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Assessment of acute and sub-acute toxicity of ethanol extract of *Pseudocedrela kotschyi* leaf in Wistar rats

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Abstract

Pseudocedrela kotschyi (meliaceae) is widely used in African traditional medicine for the management of malaria, pains, diabetes, convulsion and bacterial infection. The present study evaluates the acute and sub-acute toxicity effects of *P. kotschyi* in Wistar rats. In the acute toxic study, the leaf extract was administered to rats orally up to 5 g/kg in divided doses. The animals were then observed for signs of toxicity and mortality for 7 days. In sub-acute toxicity study, rats were orally treated daily with the extract at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg for 28 days. The control rats were given distilled water and all animals were weighed at 7 days interval. The haematological, biochemical parameters and vital organs were determined. The leaf extract was practically non-toxic showing no mortality and visible signs of toxicity on acute exposure. The extract showed a significant increase in the body weight of rats given 200 mg/kg and 400 mg/kg. The extract did not produce a significant effect on haematological indices except a significant increase in platelet count. Biochemical parameters were not significantly changed in the study while triglycerides showed a significant increase. The weight of the heart, kidneys and spleen were not significantly affected but significant changes were observed in the weight of the lungs and liver. Findings in this study revealed that *P. kotschyi* is safe when administered orally, further investigation is needed to ascertain its effect on long-term administration.

Keywords: *Psedocedrela kotschyi*, leaf extract, toxicity, haematology, biochemical, rats

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1 Introduction

Medicinal plants either as an extract, pure compound or as a derivative, offer unlimited opportunities for the discovery of new drugs. Apart from the discovery of numerous orthodox drugs from the study of traditional cures and folk knowledge, the efficacies of a number of botanicals have been proven scientifically. Globally, different medicinal plants and botanical drugs have been generally accepted as primary therapeutic supplements for treating various human diseases (Habbu et al., 2008). It has been estimated that over 70 % of indigenous populations in developing countries depend on medicinal plants (Wambebe, 1998).

Considering the challenges confronting the appropriate delivery of official health care to millions of people in rural communities which serve as an abode over 70 % of the population, including socio-economic demands for adequate pharmaceutical supplies, prevalent transportation difficulties, shortage of needed expertise for rational use of drugs, shortage and cost of orthodox products and the most viable way to bridge the gap in medicare is herbal medicines. Herbal remedies are generally regarded as safe and are promoted to the public as being natural and completely safe owing to long periods of use (Adewumi et al., 2004; Adeyemi et al., 2010). The surge in popularity and patronage of herbal medicines necessitate concern based on adverse effects of potentially toxic substances in plants. Pharmacological and toxicological evaluations of medicinal plants are important for drug development. So much has been done in investigating herbal medicines for efficacy more than the issue of safety, as reports of efficacy far outnumber those of toxicity. The toxicological studies of most medicinal plants widely used have been evaluated in vivo to ascertain their effects on organs after a short-term and long-term use.

Pseudocedrela kotschyi is a medicinal plant belonging to the family of meliaceae and is mainly found across West and Tropical Africa. It is a tree of up to 20 metres high with a wide crown fissured bark and fragrant white flowers. The plant is widely used for the treatment of various diseases by traditional healers. The antimalarial, antipyretic, dental cleaning, analgesic and anti-inflammatory and antiepileptic activities of different extracts of the plant have been scientifically investigated and reported (Akuodor et al., 2015; Kassim et al., Akuodor et al., 2013; Tabsoba and Deschamps, 2006; Musa et al., 2005; Kone et al., 2004; Anuka et al., 1990).There is paucity of information in the scientific literature on the toxicity profile of *P. kotschyi*. But since acute, sub-acute toxicity data will be needed to predict the safety or otherwise of long-term low dose exposure to medicinal products, it is necessary to provide this information in order to bridge the gap in knowledge about the toxicity profile of *P. kotschyi*.

2 Materials and Methods

Plant material collection

The fresh leaves of *P. kotschyi* were collected from Niger State, Nigeria. Plant identification and authentication was done by a taxonomist in the Department of Medicinal Plant research and Traditional Medicine, NIPRD, Abuja, Nigeria. Voucher specimen (NIPRD/H/6542) was deposited in the herbarium of the Institute for reference.

Extraction

The fresh leaves were rinsed thoroughly in distilled water and air-dried until a constant weight was maintained. The dried plant material was ground to fine powder and soaked in absolute ethanol (450 g in 2.5 L) with constant agitation. The extract was filtered 24 h later. The filtrate was evaporated to dryness on a water bath under reduced pressure of 40 ^{o}C to give a dark brown

solid yield of 17.65% (w/w). The dried extract was stored in a refrigerator at 50^{o}C and later reconstituted in distilled water before administration to experimental animals.

Animals

Male and female Wistar rats used for this study were purchased from animal house unit, Enugu, Nigeria. The animals were acclimatized for at least 14 days. Six rats were housed per cage (male and female rats were kept separate) and maintained in a well-ventilated animal room with temperatures of 25–27 0C and 12-h light/dark cycle. The animals had free access to rodent pellet and portable water ad libitum. The cage beddings and water bottles were cleaned on a daily basis. The research protocols used in this study were in accordance with the requirements of the Research Grants and Experimentation Ethics Committee of Ebonyi State University, Abakaliki, Nigeria (EBSU/UREC/TETFUND/15/14) and international guidelines on the Use and Handling of Experimental Animals (NIH, 2011).

Acute toxicity study

Oral acute toxicity test was performed using the Organization of Economic Cooperation and Development (OECD) guideline for testing of chemicals 401 (OECD, 2001). Male and female rats weighing 180-200 g were used for this study, and were conducted in two phases. Three groups of 3 rats (male separated from female) in each cage were administered 100, 600 and 1000 mg/kg of the leaf extract orally. They were observed for signs of toxicity and mortality for 24 hrs with special attention given to the first 4hrs. This was followed by administration of the extract (2000, 3000 and 5000 mg/kg) to the next three groups of 3 rats and equally observed as earlier stated, and daily for 7 days for any signs of toxicity which include salivation, paw-licking, writhing, change in body weight and mortality. The number of deaths in each group was recorded and the final LD50 values were calculated.

Sub-acute toxicity study

A total of twenty-four rats were weighed and divided into 4 groups of 6 male and 6 female rats in each cage for the study. Six rats (3 male and 3 female) were grouped based on three different treatment doses of the leaf extract with one control (distilled water) group. The rats were orally treated daily with *P. kotschyi* leaf extract at doses of 100, 200 and 400 mg/kg, and distilled water for 28 days (Ibrahim et al., 2016). All rats were individually weighed immediately before dosing on day 1 and once a week throughout the study. All animals were observed daily for mortality, general condition, and clinical signs before the test and throughout the dosing period. Clinical observations, including motor activity, appearance, and central and autonomic functions, were performed daily.

Relative organ weight measurement

On day 28, rats were fasted overnight but had free access to drinking water for 24 hrs before being sacrificed under inhaled chloroform anaesthesia. Different organs namely the heart, kidneys, liver, spleen and lungs were surgically dissected out, weighed and macroscopically examined. (absolute organ weight). The relative organ weight (ROW) of each harvested organ was calculated as: Absolute organ weight (g)/ Body weight of animal on sacrifice day (g)x100.

Haematology and serum biochemical

Blood samples were collected through cardiac puncture into EDTA and non-heparinized containers for haematological and serum biochemical analysis respectively.

Haematological measurements

Blood samples analyzed (automated haematology analyzer; Mythic 18 by Orphee, Switzerland) included the following: pack cell volume (PCV), red blood cell (RBC) count, Hemoglobin concentration (HB), platelet count (PLT), white blood cell (WBC) count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC).

Biochemical Analysis

The serum concentrations of alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), albumin (Alb), total bilirubin (T-Bili), urea (Bu), creatinine (Crea), total cholesterol (TC), triglycerides (TGs) and high density lipoprotein (HDL) cholesterol were determined using (Gesan Chem 200, USA) automated analyzer.

Statistical analysis

The data were analyzed using SPSS version 16. Results are expressed as mean ± SEM. P value < 0.05 was considered significant. One-way analysis of variance (ANOVA) and Tukey's post hoc test were used to identify differences among the groups.

3 Results

Acute oral toxicity

The leaf extract produced no untoward clinical signs in the treated rats at the dose levels tested. There were no changes in behavior patterns, nature of stool and salivation of all the rats. There was no mortality observed in the different groups after 7 days. The oral LD50 value of the plant was estimated to be >5000 mg/kg body weight.

Effect of the leaf extract on body weight

Table 1 showed the leaf extract compared to the control, caused significant increase in the percentage body weight in a dose dependent manner with the highest increase recorded at the dose of 200 and 400 mg/kg in the 28 day treatment period.

Table 1. Effect of Pseudocedrela kotschyi leaf extract on body weight of rats in subacute study.

Dose (mg/kg)	Percentage weight change
Control	24.28 ± 2.42
100	26.12 ± 3.54
200	26.44 ± 4.22
400	28.55 ± 3.41

Results are presented as Mean ± SEM (n=6)

Effect of the leaf extract on haematological parameters

The effects of sub-acute administration of P. kotschyi leaf extract on haematological parameters are presented in Table 2. Most haematological parameters assessed (packed cells volume, red blood cells, platelet count, mean corpuscular volume, mean cell haemoglobin, mean cell haemoglobin concentrate, haemoglobin, white blood cells, neutrophils, lymphocytes and monocytes) in extract treated rats were not significantly different when compared to the control group. However, the

extract showed a significant ($p<0.05$, $p<0.01$) increase in platelet count when compared to control.

Table 2. Effect of *Pseudocedrela kotschyi* leaf extract (dose:mg/kg) on haematological parameters in rats.

Parameter	Control	100	200	400
Pack cells volume (%)	45.4 ± 0.4	45.1 ± 0.5	45.5 ± 0.3	46.5 ± 0.2
Haemoglobin (g/dL)	13.0 ± 0.2	13.0 ± 0.2	13.5 ± 0.3	13.9 ± 0.1
Red blood cells (x10^{12}/L)	7.2 ± 0.2	7.5 ± 0.1	7.8 ± 0.2	8.6 ± 0.2
Platelet count(x 10^9/L)	675.8 ± 11.5	677.7 ± 14.1	696.9 ± 10.2*	730.3 ± 23.8**
Mean corpuscular volume (fl)	65.6 ± 0.3	62.0 ± 0.8	64.4 ± 0.6	66.4 ± 0.4
Mean cell haemoglobin (pg)	31.6 ± 0.2	31.8 ± 0.1	31.1 ± 0.2	33.8 ± 0.1
Mean cell haemoglobin concentration (g/dl)	32.7 ± 0.2	32.4 ± 0.3	32.8 ± 0.3	33.8 ± 0.1
White blood cells (x 10^9/L)	7.2 ± 0.2	7.5 ± 0.2	7.8 ± 0.3	7.7 ± 0.1
Neutrophils (%)	19.5 ± 0.1	19.8 ± 0.2	20.2 ± 0.1	20.2 ± 0.2
Lymphocytes (%)	76.5 ± 0.3	77.1 ± 0.5	77.0 ± 0.4	77.3 ± 0.7
Monocytes (%)	3.7 ± 0.1	3.5 ± 0.2	3.5 ± 0.2	3.8 ± 0.1

Results are presented as Mean ± SEM (n=6); * $p<0.05$ as compared to the control; ** $p<0.01$ compared to the control.

Effect of the leaf extract on biochemical parameters

Table 3 shows the value of serum biochemical parameters after a treatment period of 28 days. There were no significant ($p>0.05$) differences in liver function parameters (aspartate transaminase, alanine transaminase and alkaline phosphatase) observed in rats after treatment. The leaf extract produced no significant ($p>0.05$) changes in albumin, total cholesterol and high density level cholesterol, except for a significant ($p<0.05$, $p<0.01$) dose-dependent increase of plasma Triglycerides after 28 days treatment period.

Table 3. Effect of *Pseudocedrela kotschyi* leaf extract (dose:mg/kg) on serum biochemical parameters in rats.

Parameter	Control	100	200	400
Aspartate transaminase (U/L)	53.2 ± 0.3	58.8 ± 0.3	51.6 ± 0.5	56.8 ± 0.3
Alanine transaminase (U/L)	20.1 ± 0.2	20.3 ± 0.5	19.9 ± 0.3	23.2 ± 0.2
Alkaline phosphatase (U/L)	86.2 ± 0.3	90.0 ± 0.5	87.4 ± 0.3	89.5 ± 0.4
Albumine (mg/dL)	6.0 ± 0.2	6.6 ± 0.1	5.9 ± 0.2	5.5 ± 0.3
Total cholesterol (mg/dL)	101.8 ± 0.5	106.9 ± 1.1	107.0 ± 0.6	97.6 ± 0.2
Triglycerides (mg/dL)	35.1 ± 0.2	45.7 ± 0.3*	54.4 ± 0.2 **	37.6 ± 0.2
HDL Cholesterol (mgdL)	30.4 ± 0.3	30.5 ± 0.5	32.8 ± 0.2	31.8 ± 0.2

Results are presented as Mean ± SEM (n=6); * $p<0.05$ as compared to the control; ** $p<0.01$ compared to the control.

Effect of the leaf extract on serum electrolytes, urea and creatinine

Pseudocedrela kotschyi ethanol leaf extract did not produce a significant ($p>0.05$) effect on the serum electrolytes, sodium, potassium, chloride and bicarbonate after 28 day daily administration. Furthermore, the extract did not show significant ($p>0.05$) change in urea in treated animals

compared to the control (Table 4).

Table 4. Effect of *Pseudocedrela kotschyi* leaf extract (dose:mg/kg) on serum electrolytes, urea and creatinine in rats..

Parameter	Control	100	200	400
Na^+	140.8 ± 0.2	141.4 ± 0.5	141.0 ± 0.5	141.0 ± 0.3
K^+	4.8 ± 0.1	5.4 ± 0.2	5.4 ± 0.1	4.7 ± 0.3
Cl^-	96.6 ± 0.4	97.7 ± 0.4	97.6 ± 0.4	96.5 ± 0.3
HCO^{-3}	24.7 ± 0.3	26.0 ± 0.3	25.0 ± 0.2	25.4 ± 0.2
Urea	24.6 ± 0.2	26.7 ± 0.6	25.6 ± 0.3	24.9 ± 0.3
Creatinine	0.7 ± 0.2	0.8 ± 0.2	0.9 ±0.3	0.9 ±0.5

Results are presented as Mean ± SEM (n=6); * $p<0.05$ as compared to the control; * $p<0.01$ compared to the control

Effect of the leaf extract on organ weight

There were no significant $p>0.05$) difference in the weight of the organs between the control and the extract treated rats except for a significant elevation in the weight of the lungs at the doses of 100 mg/kg ($p<0.01$) and 400 mg/kg ($p<0.05$) when compared to the control (Table 5).

Table5. Effect of *Pseudocedrela kotschyi* leaf extract (dose:mg/kg) on rat relative organ weights.

Organs (g)	Control	100	200	400
Heart	0.62 ± 0.04	0.56 ± 0.04	0.51 ± 0.02	0.73 ± 0.08
Lungs	0.89.02± 0.04	1.08 ± 0.13*	0.99 ± 0.07	1.12 ± 0.07*
Kidneys	1.06 ± 0.06	0.99 ± 0.12	0.86 ± 0.05	1.25 ± 0.16
Liver	5.02 ± 0.25	4.25 ± 0.06	4.23 ± 0.23	5.10 ± 0.33
Spleen	0.73 ± 0.07	0.82 ± 0.17	0. 71 ± 0.04	0.82 ± 0.06

Results are presented as Mean ± SEM (n=6); * $p<0.05$ as compared to the control; * $p<0.01$ compared to the control.

4 Discussion

Herbal medicines often possess numerous active pharmacological constituents and such has been the basis for the treatment of different diseases (Ibrahim et al., 2016). Phytochemical analysis of *P. kotschyi* leaf extract showed a positive reaction to alkaloids, flavonoids, terpenoids, steroids, tannins, saponins and cardiac glycosides (Akuodor et al., 2013). However, this plant contains biological compounds with potentials to produce beneficial or adverse effects, a comprehensive toxicity study is essential to ascertain its safety. The aim of investigating the safety of any medicinal agent is to identify the nature and importance of detrimental effects and also to know the exposure level where the activity is observed. Some of the risks which may be associated with the use of herbal agents can be revealed through toxicity testing (Wanang et al., 2014; Obidike and Salawu, 2013). The lack of mortality or visible side effect in rats treated with 5g/kg dose of the ethanol leaf extract of P. kotschyi showed that the extract is practically non-toxic after an acute exposure. The high safety profile obtained may have been responsible for its wide spread use in different ethno-therapeutic interventions.

The administration of *P. kotschyi* over the period of 28 days did not significantly affect food

consumption and body weight relative to the control group, indicating that it did not suppress appetite. Changes in body weight are sensitive indicators of adverse drug effects and have been used to assess response to drugs and chemicals (Santos et al., 2009). This result showed that there was no adverse effect of the administered doses of the leaf extract on the normal growth of rats. Analysis of haematological indices can be used to determine the level of the toxic effect of plant extracts (Obidike et al., 2011). It can as well be used to determine blood- related activities of plant products (Agbaje et al., 2009). In addition, such analysis is important for risk evaluation as changes in the haematological system have higher predictive values for human toxicity when data are translated from animal studies (Yakubu et al., 2007). There was a significant increase in the platelet count which can possibly result from anaemia and induced myeloproliferative conditions. These conditions can be ruled out in this case as other haematological parameters (packed cells volume, red blood cells, mean corpuscular volume, mean cell haemoglobin, mean cell haemoglobin concentrate, haemoglobin) were not affected after administration of *P. kotschyi* leaf extract for 28 days. The non-significant effect of the extract on these parameters does not affect erythropoesis and morphology of the red blood cells (Olson et al., 2000). White blood cells are the first line defence responders to infectious agents and other inflammatory processes. More so, no significant changes were observed in neutrophils, lymphocytes and monocytes, which also confirmed our findings.

The biochemical analysis were carried out to evaluate the possible alterations in liver and kidney functions influenced by this leaf extract. Hepatic and renal function examination is very essential in the toxicity analysis of drugs and plant products as they are both important for the survival of an organism (Guyton and Hall, 2006). *P. kotschyi* leaf extract showed no significant changes in all the biochemical parameters in the 28 day treatment period except for a significant increase in plasma triglycerides. Measurement of albumin can represent nutritional status which may be used to test for and assist in the diagnosis of liver and kidney diseases (Olorunisola et al., 2012; Thierry et al., 2011). The non-significant change in serum albumin observed in the study may suggest potential hepatoprotective effect P. kotschyi leaf extract. Changes in the level of total cholesterol triglycerides and high density lipoprotein cholesterol can give important information on lipid metabolism and predisposition of the heart to atherosclerosis and other cardiovascular diseases (Ibrahim et al., 2016). The data obtained showed non-significant increase in total cholesterol and high density lipoprotein cholesterol parameters, but a significant increase in triglycerides parameter of the treated rats. Determination of serum electrolytes, sodium, potassium chloride, bicarbonate, urea and creatinine parameters are essential markers of kidney function and increase in the levels of these parameters are indicative of kidney infection (Pareick-Iwuanyanwu et al., 2012). The leaf extract caused no significant changes in the serum electrolytes in the treated compared to control rats.

Generally, decrease in internal weight of an organ is an indication of toxicity due to exposure to toxic agents (Akindele et al., 2014). In respect to the vital organs, there were no significant changes in weight relative to the control in the 28 day treatment period except for a significant increase in weight of the lungs at the small and highest doses, while the liver had significant increase at the highest dose. The weight of the lung is less important in toxicity studies probably due to its low frequency of finding weight changes that correlate with toxicity and it is less sensitive predict toxicity compared to histopathology (Raza et al., 2002; Micheal et al., 2007; Amna et al., 2013). However, since the weight of the organs for toxicity was not significantly altered, it could be said that the leaf extract does not produce a toxic effect on the vital organs of the treated compared to control rats in the study.

In conclusion, the findings from this investigation provide useful data on the acute and sub-acute toxicological studies of *P. kotschyi* leaf extract. The plant has been shown from the results

obtained to be safe on acute and sub-acute oral administration. The results observed at the treatment doses showed potential for boosting components of the immune system and protecting the cardiovascular, kidney and liver systems. However, its effects in long term use needs to be further investigated.

Conflict of interests

The authors declare that there are no competing interests.

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