OPEN ACCESS
Saudi Journal of Medical and Pharmaceutical Sciences
Abbreviated Key Title: Saudi J Med Pharm Sci
ISSN 2413-4929 (Print) |ISSN 2413-4910 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: http://scholarsmepub.com/sjmps/

Original Research Article

Sub-Chronic Oral Toxicity Study of *Pseudocedrela Kotschyi* Ethanol Leaf Extract in Wistar Rats

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DOI:10.36348/SJMPS.2019.v05i09.005 | **Received:** 20.08.2019 | **Accepted:** 27.08.2019 | **Published:** 21.09.2019

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Abstract

The leaves of *Pseudocedrela. kotschyi* are used in herbal medicine in Sub-Saharan Africa without safety concerns. Determination of its safety profile will provide supportive scientific evidence in favour of its continuous usage. To evaluate the sub-chronic toxicity activity of the ethanol extract of *Pseudocedrela. Kotschyi* leaves. Sub-chronic toxicity evaluation of the extract was determined by administering 100 mg/kg, 200 mg/kg and 400 mg/kg on Wistar rats for 40 days with distilled water as control. The haematological and biochemical parameter as well as the relative organ weights were examined. In the 40 days sub-chronic oral toxicity study, administration of 100 mg/kg, 200 mg/kg and 400 mg/kg of *P. kotschyi* leaf extract per body weight showed significant ($p<0.05$) body weight change, significant ($p<0.05$ and $p<0.01$) changes in some haematological and biochemical parameters and organ weights compared to the control group. Analyses of these results could lead to the conclusion that the oral administration of *P. kotscyi* leaf extract for 40 days does not cause sub-chronic toxicity in rats

Keywords: *Pseudocedrela kotschyi*, Maliaceae, Subchronic toxicity, Leaf extract, Herbal medicine, Rats.

INTRODUCTION

Medicinal plants have been used for decades as remedies for human diseases. Most of these natural products have strong scientific evidence with regard to their biological activities. However, little information is available regarding the possible toxicity that medicinal plants may cause to the end users [1]. Based on the long-term use of medicinal plants by humans, one might expect these plants to have low toxicity. Nevertheless, recent surveys have indicated that numerous medicinal plants applied in traditional medicine exhibited adverse effects [2, 3]. Hence, it should be emphasized that the traditional use of any plant for medicinal purposes must guarantee the safety of such plant. This calls for concern about the important toxic effects emanating from the short-term and long-term use of such medicinal plants. The values of the acute, sub-acute and sub-chronic toxicity investigations on medicinal plants should be obtained to increase the confidence in their safety to humans, especially for use in the development of pharmaceuticals products [4].

Pseudocedrela kotschyi Schweint. Harms which belonging to the family Meliaceae is a tree of up to 20 metres high with a wide crown fissured bark and fragrant white flowers. *P. kotschyi* is a very popular herb amongst practitioners of traditional medicine and is widely used as a decoction or infusion to treat various diseases. The antmalaria, antipyretic, dental cleaning, analgesic and anti-inflammatory, antibacterial and antiepileptic [5-11] activities of different extracts of the plant have been scientifically investigated and reported. The acute and sub-acute toxicity profiles of the plant leaf extract has also been reported [12]. Based on *P. kotschyi* use in herbal medicine practices and the literature references, the present study was undertaken to evaluate the sub-chronic toxicity in rats after oral dosing for 40 days.

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.

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, giving a dark brown solid with a yield of 17.65%. The dried extract was stored in a refrigerator at 4^0C 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 temperature of 25–27 ^{0}C and 12-h light/dark cycle. The animals had free access to rodent pellet and portable water *ad libitum*. The rats were handled with humane care and in accordance with Institutional guidelines of Ethics Committee of Ebonyi State University (EBSU/UREC/TETFUND/15/14) and international accepted principles for laboratory animal use and care [13].

Sub-chronic toxicity study

Wistar rats of either sex weighing between 170-200 g were used for this study and evaluation of sub-chronic toxicity was performed as the methods described by OECD guidelines 407 [14]. The animals were fed with standard pellet and had water *ad libitum*. The rats were randomly allotted to four groups of 6 animals each. The Group I rats served as control and was administered 0.2 mL of distilled water via the oral route. Group 2, 3 and 4 received an ethanol extract of *P. kotschyi* leaf orally at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg daily for 40 days.

Animal observation

All animals were weighed individually immediately before dosing on day one and thereafter, once a week. All animals were observed daily for mortality, general condition, and clinical signs of toxicity before the test and throughout the dosing period. Clinical observations including motor activity, appearance, central and autonomic functions were performed once a week. Daily food consumption was measured before the initiation of dosing and once a week thereafter. Food consumption was calculated by subtracting the amount of leftover feed from the total feed provided.

Haematology and serum biochemistry

On day 40, animals were fasted overnight and then sacrificed the following day under inhaled chloroform anaesthesia. Blood samples were collected via cardiac puncture [15] for determination of routine haematological and serum biochemistry analysis.

Haematology measurements (Mythic 18 automated haematology analyzer by Orphee, Switzerland) included the following: Red blood cell count (RBC), hemoglobin concentration (HB), packed cell volume (PCV), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), platelet count (PLT), white blood cell count (WBC), WBC differential count (neutrophil, lymphocyte and monocyte ratio).

Serum biochemistry analysis (Gesan Chem 200, USA) included: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN),creatinine (Crea), albumin (ALB), total protein (TP), total cholesterol (T-Chol), triglyceride (TG) and high density lipoprotein (HDL) of the samples were determined.

Organ weight measurement

Following the sacrifice, qualitative data on the weights of vital organs (heart, lungs, liver, kidneys and testes) were assessed by carefully dissecting each organ from sacrificed animal. The isolated organs were dried with cotton wool and weighed on a sensitive balance. Each weighed organ was standardized for 100 g body weight of each rat [16].

STATISTICAL ANALYSIS

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

RESULTS

Effect of the ethanol 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 100 and 200 mg/kg during the 40 days treatment.

Effect of the ethanol leaf extract on haematological parameters in 40-day study

Table 2 shows a non-significant ($p>0.05$) increase in RBC and HB compared to the control, whereas PLT and WBC MCV showed a significant ($p<0.05$) increase when compared to control. There was a decrease level of MCV except at 400 mg/kg where a significant ($p<0.01$) was observed compared to control. The results also showed a non-significant ($p>0.05$)

decrease in MCH compared to the control, while a significant decrease was observed in MCHC compared to the control. The lymphocytes and neutrophils percentage (%) showed a significant ($p<0.01$) increase in the group of animals administered 100 mg/kg, 200 mg/kg and 400 mg/kg, while the monocytes percentage (%) showed a significant ($p<0.05$) at 200 mg/kg and 400 mg/kg compared to the control. The PCV showed a significant ($p<0.01$) increase at both 200 mg/kg and 400 mg/kg compared with the control.

Effect of the ethanol leaf extract on serum biochemical parameters in 40-day study

The data in Table 3 show the effects of ethanol root bark extract on liver enzymes and lipid profile. Asparatate transaminase (AST) levels show a significant ($p<0.05$) increase in all the treated groups when compared to the control. However, there was a statistical significant ($p<0.01$) increase in alanine transferase (ALT) levels in all the treated groups when compared to the control. Alkaline phosphatase (ALP) and albumin levels showed statistically significant ($p<0.01$ and $p<0.05$) increases respectively, when compared to the control. In the lipid profile study, there was significant decrease in levels of total cholesterol (T Chol) while increase in triglycerides (TG) levels was observed in the treated groups compared to the control. There was non-significant ($p>0.05$) increase in the levels of High Density Lipoprotein (HDL) observed when compared to the control.

Effect of the ethanol leaf extract on serum electrolytes, urea and creatinine in 40-day study

The leaf extract of *P. kotschyi* did not show any significant ($p>0.05$) activity on the serum electrolytes, sodium, potassium, bicarbonate and urea after administration of the extract for 40 days except significant ($p<0.05$ and $p<0.01$) non-dose dependent increase in creatinine and chloride levels in treated rats compared to the control (Table 4).

Effect of the ethanol leaf extract on weights of organ of rats per 100 g body weight

There were statistical significant ($p<0.05$ and $p<0.01$) dose dependent in the weight of the organs (liver, kidneys, lungs and spleen), but there was no significant ($p>0.05$) difference in the weight of the organ (heart) per 100 g body weight when compared with control (Table 5).

Table-1: Effect of the extract on body weight of rats after 40 days of sub-chronic toxicity study Dose (mg/kg) Percentage weight change

Control	**67.85±4.79**
100	82.50±5.69
200	87.44±6.57
400	79.65±4.34

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

Table-2: Effect of the extract on haematological parameters in rats after 40 days sub-chronic toxicity study

	Dose (mg/kg)			
Parameter	**Control**	**100**	**200**	**400**
Red blood cells (x10^{12}/L)	5.91±0.27	4.92±0.16	5.65±0.25	4.65±0.40
Haemoglobin (g/dL)	10.77±0.58	12.40±0.47	12.30±0.43	12.45±0.66
Mean corpuscular volume (fl)	61.38±0.63	60.63±1.17	61.88±0.35	68.27±0.78**
Mean cell haemoglobin (pg)	21.38±0.21	21.67±0.36	21.20±0.27	21.22±0.35
Mean cell haemoglobin concentration (g/dl)	39.50±0.63	35.22±0.66	34.33±0.46	34.72±0.46
Pack cells volume (%)	30.57±1.83	33.35±2.26	38.53±1.54**	37.19±2.86**
Platelet count(x 10^{9}/L)	304.17±37.64	313.67±38.67	322.17±28.66	328.75±54.82
White blood cells (x 10^{9}/L)	3.95±0.64	4.88±0.64	4.47±0.68	4.65±0.40
Lymphocytes (%)	67.52±1.39	84.83±1.33**	82.83±2.83**	84.00±2.86**
Neutrophils (%)	6.00±1.18	11.00±1.22**	13.33±1.94**	11.83±1.80**
Monocytes (%)	4.17±0.65	4.17±0.39	5.83±0.39*	5.95±0.38*

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

Table-3: Effect of extract on serum biochemical parameters in rats after 40 days of sub-chronic toxicity study

	Dose (mg/kg)			
Parameter	**Control**	**100**	**200**	**400**
Aspartate transaminase (U/L	60.48 ± 5.34	64.52 ± 6.10	63.66 ± 5.25	66.84 ± 8.48*
Alanine transaminase (U/L)	43.19 ± 3.48	45.67 ±4.32	43.9 ± 3.92	44.64 ± 4.63
Alkaline phosphatase (U/L)	102.80 ± 6.11	120.55 ± 8.57**	110.62 ± 9.46*	108.71 ±10.38*
Albumin (mg/dL)	6.36 ± 0.50	70.52 ± 0.46*	6.89 ± 0. 48	6.95 ± 0.39
Total cholesterol (mg/dL)	134.20 ± 11.42	128.49 ± 9.19	120.64 ± 10.60	118.56 ± 10.20
Triglycerides (mg/dL)	54.43 ± 7.15	72.44 ± 7.63**	66.56 ± 8.42*	61.72 ± 8.55*
HDL Cholesterol (mg/dL)	38.43 ± 0.38	38.64 ± 0.67	37. 92± 0.55	37.84 ± 0.88

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

Table-4: Effect of the extract on serum electrolytes, urea and creatinine in rats after 40 days sub-chronic toxicity study

	Dose (mg/kg)			
Parameter	**Control**	**100**	**200**	**400**
Sodium (meq/L)	147.77 ± 3.52	148.47 ± 3.66	148.40 ± 4.18	146.87 ± 4.38
Potassium (meq/L)	5.15 ± 0.51	5.55 ± 0.76	5.77 ± 0.82	5.10 ± 0.66
Chloride (meq/L)	108.54 ±3.65	115.69 ±5.69**	113.71 ±4.75**	111 .89 ± 5.28*
Bicarbonate (meq/L)	34.85 ± 1.44	35.68 ± 2.29	34.95 ± 1.53	35.76 ± 1.66
Urea (mg/dl)	28.47 ± 4.60	28.69 ±3 .66	27.55 ± 3.42	27.88 ± 3.76
Creatinine (mg/dl)	0.80 ± 0.14	0.89 ± 0.17	1. 02. ±0.22*	0.90 ±0.33*

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

Table-5: Effect of the extract on rat relative organ weights after 40 days sub-chronic toxicity study Dose (mg/kg)

Organs (g)	**Control**	**100**	**200**	**400**
Liver	8.49±0.53	6.64±0.34*	4.55±0.17**	4.72±0.26**
Kidneys	1.13±0.67	0.95±0.17	0.65±0.63**	0.76±0.14**
Heart	0.66±0.44	0.66±0.88	0.56±0.62	0.56±0.64
Lungs	1.48±0.17	1.65±0.64	1.66±0.29	1.21±0.23**
Spleen	0.68±0.06	0.69±0.68	0.55±0.66*	0.57±0.67*

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

DISCUSSION

Medicinal plants mostly contain highly potent pharmaceutical constituents which has actually been the basis for the treatment of different diseases [17]. Although, *P. kotschyi* leaf extract contains bioactive compounds with potential to cause either beneficial or detrimental effects, which makes it imperative to carry out toxicity study to ascertain its safety and efficacy. The aim of evaluating the safety profile of any herbal agent is to identify the significant nature and the leaf extract adverse effects, also to determine the exposure level where the effect is noticed.

Variations in body weight are sensitive parameters of effects of chemicals and drugs [18]. Daily administration of the ethanol leaf extract for 40 days showed significant changes in body weight gain in extract treated rats compared to the control. The observed results showed that there was effect on rats' normal growth at the treatment of the sub-chronic oral doses of *P. kotschyi* ethanol leaf extract. The various investigated haematological indices are important parameters that can be studied to assess the toxic effect of plant extracts in both human and animals [19]. The RBC and HB are very important in transferring respiratory gases, and their non-significant effect in the test groups when compared to the control shows that there was not much change in the oxygen carrying capacity of the blood and the amount of oxygen carried to the tissues due to treatment with different doses of the leaf extract to the test animals. There was significant increase in PCV at the highest dose (400 mg/kg) in the test groups at all doses of the treated extract of *P. kosctyii* leaf when compared to control.

MCV, MCH and MCHC have been reported to be significant in diagnosing anaemia in animals and human red blood cells [20, 21]. The non-significant effect of the leaf extract at the treated doses on RBC and some of its indices (HB, MCH and MCHC) in the study could be an indication that there was no destruction of RBCs. Furthermore, this observation might equally be shown to the fact that the correlation between the production rate and destruction of the corpuscles was not significantly altered by the extract. The result also show that the leaf extract does not possess the potential to stimulate the release of erythropoietin in the kidney which is the regulator of RBC production [22, 23].

A decrease in platelet count in laboratory animals has been reported to indicate an adverse effect on the oxygen-carrying capacity of the blood as well as on thrombopoietin [24]. However, it was on the contrary in the present study as an increase in platelets was observed in the test groups compared to the control. The observed result may be due to the stimulatory effect of the extract on thrombopoietin.

However, the significant ($p<0.05$) increase observed in WBC following the administration of the plant extract may be due to increase in vascular permeability. Results also showed that other indices that relate to WBC (neutrophils, lymphocytes and monocytes) were significantly increased in the test groups compared to the control group. The lymphocytes are believed to be main effector cells of the immune system and the observed increase in the test groups compared to the control in this study may specifically be ascribed to the ability of the extract to stimulate neutrophils to promote phagocytosis [25]. High neutrophil counts can be the result of many factors that include bacterial infection, acute inflammation, stress

response effect from some drugs and splenectomy, among others [26].

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) have been determined to be markers of hepatocellular injury while alkaline phosphatase (ALP) is a marker of cholestasis [27]. Of the two, increase in ALT levels is a more specific indicator of liver injury because ALT catalyses the conversion of alanine to pyruvate and glutamate and is released in a similar manner. A non-significant decrease in AST, significant ($p<0.001$) decrease in ALT levels, as well as an insignificant ($p>0.05$) increase in ALP levels was observed in the test groups compared to the control. It is possible to speculate that this observed effect of the extract was due to its ability to stabilize the plasma membrane and may be a potential in modulating the exogenous toxic effects of agents on liver cells. The serum ALP levels are also related to the status and function of hepatic cells and according to the literature, an increase in serum ALP may occur as a result of increased synthesis, in the presence of increasing biliary pressure.

Determination of serum electrolytes, Urea and Creatinine are important markers of kidney function and elevations in the levels of these parameters are indicative of kidney injury [28, 29]. The extract caused significant increase in Chloride and creatinine with no observable significant changes in other serum electrolytes and blood urea parameters in the treated compared to control rats. These results indicated that the plant extract has no toxic effect on the kidneys.

Generally, reduction in internal weight of an organ is an indication of toxicity due to exposure to toxic substances [30]. In respect to the vital organs, significant changes in weight were observed in the liver, kidneys, lungs and spleen in the 40 days treatment period except for a no significant decrease in weight of the heart. The observed increase in weight of the liver, kidneys, lungs and spleen in sub-chronic study may however be attributed to unknown cause.

In conclusion, the findings from this study provide valuable data on the sub-chronic oral toxicological profile of *P.kotschyi* leaf extract. The plant has been reported to be non-toxic on acute exposure [12]. Further study on the chronic toxicity is needed to fully ascertain the safety profile of *P. kotschyi* leaf extract.

TRANSPARENCY DECLARATION

The authors declare that there is no conflict of interest.

ACKNOWLEDGMENTS

This project was supported by funding from Tertiary Education Trust Fund (TETFund) administered by the Directorate of Research, Innovation & Commercialization, Ebonyi State University, Abakaliki, (Ref No. EBSU/TETFund/IBR/15/14). The authors are grateful to Mr. Simon Eze Nwibo and Chibueze Nwonu for their technical assistance.

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