Toxicological evaluation of the aqueous extract of *Pseudocedrela kotschyi* (Meliaceae) stem bark in albino rats

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Abstract

Pseudocedrela kotschyi is a monoiceous plant used traditionally to treat many diseases. The present work has been designed to evaluate the toxicological profile of aqueous extract of *Pseudocedrela kotschyi* stem bark in experimental rats. Acute toxicity was conducted as Organisation for Economic Cooperation and Development guidelines 425 where limit dose of 2000 mg/kg of body weight was used. Body weight, water intake and food consumption were recorded during 14 days. In sub-chronic toxicity test, distilled water or aqueous extract at the doses of 100, 200, 400 mg/kg were daily administrated to rats for 28 days. Haematological, biochemical and histological parameters were evaluated at the end of the treatment. In acute toxicity study, *Pseudocedrela kotschyi* significantly increased ($p<0.05$) food consumption, water intake and relative body weight. In subchronic toxicity study, *Pseudocedrela kotschyi* significantly increased ($p<0.05$; $p<0.001$) water intake, food consumption, relative body weight, total protein and HDL-c, and significantly decreased($p<0.05$) LDL-c and triglycerides. Extract significantly increased ($p<0.05$; $p<0.001$) platelet at doses of 200 and 400 mg/kg. Histopathological examinations showed abnormal architecture of liver and kidney at dose of 400 mg/kg. These findings, therefore supported the traditional believes on effect of the stem barks of *Pseudocedrela kotschyi* at lower dose.

Keywords: *Pseudocedrela kotschyi*, Toxicity, Rats

Introduction

Plants play a major role in the society and are used as source of energy to heterotroph individual. Herbal medicine is universally popular in primary healthcare, particularly in undergo developing country.(1) Since many decades, human being use plants to treat illnesses due to their availability, low cost, low undesired effects. The wide use of medicinal herbs for self-medication is a result of the fact that the general public believes them to be safe and do not have any compromising health effects.(2) Many African countries still used traditional medicines. Plants herbs can be considered like source of secondary metabolites, a source of new isolated modern drug. Plants are used either processed as decoction, maceration or used to extract pure active principles.(3) Then, it is important to assess the risk associated from them during short or long-time administration.(4)

Pseudocedrela kotschyi (Meliacea) is the monoiceous plant of class Magnoliopsida with 12-20 meters of long and atleast 70 cm of large. Since centuries, stem bark of this plant are used as form of decoction or maceration to treat ulcers, rheumatism, syphilis, itches, leprosy, gingivitis, stomach ache, diarrheal, or as aphrodisiac and diuretic.(5,6) Stem bark extract was showed antibacterial activity in vitro and anti ulcers activity in rat and root extract was showed large antimicrobial spectrum, anti leishmaniasis, anti-trypanosoma, anti-plasmodium and anti-HIV activities.(7) Leaves, ethanolic roots and crude methanolic extracts are used for analgesic and anti-inflammatory activities, nephroprotective activities and antinociceptive activity respectively.(8-10)

Despite the widespread uses of this plant for treating plethora human diseases, toxicological profile of stem bark extract has not been studied. In the present study we investigated the adverse effects of acute and subchronic oral administration of the aqueous extract of *Pseudocedrela kotschyi* stem bark (AEPK) in rats.

Materials and Method

Plant material and extraction: The stem barks of *Pseudocedrela kotschyi*(PK),were collected from Foumban Sub-division, West Region of Cameroon. The plant was characterized or identified at the national herbarium of Cameroon by Letouzey R., where it was registered as plant number 7007 (8563/SRFCam). Stem bark of plant materiel was collected and driedin the laboratory at the room temperature. The dry barks were powdered and the powder (500g) was macerated into 3liters of distilled water for three days. Using the filter paper (wattman n° 1) the solution was filtered. The filtrate was dried at 45-50°C using the oven (Titanox mark). The obtained product constituted the aqueous extract (AE).

Animals: Both male and female albino rats weighing 120-150g (8-10 weeks old) were used. Rats were raised in the animal house of the Department of Animal Biology, Faculty of Sciences of the University of Dschang, Cameroon. They were fed on standard pellet diet with free access to water.

Acute toxicity study: Acute toxicity study was carried out according to the methods described by the Organisation for Economic Cooperation and Development.(11) Ten female rats were selected and grouped in two groups of five animals each. Animals were deprived of feed for 18h with free access to water. Animals of group I received 10 ml/kg of distilled water and those of group II received a single dose of 2000mg/kg body weight (bw) of plant extract. Distilled water and extract were administered orally using endo-oesophagous tube. Animals were observed continuously for 4 hours and then periodically after 12h, 24h and 48h. The general behaviour: behaviour pattern, convulsion, locomotion, coma and death, quality of faeces, aggressively and pain sensibility was recorded.(12) The

survivors were kept for 14 days. During this period, rats were weighed every day. Water and food intake were also recorded daily. Lethal doses (LD 50 and 100) were estimated using the limit test dose up and down procedure.

At 15 days post gavage, animals were sacrificed by injecting intraperitoneally the diazepam and ketamine (0.2:0.1). Organs such as kidneys, spleen, lungs, liver and heart were collected and weighed.

Sub-chronic toxicity study: Twenty four nulliparous and non-pregnant rats were randomly grouped in four groups(3 males and 3 females).The group I considered as control group and received distilled water (10 ml/kg). The three others groups II, III and IV received orally the AEPK at respective doses of 100, 200 and 400 mg/kg bw. The treatment was done for 28 days.[13] Food consumption, water intake and body weight of each animal were evaluated daily and recorded weekly.

At days 29 of treatment, animals were scarified using diazepam and ketamine. Blood samples were collected by catheterism of abdominal artery and divided into two parts: a part of blood was put into the test tubes with anticoagulant (EDTA) and the remaining into the test tubes without anticoagulant. Blood collected in tube with EDTA were used to determine the haematology parameters. While, that in tubes without EDTA was centrifuged at 5000 rpm for 15 min and serum was collected for biochemical assay.

Organs: heart, lungs, kidney, spleen and liver were collected and weighed to determine relative organ weight.[14] A part of kidney and liver was conserved into the formaldehyde 10% for further histopathological study.[15]

Haematological assay: Haematological assay concerned the evaluation of volume or percentage of the parameters such as: haemoglobin (Hb), mean corpuscular hemoglobin (MCH), platelets (PLT), mean platelet volume (MPV), red blood cell (RBC), white blood cell (WBC), hematocrit (HTC), lymphocytes, monocytes, granulocytes, packed cell volume (PCV), red cell width (RCW), platelet distribution width (PDW), mean corpuscular hemoglobin concentration (MCHC).[16]

Biochemical evaluation: Bilirubin (total and direct) and total protein content in serum were determined using analytical method and Biuret method respectively. A spectrophotometrical measurement was done to estimate the quantities of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST). Triglycerides, total cholesterol, HDL and LDL-Cholesterol were determined.[17] Creatinin concentration was also evaluated.

Histopathological examination: Organs kept into the formaldehyde 10%, were dehydrated in ethanol, clarified using xylen and impregnated with paraffin locks. Then, they were cut using the microtome into 5µm slices, stained with hematoxylin-eosin and examined using a microscope at 400X.[18]

Statistical analysis: The mean ± standard mean error (SEM) was calculated for each parameter carried out. Data analysis was carried out using GrahPad Instat Prism, version 5. The results were statistically analyzed using One-way analysis of variance (ANOVA) followed by Turkey test and two-way ANOVA. P values<0.05 was considered significant.

Results

Acute toxicity of Pseudocedrela kotschyi: In the acute toxicity studies, the AEPK did not produce any change in behaviour and lethality among the tested animals when dose 2000 mg.kg-1 bw was administered orally and observed daily for 14 days. In addition, the AEPK significantly increased ($p<0.05$) the water intake, food consumption and body weight of the treated animals compared to control rats (Table 1). Besides, the extract did not significantly affected ($p\geq0.05$) the organ's weight (liver, kidneys, lungs, heart and spleen) of treated rats compared to control (Table 2).

Table 1: Effects of aqueous stem bark extract of *Pseudocedrela kotschyi* on Relative Body weight, water intake and Food consumption during acute toxicity

	Distilled water (10ml/kg bw)			Extract (2000mg/kg bw)		
Weeks			**Food**			**Food**
	Relative Body	**Water intake**	**consumption**	**Relative Body**	**Water intake**	**consumption**
	weight (%)	**(ml/rat/week)**	**(g/rat/week)**	**weight (%)**	**(ml/rat/week)**	**(g/rat/week)**
0	100±0.00	06.35±1.09	06.15±0.73	100±0.00	5.66±0.89	08.89±1.73
1	101.66±0.87	10.91±1.1	09.51±0.67	106.94±0.89***	13.60±2.04	10.36±1.22
2	106.56±0.96	16.14±1.2	11.64±0.88	112.78±1.3***	21.57±1.53*	17.75±2.49*

Values are expressed as Mean ± SEM, (n=5)**.** *p<0.05 and ***p<0.001 are significant differences between treated animals and control.

Table 2: Effect of aqueous stem bark extract of *Pseudocedrela kotschyi* on relative organs weight (%) of rats during acute toxicity

Organs	Control	Treated rats
Liver	2.45±0.15	2.47±0.14
Kidneys	0.61±0.01	0.57±0.02
Lungs	0.78±0.07	0.61±0.03
Heart	0.32±0.01	0.32±0.02
Spleen	0.27±0.01	0.29±0.01

Values are expressed as Mean ± SEM, (n=5)**.** p≥0.05, non significant

Subchronic toxicity study of Pseudocedrela kotschyi: In the present studies, the extract did not produce any lethality among the tested animals when varying doses of 100, 200 and 400mg.kg^{-1} bw, were administered orally, daily for 28 days.The rats treated with the extract, however, recorded a significant (P<0.001) increase in water intake, food consumption and body weight compared with that of the control (Table 3-5). While, the organ's weight (kidney, liver, heart, spleen and lungs) of the animals treated with the extract showed no significant (P≥ 0.05) changes when compared with that of the control (Table 6).

Table 3: Effect of aqueous stem bark extract of *Pseudrocela kotschyi* on food consumption (g/rat/weeks) in rats during sub-chronic toxicity

Weeks	Control	100 mg/kg	200mg/kg	400mg/kg
0	12.04±0.51	16.91±0.75	20.55±0.79	20.49±1.43
1	11.28±0.34	18.15±0.54***	22.64±0.93***	20.39±1.14**
2	12.54±0.29	19.24±0.89***	26.49±1.14***	26.41±1.38***
3	12.80±0.29	20.69±0.97***	29.21±1.25***	27.58±1.51***
4	13.17±0.38	24.44±1.53***	29.13±1.58***	30.34±1.55***

Values are expressed as Mean ± SEM, (n=6)**.** **p<0.01 and ***p<0.001, significant differences between treated animals and control.

Table 4: **Effect of aqueous stem bark extract of *Pseudrocela kotschyi* on water intake (ml/rat/weeks) during sub-chronic toxicity**

Weeks	Control	100 mg/kg	200 mg/kg	400mg/kg
0	10.27±1.47	31.27±1.37	28.32±0.85	33.89±1.57
1	11.74±0.37	30.47±1.4***	33.26±1.43***	38.82±2.22***
2	16.01±0.45	30.50±0.87***	35.36±1.93***	34.64±1.61***
3	19.67±1.06	31.05±1.08***	35.71±1.25***	37.03±1.45***
4	22.42±0.77	33.67±0.81***	37.16±1.62***	38.66±1.8***

Values are expressed as Mean ± SEM, (n=6). ***p<0.001, significant difference between treated animals and control.

Table 5: Effect of aqueous stem bark extract of *Pseudocedrela kotschyi* on relative body weight (%) of rats during sub-chronic toxicity

Week	Control	100 mg/kg	200mg/kg	400mg/kg
0	100	100	100	100
1	103.77±1.65	110.39±1.88	109.51±2.23	108.64±2.53
2	105.12±1.96	116.57±2.10**	114.24±1.78*	114.99±2.80*
3	108.37±2.15	122.79±2.15***	119.98±2.41**	117.90±2.97**
4	111.84±1.48	126.24±1.43***	124.69±3.12***	119.29±3.99

Values are expressed as Mean ± SEM, (n=6)**.** *p<0.05; **p<0.01 and ***p<0.001 are significant differences between treated animals and control.

Table 6: Effect of aqueous stem bark extract of *Pseudocedrela kotschyi on relative organ weight of rats* during sub-chronic toxicity

Organ	Liver	Spleen	Heart	Lungs	Kidneys
Control	4.68±0.12	0.53±0.08	0.6±0.03	1.19±0.02	1.79±0.03
100mg/kg	4.89±0.21	0.53±0.03	0.61±0.03	1.17±0.03	1.79±0.04
200mg/kg	4.60±0.16	0.61±0.07	0.60±0.03	1.13±0.035	1.73±0.05
400mg/kg	4.75±0.16	0.66±0.05	0.62±0.01	1.27±0.04	1.89±0.05

Values are Mean ± SEM, (n=6). p≥0.05, non significant

After 28 days of treatment with AEPK at doses 100, 200 and 400 mg/kg bw, no significant variation (p≥0.05) of the level of transaminase (ALT and AST), bilirubin's (total and direct), total cholesterol and serum creatinin were not found compared with that of the control (Table 7). While there were significant increase (p<0.05; p<0.01) of total protein at dose of 400 mg/kg of bw and level HDL-Cholesterol at dose of 200 mg/kg bw and significant decrease (p<0.05) of the level of triglyceride and LDL-Cholesterol in treated animals compared with the control (Table 7).

Table 7: Effects of aqueous stem bark extract of *Pseudocedrela kotschyi* on biochemical parameters during sub-chronic toxicity

Biochemical		Concentrations of Extract		
Parameters	**Control**	**100 mg/kg**	**200mg/kg**	**400mg/kg**
ALT (U/L)	0.005±0.001	0.004±0.001	0.003±0.001	0.007±0.001
AST (U/L)	0.012±0.002	0.012±0.002	0.008±0.002	0.014±0.001
Creatinin (mg/dl)	1.75±0.17	1.5±0.22	1.7±0.23	1.96±0.26
Total Bil (mg/ml)	1.57±0.16	1.39±0.15	1.13±0.07	1.31±0.21
Direct Bil (mg/ml)	1.17±0.28	0.72±0.18	0.46±0.07	0.64±0.22
Protein (mg/ml)	69.65±4.16	72.58±1.2	78.44±2.71	81.35±2.21*
Total Chol(mg/dl)	56.17±5.003	54.17±5.19	49.33±4.05	51±3.2
HDL-Ch(mmol/l)	90.67±3.99	101±3.57	116.5±3.68**	105.2±5.04
LDL-Ch(mmol/l)	58±4.16	46.83±4.5	41±2.15*	49.5±4.5
Trigl (mg/dl)	78.67±3.44	70.67±4.07	62.67±2.741*	63.17±2.92*

Values are expressed as Mean ± SEM, (n=6)**.** *p<0.05 and **p<0.01 are significant differences between treated animals and control.

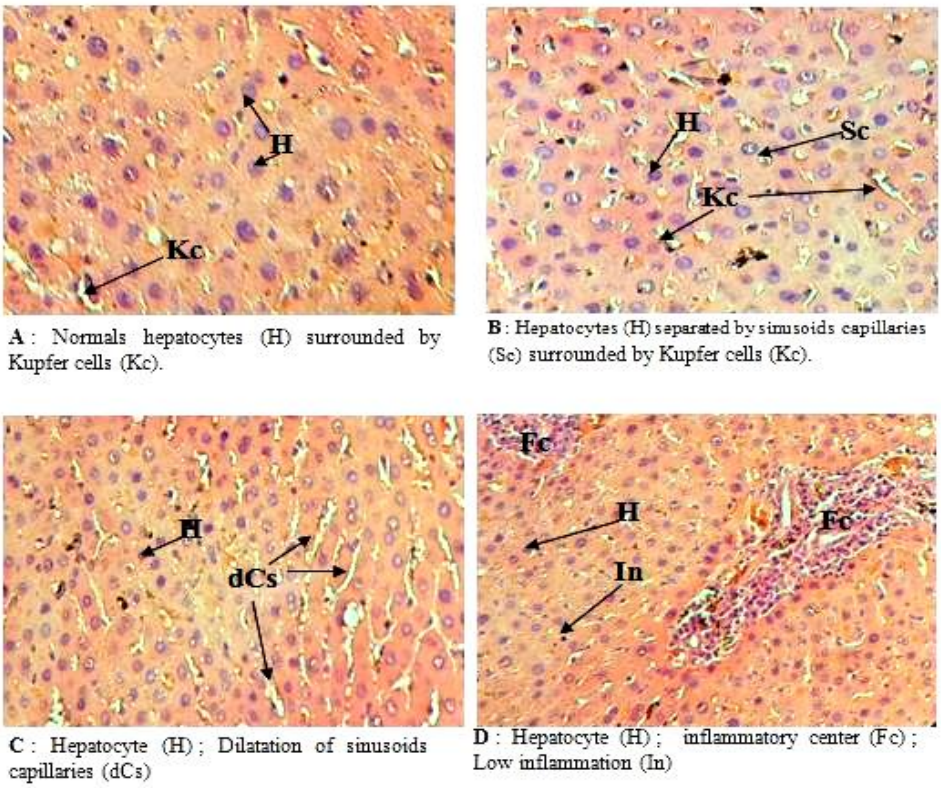
Table 8: haematological parameters of rats treated with aqueous stem bark extract of *Pseudocedrela kotchyi* during sub-chronic toxicity

	Distilled water	**Extract concentrations (mg/Kg)**		
		100mg/kg	**200mg/kg**	**400mg/kg**
	10 ml/Kg			
RBC(10^6/mm^3)	8.91 ± 0.11	7.47 ± 0.49	9.10 ± 1.11	8.68 ± 0.40
HGB(g/dL)	17.22 ± 0.52	15.07 ± 0.98	16.60 ± 0.75	17.23 ± 0.94
HCT(%)	45.88 ± 1.10	40.80 ± 2.79	53.42 ± 9.09	46.42 ± 2.40
WBC(10^3/mm^3)	10.00 ± 0.61	11.32 ± 1.06	9.50 ± 0.60	13.57 ± 2.83
PLT (10^3/mm^3)	868.00 ± 68.32	927.67 ± 33.91	983.67 ± 68.48*	1028.50 ±62.05***
MCV (fL)	52.48 ± 0.34	54.58 ± 1.14	57.48 ± 2.22	53.37 ± 0.78
MCH (pg)	19.67 ± 0.26	20.15 ± 0.38	18.97 ± 1.33	19.77 ± 0.33
MCHC (g/dL)	37.45 ± 0.32	36.93 ± 0.24	33.58 ± 3.05	37.03 ± 0.34
MPV (fL)	6.07 ± 0.12	6.28 ± 0.15	6.48 ± 0.18	6.05 ± 0.096
RDW (%)	16.97 ± 0.36	16.43 ± 0.58	19.77 ± 3.35	17.25 ± 0.73
PDW (%)	8.65 ± 0.31	8.83 ± 0.46	9.87 ± 0.70	8.38 ± 0.25
Lymphocyte (%)	4.03 ± 0.56	2.93 ± 0.49	4.37 ± 0.72	3.00 ± 0.60
Monocyte (%)	0.55 ± 0.09	0.52 ± 0.10	0.53 ± 0.08	0.55 ± 0.08
Granulocyte (%)	74.07 ± 1.76	65.75 ± 3.91	73.53 ± 2.42	65.32 ± 3.33

Values are Mean ± ESM, (n=6).*significant different (p<0.05) and ***significant different (p< 0.001) compared to control

The hematological analysis provided the results presented in Table 8. There were no significant (P≥0.05) changes in the packed cell volume (PCV), white blood cell count (WBC) and red blood cell count (RBC), hemoglobin (Hb), mean cell volume (MCV), mean corpuscular hemoglobin (MCH),mean corpuscular hemoglobin concentration (MCHC), haematocrites (HCT), lymphocytes, monocytes and granulocytes, packed cell volume (PCV), red cell width (RCW), platelet distribution width (PDW) intreated rats compared to the control. While significant augmentation (p<0.05; p<0.001) of platelets was observed at dose 200 and 400 mg/kg bw in treated rats compared to the control.

Histopathological examinations of the tissue samples taken from liver and kidneys of both the rats treated with the extract and the control rats revealed inflammation heath, low inflammation of liver, inflammation and necroses of glomerule of rats treated at dose 400 mg/kg bw compared to control structure (Fig. 1-2).



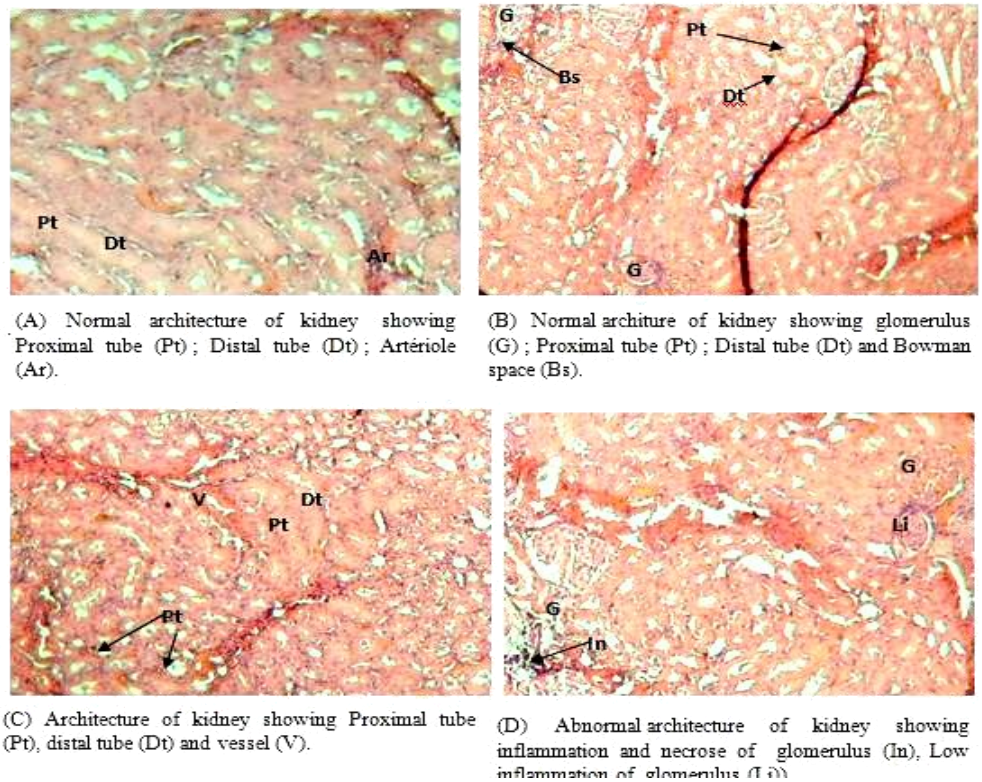
A : Normals hepatocytes (H) surrounded by Kupfer cells (Kc).

B : Hepatocytes (H) separated by sinusoids capillaries (Sc) surrounded by Kupfer cells (Kc).

C : Hepatocyte (H) ; Dilatation of sinusoids capillaries (dCs)

D : Hepatocyte (H) ; inflammatory center (Fc) ; Low inflammation (In)

Fig. 1: Histological structure of liver rats during sub-chronic toxicity seen on light microscope at 400x (A: control; B, C and D: treated rats with AEPK at dose 100, 200 and 400mg/kg bw



(A) Normal architecture of kidney showing Proximal tube (Pt) ; Distal tube (Dt) ; Artériole (Ar).

(B) Normal architure of kidney showing glomerulus (G) ; Proximal tube (Pt) ; Distal tube (Dt) and Bowman space (Bs).

(C) Architecture of kidney showing Proximal tube (Pt), distal tube (Dt) and vessel (V).

(D) Abnormal architecture of kidney showing inflammation and necrose of glomerulus (In), Low inflammation of glomerulus (Li))

Fig. 2: Histological architectureof rats kidney during sub-chronic toxicity seen on light microscope at 400x (A: control; B, C and D: treated rat with AEPK at dose 100, 200 and 400mg/kg of bw

Discussion

The preparation of *Pk* the stem barks is used as a traditional medicine in renal disorders and infectious liver diseases in the west and northern part of Cameroon. Use of herbal remedies requires deep evaluation of their efficacies and safety due to their increase use all over the world.[19] In this study, aqueous extract of *Pseudocedrela kotschyi* stem bark was used to evaluate its toxicological profile.

The AEPK was subjected to acute toxicity studies as per OECD, 425 (2008) guidelines (Up and Down procedure). The results showed that AEPK did not produce any lethality, no adverse effect on behavioural responses among the tested animals at dose 2000 mg/kg bw, was administered orally and observed daily for 14 days. These results showed that LD50 of this plant material is more than 2000mg/kg bw. With a LD50 above this value, according to OECD 425 (2008), this extract can be considered relatively safe. It was accepted as cut off dose 1/20th (100 mg/kg) and 1/5th (400 mg/kg) of doses were taken as effective lower and higher doses for sub-chronic toxicity.

Body weight is used to assess the response therapy and adverse effects of the drug.[20] Water is essential nutrient to the life and improves growth and development of body weight.[21] In the present study, administration of AEPK on rats showed significant ($p<0.001$) increase of water intake, food consumption and body weight in treated animals compared with the control. These effects can indicate that this extract at different dose, enhances appetite centre after consumption. Investigation of biochemical parameters is useful indices of evaluating toxicity of plant extract on animals.[22] Evaluation of serum transaminase gives information's on the effect and nature of pathological damage to the liver tissue, the man organ of detoxification.[23] Any injury to the hepatocytes by test substances would cause the increase of hepatic enzymes to leak into the circulation and rise in the serum level.[24] In this study, rats treated with AEPK did not develop a significant hepatic damage compared with control animals, which was observed from substantial levels of transaminase (ALT, AST), total cholesterol and Bilirubin (Total and Direct). This is indicative of no cellular leakage and loss of functional integrity of cell membrane in liver.[25] Bilirubin is an endogen substance of the liver coming from destruction of senescent hemoglobin. Hemolyse, obstruction of biliary tract and cellular necrose can cause increase of the level of total and direct bilirubin.[26] In the present study, rats treated with AEPK at different dose, showed no significant reduction of the level of direct and total bilirubin during toxicological assessment compared with the control. This result traduce that AEPK did not have any deleterious impact on the liver. Assessment of the protein level in serum gives information concerning protective effect of plant extract against liver oxydative damage, nutrutional status and diagnostic measurement of liver and kidney diseases. Increase of total protein level in rats indicates protective effect of plant extract.[27] Significant ($p<0.05$) increase of total protein level in treated rats at dose 400 mg/kg of bw compared with control was obtained suggesting that AEPK have protective effect and food was consumed. Evaluation of lipid profile as total cholesterol, HDL and LDL cholesterols and triglyceride could give information on lipid metaoilism and diseases.[28] Oral administration of plant extract on the rats showed no significant variation of serum total cholesterol but significant ($p<0.01$) increase of HDL-Cholesterol and significant ($p<0.05$) decrease of LDL-Cholesterol and triglyceride. These significant results on lipid profile may suggested that AEPK has hypolidemiant effect and no risk on cardiovascular system. Creatinin is good indicator of kidneys assessment. To evaluate kidney activity of AEPK, seum creatinin was evaluated and was not showed any significant variation of their level compared to control. Blood parameters are indicator for risk evaluation.[1] Assessment of hematological parameters is one of the most sensitive targets for toxic compounds and important index of physiological and pathological states in men and animal.[29] Oral administration of AEPK in rats at doses 100, 200 and 400 mg/kg bw during 28 days showed significant ($p<0.05$; $p<0.001$) augmentation of PLT on treated rats at dose 200 and 400 mg/kg bw compared to the control. The result hematological assessment obtained during this toxicological study showed that AEPK can enhance PLT cell blood circulation or production. Platelets have major role to maintain vascular integrity by accelerate the processes of coagulation at the site of vessel injury.[30] Thus, these results obtained during sub-chronic toxicity are further confirmed by histological assessment of liver and kidney at dose 100 and 200 mg/kg bw in which structural organisation of hepatocytes and kidney respectively showed normals hepatocytes and nephron compare to control. But at higher dose 400 mg/kg bw, structural organization of liver and kidney was not in conformity with biochemical parameters. Abnormal architecture was observed suggesting that AEPK could be toxic at high dose.

Conclusion

By the characterization of toxicological study of *Pseudocedrela kotschyi* we get some significant and insignificant impacts on different parameters. The findings suggest that oral administration of very high doses of the aqueous extract of *Pseudocedrela kotschyi* may be associated with increased risk of toxicity as demonstrated by the general architecture of liver and kidney of the animal and justified the traditional use of this plant extract at lower dose.

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