml of different concentrations (3.9 to 250 µg/ml) of test samples. The reaction was allowed to stand at room temperature for 6 min in dark

and the absorbance was immediately measured at 734 nm. Appropriate blank or negative control (ABTS solution without test sample) was used. Vitamin C was used as a reference antioxidant product (n= 3). The percentage inhibition in absorbance was calculated using the following formula:

% Inhibition = ODnc- ODts/ODnc x 100

Where ODnc is the absorbance of the negative control and ODts the absorbance of the test sample.

The IC50 of each sample was graphically determined (n= 3)

2.3. Acute toxicity

The acute toxicity of the aqueous extract of *T. gilletii* stem bark (AE-1) was evaluated in Wistar rats according to the procedure described by the Organization for Economic Cooperation and Development (OECD) guideline for testing chemicals, TG420.[23] Animals (body weight: 140– 150 g bw, aged 8–10 weeks of either sex) were divided into three groups:
Group I (5 rats) orally received 5 ml distilled water as the negative control group.

Groups II, III and IV (10 rats for each oral dose) received orally single oral dose of 1000, 2000 and 5000 mg/kg body weight of aqueous extract respectively. The animals were observed for toxic symptoms continuously for the first 4 h dosing and were daily weighed. Finally, all animals were then maintained in daily observation and the number of toxic effects and survivor was recorded for further 28 days.

2.5. Sub-acute toxicity

The sub-acute toxicity of the aqueous extract of *T. gilletii* was evaluated according to the procedure described by.[15,24] Briefly, Wistar rats (145-160 g bw, aged 8-10 weeks of either sex) were used and divided into four groups. Group I (5 rats) orally received daily normal saline solution (NaCl 0.9%) as the negative control group. Groups II, III and IV (10 rats for each oral dose) orally received daily 200, 400 and 800 mg of the extract for 28 days. Animals were observed for symptoms, behavior, alteration, digestive troubles, food and water intake. The body weight was daily recorded. They were observed twice daily for mortality during 28 days period of the investigation.

2.6. Biochemical and hematological parameters analysis

Blood from rats having received 5 g/kg in acute toxicity test was collected from tail vein on Day 28 for analysis. For biochemical parameters, blood was centrifuged at 4000g for 5 min to obtain plasma, which was stored at –20°C: glucose, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum glutamic purivic transaminase (SGPT), serum glutamo-oxaloacetate transaminase (SGOT), uric acid, total cholesterol, triglycerides, high–density lipoproteins (HDL), low–density lipoproteins (LDL), total and direct bilirubin were quantified using Architect (Abbott) automation with Boehringen Ingelheima biochemical kits. Total proteins were estimated using Biuret's method. Hematological parameters analysis was carried out using an automatic hematological analyzer (Coulter STK, Beckham) with appropriate kits. The differential leucocyte count was performed with an optical microscopy after staining and, in each case, 100 cells were counted.

For mineral elements, 10 ml of blood of animals having received 1000 and 5000 mg/kg of aqueous extract were collected and incinerated at 450°C for 24 h in a mufleand acid digest. The material for analysis was prepared by oxidizing sample with nitric/perchloric acids 2:1. The concentrations of minerals were determined with flame atomic absorption spectrophotometer (Perkin-Elmer 2880 Model) and the inorganic phosphorous was estimated by phosphomolybdovanate method.[25]

2.7. Histopathological study

The histopathological study of vital organs such as heart, kidney, liver, spleen, large intestine and lungs was carried out according to the procedure previously described by[26] after acute toxicity. The vital organ pieces (5-8 µm) were removed after sacrification of treated animals with 5000 mg of extract and fixed in 10% formalin for 24 h and washed with distilled water 24h. After dehydration in an autotechnicon, the cleared organs were embedded by passing through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtone and the slides were stained with haemtoxylin/eosin and observed under electronic microscopic. The dried organs were weighed.

2.8. Statistical analysis

The values were expressed as mean ± SEM from three separate experiments. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Student-t's test to analyze the differences among IC50 values of various tested samples for antioxidant assays. P values < 0.05 were considered as significant.

3. RESULTS AND DISCUSSION

3.1. Phytochemical screening

Results of this study revealed the presence of alkaloids, steroids and terpenes, aminated compounds, saponins, coumarins, reducing sugars and polyphenolic compounds such flavonoids and tannins (gallic, cathechic and proaanthocyanidins) in the aqueous extract.

Anthocyanins, anthraquinones and cardiotonic heterosids (glycosids) were not detected in our experiment conditions in aqueous extract and its fractions in the present study. Our results are in good agreement with.[27]

3.2. DPPH radical scavenging activity

The free radical scavenging activity of *T. gilletii* stem bark aqueous extract and soluble fractions was evaluated by their ability to reduce in vitro DPPH (1,1-diphenyl-2-picryhydrazl), a free stable radical. This method depends on the reduction of purple DPPH radical by antioxidant agents to a yellow colored diphenylpicrylhydrazine and the remaining DPPH radical that show maximum absorption at 517 nm as measured.[28,29] For the present study, the following criteria were adopted to appreciate more the level of antioxidant activity against DPPH and ABTS radicals: IC50 ≤ 10 µg/ml: strong activity, 10 < IC50 ≤ 30 µg/ml: good activity, 30 < IC50 ≤ 50: weak activity,, 50 < IC50 ≤ 100: very weak activity, IC50 100 µg/ml: inactive.

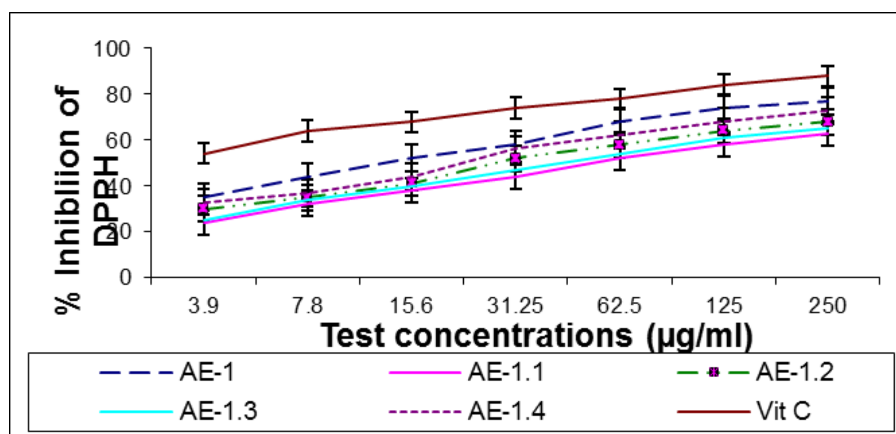
Results from the present study indicated that aqueous extract AE-1exhibited strong antioxidant activity against DPPH with IC50 value of 9.75 ± 0.2 µg/ml fractions AE-1.2 and AE-1.4 rich respectively in flavonoids and phenolic compounds showed good activity (16 < IC50 < 20 µg/ml). Fractions AE-1.1 and AE-1.3 rich in terpenes and steroids, and saponins respectively exhibited low activity (Table 1).

Table 1: DPPH and ABTS radical scavenging activity of *T. gilletii* stem bark samples (IC_{50}, µg/ml).

Code samples	DPPH	ABTS
AE-1	9.75 ± 0.50	11.44 ± 0.24
AE-1.1	39.06 ± 0.31	52.08 ± 0.41
AE-1.2	19.50 ± 0.22	23.92 ± 0.62
AE-1.3	33.85 ± 0.30	45.83 ± 0.11
AE-1.4	16.90 ± 0.73	21.84 ± 0.85
Vitamin C	< 39	< 39

AE-1: aqueous extract (decoction), AE-1.1, AE-1.2, AE-1.3 and AE-1.4: chloroform, ethylacetate, *n*-butanol and aqueous soluble fractions from the partition of Extract AE-1.

In figure 1, at the highest tested concentration of 250 µg/ml, it was shown that acid ascorbic produced 83% inhibition of DPPH, aqueous extract 75% and fractions showed between 57 and 67% inhibition of this radical with the **highest percentage** inhibition of AE-1.4 fraction (Fig.1). Results in Table 1 and figure 1 indicated that the aqueous extract had high antioxidant activity compared to its fractions. This finding suggested a probable existence of synergistic effect between different components present in the crude extract.[27] Moreover,the antioxidant capacity of various samples of *T. gilletii* stem bark was found to decrease in the following order: AE-1 > AE-1.4 > AE-1.2 > AE-1.1 > AE-1.3 (Fig.1 and Table 1).



See: Vit C: Vitamin C, Table 1,

Figure 1: Inhibition percentages of different samples from *T. gilletii* stem bark against DPPH radical at tested concentrations. AE-1; aqueous extract, AE-1.1 to AE-1.4: chloroform, ethylacetate, n-butanol and residual aqueous phase respectively from the partition of extract AE-1.

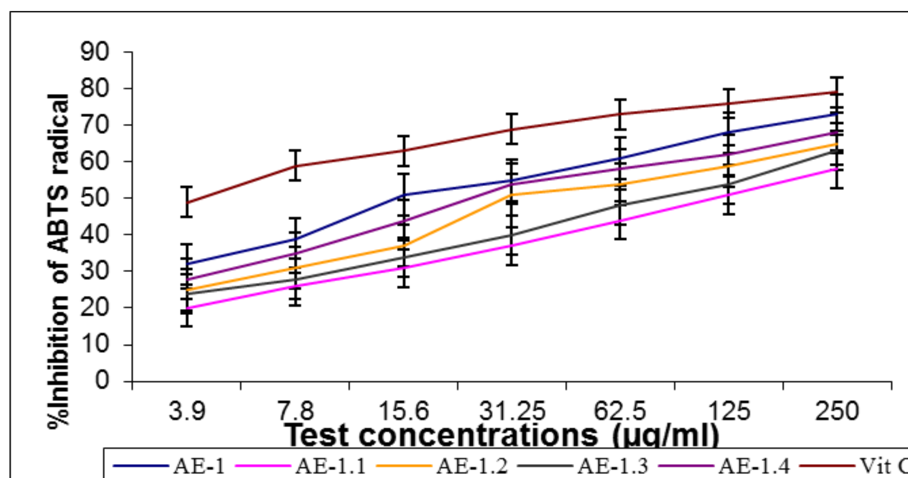


Fig. 2: Inhibition percentages of different samples from *T. gilletii* stem bark against ABTS radical at tested concentrations.

See Table 1 and Fig. 1

In addition, the reduction level in the number of DPPH molecule by these samples can be correlated with the presence of available phytochemical constituents mainly flavonoids, steroids, terpenes, saponins and phenols in respective sample. Some of them such as phenolic compounds are powerful chain breaking antioxidants, effect due to their hydroxyl group.[27]

3.3. ABTS scavenging activity

The ABTS assay is based on the inhibition of the radical cation ABTS+, which has a characteristic long wavelength absorption.[30] Its chemistry involves direct generation of ABTS radical mono cation with no involvement of any intermediary radical.[22] Against this radical, aqueous extract AE-1 exhibited good **activity** with IC_{50} value of 11.44 ±0.4 µg/ml, fractions **AE**-1.2 and AE-1.4 showed good activity (21 < IC50 < 24 µg/ml), fractions **AE**-1.1 and **AE**-1.1 exhibited low activity (Table 1).

The radical scavenging activity of Extract AE-1 was ranging from 32.09 to 73.16% and from 20.66 to 68.38% for soluble fractions when tested at concentrations from 3.9 to 250 µg/ml (Fig. 2). The ABTS scavenging activity of soluble fractions from Extract AE-1 was ranging from 20.17 to 68.54 % with the residual aqueous soluble fraction as the most active (IC50 = 21.84 ±0.85) followed by the ethyl acetate\ soluble fraction (IC50 = 39.57 ± 0.7). The wide range of antioxidant activity displayed by these samples may be attributed to the presence of a wide variety of bioactive compounds of different nature (phenols, steroids, terpenes, saponins, tannins, alkaloids) al already mentioned above.

The ABTS radical scavenging activity of Extract AE-1 from *T. gilletii* stem bark and its derived soluble fractions can be ranked as AE-1 > AE-1.4 > AE-1.2 > AE-1.3 > AE-1.1

(Fig.2 and Table 1). In both antioxidant activity assays, the radical scavenging effect of all samples from *T. gilletii* stem bark was lesser than that of Vitamin C used as a reference antioxidant product (Table 1 and Fig. 1 and 2). All samples displayed these biological activities in dose dependent manner (Fig.1 and 2) and showed their capacity to donate electron to neutralize free radicals and form a stable product.[21] The markedly strong ($p < 0.05$) antioxidant response of Extract AE-1, and good activity of AE-1.2 and AE-1.4 soluble fractions might be helpful in characterizing good sources of natural antioxidant reactions. The highest and lowest activity against both radicals for all tested samples was seen at 3.9 and 250 µg/ml respectively (Fig. 1 and 2). This finding showed the capacity of these tested samples from *T. gilletii* stem bark to scavenge different free radicals suggesting that they may be useful therapeutic agents for treating radical-related pathological diseases. For both antioxidant activities, our results are only qualitatively in good agreement with other reported studies.[21,27,28]

3.4. Acute toxicity of Extract AE-1 of T. gilletii stem bark

The acute toxicity investigation of the aqueous extract of ***T. gilletii*** stem bark revealed that no sign of toxic effects such as alteration of the locomotion activity, no changes in behaviour, physiological activities, gastrointestinal disturbances appearance, sensory nervous system responses or other abnormalities in treated animals with a single oral dose respectively of 500, 1000, and 5000 mg/kg in acute toxicity were observed after 28 days of observation.

Moreover, at high dose, crude medicinal plant extracts may be metabolised to a toxic product, which could interfere with gastric function and decreased food conversion efficiency.[31] Interestingly, this was not observed in the present study. The effect of the Extract AE-1 on the body weight variation was significantly remarkable ($p < 0.05$) in the treated animals receiving all tested oral doses because normal body weight gains were observed in treated animal groups during the period of treatment compared to negative control group (Fig. 3) and could be attributed to the nutritive components in

Extract AE-1 of *T. gilletii.*[32,33] According to,[34] the progressive increase in body weight during the period of treatment may indicate the improvement of the nutritional state of animals and the observed increase of body weights might be attributed to the appetite stimulation of the extract on the animal[1] and could be a result of increased food and water intake since, the significant increment in food and water intake is considered as being responsible for the increment of body weight gain.[30]

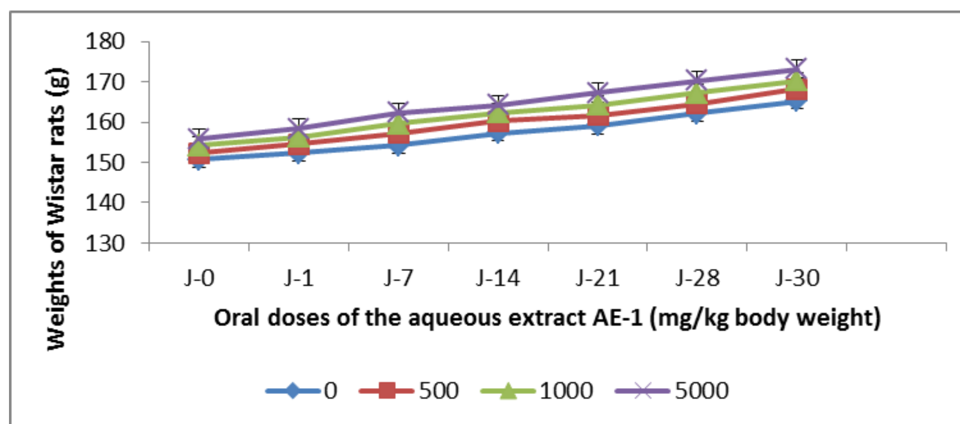


Fig. 3: Effect of extract AE-1 of *T. gilletii* on body weights of Wistar rats in acute toxicity. ***NC: negative control, AE-1: aqueous extract.***

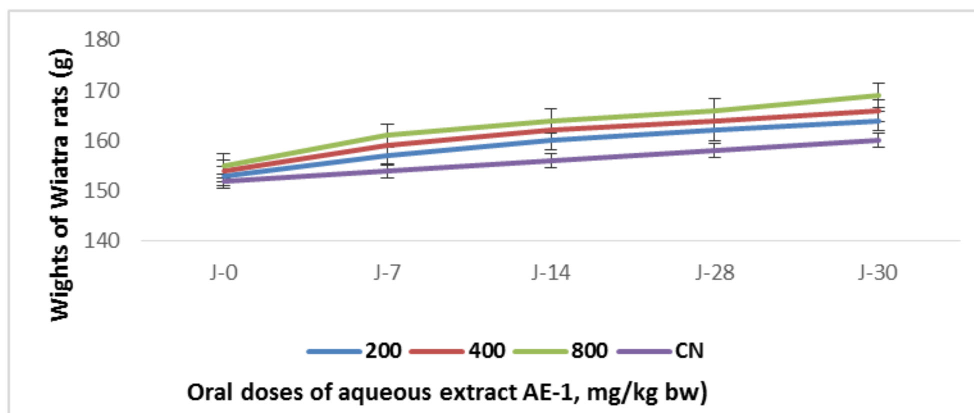


Fig. 4. Effects of extract AE-1 of *T. gilletii* on body weights of Wistar rats in sub-acute toxicity. NC: negative control.

No death of animals was observed after 28 days of observation. Therefore, the LD50 of the extract was estimated to be greater than 5000 mg/kg body weight. Thus, according to[23] and,[35] substances that present LD50 higher than 2000 mg/kg or 5000 mg/kg body weight respectively via oral route, may be considered non-toxic and this suggested that the aqueous extract of *T. gilletii* stem bark is practically non-toxic by oral route.

3.5. Sub-acute toxicity of Extract AE-1 of T. gilletii stem bark

In this test, it was observed that the animals fed the aqueous extract of ***T. gilletii*** stem bark at all administered oral doses were healthy. No unusual changes in behaviours, locomotion activity as well as no ataxis and no others signs of intoxication were observed during the 28-Day period of observation. There was no significant difference in food and water consumption between treated and untreated animals, but animals which have received daily aqueous extract at oral doses of 200, 400 and 800 mg/kg gained body weight compared to the negative control group (Fig.4). No death of animals was recorded at all administered oral doses in this toxicity test.

3.6. Effect of Extract AE-1 of T. gilletii stem bark on some hematological parameters

The concentrations of evaluated hematological parameters are presented in Table 2.

Table 2: Effect of the aqueous extract AE-1 of *T. gill*etii stem bark on some hematological parameters at oral dose of 5000 mg/kg body weight.

Parameters	Negative control	*T. gilletii*: 5000 mg/kg	*Reference values* (8,9, 40, 47)
RBC (x 10^6 µL^{-1})	8.5 ± 0.7	10.2 ± 0.5	7.6-10.29
Hemoglobin (g/dL)	16.6 ± 0.2	17.8 ± 0.2	15-18.2
Hematocrit (%)	47.2 ± 0.3	48.9 ± 2.2	40.7-50
Platelets (x 10^3 µL^{-1})	1261.0 ± 0.6	1387.2 ± 0.2	995-1713
WBC (x 10^3 µL^{-1})	15.7 ± 0.3	16.9 ± 0.5	6.6-20.5
Neutrophils (%)	21.2 ± 0.3	22.4 ± 1.3	3-24.7
Basophils (%)	0.0±0.0	0.0 ± 0.0	0.0 ± 0.0
Eosinophils (%)	0.9 ± 0.1	1.5 ± 0.6	0-2
Lympocytes (%)	87.2 ± 1.6	91.3 ± 0.1	58.8-94
Monocytes (%)	3.0 ± 1.1	3.5 ± 1.2	0-4
Segmented leucocytes s (%)	16.6 ± 0.6	23.2 ± 2.2	-

RBC: Red blood cells, WBC: White blood cells.

Table 3: Effect of the aqueous extract (AE-1) of *T. gill*etii stem bark on biochemical parameters at oral dose of 5 g/kg body weight.

Parameters	Negative control	Extract AE-1 (5000 mg/kg)
Glucose (mg/dL)	242.5 ± 0.4	235.3 ± 1.4
Creatinine (mg/dL)	0.87 ± 0.05	0.86 ± 0.02
AST (IU/L)	177.6 ± 0.3	178.2 ± 0.5
ALT (IU/L)	50.2 ± 2.2	49.5 ± 1.2
Total cholesterol (mg/dL)	101.2 ± 1.3	100.6 ± 2.2
Triglycerides (mg/dL)	43.7 ± 1.8	43.3 ± 3.5
Total bilirubin (mg/dL)	0.5 ± 0.1	0.4 ± 0.7
Direct bilirubin (mg/dL)	0.2 ± 0.0	0.2 ± 0.0
Total Proteins (g/dL)	7.6 ± 0.3	8.1 ± 1.1
Albumin (g/dL)	3.4 ± 0.5	3.5 ± 0.6
ALP (IU/L)	145.4 ± 1.6	146.3 ± 2.4
HDL- cholesterol (mg/dL)	62.3 ± 1.3	63.6 ± 1.3
LDL- cholesterol (mg/dL)	37.5 ± 2.1	36.2 ± 0.4
Uric acid (mg/dL)	1.91 ± 0.1	2.1 ± 0.5
SGOT (IU/L)	127.3 ± 1.6	126.4 ± 0.2
SGPT (IU/L)	30.7 ± 2.3	32.1 ± 1.2
Urea (mmol/L)	5.1 ± 0.8	6.9 ± 1.6

AST: aspartate aminotransferase or aspartate transaminase, ALT: alanine aminotransferase or alanine transaminase, ALP: alkaline phosphate, HDL: hight-density lipoproteins, LDL: low-density lipoproteins, SGOT: serum glutamate oxaloacetate transaminase, SGPT: serum glutamic pyruvic transaminase.

Results showed an increase in the haemoglobin and red blood cells (RBC) concentration in treated rat group at the highest administered oral dose of 5000 mg/kg bw, and showed significant difference for both parameters compared to that seen in untreated group (p < This increase might be due to the increased absorption of iron and to the immunopotentiating effect of the extract as also previously reported for some medicinal plant extracts.[36]

The concentration of haematocrit did not show significant changes compared to control group ($p > 0.05$) while it was observed significant increase of WBC and platelets in treated animals compared to untreated group ($p < 0.001$) and significant difference was deduced. The recorded the concentration values of these haematological parameters remained in the acceptable limits (Table 2).[8,9] Segmented leucocytes are the first line of cellular defense that respond to infectious agents, tissue injury or inflammation process.[39] Furthermore, in the present study, significant increases were observed in neutrophils, lymphocytes, monocytes and eosinophils in treated animals compared to the negative control ($p < 0.05$) and their concentration remained in the acceptable limits.

3.7. Effect of Extract AE-1 of T. gilletii stem bark on biochemical parameters

The serum biochemical and clinical biochemistry analyses were carried out to evaluate the possible alteration of the hepatic and renal function of treated rats by the administered extract. Liver and kidney function analysis is very important in the toxicity assessment of drugs and medicinal plant extracts as they are both necessary for the survival of an organism.[36]

Results indicated that the oral administration of the extract at the highest oral dose of 5g/kg body weight in

acute toxicity induced significant decrease of the concentration of glucose in treated group compared to untreated group ($p < 0.05$). This decrease may be due to the hypoglycaemic potentials of the extract as also previously reported for other medicinal plant extracts.[8,9,24,38]

ALT and AST are two liver enzymes associated with hepatocellular damage and are thus indicators of liver damage. Although both AST and ALT are common liver enzymes, only ALT is remarkably specific for liver function and AST is mostly present in a wide variety of tissues including the heart, the myocardium, skeletal muscle, kidneys, liver and Brain.[24,41] The analysis of these parameters is important since there are several reports of liver and kidneys toxicity related to the use of phytotherapeutic products.[24,42,43] Results reported here indicated that there was slight increase of the concentration of both enzymes, but there was no significant difference compared to the negative control ($p > 0.05$). The concentrations of biochemical parameters of treated and untreated Wistar rats are presented in Table 3.

According to,[1,33,44] this finding implies that the extract at the tested oral dose of 5g/kg bw may not cause damage to the organs cited above and no deleterious effects of other organs in treated animals was observed as reported for other medicinal plant extracts.[8,9,14] Therefore, it suggested that hepatocytes of the treated rats were not damaged, and the hepatic and renal functions of the treated animals were maintained. The concentration of creatinine, SGPT and SGOT of treated groups did not show significant difference compared to untreated groups ($p > 0.05$), and support this observation, because these biochemical parameters are also considered as indicators for good renal and hepatic functions.[14,24]

Serum ALP is a sensitive detector for intrahepatic and extrahepatic bile obstruction.

From the obtained results, no significant difference in the concentration level of ALP in treated rat groups compared to untreated group (Table 2). As the presence of infiltrative diseases of the liver and all bones diseases is associated with osteoplastic activity,[46] it is likely that the oral dose used in this study for the aqueous extract of *T. gilletii* stem bark did not abnormally interfere with the calcification or metabolic activities involving the liver. This finding is in good agreement with[33,47] concerning the effect of other plant extracts on ALP concentration level in animals. The non-significant changes in ALT, AST and ALP in treated animals compared to untreated group suggested that the administration of Extract AE-of *T. gilletii* stem bark did not affect the hepatic and renal functions in treated animals. Our finding is in good agreement with.[8,9,24,30,33]

The concentration of ALT, AST, creatinine, SGPT, SGOT and ALP reported in the present study in treated animals suggested that the administered aqueous extract **AE-1** of *T. gilletii* stem bark did not affect the hepatic function. The same conclusion can be made for the renal function. Our observation is in good agreement with other studies on various medicinal plant extracts for their effects on these functions.[8,9,15,16,30]

The observed slight decrease of the concentration of cholesterol, LDL and triglycerides ($p < 0.05$) in treated rat group compared to untreated rat group, may be due to the hypolipidemic properties of the extract and sometimes to the increase of the secretion of thyroid hormones T3 and T4.[45,46] Also significant increase in HDL concentration ($p < 0.05$) in treated animals compared to untreated animals was observed, but a statistically significant difference was not deduced ($p > 0.05$). These results suggest that the extract can be beneficial in preventing cardiovascular risk factors which contribute to the death of mainly diabetic patients.[16,38,39]

Albumin is a protein with high concentration in plasma. Since it is produced in the liver, its decrease in serum may arise from liver and kidney diseases.[43] Fortunately, this was not observed in the treated rats in the present study, the level of albumin in treated animals was comparable to that of untreated rat groups and did not show a statistically significant difference ($p > 0.05$). In addition, there was not significant changes in the concentrations of the total and direct bilirubin in treated animals compared to control groups ($p > 0.05$). , The level of total proteins significantly increased in treated rats compared to untreated group ($p < 0.05$) suggesting an increase from external supply or increased synthesis in the liver.

The urea concentration significantly increased at used oral dose in treated group compared to untreated group ($p < 0.01$), but this last observation was not found as a sign of deficiency renal because its concentration remained within the normal limits (2.5-7.5 mmol/L).[8,9] As urea production in mammals occurs specially in liver, its concentration level could also be used as an indicator of hepatic function.[40] Thus, our results more suggested and confirmed good state of hepatic function of treated animals as already demonstrated with the concentration levels of other hepatic biomarkers cited above. In general, all concentrations of hematological and biochemical parameters evaluated in the present study were within the normal physiological ranges.[8,9,47,48]

2.10: Effects of the aqueous extract (AE-1) of T. gilletii stem bark on electrolytes and weights of organs of Wistar rats

Table 4 shows the effects of the aqueous extract of *T.* ***gilletii*** (5000 mg/kg bw) on some electrolytes. Results showed that the administration of the extract at the highest oral dose of 5000 mg/kg bw induced a significant increase of calcium, chloride, iron, potassium and

sodium in treated animals compared to untreated animals ($p < 0.05$) and significant difference between both groups was deduced ($p < 0.05$).

Significant difference in decrease ($p < 0.05$) of inorganic phosphor concentration was also observed in treated animals compared to untreated animals (Table 4). In addition, no significant difference between the weights of all organs of treated animals compared to untreated animals was observed at all administrated oral doses ($p > 0.05$) (Table 5). This observation suggested that the extract has no significant effect on these animal organs and mainly on the normal growth of treated animals. Study on histopathological examination of vital organs showed normal architecture suggesting no morphological disturbances in treated group compared to untreated group. Our finding is in good agreement with.[2,49]

Table 4: Effect of the aqueous extract (AE-1) of *T. gill*etii stem bark on some hematological parameters at oral dose of 5000 mg/kg body weight.

Parameters	Negative control	*T. gilletii*: 5000 mg/kg	*Reference values* (8,9, 40, 47)
RBC (x 10^6 μL^{-1})	8.5 ± 0.7	10.2 ± 0.5	7.6-10.29
Hemoglobin (g/dL)	16.6 ± 0.2	17.8 ± 0.2	15-18.2
Hematocrit (%)	47.2 ± 0.3	48.9 ± 2.2	40.7-50
Platelets (x 10^3 μL^{-1})	1261.0 ± 0.6	1387.2 ± 0.2	995-1713
WBC (x 10^3 μL^{-1})	15.7 ± 0.3	16.9 ± 0.5	6.6-20.5
Neutrophils (%)	21.2 ± 0.3	22.4 ± 1.3	3-24.7
Basophils (%)	0.0±0.0	0.0 ± 0.0	0.0 ± 0.0
Eosinophils (%)	0.9 ± 0.1	1.5 ± 0.6	0-2
Lympocytes (%)	87.2 ± 1.6	91.3 ± 0.1	58.8-94
Monocytes (%)	3.0 ± 1.1	3.5 ± 1.2	0-4
Segmented leucocytes s (%)	16.6 ± 0.6	23.2 ± 2.2	-

RBC: Red blood cells, WBC: White blood cells

Table 5: Effect of the aqueous extract (AE-1) of *T. gill*etii stem bark on biochemical parameters at oral dose of 5000 mg/kg body weight.

Parameters	Negative control	Extract AE-1 (5000 mg/kg)
Glucose (mg/dL)	242.5 ± 0.4	235.3 ± 1.4
Creatinine (mg/dL)	0.87 ± 0.05	0.86 ± 0.02
AST (IU/L)	177.6 ± 0.3	178.2 ± 0.5
ALT (IU/L)	50.2 ± 2.2	49.5 ± 1.2
Total cholesterol (mg/dL)	101.2 ± 1.3	100.6 ± 2.2
Triglycerides (mg/dL)	43.7 ± 1.8	43.3 ± 3.5
Total bilirubin (mg/dL)	0.5 ± 0.1	0.4 ± 0.7
Direct bilirubin (mg/dL)	0.2 ± 0.0	0.2 ± 0.0
Total Proteins (g/dL)	7.6 ± 0.3	8.1 ± 1.1
Albumin (g/dL)	3.4 ± 0.5	3.5 ± 0.6
ALP (IU/L)	145.4 ± 1.6	146.3 ± 2.4
HDL- cholesterol (mg/dL)	62.3 ± 1.3	63.6 ± 1.3
LDL- cholesterol (mg/dL)	37.5 ± 2.1	36.2 ± 0.4
Uric acid (mg/dL)	1.91 ± 0.1	2.1 ± 0.5
SGOT (IU/L)	127.3 ± 1.6	126.4 ± 0.2
SGPT (IU/L)	30.7 ± 2.3	32.1 ± 1.2
Urea (mmol/L)	5.1 ± 0.8	6.9 ± 1.6

AST: aspartate aminotransferase or aspartate transaminase, ALT: alanine aminotransferase or alanine transaminase, ALP: alkaline phosphate, HDL: hight-density lipoproteins, LDL: low-density lipoproteins, SGOT: serum glutamate oxaloacetate transaminase, SGPT: serum glutamic pyruvic transaminase

4. CONCLUSION

In conclusion, this is the first report of acute and sub-acute toxicity of aqueous extract of ***T. gilletii*** stem bark. The extract was considered as safe and well tolerated in animals without toxic effects and did not induced mortality in animals. It was found to have no significant influence on the concentration of biochemical and hematological parameters. It caused significant increase or decrease of some electrolytes in treated animals and did not significantly modify the weights of some organs of treated animals. Thus, these observations clearly demonstrates that the aqueous extract AE-1 is pratically non-toxic.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

CONFLICTS
The authors declare that there is no conflict of interests regarding the publication of the present paper.

REFERENCES

1. Ogbonnia SO, Olayenis SO, Anyika EN, Anurwu WN, Polayi OO. Evaluation of acute toxicity in mice and subchronic toxicity of hydroethanolic extract of *Parinari curatellifolia* Planch (Chrysobalanaceae) seeds in rats. Afr J Biotechnol, 2009; 8(9): 1800-18006.
2. Pillai PG, Suesh, P, Mishro G, Annapurnam. M. Evaluation of the acute and subacute toxicity of the methanolic leaf extract of *Plectranthus ambionicus* (Lour) Spreng in Balb C mice. Eur J Exp Biol, 2011; 1: 236-45.
3. Kamboji VP. Herbal medicine. Curr Sci, 2005; 78(1): 35-39.
4. Akah PA. Indigenous knowledge and medical practices in ethnopharmacology. *In* .Akah PA, (Ed). Research Stgnpost Kerala, India, 2008; 1-13.
5. Saha P, Kantio Haldar P, Mazumber VK, Suresh Ku RB. Evaluation of acute and subchronic toxicity of *Lagenaria siceraria* aerial parts. Int J Pharm Sci Res, 2011; 2(6): 1507-1512.
6. Anupata T, Manu C, Rajesh S. A 28-Day sub-acute toxicity study in Swiss albino mice to evaluate toxicity profile of neurotol plus (Mannitol and glycerol combination). Int J Biomed Sci, 2009; 5(4): 428-433.
7. Rajasekaran M, Kannabiran S. The study on acute, sub-acute toxicity and hematinic activity of *Nimilai chenduram* (Sidda formulation) in Wistar rats. Int J Pharm Res, 2012; 4(4): 1498-1503.
8. Nsaka Lumpu, S, Tshodi Ehata M, Kambu Kabangu, O., Tona Lutete G, Apers S, Pieters, L., Vlietinck, AJ, Cimanga Kanyanga R. Assessment of the safety and tolerability of the aqueous extract of the stem bark of *Alstonia congensis* Engl. (Apocynaceae) in Wistar rats. In J.N Govil (Ed.), *In* Recent Progress of Medicinal Plants, Volume 35: Phytoconstituents and Biochemical Processes, New Dehli-India, 2013; 201-210.
9. Cimanga Kanyanga, R., Tshodi Ehata M, Nsaka Lumpu S, Kikweta Munduku C, Mbenza Phuati A, Cos P, Apers S, Vlietinck AJ, Pieters L. Antiprotozoal and cytotoxic activities and the acute toxicity of extracts from *Brucea sumatrana* Roxb. (Simaroubaceae) stem bark collected in Mai-Ndombe, Democratic Republic of Congo. J. Pharmcogn. Phytother, 2015; 7(4): 35-44.
10. Hutadilok-Towatana N, Chatchar W, Reanmaghd W, Bunkrongkepo R. Acute and subchronic toxicity evaluation of the hydroethanolic extract of mangosteen pericarp. J. Med. Plants Res, 2010; 4(10): 969-974.
11. Mukinda JT, Eagle FK. Acute and sub-chronic oral toxicity of the aqueous extract of *Polygala fruticosa* in female mice and rats. J Ethnopharmacol, 2010; 128(1): 236-240.
12. Li X, Luo Y, Wang L, Li Y, Shi Y, Cui Y, Xue M. Acute and subacute toxicity of ethanol extracts from *Salvia przewalskii* Mawim in rodents. J. Ethnopharmacol, 2010; 131(1): 110-115.
13. Wazis CH, Anaka JA, Timothy SY, Zeze AU, Mohammed GT, Hussami IM. Acute toxicity and in-vivo effects of leaf extracts of *Byrsocarpus coccineus* Shum & Thonn in pregnant rat uterus. J Appl Pharm Sci, 2012; 2(12): 130-136.
14. Akeyemonglami OA, Makinde JM. Acute and sub-acute toxicity studies of the methanol extract of the leaves of *Paullinia pinnata* (Linn.) in Wistar albino mice and rats. Afr J Med Sci, 2013; 42(1): 81-90.
15. Gandliares, B, Kavimani S, Rajekapoor B. Acute and subacute toxicity study of methanolic extract of *Ceiba pentandra* (Lam.) Gaerth. on rats. J Sci Res, 2013; 5(2): 315-324.
16. Akpanabiattu MI, Ekpo NB, Ufoto, F, udoh NM, Akpan, EJ, Etuk EO. Acute toxicity, biochemical and hematological study of *Afromomun melegeuta* seed oil in male Wistar albino rats. J Ethnopharmacol, 2012; 150(2): 590-594.
17. Abdillah S, Citaz YP, Ahmad BW, Noor NM. Evaluation of antimalarial activity and acute toxicity of Xetospongia sp Am J Pharmacol Toxicol, 2013; 8(2): 83-88.
18. Adjanhoun EJ, Ehyi MRA, Aké Asi, Baniakina J, Chibon, P, Cusset G, Doulou V, Enzanza A, Eymé J, Goudoté E, Keita A, Mbemba C, Mollet J, Moutsamboté JM, Mpati J, Sita P. *Médecine Traditionnelle et Pharmacopée. Contribution aux études ethnobotaniques et floristiques en République Populaire du Congo*. Agence de Coopération Culturelle et Technique ISBN 92-9028 123-5. Paris, France, 1988; 245.
19. Neuwinger HD. *African Traditional Medicine. A Dictionary of Plant Use and Applications*. Medipharm Scientific Publishers, Stuttgart, 2000; 532-533.
20. Harborne JB. *Phytochemical Methods. A guide to modern techniques of plants analysis*. Chapman & Hall, London, 1998; 160-253.
21. Wangia CO, Orwa JA, Muregi FW, Kareru PG, Cheruiyot K, Kibet J. Coperative antioxidant activity of aqueous and organic extracts from Kenyan *Ruellia linearibracteolata* and *Ruellia bignoniiflora*. Eur J Med Plants, 2016; 17(1): 1-7.
22. Rajamurugan R, Deepa V, Sivashanmugam M, raghavan CM. Phytochemistry, antioxidant and antibacterial activities of medicinal plants - A comparative study. Int J Cur Res Rev, 2013; 5(2): 8-19.
23. Soni AP, Chauhan GN. Study of antioxidant and antimicrobial activity of medicinal plants utilized in cancer treatment. Res J Recent Sci, 2015; 4(1): 15-21.
24. The Organisation of Economic Co-operation Development (OECD). The OECD Guideline for the Testing Chemical: 407 Repeated Dose Oral

Toxicity-Rodent 28-Day or 14 Day Study. OECD, Paris, 2001; 1-7.

25. Kripa KG, Chamundeeswan O, Thanka J. Acute and sub-acute toxicity evaluation of ethanolic extract of *Leucas aspera* (Lamiaceae) in experimental rats. Int J Drug Dev Res, 2011; 3(3): 339-347.
26. AOAC. *Official Methods of Analysis* 15th edition in Herich (Ed.). Association of Official Analytical Chemists. Arlington, VA/Washington, DC, USA.
27. Biswas M, Kar B, Karan TK, Biattacharya S, Suresh Kumpar RB, Ghosh AK, Haldar PK. Acute and sub-chronic toxicity study of *Dregea volubilis* fruit in mice. J Phytol, 2010; 2(8): 6-10.
28. Padmanabhan P, Jangle SN. Evaluation of DPPH radical scavenging activity and reducing power of four selected medicinal plants and their combinations. Int J Pharm Sci Drug Res, 2012; 4(2): 143-146.
29. Pazhanisamy M, Abraham Immanuel Ebenezer G. Antioxidant activity of leaves of an important medicinal plant *Ormocarpum cochinchinese* (Lour.) Merr. J Modern Biotechnol, 2013; 2(5): 89-94.
30. Sanchez-Moreno C. Methods used to evaluate the free radical scavenging activity in foods and biological systems. Food Sci Tech Int, 8(1): 121-137.
31. Ping KY, Darah I, Chen Y, Screeramanam S, Sasidaharan S. Acute and subchronic toxicity study of *Euphorbia hirta* L. methanol extract in rats. Biomed res Int, 2013; doi: 10.1155/2013/182064.
32. Duke JA. *The Green Pharmacy: New Discoveries in Herbal Remedies for Common Diseases and Conditions from the World's Foremost Authorities on healing Herbs*. St Martins Press, 1997; 35-50.
33. Ezeonwumelu JOC, Julius AK, Muhoho, CN, Ajayi AA, Oyewale AA, Tanayen JK, Balogun, SO, Ibrahim A, Adzu B, Adiukwu CP, Oloro J, Kiplayat DM, Goji ADT, Okoruwa AG, Onchwesi AN, Erddy PMK. Biochemical and histological studies of aqueous of *Bidens pilosa* from Ugandan Rift Valley in rats. British J Pharmacol Toxicol, 2011; 2(6): 303-309.
34. Pieme CA, Penlap VN, Nkeyoum B, Taziebou CL, Tekuwu EM, Etoa FX, Ngongang J. Evaluation of acute and subacute toxicity of aqueous ethanolic extract of stem bark of *Senna alata* (L.) Roxb (Ceasalpiniaceae). Afr J Biotechnol, 2006; 5(3): 283-289.
35. Kennedy GLJr, e BL, Burgers BA. Estimation of acute oral toxicity in rats by determination of the approximative lethal dose rather the LD50. J. Appl. Toxicol, 1986; 4: 6(3): 145–148.
36. Olurunnisola OS, Braddly G, Afolayan AJ. Acute and sub-chronic toxicity studies of methanolic extract of *Thulbaghia violacea* rhizomes in Wistar rats. Afr J Biotechnol, 2012; 11(83): 14934-14940.
37. Ali KZ, Hazikawati H, Hutheyfa S, Mohd RS, Shanmugavelu S, Hemn HO. Acute and sub-acute dermal toxicity studies of *Morinda citrifolia* L. fruit extract in Sprague Dawley rats. Asian J Pharm Clin Res, 2005; 8(2): 400-408.
38. Rajeh MB, Kwam YP, Zakorin Z, Lasha LY. Acute toxicity impacts of *Euphorbia hirta* L extract on behavior, organs body weight index and histopathology of organs of the mice and *Artemia salina*. Pharmacogn Res, 2012; 4(3): 170–177.
39. Ogbonnia S, Adekunde AA, Bosa MK, Enwuru WN. Evaluation of acute and subacute toxicity of *Alstonia congensis* (Apocynaceae) bark and *Xylopia aethiopica* (Dunal) A. Rich (Annonaceae) fruits mixtures used in the treatment of diabetes. Afr J Biotechnol, 2008; 7(6): 703-705.
40. Wasan KM, Najafi, S, Wanh J, Kwong M. Assessing plasma lipid levels body weight and hepatic and renal toxicity following chronic oral administration of a water soluble phytostanol compound FM–VP4, to gerbils. J Pharmacol Sci, 2001; 4(3): 228–239.
41. Crook MA. *Clinical Chemistry and Metabolic Medicine*. 7**th** Edition. Hoker Arnold, London, 2006; 42-43.
42. Ozolua RI, Anaka O, Okpo S, Idogum S. Acute and sub-acute toxicological assessment of the aqueous seed extract of *Persea americana* Mill (Lauraceae) in rats. Afr J Compl Altern Med, 2009; 6: 573-578.
43. Lima LB, Vascunulos CFB, Maranhao HML, Leita PA, Ferreira PA, Andrale BL, Aranjo EL, Xavier HS, Lafayette SSL, Wanderly AG. Acute and subacute toxicity of *Schumus terenbinthifolius* bark extract. J. Ethnopharmacol, 2009; 126(3): 468-475.
44. Arüjo EJA, Sant'Ana DMG, Manilri, SL, Neto HHM. Hematological and biochemical parameters of rats subjected to hypotropic and hypertropic diet. Unuarana, 2005; 8(1): 139-146.
45. Vasudevan MM, Screekumari S. Textbook of Biochemistry (for Medical Su00dents). 4th Edition, Jaygne Bryhers. Medical Publishers (P) Ltd., New Dehli. India, ISBN, 81- 8061-369-0, 2005; 502-503.
46. Edem DO, Usoh IF. Biochemical changes in Wistar rats and oral doses of Mistletoe (*Loranthus micranthus*). Am J Pharm. Toxicol 2009: 4 (3) 94-97.
47. Barry SL. *Animal clinical pathology*. In Michael, J.D., Manfred, A.H. eds., CRC Handbook of Toxicology. CRC Press, Inc, USA, 1995: 517-537.
48. Feldamn, BV, Zinkji JG, Jain NC. *Scham's Veterinary Hematology*, 5th Edition. Les Febiger, Philadephia, 1997; 1210-1218.
49. Varadarason MM, Subramanian K, Gnanapragasam S, Quine SD, Subramani, K.et al. Evaluation of acute and subacute toxicity of ethanol extracts of *Cansjera rheedii* J. Gmelin (Opiliaceae) J Brew Dist, 2010; 1(1): 011-014.