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A 28- Day Oral Toxicity Study of *Pseudocedrela kotschyi* Methanol Extract in Sprague-Dawley Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AK, MBB, AJA and DNM designed the study and performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Authors YMF, YA, HSY and AK performed the experiment managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate the safety profile of *Pseudocedela kotschyi* which is used in traditional medicine for the treatment of epilepsy, malaria, diarrhoea and pains, a 28 day sub chronic toxicity study was conducted was evaluated using the 28 day subchronic toxicity study.
Place and Duration of Study: This study was conducted in the Departments of Pharmacology, Faculty of Pharmacy, University Sains Malaysia, during the period between January 2013 and February 2014.
Methodology: The methanolic extract of *P. kotschyi* stem bark was evaluated for acute and sub chronic toxicity in female and male rats. In the acute oral toxicity study, a limit dose of 2000 mg/kg

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was administered to five non-pregnant female rats by oral gavage. They were observed for signs of toxicity/mortality for 14 days. In the sub chronic toxicity study 48 rats of both sexes were grouped into 4 groups of 12 animals (6 males, 6 females) and treated with *P. kotschyi* extract at a dose of 40, 200 and 1000 mg/kg respectively, the fourth group was considered as a control.

Results: The 28 days acute oral toxicity study of *P. kotschyi* demonstrated a lack of toxicity of the methanol extract. Parameters such as general behavioural changes were observed to be normal; moreover no death was recorded at the end of the study period. Assessment for signs of chronic toxicity indicated no abnormalities in the test groups as compared to the controls. Haematological and biochemical values in treated groups were normal in comparison with the control group. Insignificant changes in body weight, internal organ weight and general behaviour were considered to be incidental.

Conclusion: The stem bark methanol extract of *P. kotschyi* administered orally to female and male rats was relatively safe at the doses administered. We therefore conclude that toxic effects, if any occurred at doses higher than those used in our country.

Keywords: Pseudocedrela kotschyi; acute toxicity; rats; subchronic toxicity; meliaceae.

1. INTRODUCTION

Pseudocedrela kotschyi (meliaceae) is a medium sized tree, sometimes up to 60 ft high. It is well distributed in Senegal, Congo basin, Uganda and Nigeria. The plant is known as t*una* in Hausa and *Emi gbegi* in Yoruba [1]. The plant is used traditionally in Ghana to treat leprosy and epilepsy [2]. The twigs and leaves are important in the management of malaria and stomach aches [3]. A decoction of both the fresh and dried leaves is used for the treatment of malaria in Ogun State Nigeria [4]. The roots and leaves are used to treat rheumatism and dysentery [5]. In northern Nigeria, the plant serves as an infrequent constituent for use in arrow poison and treatment of insomnia [6]. In West Africa, it has been established that the root of *P. kotschyi* is widely used as chewing sticks for dental cleaning [7-10]; and in North Côte d'Ivoire, it is of value in the treatment of toothache and internal wounds. The antibacterial potential of the root was reported by [11] in addition to its use in the treatment of intestinal helminthosis.

Chemical constituents of *P. kotschyi* includes the stem and root barks (essential oils, mainly sesquiterpenoids [12] and pseudocedrelin [13]). Limonoids, 7-deacetoxy-7-oxogedunin and pseudrelones A, B and C were isolated from the wood oil [14-16].

Some pharmacological activities previously investigated include anti-nociceptive and anti-inflammatory activities [17], effect on Phenobarbitone induced sleep [18] and antimalarial potentials [10].

Despite its widespread use, little toxicological data are available regarding the safety of repeated exposure to *Pseudocedrela kotschyi*. As part of a safety evaluation of *P. kotschyi*, a toxicological study of its potential toxicity after single and 28-day repeated oral dosing of the methanol extract of in Sprague Dawley rats was inevitable.

2. MATERIALS AND METHODS

2.1 Experimental Samples

Pseudocedrela kotschyii stem bark was collected from Zuru Local Government area of Kebbi State, Nigeria in the month of July 2012. It was authenticated by Dr E.M Mshelia of the Department of Pharmacognosy and Ethno pharmacy, Faculty of Pharmaceutical Sciences Usmanu Danfodiyo University Sokoto. A voucher specimen (PK509-10) was deposited at the Departmental herbarium. Four kilograms of previously cleaned and shade dried plant material was pulverized into a fine powder. The dried powder was then extracted with methanol, using a soxhlet apparatus maintained at a temperature not exceeding 40°C for duration of 16-18 hours. The filtrates were concentrated under vacuum in a rotary evaporator. The concentrated filtrates were then freeze-dried to produce a powder that was used in the study (all the equipment and apparatus for extraction and freeze-drying were cleaned and disinfected with 70% ethanol prior to use). The yield obtained was 20% (w/w). The dried extract was sealed in a bottle and stored in the refrigerator at -20°C until further use. The powder was subsequently reconstituted in distilled water to the final concentrations required for the experiments. The extract was administered to both female and male rats by oral gavage, at a volume of 10 ml/kg.

2.2 Experimental Animals

Male and female Sprague-Dawley (SD) rats at 8 weeks of age (male, 200–220 g; female, 160–190 g) were used for the acute and sub chronic toxicity studies. The rats were obtained from the Animal Research and Service Centre, Universiti Sains Malaysia. The animals were acclimatized to laboratory conditions for 7 days prior to the experiments. During acclimatization, three rats were housed per polycarbonate cage, with free access to normal diet [48% carbohydrate, 23% crude protein, 3% crude fat, 8% crude ash, 5% crude fibre and 13% moisture] and tap water *ad libitum*. The food pellets for the experimental animals were purchased from Gold Coin Holdings Sdn. Bhd. (Malaysia). The rats were maintained at 26±4°C under a light/dark cycle of 12 h and relative humidity 70%±10%. All procedures in this study were performed according to the requirements of the Animals Ethics Committee, Universiti Sains Malaysia.

2.3 Acute Toxicity Study in Rats

Five female rats (nulliparous and non-pregnant) weight (160-190 g) were used for the acute oral toxicity study. The rats were fasted overnight and administered with a single oral dose of (2000 mg/kg) one at a time. Each rat was observed for signs of toxicity and mortality every 30 minutes for the first four hours and then two hourly for fourty eight hours. Each animal was further observed daily for fourteen days. Since all the animals survived, the limit dose was terminated and the LD_{50} was assumed to be greater than 2000 mg/kg.

2.4 Sub Chronic Toxicity Study in Rats

The sub chronic oral toxicity study of the methanol stem bark extract of *Pseudocedrela kotschyi* was conducted according to the protocols described by OECD Guideline 407 [19], with minimum modification. Sprague Dawley rats of both sexes were randomly assigned into four groups; a control group and three treatment groups (n = 12; 6 males and 6 females). *P. kotschyi* methanol extract was dissolved in distilled water and administered orally on a daily basis for 28 days at single dose of 40, 200 and 1000 mg/kg (the volume of the extract was given at 10 ml/kg), while the control group received only distilled water. The behaviour of the rats was observed daily, and their weights were recorded once per week. At the end of experiment (28 days), all the rats were anesthetized under diethyl ether inhalation, and their blood samples were collected via cardiac puncture into non-heparinised and EDTA containing tubes for biochemical and haematological analyses, respectively [20,21]. After cardiac puncture, the rats were killed by cervical dislocation. The liver, kidney, adrenal gland, lung, brain, spleen, heart, testes, ovaries, uterus, thymus and gut were excised, weighed, and examined macroscopically. The relative organ weight was calculated as (organ/body weight)×100%. Vital organs such as liver, kidneys and lungs were preserved for histopathological analysis. The organs were fixed in 10% formalin before being processed using Citadel 1000 histokinette (Shandon Scientific Ltd., Cheshire, UK). After processing, the tissues were embedded in paraffin with Histo-Centre II-N (Barnstead/Thermolyne Corp., Dubuque, USA) and sectioned to a thickness of 5 µm using a Reichert-Jung Histocut 820 II (Cambridge Instrument GmBH, Nussloch, Germany). The sections were stained with haematoxylin and eosin. The tissues were examined under a microscope in a random order and without knowledge of animal or group. The renal injury was based on degeneration of Bowman space and glomeruli, degeneration of proximal and distal tubules, vascular congestion and interstitial edema. The criteria for liver injury were vacuolization of hepatocytes and pyknotic hepatocyte nuclei, number of Kupffer cells and enlargement of sinusoids.

2.5 Blood Analyses

Haematological and biochemical analyses were performed at Gribbles Pathology Laboratory Malaysia Sdn. Bhd., Penang. Full blood cell counts were determined using a ABX Micros 60 Haematology Analyzer (Diamond Diagnostics, USA) and serum biochemistry tests were performed using an automated analyser (Selectra Junior, Vital Scientific B.V., Netherlands), according to the manufacturer's instruction using reagents purchased from Fortress Diagnostics (United Kingdom).

2.6 Statistical Analysis

The results of the study were presented as mean±S.E.M; statistics were performed using one-way analysis of variance (ANOVA), by using the statistical package for social sciences (SPSS) version 19. Significant differences between the control and treatments groups were determined using Dunnett's test and $P < 0.05$ was considered significant.

3. RESULTS

3.1 Acute Toxicity Study in Rat

No death was recorded following oral administration of *P. kotschyi* methanol extract (2000 mg/kg) after the fourteenth day. There were no significant changes in the body weight, food intake, appearance and behaviour of the animals. This suggests that the acute oral LD_{50} of the extract is greater than 2000 mg/kg.

3.2 Sub Chronic Toxicity Study in Rats

3.2.1 Effect of oral administration of *P. kotschyi* extracts on general behavior

There were no significant changes in the general behaviour of the animals relative to the control group. Respiratory pattern, cardiovascular signs, motor activities, reflexes, and normal change in skin and fur, all appeared to be normal [23].

3.2.2 Effect of oral administration of *P. kotschyi* extract on the body and organ weights

The weekly body weights and body weight gain of both female and male rats at various concentrations (40,200 and 1000 mg/kg body weight) are presented in Fig. 1. Both male and female rats showed a slight initial increase in body weight during the first two weeks and then, there was a decrease of the body weights towards the third and fourth weeks. All animals showed gain in weight at the end of the study but these weight changes were not statistically significantly from those of the control. As shown in Table 1, the male rats treated with the extract at a dose of 1000 mg/kg body weight, had their adrenal gland, lung and stomach weights slightly greater than that of the control. All other organ weights were slightly decreased compared to the control group. The female rats Table 1 showed a slight decrease in the weight of the heart, spleen, lung and stomach relative to the control. The weights of the liver, thymus, kidney, ovary, uterus, and gut were slightly increased.

3.2.3 Result of oral administration of *P. kotschyi* extract on the haematological indices

Haematological parameters of female and male rats were examined as shown in Table 2. Haemoglobin concentration, total red blood cell count, packed cell volume, mean corpuscular haemoglobin concentration and red blood cell distribution width were not significantly decreased at the dose of 1000 mg/kg in both sexes. There was insignificant difference in the values of the mean corpuscular volume (MCV) and the mean corpuscular haemoglobin (MCH) in female rats treated with 40 and 1000 mg/kg body weight. The dose of 200 mg/kg induced a significant reduction in the MCV and MCH values relative to the control group.

In the male rats, the red blood cell (RBC), and haemoglobin values in the group treated with 200 mg/kg body weight were significantly higher than those in the control group. In contrast the mean corpuscular volume and mean corpuscular haemoglobin concentration was significantly reduced. There was no observed difference in the values obtained between the control and the group treated with 1000 mg/kg. Nonetheless, the variation of these values was minor and remained within the normal ranges.

The differential white blood cell counts of the female and male rats are presented in Table 3. A significant decrease in eosinophils was observed with 40,200 and 1000 mg/kg body weight. Significant increase in lymphocytes was observed in the male rats at 200 and 1000 mg/kg, in contrast lymphocyte concentration was significantly reduced in the female rats at 40, 200 and 1000 mg/kg. A significant decrease in neutrophils was observed in the female groups treated with *P. kotschyi*, but such was not the case in the male group. Furthermore, a significant decrease in monocytes were observed in the females treated with 40, 200 and 1000 mg/kg body weight, similar to the control group (p<0.05).

3.2.4 Effect of oral administration of *P. kotschyi* extract on the biochemical parameters

The results of the biochemical tests of the female and male rats are summarized in Table 4. In the male rats treated with 40 mg/kg body weight, the concentrations of total protein and alanine aminotransferase (ALT) were significantly higher than that of the control group. In female rats treated with 40 mg/kg, aspartate aminotransferase (AST) and globulin were significantly increased. Furthermore, there was a significant reduction in alkaline phosphatase (ALP) level in the males treated with 200mg/kg with a corresponding elevation in creatinine concentration and ALT. AST, ALT and globulin levels were significantly increased in the female rats treated with 200 mg/kg of *P. kotschyi* extract. The male group treated with 1000 mg/kg had a

significant decrease in alkaline phosphatase and ALT activity levels. Similar observations were made for the 1000 mg/kg female group that had significant decreases in AST and ALP activities and, albumin and total protein concentrations as compared with the control.

3.3 Histopathological Study

Macroscopic and microscopic observation of the internal organs showed negligible structural changes. None of the macroscopic observations were considered to be treatment related. No gross abnormalities were attributed to treatment with *P. kotschyi* stem bark extract. The histopathological analyses of the liver and kidney showed no histological changes indicating abnormalities (data not shown).

4. DISCUSSION

Plant based medicines have attained greater relevance as adjuncts or alternatives to orthodox medicines. To rationalize the safe use of a plant-based medicine, the ethno botanical and ethno medical history of the plant has to be considered, in addition to its toxicological data if available. Alternatively the plant's toxicity profile has to be investigated using acute, sub chronic and chronic toxicity studies [22]. Since *P. kotschyi* has a history of long time traditional use, a limit test was performed in the acute oral toxicity study. According to the OECD test guideline 423, when there is information in support of low or non-toxicity, then a limit test can be performed. A limit test at the highest starting dose level (2000 mg/kg body weight) was conducted. There were no mortality or toxicity signs observed at

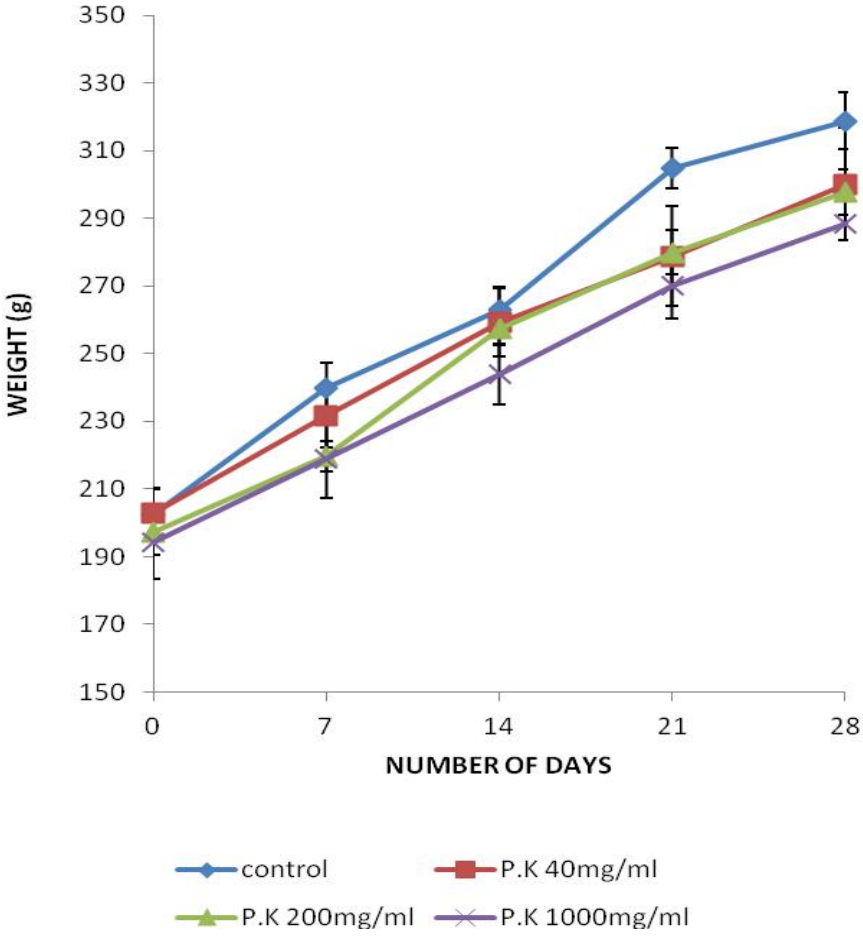

Fig. 1. Effect of sub chronic exposure to *Pseudocedrela kotschyi* extract on male rat body weights (expressed as mean +/- standard error of mean)

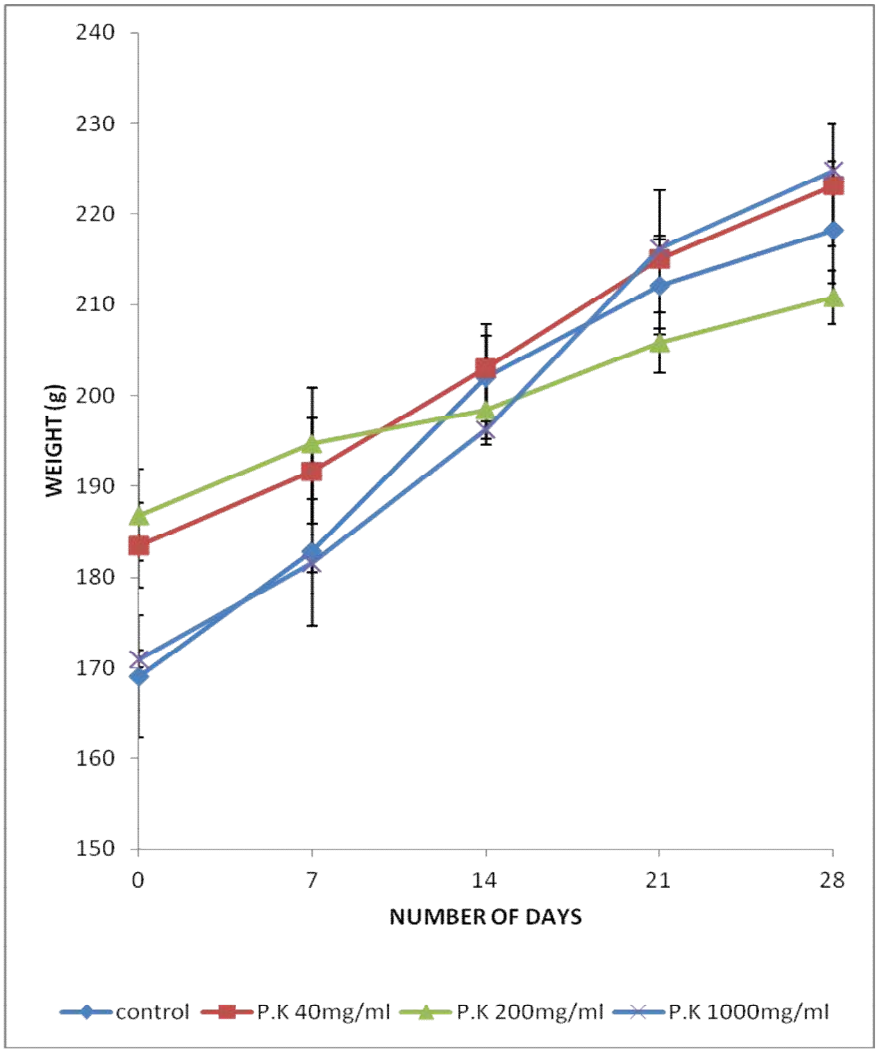

Fig. 2. Effect of sub chronic exposure to *Pseudocedrela kotschyi* extract on female rat body weights

2000 mg/kg. Based on these findings *P. kotschyi* can be classified under category-5 and LD50 value was greater than 2000 mg/kg in accordance with Globally Harmonised System (GHS) of Classification and Labelling of chemicals. This provides us a direct relevance for protecting human and animal health. It is therefore safe to say that the LD50 of the extract of *P. kotschyi* extract is greater than the 2000 mg/kg limit dose.

The 28-days oral toxicity study has been recommended as a basic test to assess the safety of a drug or chemical substance. Previous studies employing this test include studies by [20,21,23,24]. In the present study there were no behavioural or toxic effects attributed to the plant over the study period. There was no significant difference in body weight gain in the male group. The highest dose of 1000 mg/kg caused a slight significant difference compared to the control group. This effect may be due to a decrease in food intake observed during the first 1-2 weeks of the study. There was no significant difference in the body weight gain between the control and extract treated groups in the female rats. Moreover, an increase or decrease in the body weight of an animal after certain exposure period is indicative of the noxious nature of a drug or chemical [25]. In the present study there were no significant difference ($p< 0.05$) observed in either sexes of rats treated orally at 40,200 and 1000 mg/kg. The relative organ weights of all the tested animals, both control and treated

Table 1. Effect of sub chronic exposure to *Pseudocedrela kotschyi* on organ weights (g) on male and female rat body weights

	Treatment			
	Pseudocedrela	*kotschyi*		
	Control	40 mg/kg	200 mg/kg	1000 mg/kg
Males				
Heart	1.07±0.08	0.90±0.04	1.08±0.06	1.05±0.03
Liver	11.13±0.63	9.70±0.93	9.71±0.41	10.07±0.35
Thymus	0.43±0.08	0.37±0.05	0.42±0.04	0.38±0.06
Spleen	0.91±0.06	0.87±0.13	0.80±0.09	0.85±0.06
R-kidney	1.19±0.07	1.03±0.06	1.02±0.04	0.97±0.03
L-kidney	1.21±0.10	1.02±0.06	1.04±0.05	0.97±0.03
R-adr gland	0.03±0.00	0.04±0.00	0.045±0.00	0.05±0.01
L-adr gland	0.036±0.00	0.04±0.00	0.04±0.00	0.05±0.01
Lungs	1.83±0.09	1.72±0.12	1.78±0.11	1.88±0.06
R testis	1.67±0.11	1.43±0.06	1.39±0.09	1.51±0.08
L-testis	1.83±0.24	1.32±0.06	1.39±0.09	1.50±0.12
F stomach	3.30±0.76	2.86±0.55	3.53±0.87	3.98±1.17
E-stomach	1.41±0.14	1.31±0.19	1.45±0.21	1.54±0.27
F- gut	15.48±0.54	15.33±0.50	14.27±0.47	15.26±0.65
E-gut	8.08±0.48	8.27±0.70	8.33±0.86	7.82±0.68
Female				
Heart	0.80±0.03	0.82±0.04	0.76±0.03	0.76±0.03
Liver	8.09±0.50	8.47±0.56	8.30±0.38	8.42±0.36
Thymus	0.27±0.06	0.43±0.03	0.28±0.04	0.32±0.05
Spleen	0.68±0.04	0.65±0.04	0.60±0.06	0.58±0.03
R-kidney	0.68±0.04	0.72±0.05	0.66±0.03	0.69±0.01
L-kidney	0.71±0.04	0.70±0.04	0.69±0.03	0.70±0.02
R-adr gland	0.05±0.00	0.05±0.00	0.05±0.01	0.05±0.01
L-adr gland	0.05±0.00	0.05±0.00	0.04±0.00	0.04±0.00
Lungs	1.79±0.11	1.73±0.06	1.88±0.21	1.52±0.09
Ovaries	0.20±0.02	0.23±0.01	0.18±0.03	0.16±0.03
Uterus	0.43±0.05	0.55±0.05	0.50±0.04	0.50±0.06
F stomach	3.82±0.64	3.71±0.62	3.99±0.64	3.74±0.45
E-stomach	1.39±0.18	1.48±0.24	1.76±0.39	1.25±0.06
F- gut	13.06±0.09	15.57±1.11	15.24±0,79	16.83±1.30
E-gut	6.79±1.07	9.03±1.12	9.03±0.45	10.85±0.67

Values are expressed as mean ± S.E.M., n = 6

group continued to increase throughout the 28 days study period. This effect indicates that the LD50 of *P. kotschyi* methanol extract is greater than 2000 mg/kg.

Haematological parameters such as haemoglobin concentration, red blood cells number, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, mean platelet volume and red cell width were found to fall within the clinical range of rats [26]. The haematopoietic system is very sensitive to the presence of toxins; therefore values obtained after exposure of an animal to toxic compounds can greatly indicate the pathological and/or physiological status of the test animals [27]. The differential white blood cell count (Table 3) indicated a significant decrease in the total white blood cell, neutrophils, lymphocytes and monocytes in the female rats treated with *P. kotschyi* extract. Although some hematologic values differ significantly from those of the control group, they were still observed to fall within the normal ranges for rats [23].The variations may be due to normal variations within animals individually or in group, therefore it is safe to say from the haematological parameters that *P. kotschyi* methanol extract did not cause toxic effects in rats [28].

Table 2. Effect of sub chronic exposure to *Pseudocedrela kotschyi* on haematologic parameters of male and female rat

Parameter	Units	Control	P.K 40 mg/kg	P.K 200 mg/kg	P.K 1000 mg/kg
Male					
Haemoglobin	g/L	158.17±4.00	160±5.66	162±4.43	156.67±3.77
Total red blood cell count	10^{12}/L	8.36±0.19	8.76±0.45	9.01±0.38*	8.21±0.15
Packed cell volume	L/L	0.49±0.01	0.49±0.02	0.50±0.02	0.47±0.01
Mean corpuscular vol.	fl	57.17±0.89	57.17±3.19	55.67±2.66*	57.5±0.67
Mean corpuscular Hb	pg	18.33±0.31	18.33±0.52	17.83±0.75*	18.3±0.17
Mean corpuscular Hb conc. g/L		327.5±0.19	319.83±9.47	323.67±7.99	326.5±4.24
Red blood cell width	%	16.3±0.22	16.58±1.13	17.62±1.29	15.68±0.26
Platelet count	10^9/L	872.83±102,28	872.83±37.78	976.33±36.40	965.50±27.49
Female					
Haemoglobin	g/L	143.33±6.97	140.83±6.11	138±7.05	152.33±3.19
Tot.red blood cell count	10^{12}/L	8.03±0.43	7.45±0.35	7.53±0.40	8.35±0.19
Packed cell volume	L/L	0.49±0.01	0.41±0.02	0.41±0.02	0.44±0.01
Mean corpuscular vol	fl	55.33±1.23	55.33±0.21	54±1.15	53.33±0.88
Mean corpuscular Hb	pg	16.5±1.20	19.00±0.26	18.33±0.49	18±0.26
Mean corp. Hb conc.	g/L	295.17±19.23	342.00±4.65	340.67±9.45	341.33±4.59
Red blood cell width	%	15.57±0.95	11.47±1.78	13.47±0.54	14.45±0.52
Platelet count	10^9/L	961.83±23.12	376.83±100.08	506.83±172.69	1028±27.49

*Values are expressed as Mean±S.E.M., n = 6; *= Statistically significant compared to control (P < 0.05)*

Table 3. Effect of sub chronic exposure to *Pseudocedrela kotschyi* on differential white blood cell count of male and female rats

Parameters	Units	Treatment			
		Control	*Pseudocedrela*	*kotschyi*	
			40 mg/kg	200 mg/kg	1000 mg/kg
Male					
Total WBC	10^9/L	7.8±1.65	5.23±0.63	8.75±0.99	8.13±1.61
Neutrophils	10^9/L	2.7±0.84	1.35±0.14	2.47±0.39	1.77±0.23
Lymphocytes	10^9/L	4.87±0.82	3.63±0.53	5.95±0.77*	6.07±1.37*
Monocytes	10^9/L	0.27±0.09	0.26±0.05	0.3±0.03	0.23±0.11
Eosinophils	10^9/L	0.45±0.14	0.1±0.0*	0.1±0.0*	0.15±0.02*
Female					
Total WBC	10^9/L	8.79±0.58	5.7±0.55	4.85±1.55	6.08±0.72
Neutrophils	10^9/L	2.63±0.35	1.43±0.20*	1.13±0.29*	1.4±0.17*
Lymphocytes	10^9/L	7.35±0.94	3.72±0.43	3.48±1.22	4.4±0.49
Monocytes	10^9/L	0.6±0.03	0.33±0.08*	0.2±0.04*	0.3±0.09*
Eosinophils	10^9/L	0.1±0.0	0.22±0.06	0.1±0.0	0.1±0.0

*Values are expressed as Mean ± S.E.M., n = 6; *= Statistically significant compared to control (P < 0.05)*

Table 4. Effect of sub chronic exposure to *Pseudocedrela kotschyi* on biochemical parameters of male and female rat

Parameters	Units	Treatment			
		Control	*Pseudocedrela*	*kotschyi*	
			40 mg/kg	200 mg/kg	1000 mg/kg
Males					
Sodium	mmol/l	143.83±0.31	143.83±0.60	143±0.37	142±0.73
Potassium	mmol/l	6.32±0.38	5.98±0.22	6.85±0.42	6.73±0.12
Chloride	mmol/l	100.83±0.48	101.5±0.50	100.33±0.42	101.33±0.56
Urea	mmol/l	7.98±0.51	7.4±0.26	7.88±0.41	7.57±0.24
Creatinine	µmol/l	38.5±2.17	36.02±6.58	41.17±2.87*	37.5±2.14
Uric acid	µmol/l	0.14±0.02	0.14±0.01	0.16±0.03	0.11±0.01
Tot.prot	g/l	70.83±0.79	74.17±1.30*	73.5±1.31	72±0.82
Albumin	g/l	37.67±0.80	38.67±0.95	38.33±0.76	38.17±0.79
Globulin	g/l	33.17±1.25	35.5±0.76	35.17±1.13	33.83±1.35
Alb/glo	µmol/ls	1.15±0.06	1.12±0.03	1.12±0.05	1.15±0.06
Alk.phos	iu/l	333.5±27.69	338.5±26.63	298.33±24.69*	239.83±28.44*
Ast	Iu/l	200.33±12.15	201.83±20.53	198±16.38	179.33±28.87
Alt	Iu/l	60.5±3.96	67±2.66	70±7.30	61±3.93*
Female					
Sodium	mmol/l	140.33±0.88	139±1.00	138±1.21	141±1.21
Potassium	mmol/l	5.65±0.15	5.67±0.30	6.62±0.70	5.6±0.17
Chloride	mmol/l	100.67±0.88	100±0.58	101.33±0.61	102±0.86
Urea	mmol/l	7.13±0.32	8.4±0.53	7.2±0.34	6.84±0.42
Creatine	µmol/l	45.33±2.95	34±6.34	37.33±2.26	46.5±10.99
Uric acid	µmol/l	0.12±0.02	0.11±0.02	0.13±0.03	1.24±1.13*
Tot.prot	g/l	75.67±1.15	78.5±2.64	78.67±2.80	68.67±7.34*
Albumin	g/l	40±0.63	36±1.21	35.83±1.13	33.01±6.70*
Globulin	g/l	36±0.77	42.5±3.04	42.83±3.62*	44.5±8.29*
Alb/glo		1.1±0.0	0.88±0.09	0.88±0.10	6.6±5.48*
Alk.phos	Iu/l	273.17±30.33	238±28.88	246.5±15.87	204.83±42.71*
Ast	Iu/l	176.67±11.04	186.67±12.22*	221.67±44.97*	146±20.52*
Alt	Iu/l	59±1.69	63.83±4.63	83±13.73*	60.5±6.10

*Values are expressed as Mean ± S.E.M., n = 6; *=Statistically significant compared to control (P < 0.05)*

Biochemical examination was performed in order to assess any toxic effects on the liver and kidney because clinically significant values indicate toxicity [25]. Statistically insignificant values were obtained for biochemical parameters in both sexes. However, ALT and ALK activities were decreased significantly (P< 0.05). Serum enzymes activities in human livers are used as an index of functionality of the liver. These enzymes include ALK, ALT and AST [29,30]. Globulin concentration increased in the female rats at all the test doses. In contrast albumin concentrations decreased resulting in a decrease in the albumin/globulin ratio. Serum proteins are produced by the hepatocytes, therefore increase in their value suggest tissue injury [30]. However, the changes were regarded as toxicologically irrelevant because the adverse results did not appear in both sexes, were not dose-related, and were not reflected by changes in other related parameters. Moreover, these changes were within normal laboratory range [31], and were considered as incidental.

Histopathological studies were conducted verify whether or not the organs or tissues had been damaged. The results showed no macroscopic or microscopic changes in these internal organs or tissues in any treated rats.

5. CONCLUSION

From the results of the study it could be observed that 28 days oral administration of 40, 200 and 1000 mg/kg/day of *P. kotschyi* to both male and female rats did not result in mortality or gross changes. Furthermore, the growth, organ weight, haematological and biochemical parameters were not affected. Slight increase/decrease in some observed values were still within the clinical range of rats. The lethal oral dose for male and female rats exceeds

2000 mg/kg based on the result of the acute toxicity study. According to [31], the no-observed-adverse-effect level (NOAEL) is the highest exposure level at which there is no statistically or biologically significant increase in the frequency or severity of adverse effects between exposed and control groups. Therefore the NOAEL for rats in the present study was 2000 mg/kg per day. This study provides valuable preliminary data on the toxicity profile of *P. kotschyi*. Since the toxicity studies have only been assayed using laboratory animals, there is a need to determine the safe dose in human considering the widespread use of medicinal plants. Therefore, the results obtained may not necessarily be extrapolated to the situation in humans.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All procedures in this study were performed according to the requirements of the Animals Ethics Committee, Universiti Sains Malaysia.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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