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SUBACUTE TOXICITY OF AQUEOUS AND ETHANOLIC EXTRACTS OF STEM BARK FROM *Trichilia emetica* (Meliaceae)

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ABSTRACT

Trichilia emetica is a tropical plant widly found in sub-saharan Africa. Therapeutic use of various parts of this plant has been mentioned in many traditional medicinal systems. Owing to their safety profiles, the aim of the present study was to evaluate subacute toxicity of ethanolic and aqueous extracts of steem bark of *Trichilia emetica*. Serial extraction was done using ethanol and water as solvents. The study was evaluated by daily doses of extracts 500, 800 and 1100 mg/kg orally for 28 days to groups of 6 animals per group including 3 males and 3 females. The control group received no extract. Animals were observed for general behavioral and signs of abnormalities during the experiment duration. The last day, blood was taken for hematological and biochemical analysis. The liver, kidney, and heart tissues were weighed. The results showed that, there were no significant ($p>0,05$) changes in both the asolute and relative organ weights between the control and the test groups. Biochemical parameters were statistically equal in all groups. In addition, both extracts did not cause any significant effect on RBC and indices relating to it (Hb, PCV, MCV, MCH and MCHC) throughout the experimental period. But, there was a significant decrease on WBC and percentage of total lymphocytes cells with ethanolic extract at dose of 1100 mg/kg b.w. In conclusion, the ethanolic and aqueous extracts of steem bark from *Trichilia emetica* did not produce any toxicity in oral suba-cute toxicity study. However, further studies are needed to confirm long term toxicities.

KEYSWORDS: subacuty, *Trichilia emetica*, Haematological and biochemical parameters.

INTRODUCTION

Nowadays, there has been increasing interest in the use of herbal medicines and natural products for the treatment of a variety of disorders (Talha *et al*., 2011). Traditional medicine has maintained greater popularity all over the world and the use is rapidly on the increase (Ogbonnia *et al*., 2008). One reason for the widespread use of medicinal species is the belief that these products from medicinal plants are risk free and considered by patients to be a safe alternative for the treatment of several diseases (Marrone, 1999)**.** The use of herbs in treatment of disease has declined in the west, but it continues to exist throughout the developing countries (Rahma & Choudhary, 1999)**.** However, some medicinal plants must be used with caution since they can be potentially harmful at high doses and can interact with modern drugs (Inamul, 2004). *Trichilia emetica* is a tree 8 to 10 m high. It is widely distributed, especially in the forest and Savannah zones of Tropical Africa. The name 'Trichilia' is Greek for 'in 3 parts', referring to the 3-lobed fruit,and 'emetica' means with emetic properties (Orwa *et al*., 2009). It has many different traditional uses (Diallo *et al*., 2003) including treatment of convulsion, fever, jaundice, cold, epilepsies, scabies, pneumonia and also as purgative, diuretic agents (Sanogo, 2011). The decoction of *T. roka* possesses antioxidant (Germano *et al*., 2006), anticancer, antimutagenic (Verschaeve & van Staden, 2008), antidiabetic and hepatoprotective activities (Lindsey *et al*., 2002). However, the production, prescription, packaging, distribution and use of herbal medicines are still poorly regulated (Mugisha *et al*., 2014). Also, the use of a medicinal plant on its entire form macerate, infusion or decoction can induced some side effect or allergic reactions of short, middle and long term. Therefore, the present study evaluated the subacute toxicity effect of aqueous and ethanolic extracts of steem bark from *Trichilia emetica.*

MATERIAL AND METHODS

Plant materials

The fresh barks of *Trichilia emetica* were collected in February 2014 in the region of Mankono, Northen of Côte d'Ivoire. The plant was identified at the National Floristic Centre of Felix Houphouet-Boigny University of Cocody (Abidjan).

Experimental animals

Albinos Wistar healthy rats, weighing 90 to 120 g were obtained from Animal House. The entire process was approved by OECD Guidelines- Guideline 407. The animals were kept in plastic cages in environmental conditions and allowed to drink water ad libitum without distraction. Animal care and handling conformed to international guidelines(OECD).

Preparation of extract

Barks of the plant were dried at room temperature during 14 days, ground coarsely in a grinder (IKAMAG RCT®) and then stored for further use. The extracts were prepared according to the method described by Zihiri et Kra (2003). The preparation of the total aqueous extract and ethanolic extract 70%, 100 g of plant powder were extracted in one liter of distilled water or ethanol-water (70/30, v/v) by maceration using a magnetic agitator (the process was repeated 3 times). The homogenate obtained is filtered twice successively on cotton wool and once on Whatman filter paper (3 mm). The filtrate was concentrated using a rotary evaporator at 60°C. The concentrate have been evaporated at 50°C in an oven for 48 hours giving a dry ethanolic and aqueous extract. The powder obtained after drying was dissolved in distilled water to give the aqueous and ethanolic extracts of bark from *Trichilia emetica*. Thus, different concentrations were prepared to carry out the experiments.

Subacuty study

Forty-two albinos Wistar healthy rats, weighing 103 to 109 g were divided into seven groups of 6 animals each (3:3 ; males:females). They were grouped according to the dose rates (500, 800 and 1100 mg/kg body weight) of ethanolic and aqueous extracts of steem bark of *Trichilia emetica* and treated orally with a single dose daily for day. The groups of treatment were designed as follows: group I served as control and received distilled water, group II, group III and group IV were administered aqueous extract at a doses of 500, 800 and 1100mg/kg body weight (b.w.) respectivly. Groups V, VI and VII received ethanolic extratc at a doses of 500, 800 and 1100mg/kg body weight (b.w.) respectivly. Treatments have been done 28 consecutive days and the body weight was determined once before commencement of dosing, once weekly during the dosing period and once on the day of sacrifice. The repeated doses for this study were carried out according to OECD guideline 407. The animals were monitored closely for signs of toxicity. Appearance and behaviour pattern were assessed daily and any abnormalities in food and water intake were registered. After 28 days of treatment, animals were fasted overnight but allowed access to water ad libitum. At the end of the study (on day 29), rats were then anesthetized with ether and blood samples were obtained into sterile tubes with anticoagulant EDTA (ethylene diamine tetra acetic acid) for hematological tests and without anticoagulant tubes for biochemical tests.

Organ weight

Immediately after blood collection the animals were sacrificed. The organs of rats in the various groups were weighed: liver, kidney and heart (paired organs were weighed together). The organ weight ratio was calculated (relative organ weights was calcumated).

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on sacrifice day (g)}} \text{X } 100$$

Hematological and biochemical analysis

For biochemical analysis, blood was centrifuged at 2500 rpm for 15 min and serum was also obtained and stored at −40°C. The serum was analyzed for various parameters such as Aspartase amino Transferase (ASAT), Alanine aminotransferase (ALAT), LDH, total protein, serum urea, serum creatinine. Dosages were made using Cobas integras (Abott ®) automation with Biolabo biochemical kits.

For hematological study, the EDTA tube blood was used and red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC) and percentage of total lymphocytes (LYMP) were assessed with an automatic hematological analyzer.

STATISTICAL ANALYSIS

The graphical representation of the data was performed using the Graph Pad Prism 5.0 software (Microsoft, USA). Results are expressed as mean ± SEM. The difference between two values is considered significant when $P < 0.05$.

RESULTS

The results showed that, all the animals which were given subacute doses 500, 800 and 1100 mg/kg body weight, orally for 28 days remained active and healthy throughout the period of study. The animals did not show any changes in general behavior or other physiological activities and no symptoms of adverse effects were recorded during the study. Also, there was no significant difference in food and water consumption between treatment and control groups. The body and organ weights and organ weight/body weight of rats are given in Table 1. This result shows that there were no significant differences ($p>0.05$) in the body and organ weights between control and treated animals. All vital organs (kidney, liver and heart,) showed no significant changes in the organ weight/body weight ratios in treated groups in comparison to controls.

Similarly, in case of hematological parameters, no significant changes were observed in Haemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV), Red Blood Cell Count (RBC) with extracts treated groups when compared to control. But at a doses of 800 and 1100 mg/kg body weight, aqueous extract

induced significant increase in White Blood Cell Count (WBC) and percentage of lymphocytes. However, ethanolic extract at hight dose (1100 mg/kg body weight) showed any significant decrease in White Blood Cell (WBC) and the percentage of total lymphocytes compared with the control group.

In addition, the table 3 shows that treatment with the extracts at various doses for 28 days did not alter the serum biochemical parameters like as ASAT, ALAT, LDH, creatinine, urea and CK-MB.

Table 1 : Body and organ weights of rats in subacute toxicity study in control and groups treated with different doses of ethanolic and aqueous extracts of *Trichilia emetica*

		Aqueous extract			Ethanolic extract		
parameters	**Control**	**500 mg/kg bw**	**800 mg/kg bw**	**1100 mg/kg bw**	**500 mg/kg bw**	**800 mg/kg bw**	**1100 mg/kg bw**
Body weight							
Day0	107.00±2.00	107.2±2.09	104.33±1.84	103.83±1.88	109.5±2.57	105.50±2.17	106.80±3.19
Day7	122.70±2.75	123.2±2.49	120.0±2.28	120.5±1.89	122.5±1.33	122.80±2.49	121.30±1.54
Day14	131.00±1.37	130.7±1.39	131.8±1.37	130.0±1.70	130.5±1.57	131.80±1.70	130.00±1.83
Day21	149.80±1.25	148.8±1.30	147.8±1.19	148.5±1.20	146.2±1.19	148.30±1.45	148.80±1.30
Day28	149.30±1.65	147.2±1.01	146.2±1.19	146.8±1.11	146.8±1.45	146.70±1.05	147.20±1.01
Absolute weight (g)							
Liver	6.18±1.28	6.37±1.10	4.78±1.14	5.42±1.40	5.20±1.76	5.37±1.13	4.78±1.28
kidney	1.04±0.11	1.15±0.15	1.00±0.12	0.93±0.12	1.04±0.18	1.02±0.14	1.52±0.18
Heart	0.61±0.05	0.68±0.06	0.62±0.04	0.60±0.06	0.62±0.06	0.67±0,06	0.69±0.05
Relative weight (%)							
Liver	4.07±1.21	4.14±1.22	3.42±1.42	3.55±1.38	3.49±1.40	3.53±1.39	3.42±1.22
kidney	0.70±0.12	0.78±0.12	0.68±0.11	0.63±0.12	0.71±0.12	0.70±0.11	0.71±0.11
Heart	0.41±0.12	0.46±0.12	0.42±0.12	0.41±0.12	0.42±0.12	0.46±0.12	0.47±0.12

Data are expressed as mean ± SEM, n=6 for each group. No statistical difference was found between the control and extracts of Trichilia emetica treated groups (P>0.05).

Table 2 : Hematological values of rats in subacute toxicity study in control and groups treated with different doses of ethanolic and aqueous aqueous extracts of *Trichilia.*

	WBC	RBC	HBG	HCT	VGM	TCMH	CCMH	%LYMPH
Control	15.06±1.12	8.60±0.93	14.64±0.63	44.65±2.58	54.15±1.21	16.87±0.68	32.57±0.12	58.67±1,09
Aqueous extract (mg/kg b.w.)								
500	16.47±1.35a	7.28±0.93a	13.62±0.76a	43.78±2.03a	56.13±1.03a	17.73±0.68a	32.30±1.04a	58.33±1.86a
800	17.59±1.53b	8.14±0.40a	14.36±0.62a	45.65±2.47a	56.08±1.44a	17.62±0.59a	31.40±0.56a	61.03±1.16b
1100	18.05±1.54b	8.82±0.49a	13.73±0.66a	43.21±2.04a	56.03±109a	18.08±0.56a	32.17±0.49a	61.00±1.15b
Ethanolic extract (mg/kg pc b.w.)								
500	13.78±1.05a	8.21±0.66a	13.93±0.83a	44.10±2.37a	53.40±1.41a	16.83±0.58a	31.33±0.64a	59.75±1.15a
800	13.85±1.45a	8.48±0.44a	14.85±0.77a	46.70±2.13a	55.12±1.88a	17.53±0.61a	31.33±0.55a	59.60±1.73a
1100	09.71±1.10b	8.40±0.52a	14.97±0.78a	46.80±2.09a	56.02±1.03a	17.85±0.69a	31.77±0.78a	48.23±1.73b

Data are expressed as mean±SEM, n=6 for each group. [a] p>0,05 : No statistical difference was found between the control and extracts of Trichilia emetica treated groups; [b] Significant at P<0.05 as compared with control.

Table 3: Blood chemistry values of rats in subacute toxicity study in control and groups treated with different doses of ethanolic and aqueous extracts of *Trichilia emetica.*

		AQUEOUS EXTRACT (mg /kg) B.W.			ETHANOLIC EXTRACT (mg /kg) B.W.		
	control	**500**	**800**	**1100**	**500**	**800**	**1100**
UREA (g/l)	0.54±0.12	0.71±0.15	0.63±0.15	0.55±0.14	0.73±0.13	0.50±0.13	0.50±0.14
CREAT. (g/l)	4.00±0.48	4±0.45	4±0.80	5±0.68	4±0.42	4±0.42	5±0.54
TOTAL PROT.(g/l)	71±1.57	71±1.55	70±1.61	71±1.51	73±1.63	75±1.54	74±1.61
ALAT (UI/l)	49±2.04	52±1.81	53±1.77	55±2.46	48±1.72	49±1.72	51±2.49
ASAT (UI/l)	139±4.97	156±5.09	145±5.09	150±6.41	154±5.25	143.0±4.95	132±5.64
LDH (UI/l)	1755±8.51	1774±9.24	1745±8.37	1756±8.08	1747±7.03	1748±7.49	1785±9.61
CK-MB (UI/l)	1386±11.99	1381±11.96	1403±11.84	1413±12.47	1368±12.10	1417±12.04	1414±11.88

Data are expressed as mean±SEM, n=6 for each group. No significant difference compared was found between the control and extracts of Trichilia emetica treated groups (P>0.05).

DISCUSSION

Herbal medicines are often wrongly regarded as safe because they are "natural". Nevertheless, those products contain bioactive principles with potential to cause adverse effects (Bent, 2004). It is therefore important that all herbal medicines are subjected to efficacy and safety tests by the same methods used for new synthetic drugs (Talalay, 2001). In repeated dose studies, daily clinical observations are of major importance as well as the final observations (Feres *et al*., 2006). Thus, no significant changes were observed in water and food consumption. In addition, body weight changes are an indicator of adverse side effects, because the animals that survive cannot lose more than 10% of the initial body weight **(**Feres *et al*., 2006). No physical changes were observed throughout the dosing period. All rats showed significant increase in body weight compared to their initial values. However there was no significant difference in body weight between the different treatment groups and the control, indicating that both extracts did not have any adverse effects on the body weight. There is a very high possibility that herbal products, when ingested into the body may be toxic to important organs such as the kidney, liver and heart because of their diverse roles in the human body (Abotsi *et al*., 2011). The absence of any significant differences in weights of the liver, kidney and heart provides support for the safety of aqueous and ethanolic extracts of stem bark from *Trichilia emetica*. The results not show significant differences of the organ weight/ body weight ratio in the treatment and the control group demonstrate that the extracts did not produce organ swelling, atrophy or hypertrophy (Singh *et al*, 2014).

The biochemical parameters evaluation is important since there are several reports of toxicity liver and kidney related to the use of phytotherapeutic products (Obici *et al*., 2008; Rhiouani *et al*., 2008). In preclinical toxicity studies, renal changes are particularly liable to occur because of the high doses given and the fact that the kidneys eliminate many drugs and their metabolites (Greaves, 2007). So to speak, determinations creatinine and urea are used as markers of kidney function (Arneson & Brickell, 2007). In the present study, no significant differences was observe in serum levels of creatinine and urea in the extracts treated groups compared to controls. This indicate that, there is no adverse effect on kidney functions after 28 days oral administration of extracts. Similary, hepatic function is well preserved by the administration of *Trichilia emetica* extracts in rats. This can be explained by serum enzyme levels of ASAT and ALAT that was comparable to control values. Determination of tota l protein may act as an indicator of synthetic capacity of liver (Rasekh *et al*., 2008). Unchanged total protein therefore suggests absence of any abnormality in the synthetic capacity of liver.

The creatine kinase MB isoenzyme (CK-MB) is one of three creatine kinase (CK) isoenzymes. Unlike CK, which is found in many tissues of the body, CK-MB is present in a relatively high concentration in the myocardium (about 20% of the total myocardial CK), whereas only up to 2% can be found in healthy skeletal muscle. This fact makes CK-MB as specific biomarker to evaluate the evidence and severity of myocardial injury after cardiac troponin (Roza *et al*., 2015). Creatine phosphokinase (CPK) is one of the indicators available for the diagnosis of cellular damage to the heart (OECD, 2001). The data showed no change in the level of CK-MB isoenzyme (CK-MB) and creatine phosphokinase in control and all treated groups. This indicates that, the subacute treatment of *Trichilia emetica* extracts at the above mentioned doses did not induce any damage to the heart.

Assessment of the haematological indices showed that, the extracts of *Trichilia emetica* at various doses did not cause any significant effect on RBC and indices relating to it (Hb, PCV, MCV, MCH and MCHC) throughout the experimental period is an indication that there was no destruction of matured RBC's and no change in the rate of production RBCs (erythropoiesis) (Udut *et al*, 2005). RBC and Hb are very important in transferring respiratory gases; and the non-significant effect of extracts on the RBC and Hb indicates that there has been no change in the oxygen-carrying capacity and amount of oxygen delivered to the tissues.

In addition, the normal levels of MCV and MCHC indicates that the morphology and osmotic fragility of the red blood cells were not affected (Guyton & Hall, 2000). The determination of blood indices MCV, MCH and MCHC have a particular importance in anaemia diagnosis in most animals (Oyewo & Akanji, 2011). The non-significant effects on these indices relating to RBC suggest that there was no effect on the average size of RBC (microcytes) and also in the haemoglobin weight per RBC. This implies that extracts does not possess any potential of inducing anaemia throughout the 28 days period of oral administration. The decrease in WBC and percentage of total lymphocytes observed with the ethanolic extract at a dose 1100 mg /kg body weigth may imply reduction in the ability of the body to respond to infection.

CONCLUSION

The overall data of this study suggest that the oral administration of both extratcs of stem bark from *Trichilia emetica* does not induce any toxic effects. This could stand as an assurance for the use of "*Trichilia emetica*" in folk medicine. Further investigation is needed to evaluate the long-term safety of this plant extracts.

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