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Research Article

Assessment of acute and subacute toxicological profiles of the aqueous extract (decoction) from the leaves of *Triclisia gilletii* (De Wild.) Staner (Menispermaceae) in Wstar rats

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Abstract: The present study was undertaken to evaluate the acute and subacute toxicity of the aqueous extra*ct of Triclisia gilletii leaves in experimental animals. Wistar rats in acute toxicity were used and given singleoral doses of 1 and 5 g/kg body weight respectively and were observed for 28 days. Results indicated that t*here was no evidence of the administered extract-induced mortality or other toxic symptoms in animals leading to consider the lethal dose 50 (LD_{50}) of the extract being greater than 5 g/kg body weight. These observations were also deduced during the subacute toxicity test where animals orally received daily doses of 100, 400 and 800 mg/kg body weight of the extract respectively. The concentration levels of all evaluated hematological parameters such hematocrit, RBC, RWC, hemoglobin in treated rats receiving orally 5g/kg of the aqueous extract of *T. gillettii* leaves did not show statistically significant difference compared to untreated animals. Only the concentration level of platelets in treated rats significantly increased in treated rats compared to untreated rats and a significant difference was deduced. For biochemical parameters, a significant reduction of the concentration level of glucosein treated animals compared to untreated animal was observed and this effect suggested the hypoglycemic and antidiabetic properties of the extract. A significant decrease of the concentration levels of cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerideurea and protein in treated animals compared to untreated animals was also observed, but did not show significant difference. On the other hand, it was observed slight increase or decrease of ALT, AST, PAL, SGOT, SGPT, albumin, total bilirubin concentration level according to the case in treated animals compared to untreated animals, but no significant difference was deduced. The extract caused significant increase or decrease of some electrolytes according to the case and did not affect the weight of some organs of treated animals compared to untreated animals. From these results, it was concluded that the acute and subacute toxicity of the aqueous extract of *T. gillettii* by oral route is practically null or neglected since the extract was found to havegood safe and was well tolerated by animals.

Keywords: *Triclisia gilletii*, Menispermaceae, aqueous extract, leaves, acute and subacute toxicity.

INTRODUCTION

Traditional use of plant medicines popularly known as herbal remedies for the treatment a series of various diseases is widely practiced not only in Democratic Republic of Congo, but also in other developing countries. Herbal medicines used in traditional medicine for the treating various diseases contain active organic natural compounds belonging to different chemical groups. Thus medicinal plants therefore remain the principal source of active natural metabolites which contribute in traditional medicine for treating a number of various ailments[1].

It well known that 80%of the world's population relive to traditional medicine for health care delivery[2,3]. Since the access to traditional medicine is very easy and show better compatibility for economic and social reasons[4, 5]

Nowadays, several studies are conducted on various medicinal plants belonging to different botanical families using scientific approaches to demonstrate their various biological activities of crude extracts claimed by practitioners [6, 7, 8,]. However other studies have been carried out to evaluate the acute, sub-acute, sub chronic or chronic toxicity of herbal medicine leading to prove their safe and

tolerability in animals and subconsequent in human [9, 10, 11, 12, 13].

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 the valuable information on the cumulative toxicity of a substance, forget organs, physiological organs and metabolic of a product at low dose prolonged exposure and wide variety of adverse effects can be detected [12].

Hence, systematic scientific studies of medicinal plants should include a trough toxicity study beforetheir use in the treating of any disease or disorder to confirm its safe and tolerability and to report some adverse effects if these occur after oral administration of the studied plant extracts.

Triclisia gillettii is a medicinal plant largely used in traditional medicine to treat various ailments. Its root, stem bark and leaves have some medicinal values. Concerning particularly the leaves, Musuyu Muganza et al. [22] have previously reported the antiprotozoal activity of the lyophilized aqueous extract of *T. gilletii* leaves (decoction) against *Trypanosoma brucei brucei*, *T. cruzi*, *Leishmania infantum* and chloroquine and pyrimethamine resistant K1 strain of *Plasmodium falciparum.* The extract was found to be active against all selected protozoa with IC_{50} values ranging from5.12 to 32 µg/ml with *L. infantum* as the resistant parasite (IC_{50} = 32 µg/ml). In addition, the extract was found to be non-cytotoxic against MRC-5 cell line (CC_{50} > 64 µg/ml. On the other hand, Kiku *et al.* [23] have also reported the antimalarial activity of the aqueous, 80% methanol and total alkaloid extracts *in vitro* against Congolese chloroquine-sensitive and chloroquine and pyrimethamine resistant K1 strain of *P. falciparum.* All tested extracts were found to inhibit the growth of both parasites with IC_{50} ranging from < 0.02 to 1.55 µg/ml with the 80% methanol and total alkaloid extracts as the most active samples. *In vivo* test against *Plasmodium berghei berghei* infecting Wistar rats, all extracts from *T. gilletti* leaves produced more than 70% chemosuppession. In cytotoxic testing against MRC-5 cell line, the aqueous extract and total alkaloid extracts were found to be cytotoxic against this cell line with cytotoxic concentration 50 (CC_{50}) values of 4.79 and 2.77 µg/ml respectively against MCR-5 cell line. This result was not in good agreement with Musuyu Muganza et al[22] concerning the effect of the aqueous extract against this cell line. Unfortunately, until now no active constituent of *T. gillettii* leaves for these biological activities is not yet known.

Taking account of the current use of the aqueous extract (decoction) of this medicinal plant in traditional medicine, the present study deals with the assessment of the acute and subacute toxicity of the aqueous extract (decoction) of *T. gillettii* leaves as the typical traditional preparation used by people.

MATERIALS AND METHODS

Plant material

Leaves of *Triclisia gilletii*(De Wild) Staner (Synonyme: *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 (NL29052011ACL) has been deposited in the herbarium of this institute. The plant part was dried at room temperature and reduced to powder.

Preparation of the aqueous extract

20 g of each powdered plant material were mixed with 150 ml distilled water. The mixture was boiled for 15 minutes. After cooling and filtration, the filtrate was evaporated *in vacuo* yielding corresponding dried extract denoted as AE-1 (5.32 g).

Phytochemical screening

The phytochemical screening was carried out by TLC on precoated silica gel plates (thickness layer 0.25 mm, Merck) using 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-amylic alchool, Stiasny's reagent (formol + conc. HCl) were used for the identification of saponins, anthocyanins and tannins respectively [14].

Acute toxicity

The acute toxicity of the aqueous extract of *T. gilletii* leaves (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 (OECD, 2001).Animals (body weight: 140– 150 g, aged 8–10 weeks, of either sex) were divided into three groups: Group I(2 rats) orally received 5 ml distilled water and constituted the negative control group. Groups II, III and IV(10 rats each) received orally single oral dose of 1, 2.5 and 5 g/kg body weight of the 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[1, 12, 15].

Subacute toxicity

The subacute toxicity of the aqueous extract of *T. gilletii* was evaluated according to the procedure described by Kripa *et al.* [15] and Gandlare *et al.* [12]. Briefly, Wistar rats were used and divided into four groups. Group I (2 rats) orally received daily normal saline solution (NaCl 0.9%) and constitute the negative control. Groups II, III and IV (10 rats each) 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.

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 (ASAT), alanine aminotransferase (ALAT), serum glutamopyruvate transferase (SGPT), serum glutamooxalate transferase (SGOT), uric acid, total cholesterol, triglycerides, high–density lipoproteins (HDL), low–density lipoproteins (LDL), total and direct bilirubin were quantified using Architect (Abottâ) automation with Boehringen Ingelheimâ biochemical kits. Total proteins were estimated using Biuret's method. Hematological parameters analysis was carried out using an automatic hematological analyzer (Coulter STK, Beckam) with appropriated 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 5g:kg of the aqueous extract was 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 concentration levels of minerals were determined with flame atomic absorption spectrophotometer (Perkin-Elmer2880 Model) and the inorganic phosphourous was estimated by phosmolybdovanate method.

Histopathological study

Histopatological study of vital organs such as heart, kidney, liver, spleen, large intestine and lungs was carried out according to the procedure previously described by Lima[27]. The organ pieces (5-8 µm) were fixed in 10% formalin for 24 h and washed in running distilled water for against 24h. After dehydratation in an autotechnicon,, the cleared organs were embeddedby passing through three cups containing moltenparaffin 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 haemtoxyllin-eosin and observed under electronic microscopic. The dried organs were weighted.

Statistical analysis

Results were expressed as the mean of parameters ± standards error of the mean (SE). Differences between means were evaluated using the Student-t test. ANNOVA tests to determine multiple comparisons were also used. Differences are significant at $p < 0.05$

RESULTS AND DISCUSSION

Phytochemical screening

Results of this study had revealed the presence of alkaloids, steroids and terpenes, amined compounds, saponins, coumarins, reductor sugars and polyphenolic compounds such flavonoidsand tannins (gallics, cathechic and proanthocyanidins). Anthocyanins, anthraquinones and glycosidic heterosids were not detected in our experiment conditions in *T. gilletii* leaves in the present study.

On the acute toxicity of the aqueous extract of T. gilletii leaves

The acute toxicity investigation of the aqueous extract of *T. gilletii* leaves revealed that no sign of toxic effects such as alteration of the locomotion activity change in behaviour, physiological activities, gastrointestinal disturbances appearence, sensory nervous system responsesor other abnormalities in treated animals with the oral dose of 5g/kgwere observed after 28 days of observation. There was no change in intake food and water consumption. The determination of these parameters seems to be important to the study of the safety of a therapeutic substance, as proper intake of nutrients and water which are essential to the physiological status of the treated animals and to the accomplishment of proper response to the product tested [17]. In this case,the effect of the extract on the body weight variation of the treated rats was significantly remarkable ($p < 0.05$) on the treated animals receiving all tested oral doses since they gained body weight compared to negative control groups. According to Pieme *et al.* [24], the progressive increase in body weight during the period of treatment may indicate the improvement of the nutritional state of animals and in some case, the observed increase of body weighs might be attributed to the appetite stimulation of the extract on the animal [1]. The growth response effect could be considered as a result of the increased food intake and water consumption. The death of animal was not observed after 28 days of observation. Therefore, the LD_{50} of the extract was estimated to be greater than 5.0 g/kg body weight. Thus, according to Kennedy *et al*., [25], substances that present LD_{50} higher than 5.0 g/kg body weight via oral route, may be considered as practically non-toxic and this suggested that the aqueous extract of *T. gilletii* is practically non-toxic by oral route.

On the subacute toxicity of the aqueous extract of T. gilletii leaves

In this test, it was observed that the animals fed the aqueous extract of *T. gilletii* leaves at all administered oral doses were healthy. No unusual changes in behaviour, locomotion activity as well as no ataxis and no signs of intoxication were observed during the 28-Days period of observation. There was no significant difference in intake food and consumption of water between treated and untreated animal, but animals which have received extracts at all tested oral doses gained body weight compared to the negative control group for the same reasons evoked above. No death of animal was recoded at all administered daily oral doses.

Effects of the aqueous extract of T. gilletii leaves on some hematological parameters

The hematological parameter profile is presented in Table 1. Results showed a little increase in the haemoglobin and red blood cells (RBC) concentration level in treated rat groups with the administered oral dose, but this did not show a significant difference for haemoglobin and significant difference for RBC compared to that seen in untreated groups ($p > 0.05$).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 plant extracts [18]. (Table 2).

The concentration level of haematocrit did not show significant change compared to control group ($p > 0.05$) while it was observed significant increase of WBC and platelets in treated animals compared to untreated group ($p < 0.001$), but the concentration values of these haematological parameters remained in the acceptable limits (Table1).The remaining evaluated hematological parameters shown slight increase or decrease in treated animals according to the case, with no significant difference compared to that seen in control group ($p > 0.05$). in general the reported concentration levels of these selected haematological parameters remanded in acceptable ranges (Table 1).

Table 1. Effects of the aqueous extract of *T. gill*etii leaves on the concentrations of some hematological parameters at oral dose of 5g/kg body weight

Parameters	Negative control	*T. gilletii*: 5g/kg	*Reference values*
RBC (x 10^6 μL^{-1})	8.50 ± 0.70	9.17 ± 0.55	7.6-10.29
Hemoglobin (g/dL)	16.6 ± 0.20	17.51 ± 0.22	15-18.2
Hematocrit (%)	47.22 ± 0.31	48.90 ± 2.23	40.7-50
Platelets (x 10^3 μL^{-1})	1261.04± 0.60	1387.20± 0.20	995-1713
WBC (x 10^3 μL^{-1})	15.71± 0.34	16.91± 0.52	6.6-20.50
Neutrophils (%)	21.23± 0.33	22.41± 1.33	3-24.70
Basophils (%)	0.00	0.01	0.0
Eosinophils (%)	0.90± 0.11	1.52± 0.65	0-2.00
Lympocytes (%)	87.24± 1.64	91.35± 0.12	58.8-94.00
Monocytes (%)	3.00± 1.14	3.57± 1.20	0-4.00
Segmented leucocytes s (%)	16.65 ± 0.62	23.28 ± 2.22	-

RBC ; red blood cells, WBC : white blood cells

Effects of the aqueous extract of T. gilletii leaves on some biochemical parameters

Table 2 shows the effects of the oral administration of the aqueous extract (decoction) of *T. gilletii* leaves on the concentration levels of some biochemical parameters of Wistar rats. 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 level of glucose in treated groups compared to untreated groups ($p<0.05$). This decrease may be du probably to the hypoglycaemic and antidiabetic properties of the extract as also previously reported for other medicinal plant extracts in animals [1, 7, 8, 15].

ALAT and ASAT are two liver enzymes associated in the hepatocellular damages and are thus considered as indicators of liver damages. The analysis of these parameters is important since there are several reports of liver and kidneys toxicity related to the use of phytotherapeutic produicts [15]. Results reported here indicated that there was slight increase of the concentration of these both enzymes, but no significant difference compared to the negative control was deduced ($p > 0.05$). According to Pieme et al.[24], Ogbonia et al.[1], this finding implies that the extract at the highest tested oral dose may not cause damages of the organs cited above. Based on this observation, it was suggested that hepatocytes of the treated rats were not damaged, the hepatic and renalfunctions of the treated animals were maintained safesince the extract does not possess significant deleterious effects in treated animals on these functions as also previously reported for other medicinal plant extracts in animals[7, 8, 11]

The concentration levels of creatinine, SGPT and SGOT of treated groups did not show a significant

difference compared to untreated groups ($p > 0.05$), and more support this above observation since these biochemical parameters are also considered as indicators for the good renal and hepatic functions [11, 15].

The observed slight decrease of the concentration level of cholesterol, LDL and triglyceride ($p < 0.05$)in treated rat groups compared to untreated rat groups, may be due to the hyperlipidimic properties of the extract and in some times to the increase of the secretion of thyroid hormones T3 and T4 [26], and also a significant increase in HDL concentrations ($p < 0.05$) 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 has some beneficial effects by reducing cardiovascular risk factors which contribute to the death of mainly diabetic patients [1, 13, 18].

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 [27]. 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 untreatedrat groups and did not show a statistically significant difference ($p > 0.05$). In addition, there was not significant change observed in the concentration levels 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 apport of an exterior supply of this element.

The urea concentration level significantly increased at all used oral doses in treated groups compared to untreated groups ($p < 0.01$), but this last observation was not found as a sign of insufficiency renal because its concentration level remained within the normal limits (2.5– 7.5 mmol/L). As urea production in mammals occurs specially in liver, its concentration level could also be used as an indicator of hepatic function [19].Thus, our results more suggest and confirm good hepatic function of treated animals as already demonstrated above with the concentration levels of other hepatic biomarkers.

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 was recorded although an increase was observed in treated groups ($p > 005$) (Table 1). As the presence of infiltrative diseases of the liver and all bones diseases is associated with osteoplastic activity, it is likely that the oral dose used in this study for the aqueous extract of *T. gilletii* leaves did not abnormally interfere with the calcification or metabolic activities involving the liver. This finding is in good agreement with Pieme et al. [24] concerning the effect of other plant extracts on ALP concentration level in animals.

In general, all concentration levels of hematological and biochemical parameters evaluated in the present study were within the normal ranges [20, 21].

Table 2. Effects of the aqueous extract of *T. gille*tii leaves on the concentrations of some biochemical parameters at. oral doses of 0.5 et 1g/kg body weight

Parameters	Negative Control	*T. gilletii*: 5g/kg
Glucose (mg/dL)	242.54 ± 0.40	235.34 ± 1.41
Creatinine (mg/dL)	0.871 ± 0.052	0.86 ± 0.02
AST (UI/L)	177.62 ± 0.33	178.24 ± 0.50
ALT (UI/L)	50.22± 2.27	49.55 ± 1.22
Total cholestérol (mg/dL)	101.24 ± 1.31	100.62 ± 2.2
Triglycerides (mg/dL)	43.72 ± 1.80	43.3 ± 3.54
Total bilurbin (mg/dL)	0.54 ± 0.11	0.44 ± 0.72
Direct bilurbin (mg/dL)	0.20 ± 0.00	0.20 ± 0.00
Total Proteins (g/dL)	7.60 ± 0.31	8.10 ± 1.10
Albumin (g/dL)	3.44 ± 0.52	3.52 ± 0.62
ALP (IU/L)	145.44 ± 1.62	146.32 ± 2.41
HD L- cholesterol (mg/dL)	62.37 ± 1.32	63.60 ± 1.30
LDL- cholesterol (mg/dL)	37.55 ± 2.12	36.21 ± 0.44
Uric acid (mg/dL)	1.91 ± 0.12	2.10 ± 0.50
SGOT (UI/L)	127.34 ± 1.63	126.47 ± 0.22
SGPT (UI/L)	30.77 ± 2.32	32.14 ± 1.23
Urea (mmol/L)	5.10 ± 0.82	6.90 ± 1.61

AST : aspartate transferase, ALT : alanine transferase, ALP : alkaline phospahte, HDL : hight-density lipoproteins, LDl : low-density lipoproteins, SGOT : serum glutamoxalate transferase, SGPT : serum glutamopyruvate transferase.

Effects of the aqueous extract of T. gillettii on éléctrolytes and organ weights

Table 3 shows the effects of the aqueous extract of *T. gilletii* on some electrolytes. Results indicated that the administration of the extract at oral doses induced significant increase of calcium, chloride, iron, potassium and sodium in treated animals compared to untreated animals ($p < 0.05$). This increase shows statistically significant difference between both groups ($p < 0.05$). A statistically significant difference in decrease of inorganic phosphor concentration level was also observed in treated animals compared to untreated animals. For the remaining electrolytes, no significant diference between traited and untreated groups was deduced (Table 3).

In addition, the administration of the extract at all tested oral doses did not show significant difference between the weights of all organs of treated animals compared to untreated animals ($p > 0.05$). This observation suggested that the extract has no significant effect on these animal organs (Table 4).

Table 3. Effets of the aqueuous extract of *T. gillettii* leaves on the concentration levels on some electrolytes (mg/dL) in Wistar rats in acute toxicity

Electrolytes	Negative control	*T.gilletti* 1 g/kg	*T. gilletii* 5 g/kg
Calcium	9.80 ± 0.20	10.64 ± 0.12	10.84 ±0.31
Chloride	70.21 ± 0.42	75.34 ±0.22	78.42 ± 0.43
Inorganic phosphor	4.24 ±0.71	3.77 ± 0.40	3.90 ± 0.32
Iron	7.34 ±0.11	8.52 ± 0.48	9.22 ± 0.34
Potassium	69.57 ± 0.12	73.24 ± 0.52	77.37 ± 0.80
Sodium	72.52 ± 0.44	76.37 ± 0.71	80.28 ± 0.34
Iodure	67..32 ± 0.02	68.12 ± 0.12	70.02 ± 0.07

Table 4. Effects of the aqueuous extract of *T. gillettii*leaves on the organ weights (g/kg)of Wistar rats

Organs	Negative control	*T. gillettii* 1g/kg	*T. gillettii* 5g/kg
Brain	3.71 ± 0.11	3.72 ± 0.36	3.75 ± 0.48
Heart	0.94 ± 0.40	0.97 ± 0.25	0.99 ± 0.42
Kidneys	2.34 ± 0.12	2.24 ± 0.44	2.31 ± 0.21
Lungs	3.01 ± 0.71	2.97 ± 0.42	2.99 ± 0.40
Pancreas	1..57 ± 0.43	1.58 ±0.20	1.59 ± 0.73
Spleen	0.71 ± 0.52	0.73 ±0.22	0.75 ± 0.72
Testicules	9.12 ± 0.42	9.24 ± 0.73	9.26 ± 0.37
Ovaries	0.24 ± 0.37	0.26 ±0.11	0.29 ± 0.04

CONCLUSION

In conclusion, this is the first report of the acute and subacute toxicity of the aqueous extract of *T. gilletii* leaves. 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 levels of biochemical and hematological parameters. It caused significant increase or decrease of some electrolytes in treated animals and did not significantly modify the weight of some organs of treated animals. Thus, its toxicity by oral route was considered practically null or neglected.

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