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ACUTE AND SUBACUTE TOXICITY STUDIES ON METHANOL LEAF EXTRACT OF *TURRAEA VOGELII* HOOK. F. EX. BENTH.

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ABSTRACT

In Africa, majority of people rely on medicinal plants for their health care needs as well as for food. There are numerous scientific reports on efficacy of medicinal plants, with limited reports on the safety of these plants. *Turraea vogelii* Hook. f. ex. Benth. is an ethno medicinal plant indigenous to Tropical Africa. It is used as food, drinks and widely explored in ethno medicine for treatment of various ailments including; wounds, stomach ache, malaria fever, intestinal worms and urogenital infections. The aim of this study was to evaluate the acute and subacute oral toxicity of methanol leaf extract of *T. vogelii* in experimental animals. Acute oral toxicity was determined following OECD guideline 423. Doses of 125, 250, 500 mg/kg were administered for 28 days in the subacute oral toxicity study. Effect of extract on haematological parameters, liver and kidney function markers were determined. The acute oral toxicity of *Turraea vogelii* was estimated to be greater than 2000 mg/kg. The extract had no significant effect on haematological parameters, liver and renal function parameters when compared with the control. The extract however produced inflammatory changes and alterations in morphology of the liver. The results from this study shows that the methanol leaf extract of *Turraea vogelii* produced toxic effects on the liver when repeated oral doses were administered.

Keywords: *Turraea vogelii*; subacute; toxicity; haematology; kidney; liver

INTRODUCTION

Traditional and complementary medicine is an important part of health-care and is found in almost every country with increasing demand for its services (WHO, 2013). In Africa, majority of people use herb for their health needs as well as food (WHO, 2002; Abalaka, 2009).There are numerous scientific reports on efficacy of medicinal plants, with limited reports on the safety of these plants (Ekor, 2014). Toxicological evaluation of medicinal plants is essential as *in vivo* exposure of experimental animals to repeated oral dosing reveals organ and dose-specific toxic effects (Parasuraman, 2011). Determination of selected haematological and biochemical parameters in addition to histologic examination of tissues from vital organs such as kidneys and liver, may provide information on the mechanism of toxicity of ethnomedicines (Yamthe *et al*., 2012).

Turraea vogelii Hook. f. ex. Benth. is an ethno medicinal plant indigenous to Tropical

Africa. Local names include; Ovioza in Edo and aha omode in Yoruba. It is used as food, drinks and widely explored in ethno medicine for treatment of various ailments including; wounds, stomach aches, malaria fever, intestinal worms and urogenital infections (Burkill, 1985). A study reported its anti-proliferative activity on cancer cells (Hamid *et al*., 2015). In a recent study, we carried out a preliminary qualitative phytochemical screening and also evaluated the antinociceptive and antiinfammatory activity of the hydro methanol leaf extract of *T. vogelii* (unpublished work). There are limited scientific reports on the toxicity profile of this plant. The aim of this study was to evaluate the acute and subacute oral toxicity of methanol leaf extract of *T. vogelii* in experimental animals

METHODS

Experimental Animals

Female albino rats (100-120 g) obtained from the central animal house of University of Ilorin were used for the study. The animals were allowed a period of acclimatization with free access to food and water *ad libitum* in the animal house at the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical sciences, University of Ilorin, Ilorin. Ethics clearance was obtained from the University of Ilorin Ethics Review Committee with an approval number UERC/ASN/2017/915. All experiments were carried out in accordance with the Guidelines for laboratory Procedures laid down by the University of Ilorin Ethics Committee on Research as well as the International Animal Care and Use Committee (IACUC) in Nigeria.

Collection and Identification of Plant Material

The fresh leaves of *Turraea vogelii* were collected in January 2017 from Onigambari Plantation Reserve, Ibadan. Identification and authentication of the plant was done by Mr. S.A. Odewo of the Forestry Research Institute of Nigeria (FRIN). A voucher specimen with reference number FHI 111265 was deposited at the herbarium of FRIN.

Preparation of Plant Extract

The leaves were removed from stem and dried under the shade for 2 weeks. The dried leaves were size reduced with a laboratory mill. A 200 g quantity of the powdered leaves was weighed into a clean jar and macerated in 2 L of methanol and water in the ratio of 70:30 for 48 h. The resultant mixture was filtered using a Whatman filter paper (No.1). The filtrate was evaporated to dryness on a water bath at a temperature of 45^{o}C and the percentage yield was calculated. The concentrated extract was stored in the refrigerator at -4^{o}C.

Oral Acute Toxicity

Acute oral toxicity of *Turraea vogelii* extract was determined following OECD guideline 423 which stipulates the use of three animals (Jonsson *et al*., 2013; Sankhari *et al*., 2010). In the first phase, three female albino rats were fasted overnight for 12 hours and weighed. Methanol leaf extract of *T. vogelii* (2000 mg/kg) body weight was administered to the rats via oral cannula. The animals were monitored for signs of toxicity and mortality during the first 6 hours, then for 24 hours. Daily observation for signs of toxicity was continued for 14 days (Nana *et al*., 2011).When no mortality was recorded after 24 hours the second phase of the experiment was carried out using another set of 3 rats. Signs of toxicity and mortality were observed during the first 6 hours, then for 24 hours. Daily observation for signs of toxicity was continued for 14 days.

Subacute Toxicity Study

Twenty (20) male Albino rats were randomised into four groups of five rats

each. Rats in control group were given normal saline (1 mL/kg), while those in the treatment groups received 125, 250 and 500 mg/kg body weight of the extract via the oral route for 28 days. The body weight of the rats was measured twice a week and behavioural parameters observed. On day 29, the rats were anaesthetized with intraperitoneal injection of ketamine (60 mg/kg) and xylazine (7.5 mg/kg).

Collection of blood and organ samples

Blood samples were collected via cardiac puncture with a needle and syringe into ethylenediaminetetraacetic acid (EDTA) bottles for analysis of haematological parameters. Blood for serum biochemical analysis was collected into lithium heparin bottles. The liver and kidneys were removed, rinsed in 0.9 % normal saline and fixed in 10 % formo-saline for histology.The relative organ body weight ratio (ROW) for each rat was determined using:

ROW = weight of organ (g)/body weight of animal (g)

Determination of Heamatological and Serum Biochemical Parameters

Red blood cells (RBC), white blood cells (WBC), haemoglobin (HGB), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelets (PLT) and lymphocytes (LYM) were determined using Sysmex KX-21N automated haematology analyzer (Sysmex America Inc, USA). The blood samples for biochemical analysis were centrifuged at 3000 rpm for 15 minutes. Diagnostic kits were used to determine the concentration of biomarkers for liver enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP)], creatinine, urea and serum electrolytes (sodium, potassium and chloride).

Tissue Histology

The kidney and liver tissues were processed and embedded in paraffin wax. Sections were cut, stained with haematoxylin and eosin and examined under a light microscope.

Statistical Analysis

Data was expressed as mean ± S.E.M. The value obtained for the control group were used as reference values. Data was analyzed using GraphPad Prism (Version 7.03). Statistical analysis was carried out using one-way ANOVA and Dunnett's Test for multiple comparisons between the control and treatment groups. Statistical significance was taken at $p<0.05$.

RESULTS

Acute Toxicity

No toxic reactions or mortality was observed in the animals after administering an oral dose of 2000 mg/kg to 3 animals in the first and second phase of the study. The median lethal dose (LD_{50}) of the methanol leaf extract of *T. vogelii* was estimated to be greater than 2000 mg/kg in rats. All the animals were active and healthy during the observation period of 14 days.

Subacute Toxicity

Effect of Methanol Leaf Extract of *T. vogelii* on Body Weight and Relative Organ Body Weight (ROW) in Rats

The percentage weight gain of groups treated with (125, 250 and 500 mg/kg) of extract was lower but not significantly different ($p>0.05$) from the control group (Table 1). There was no significant difference ($p>0.05$) in the relative organ body weight ratio (kidney and liver) of groups treated with (125 and 500 mg/kg) of extract when compared with control. However, there was a significant increase ($p<0.05$) in the ROW of liver in group treated with 250 mg/kg of the extract (Table 2).

Table 1: Effect of methanol leaf extract of *T. vogelii* on body weight of rats

Weight of rats	Control	125 mg/kg	250 mg/kg	500 mg/kg
Day 0 (g)	102.95±9.00	105.60±4.09	84.10±4.43	104.3±9.97
Day 28 (g)	137.60±8.61	129.72±5.80	111.04±7.60	134.95±10.54
Weight gain	34.65	24.12	26.94	30.65
Percent weight gain	25.18	18.59	20.76	22.71

n=5 per group, Data are mean ± SEM, statistically significant $^{\#}$ $p < 0.05$, (ANOVA, followed by Dunnett's post hoc test).

Table 2: Effect of methanol leaf extract of *T. vogelii* on Relative Organ Weight (ROW) in rats

Organ	Control Normal Saline	METV (125 mg/kg)	METV (250 mg/kg)	METV (500 mg/kg)
Kidney (x 10^{-3})	7.46 ± 0.46	7.62 ± 0.16	8.24 ± 0.43	7.20 ± 0.53
Liver (x 10^{-3})	34.21 ± 1.93	34.52 ± 1.99	45.47 ± 1.30$^{\#}$	35.42 ± 0.91

n=5 per group, Data are mean ± SEM, statistically significant $^{\#}p<0.05$, (ANOVA, followed by Dunnett's post hoc test).

Effect of methanol leaf extract of *T. vogelii* on haematological parameters

Administration of leaf extract of *T. vogeliii* at different doses for 28 days produced no significant effect ($p > 0.05$) on haematological parameters compared with control group (Table 3).

Table 3: Effect of methanol leaf extract of *T.vogelii* on haematological parameters in rats

		Methanol extract of *T. vogelii* (mg/kg)		
Parameters	Control	125	250	500
WBC(x 10^3/µL)	11.15 ± 1.16	12.63 ± 0.53	13.83 ± 1.71	13.85 ± 1.38
RBC(x 10^6/µL)	7.76 ± 0.29	7.72 ± 0.24	7.00 ± 0.24	7.46 ± 0.17
Hb(g/dL)	12.08 ± 0.11	11.73 ± 0.30	11.33 ± 0.33	11.98 ± 0.28
HCT (%)	40.75 ± 0.82	40.85 ± 0.97	39.90 ± 0.60	41.00 ± 0.76
MCV(fL)	52.63 ± 1.07	53.03 ± 1.30	57.15 ± 1.82	55.15 ± 2.23
MCH(pg)	15.63 ± 0.53	15.23 ± 0.25	16.20 ± 0.39	16.10 ± 0.69
MCHC(g/dL)	29.65 ± 0.40	28.73 ± 0.23	28.40 ± 1.06	29.23 ± 0.46
Platelets(x10^3/µL)	866.00 ± 46.97	753.30 ± 47.30	655.80 ± 55.70	775.00 ± 39.31
Lymphocytes (%)	79.33 ± 10.60	91.95 ± 0.94	83.30 ± 3.45	91.20 ± 2.00

n=5 per group, Data are mean±SEM, statistically significant $^{\#}p<0.05$, (ANOVA, followed by Dunnett's post hoc test).

Effect of methanol leaf extract of *T. vogelii* on liver enzymes in rats

There was no significant difference ($p>0.05$) in the value of AST and ALT in all the treatment groups compared to control group. However, the value of ALP in the group treated with 500 mg/kg was significantly ($p<0.05$) lower than the control group.

Effect of methanol leaf extract of *T. vogelii* on renal function markers in rats

There was no statistical significant difference ($p>0.05$) in concentration of creatinine, and serum electrolytes. A significant increase ($p<0.05$) in the concentration of urea was observed in the group treated with 250 mg/kg of the leaf extract (Table 5).

Table 4: Effect of Methanol Leaf Extract of *T. vogelii* on Liver Enzymes in Rats

		Methanol extract of *T. vogelii* (mg/kg)		
Enzymes Control		**125**	**250**	**500**
AST (U/L)	11.23 ± 0.45	9.69 ± 0.83	9.11 ± 0. 81	9.84 ± 0.88
ALT (U/L)	13.05 ± 0.57	9.51 ± 0.58	8.98 ± 0.83	12.7 ± 3.42
ALP (U/L)	93.59± 17.61	74.10 ± 17.41	61.86 ± 12.15	57.85±8.90$^{\#}$

n=5 per group, Data are mean ± SEM, statistically significant $^{\#}p<0.05$, (ANOVA, followed by Dunnett's post hoc test).

Table 5: Effect of Methanol Leaf Extract of *T. vogelii* on Renal Function Markers in Rats

Parameters	**Normal Saline**	**125 mg/kg**	**250 mg/kg**	**500 mg/kg**
Creatinine (mmol/L)	1.01 ± 0.58	1.55 ±0.46	1.145±0.20	1.11 ±0.16
Urea (mmol/L)	7.95 ± 3.04	8.25 ± 1.34	11.76 ± 3.59$^{\#}$	7.95 ± 2.73
Sodium(mEq/L)	140.00 ± 13.62	125.85 ± 18.35	128.40 ± 24.99	148.15 ± 24.02
Potassium (mEq/L)	9.30 ± 0.95	7.83 ± 0.33	7.82 ± 0.79	6.41 ± 0.22
Chloride(mEq/L)	93.96 ± 9.26	106.93 ± 7.95	81.21 ± 11.80	92.84 ± 16.72

n=5 per group, Data are mean ± SEM, statistically significant *$p<0.05$, (ANOVA, followed by Dunnett's post hoc test).

Histopathological Findings

Micrograph section of liver showed hexagonal plates of hepatocytes with central vein for rats in control group (Plate 1A). The plates of hepatocyte appeared normal with no abnormalities. The liver section for groups treated with 125, 250 and 500 mg/kg showed irregular shaped hexagonal plates of hepatocyte admixed with central vein and portal traid (Plates 1B, 1C and 1D). Micrograph showing kidney tissues composed of glomeruli, tubules, arterioles and interstitial spaces. The glomeruli demonstrate no abnormalities and the tubules are lined by cuboidal epithelium in the control and groups treated with the extract (Plate 2).

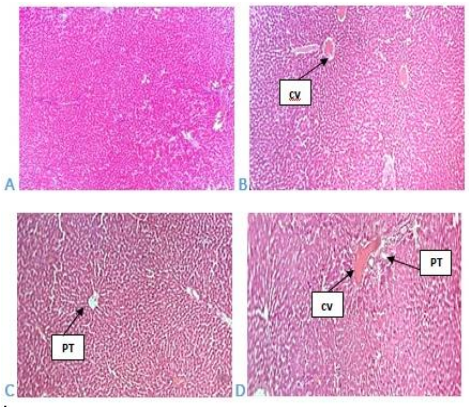

Plate 1: Micrographs of liver sections from rats treated with different doses of *T. vogelii*. 40× Magnification. A) control, B) 125 mg/kg, C) 250 mg/kg, D: 500 mg/kg. Portal Traid (PT), Central Vein (CV).

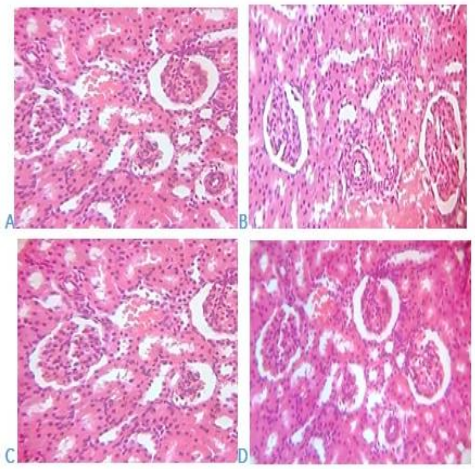

Plate 2: Micrographs of kidney sections from rats treated with different doses of *T. vogelii*. 40× Magnification. A) control, B) 125 mg/kg, C) 250 mg/kg, D: 500 mg/kg.

DISCUSSION

In the acute oral toxicity study, no sign of toxicity was observed in all the animals during the two phases of the toxicity study. The animals were active and alive during the study. The median lethal dose (LD_{50}) for methanol extract of *T. vogelii* was estimated to be greater than 2000 mg/kg in rats (Lu *et al*., 1965). Based on the finding from oral acute toxicity, doses of 125, 250, 500 mg/kg were administered for the subacute toxicity study. Feed intake for animals in the test groups was similar to those in control group and repeated oral dosing of the extract produced no change in weight. This may suggest the extract did not alter metabolic processes or hormonal balance that affects body weight (Cajuday and Poscidio, 2010). The relative organ body weight ratio for kidney in the treated and control group was not significantly different. The increase in size of liver observed in this study may be attributed to inflammation. An increase in organ-body weight ratio has been reported to be caused by inflammatory reactions in the target organ (Moore and Dalley, 1999). The extract has no toxic effect on blood forming constituents since the haematological indices evaluated in this study were all within the normal limit for experimental animals. The slight increase in white blood cell concentration at all the doses (125, 250 and 500 mg/kg body weight) may suggest an increased activity of the immune system (Ashafa *et al*., 2009).

This study further showed that liver enzymes (ALT and AST) were not significantly altered with repeated dosing of different concentrations of the extract when compared with control group. The inflammation and abnormal morphology observed in the liver cells suggests the extract is toxic to the liver with subacute administration of repeated doses.

The concentration of creatinine, potassium and chloride ion in the treated groups were also within the normal limits. The values obtained for these renal function markers support the histological findings in the kidney tissues which revealed no abnormalities glomeruli and tubules. Creatinine has been reported to be a more accurate marker of kidney function when compared to urea (Blann, 2014). The significant increase in the concentration of urea alone observed in this study may not interfere with kidney function and structure since very high concentrations of both serum creatinine and urea are biomarkers of kidney function abnormality (Palm and Lundblad, 2005). Evaluation of liver and kidney function markers with histologic investigations are important in toxicological assessment of plant extracts due to the involvement of these organs in metabolism and excretion of xenobiotics (Fouche *et al*., 2015). A derangement in function of one or both of these vital organs may also result in accumulation and subsequently toxicity from administered drugs.

CONCLUSION

Repeated oral dosing of methanol leaf extract of *Turraea vogelii* for a period of 28 days produced inflammation and alterations in liver morphology. Chronic toxicity studies will be carried out to evaluate the long-term effect of this medicinal plant.

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