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Acute and Sub-Acute Toxicity of Dichloromethane-Methanol Root Bark Extract of *Teclea trichocarpa* Engl. (Rutaceae) in Rats

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The *in vivo* toxicity profile of dichloromethane-methanol (50:50 % v/v) extract of *Teclea trichocarpa* Engl. (Rutaceae) root bark using Wister rats is reported. No death occurred in the oral acute and sub-acute toxicity studies. In the acute intraperitoneal test, all the animals at 2000 mg/kg developed convulsions followed by death within 3 min; at 300 mg/kg, death occurred within 4-48 h, but there was no death at 50 mg/kg. In the acute oral, sub-acute oral and 50 mg/kg acute intraperitoneal tests, all haematological and biochemistry parameters fluctuated but remained within normal limits, suggesting that *T. trichocarpa* root bark extract is practically non-toxic and supports the safety of this plant as a traditional herbal remedy. However, toxicity of the extract on intraperitoneal administration requires further study.

Key words: *Teclea trichocarpa*, root bark extract, acute toxicity, sub-acute toxicity

INTRODUCTION

Medicinal plants have been used for centuries to treat illness and improve health, and they account for approximately 80% of medical treatments in the developing world [1,2]. *Teclea trichocarpa* Engl. (Rutaceae) is commonly used herbal remedy in Kenya in the treatment of malaria, helminthiasis and fever [3]. The ethnopharmacological claims of the medicinal properties of *T. trichocarpa* have been scientifically validated while phytochemical studies have led to isolation of several alkaloids such as melicopicine, normelicopicine, arborinine, skimmianine, diatamnine, tecleanthine and 6-methyltecleanthine [3].

In one study, crude methanol extract of *T. trichocarpa* leaves and the isolated compounds showed *in vitro* activity against parasitic protozoa *Plasmodium falciparum*, *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi* and *Leishmania donovani* [4]. A recent study demonstrated the anthelmintic activity of its root bark extract [5]. The World Health Organization has emphasized that any method used in alternative/complementary medicine must satisfy the criteria of safety, efficacy and quality [6]. Despite its widespread use in traditional medicine and scientific evidence of its pharmacological efficacy, safety of *T. trichocarpa* to humans and animals has not been established. Thus, this work aimed to investigate the acute and sub-acute toxicity of *T. trichocarpa* root bark extract.

METHODOLOGY

Experimental animals

Wistar rats (*Rattus norvegicus*) were bred at the National Public Laboratories, Nairobi, and used at the age of 6-8 weeks. All handling of the rats was conducted as per the recommendation of the National Institutes of Animal Health [7].

Plant material

The roots of *T. trichocarpa* were collected from Ngong Hills Forest, Kajiado County, Kenya in January 2012. The plant was authenticated at site by a taxonomist and voucher specimen deposited

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at the School of Biological Sciences Herbarium, University of Nairobi (voucher number BMDK04). The root barks were air-dried, milled and extracted with dichloromethane-methanol (1:1).

Reagents

The clinical chemistry kits for total protein, creatine kinase (CK) and creatinine were from Diagnostic Systems International (Holzheim, Germany) while kits for aspartate aminotransferase (AST), alanine aminotransferase (ALT) and albumin were from Thermo Electron (Scoresby Vic, Victoria, Australia) and Fischer Diagnostics (Massachusetts, USA). Eosin and haematoxylin dyes were sourced from Kobian Limited (Nairobi, Kenya). Solvents used in this work included diethylether (Lobachemie, Mumbai) and phosphate buffered saline (PBS).

Equipment

A Visual® spectrophotometer (Biomerieux, Paris) for clinical chemistry analysis, a Leica DM 750 LED biological microscope (Leica, Wetzlar, Germany) for histopathological work, MS4 Vet® haematology blood counter (Melet Schloesing Laboratories, Cergy-Pontoise Cedex, France) and a locally fabricated anaesthetizing chamber were used.

Acute toxicity after oral administration

Rats were randomly allocated and fasted overnight prior to dosing but water was not withheld. Extract were suspended in 2.5 % Tween 80 in normal saline and administered using suitable intubation cannula. Food was withheld for a further 3-4 h after dosing. For each dose used, the volume administered was calculated using an equation described by Tendong [8]. The Globally Harmonized Classification System (GHS) in acute toxicity category (ATC) was used to determine the LD_{50} range [9]. Since no death occurred at the starting dose level of 300 mg/kg, the next higher dose, 2000 mg/kg body weight, was used. The animals were observed for 5 days.

Acute toxicity after intraperitoneal administration

The extract was dissolved in PBS with 3 % v/v dimethyl sulphoxide (DMSO). The extract was first filtered through Whatman filter paper No. 40 and then through 0.2 µm millipore filters to ensure sterility of the solution. The solution (1-2 ml) was then injected intraperitoneally starting with the 2000 mg/kg body weight dose level. The GHS/ATC method was then used to estimate the LD_{50} range [9].

Sub-acute toxicity oral administration

Forty Wistar rats were randomized to three treatment groups and one control group each of 5 rats per sex. The dose levels used were 100 mg/kg, 300 mg/kg and 1000 mg/kg body weight. The vehicle comprising 2.5% Tween 80 in normal saline was used as the control. Animals were dosed daily for 28 days by gavage on the basis of weekly mean group weight. Clinical observations were done twice a day. Blood for haematological and clinical chemistry tests was collected before treatment, on day 14 and 28. A complete necropsy was performed on all treated and control animals that either died or were sacrificed in extremis. The SPSS Programme version 17 (SPSS Inc., Chicago) was used for all other data analyses.

RESULTS AND DISCUSSION

Acute toxicity after oral administration

In the acute oral toxicity testing at 2000 mg/kg body weight, no death was observed. All the animals showed clinical signs such as piroerectile, rubbing of nose and mouth, and avoided feeds for 10 min post-dosing. All these symptoms disappeared completely 30 min post-dosing. All treated animals showed a stable increase in body weight.

Acute toxicity after intraperitoneal administration

All the rats given *T. trichocarpa* root bark extract at 2000 mg/kg body weight

intraperitoneally died within 2-3 min. Soon after dosing, the animals became restless, developed uncoordinated jerky movements and convulsed with their tails stretched upward. The death mimicked that of strychnine poisoning. In the 300 mg/kg body weight category, the first, second and third rats died after 45 min, 4 h and 48 h, respectively. The symptoms were similar to 2000 mg/kg category but milder. All the rats given the extract at 50 mg/kg body weight exhibited no major symptoms. Dosing was continued for the next 5 days until the cumulative dose was equal to the next toxic dose of 300 mg/kg body weight but the rats survived.

Sub-acute toxicity after oral administration

Clinical signs

All the animals in the 100 mg/kg, 300 mg/kg and 1000 mg/kg dose categories did not exhibit any abnormality throughout the 28 days oral administration of the extract.

Weight gains

All the treatment groups gained weight progressively over the weeks but the gain was not dose related. Figure 1 shows percentage body weight changes in the rats treated with *T. trichocarpa* root bark extract in respect to weight at week 0. The Organ weight index (OWI) for various organs remained constant across the three treatment groups and in the control group implying that the oral doses used had little or no impact on major body organs thus suggesting that there was insignificant organotoxicity. Any alteration in liver weight is usually suggestive of treatment-related adverse effects including hepatocellular hypertrophy either due to enzyme induction or peroxisome proliferation [10]. Similarly, elevated heart weight may be the only evidence of myocardial hypertrophy that is often macroscopically and microscopically difficult to recognize. Changes in kidney weight may reflect renal toxicity, tubular hypertrophy or chronic progressive nephropathy [10]. The histopathology of the kidney during acute toxicity study and the gross pathology at all dose levels during sub-acute toxicity study showed no signs of toxicity. Variations in adrenal gland weight which may indicate hypertrophy, hyperplasia, or atrophy of the organ associated with stress, endocrinopathies, or test article effects [10], did not occur in this study (Table 1).

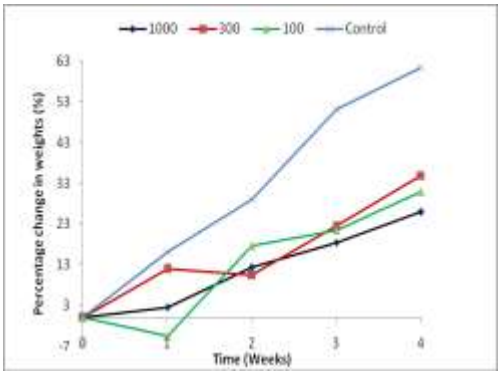

Figure 1. Percentage body weight changes in rats treated with dichloromethane-methanol root bark extract of *Teclea trichocarpa*.

Table 1: Effects of dichloromethane-methanol root bark extract of *Teclea trichocarpa* on actual weights and organ weight indices in rats

Dosage	1000 mg/kg		300 mg/kg		100 mg/kg		Control	
Organ	**AMW (mg)**	**OWI**	**AMW (mg)**	**OWI**	**AMW (mg)**	**OWI**	**AMW (mg)**	**OWI**
Liver	7600 ± 710	3.62	7130 ±540	3.36	7800 ±670	3.67	7040 ±280	2.98
Kidney	1290 ±09	0.61	1. 22 ±90	0.57	1.24 ±80	0.58	1.40 ±540	0.59
Adrenals	50 ± 10	0.02	60 ±10	0.03	60 ±0	0.03	60 ±10	0.03
Heart	630±20	0.30	570 ±40	0.28	590 ±20	0.26	490 ±20	0.21
Spleen	740±240	0.35	780±90	0.37	800±40	0.36	770±30	0.33
Thymus	240±30	0.11	240±30	0.11	290±30	0.13	180 ±10	0.08
Testis	1680 ±280	0.80	2000 ±260	0.90	1690±760	0.78	2090±120	0.89
Ovaries	100±20	0.04	110±10	0.05	120±10	0.05	70±10	0.03

OWI = Organ Weight Index; AMW = Actual Mean Weight. Values are expressed as mean ± standard error of the mean.

Haematology

The haematological parameters during the sub-acute oral toxicity study are summarized in Table 2. Haematological parameters did not show any significant variations associated with toxicity to haemopoietic organs. The modest variations noted in any parameter were well within the normal biological ranges [11]. There was a small dose independent increase in the mean haemoglobin levels and thrombocytes count with time in all treatment groups as well as the control. In a situation where the haemopoietic organs are affected by toxic agents, the blood film usually shows elevated levels of immature neutrophils and nucleated RBCs. Absence of such a phenomenon corroborates non-toxicity of the extract to the rats at tested doses.

Clinical chemistry

The effects of the *T. trichocarpa* root bark extract on clinical chemistry parameters are summarized in Table 3.

Table 2: Effects of dichloromethane-methanol extract of *Teclea trichocarpa* root bark on haematological parameters in rats at week 4

Haematological parameters	1000 mg/kg	300 mg/kg	100 mg/kg	Control
WBC (μL^{-1})	24057±3170	24122±3553	12112±5410	28092±4016
RBC ($\times 10^6/\mu L$)	6.63±0.4	6.9±0.2	6.6±0.3	6.7±0.2
PVC (%)	40.16±2.2	42.2±1	39.6±2.1	41.4±1
Hb (g/dL)	15.84±0.4	15.6±0.2	15.5±0.7	16±0.3
MCV (fL)	60.9±1.1	60.9±1.1	60.3±1	61.6±1.2
MCHC (g/dL)	39.95±2.6	37.1±0.9	39.5±1.3	38.6±0.9
Thrombocytes ($\times 10^3/\mu L$)	433.3±46.1	448.5±55.5	415.6±55.4	430±30.9
Total neutrophils (%)	22.7±4.7	28.8±5.5	25.2±4.3	25±4.2
MCH (pg)	23.3 ±4.2	28.4 ±4.7	23.8 ±3.8	23.7 ±3.3
Lymphocytes (%)	77 ±2.3	70 ±2.1	74.4 ±2.4	73.3 ±2.0
Immature neutrophils (%)	0.1	0	0.2	0
Eosinophils (%)	0	0	0	0
Monocytes (%)	0	0	0	0
Basophils (%)	0	0	0	0
Nucleated RBCs (%)	0.8	0.1	0.3	0

Normal ranges were adopted from ref. [11].

Table 3: Effects of dichloromethane-methanol extract of *Teclea trichocarpa* root bark on haematological parameters in rats at week 4

Dose of extract (mg/kg)	Clinical chemistry parameters					
	Total protein g/dL	Albumin g/dL	Creatinine g/dL	AST (IU/L)	ALT (IU/L)	CK (IU/L)
1000	7.2	3.4	0.5	10.1	4.4	50.5
300	7.2	3.7	0.4	6.9	9.1	78
100	7.5	3.7	0.4	6.9	9.1	78
Control	7.3	3.3	0.5	9.3	9.7	47.5

IU = international units; normal ranges were adopted from ref. [11].

There were no significant variations in total protein, albumin and creatinine levels in all groups over the treatment period and amongst the groups. On the other hand, AST levels

dropped steadily from week 1 to week 4 but levels in control group rose moderately between weeks 2 and 4 (Figure 2). The drop in AST was dose-related, being highest in the 1000 mg/kg group and lowest in 100 mg/kg group. There was a significant difference ($p<0.002$) in all treatment groups as compared to the control.

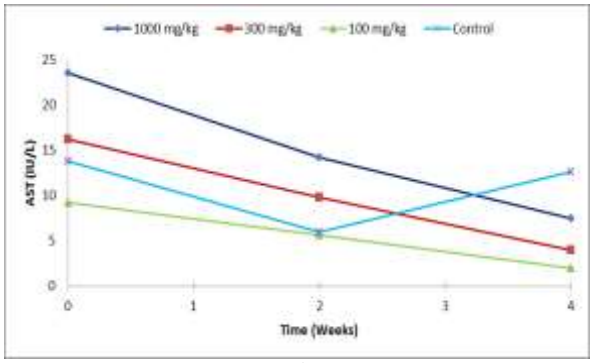

Figure 2. Aspartate aminotransferase levels in rats dosed with *Teclea trichocarpa* root bark extract.

Alanine aminotransferase levels also showed a decreasing trend. The levels of ALT in the control group decreased in week 1 and 2 then rose gradually between weeks 2 and 4. ALT levels decreased less than AST levels. The ALT and AST enzyme activities are the commonly used surrogate markers of liver function. The two enzymes are only released from cytosol and sub-cellular organelles during cell injury. Alanine aminotransferase is more hepatocellular specific whereas creatinine is an indicator of muscle-wasting.

Alanine aminotransferase is a critical parameter for identification of potential drug-induced injury in both pre-clinical studies and human patients [12,13]. This study showed an unexpected apparent decrease in ALT and AST values as opposed to an increase. The levels of CK experienced a rapid drop from the pre-treatment level to the levels in week 2, and then rose between weeks 2 and 4. The trend in CK levels for all the 3 test groups was very close at all dose levels and the control (Figure 3).

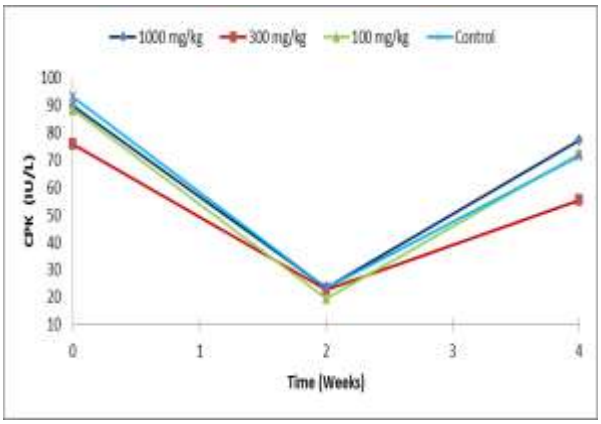

Figure 3. Creatine kinase levels in rats dosed with *Teclea trichocarpa* root bark extract.

Histopathology

All organs showed no lesions when observed under the microscope. The 28 days oral sub-acute dosing of the extract had no effects on the cellular integrity of body organs. The fact that the non-enzyme parameters such as creatinine, protein and albumin were within normal limits corroborates the absence of pathological lesions. The extract did not induce any notable pathological changes in rats at the highest dose tested. The gross pictures of the internal organs such as the liver, lungs, kidney, adrenal glands and the gastrointestinal tract were normal in both colour and architecture. However, one animal in the 100 mg/kg body weight group had an abscess in one half of the spleen. The animal was not sickly and this may have been an incidental finding.

CONCLUSION

Absence of clinical, haematological, clinical chemistry and pathological effects of *T. Trichocarpa* root bark extract supports the safety of this plant in its use as a herbal remedy.

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