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Research Article

Oral Toxicity Study of X_{42} Fraction of *Terminalia ivorensis* A.Chev. (Combretaceae) in Rats

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ABSTRACT
This study investigated the acute and sub acute toxicity effects of X42 fraction of *Terminalia ivorensis* in rat. In the acute test, the limit test dose of 5000mg/kg was administered to Wistar rats and then observed for the first 24h and daily for 14 days. Sub-acute toxicity was evalued after administering daily oral doses of 250; 500 and 1000mg/kg body weight for 28 days. Adverse effects and mortality were observed throughout the experimental period. Body weight, organ weight, Hematological and biochemical assessments were evaluated. The dose of 5000 mg/kg did not cause any mortality. In the sub-acute tests, the results did not show any treatment related abnormalities in terms of hematological and biochemical parameters. There were no significant differences in body and organs weight between control and treated groups. The X_{42} fraction of *T. ivorensis* could not cause any mortality and signs of toxicity, it would be well tolerated.

Keywords: Acute toxicity, Biochemical parameters, Hematological parameters, subacute toxicity, Terminalia *ivorensis*, X_{42}fraction

INTRODUCTION

Traditional medicine has been practiced for many centuries to treat diseases in low cost in many parts of the world, including Africa especially in rural areas. Nature has provided a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their used in traditional medicine[8]. The present accepted modern medicine has gradually developed in the recent years by various efforts done by the researchers. However, traditional medicine still remains as basis in the development of new drugs[24]. In recent years, herbs and herbal medicines have continued to receive interest and attention from the people as these products are safe and free from side effects[28]. The growing num herbal products make it necessary to conduct toxicity study of herbal products[29]. Toxicity ber of herbal drug users around the globe and lack of scientific data on the safety profile of associated with herbal products has alerted many national and international regulatory authorities to develop and implement various set of guidelines for assessing, monitoring and preventing the toxicity associated with the herbal products. For example, Organization for Economic Cooperation and Development (OECD) sets guidelines for conducting various toxicity studies. Toxicity tests are most widely used to examine specific adverse events or specific endpoints such as cancer, cardiotoxicity and skin/eye irritation. Toxicity testing also helpful in determining the No-Observed Adverse Effect Level (NOAEL) dose and is helpful for further clinical trials[30]. Acute, sub-acute and chronic toxicity tests are routine toxicity tests carried out by the pharmaceutical companies in the development of new medicines[3]. Acute toxicity testing involves the determination of lethal dose, whereas sub-acute and chronic toxicity testing involves the determination of long term effects of the test compound upon repeated administration.

Terminalia ivorensis A. Chev. (Combretaceae) is used against diarrhea, diabetes, hypertension, parasites and coughs. This plant is also used in treatment of cutaneous infections, buccal and teeth infections. In Cote d'Ivoire, the roots of this plant are used much in the traditional pharmacopeia like toothpick against voices extinctions[6]. The *T. ivorensis* bark extract possessed in vitro antifungal property against *Candida albicans andAspergillus fumigatus*. The hydroalcoholic extract exhibited strongest inhibitory effect on *C. albicans* and *A.fumigatus* as compared to standard antibiotics (Ketoconazole)[23].

The studies carried out in Nigeria showed that the plant is an anti-inflammatory drug and antiarthritic[14]. In the veterinary medicine, the plant was proved having trypanocides and pesticides properties[2]. Despite the various uses over long time periods, no toxicological data is available regarding the safety of repeated exposure to *T. ivorensis*. As a part of safety evaluation, acute and sub-acute oral dose toxicity studies were carried out to

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investigate the potential toxicity after single oral dosing and 28- days repeated oral dosing of extract in rats.

MATERIALS AND METHODS

Plant material

The barks of *T. ivorensis* were collected in June, 2014 from the site of Pasteur institute, Abidjan, Cote d'ivoire western Africa and identified by comparison with specimens: forest of Adiopodoumé, Cote d'ivoire, May 17th 1966, Aké-Assi 8855 available at the herbarium of the floristic national center, Felix Houphouet Boigny University, Abidjan, Cote d'ivoire.

Preparation of extract

The barks were collected, washed, dried with sun's shelter at a temperature between 25 and 27°C and were returned out of powder fine with an electric crusher (IKA-MAG). One hundred (100) grams of this powder were extracted in a mixture from solvent with 700 ml from ethanol 96° and 300 ml from water by homogenization in blender. After six[6] cycles of homogenization, the homogenate obtained was dried in a white fabric and was filtered successively twice on cotton and once on whatman filter (3mm).The filtrate was concentrated with a rotary evaporator BUCHI at 60°C[35]. It gave hydro alcoholic extract. Then, a portion of this extract had been delipided with the soxhlet with hexane. A residue non hexane-soluble called X42 is obtained[22].

Experimental Animals

Adult male and female Wistar rats (Rattus norvegicus), aged 2 to 3 months, weighing 100–130 g, were obtained from vivarium of Superior Normal School Abidjan. The animals were maintained in standard conditions (22–24°C; 12:12 hdark/light cycle). Water and food were available ad libitum. The experimental protocols were approved by the Ethical Committee of Health Sciences; University Felix Houphouet Boigny-Abidjan. These guidelines were in accordance with the European Council Legislation 87/607/EEC for the protection of experimental animals[7].

Acute toxicity assay

Acute toxicity study of X42 fraction of *T. ivorensis* was carried out in female rats by using Organization for Economic Co-operation and Development (OECD)[20]. Before oral administration, the rats were deprived of food for 12hours.A single high dose of 5000mg/kg of X42 extract was administered to both three female rats in the treatment group by the oral route. The control group received a vehicle (distilled water) at a volume of 2ml/100g of body weight. The rats were observed in detail for any indications of toxicity effect withing the first six hours after the treatment period, and daily further for a period of 14 days.

Sub-acute toxicity studies

Sub-acute toxicity studies (28-days repeated oral toxicity study) was carried out according to OECD 407 guidelines [19]. Both sexes of rats were divided into four groups of 10 animals (5 males and 5 females).The group I received vehicle orally at 2 ml/100g body weight and served as a control group whereas groupII, group III and group IV received X42 fraction at 250; 500 and 1000 mg/kg body weight pers os respectively. The extract was administered daily for 28 days the same time daily and observed at least twice daily for morbidity and mortality. All the animals were observed for clinical signs and the time of onset, duration of these symptoms, if any were recorded. Body weights of the rats in all groups were recorded once before the start of dosing, once weekly during the treatment period and finally on the day of sacrifice.

Table 1: Potential toxic effects of X42 in rats

	Control	5000mg/kg
Number of rats	3	3
Number of dead	0	0
Mortality (%)	0	0
LD_{50}		>5000 mg/kg

LD_{50}: Mean Lethal Dose

On the 29th day, after an overnight fast (only water allowed), the rats were anaesthetized with ether and blood sample for hematological analysis were collected into tubes with EDTA and into tubes without EDTA for biochemical analysis.

Hematological parameters

Hematological analyses were performed using an automated hematology analyzer (Sysmex KX21) with regard to the following parameters: 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), platelet count (PLT) and leukocytes formula (neutrophiles, eosinophilic lymphocytes, monocytes)[18].

Biochemical parameters

The blood samples were collected in dry tubes without anticoagulant and allowed to stand for complete clotting. The clotted blood samples were centrifuged at 3000 rpm for 15 min, after which point the serum samples were aspirated and stored at –20°C. The serum samples were analyzed to determine the levels of Blood glucose, blood urea, creatinine (Crea), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphate(ALP), uric acid(UA), triglycerids (TG), Total protein (PT), total cholesterol(CT), and the blood electrolytes: sodium(Na^+), potassium(K^+), chlorine (Cl^-), calcium (Ca^{2+}) and magnesium(Mg) using an automated biochemistry analyzer (ADVIA 2400, Japan). The protocol for each assay was preset and then incorporated into the device during the assays[18].

Statistical analysis

The results are expressed as mean ± standard error of the mean (SEM).Data obtained was analyzed by using one way ANOVA followed by Dunnett's test and $p<0.05$ was considered as statistically significant. All statistical analyzes were carried out using the instat statistical package (Graph Pad Software, In, USA).

RESULTS AND DISCUSSION

Extraction

The hydoalcoholic extract was obtained with an average percentage of 32,2%. When the X42 fraction, it was obtained with an percentage of 90% from hydroalcoholic extract by délipidation. The powder is brown.

Table 2: Body weight of rats after 28 days of treatment with X42

Groups		Body weight (g)				
	Sex	Week0	Week1	Week2	Week3	Week4
Control	M	101±1.45	107±2.25	115±2.02	121±1.5	128±3.0
	F	104±1.73	110±1.66	118±2.33	125±1.5	132±2.0
250mg/kg	M	118±2.02	125±2.06	130±1.45	134±2.0	141±2.0
	F	110±2.03	116±1.15	122±1.73	130±1.5	136±1.5
500mg/kg	M	122±1.45	125±2.01	129±1.15	135±1.0	139±2.0
	F	114±1.76	118±2.20	123±1.45	129±1.5	134±1.5
1000mg/kg	M	129±2.02	134±1.0	139±2.02	146±1.5	151±1.5
	F	118±2.02	123±2.0	129±1.45	135±0.5	140±1.5

Values are expressed as mean±SEM, n=5 males and 5 female For the control group, the value of body mass evolved from 102.5±1.59 (W0) to 130±2.5(W4) an increase of 26.82%.In groups 250; 500 and 1000 mg (treated groups) values of body weight have evolved 114±2.25 (W0) to 138.5±1.75 (W4); 118±1.6 (W0) to 136.5±1.75(W4) 123.5±2.02 (W0) to 145.5±1.5 (W4) respectively. An increase is 21.56%, 15.73% and 17.84%

Table 3: Organ weight of rats after 28 days of treatment with X42

Organs weight (g)	Groups							
	Control		250mg/kg		500mg/kg		1000mg/kg	
	M	F	M	F	M	F	M	F
Liver	3.90±0.5	3.89±0.18	4.16±0.10	3.98±0.14	4.13±0.31	3.72±0.31	4.27±0.37	4.19±0.5
Heart	0.63±0.04	0.62±0.09	0.73±0.00	0.65±0.04	0.65±0.25	0.60±0.04	0.73±0.04	0.67±0.07
Kidney	0.99±0.21	0.94±0.05	1.03±0.0	0.92±0.04	0.94±0.07	0.90±0.07	1.04±0.1	0.99±0.12

Values are expressed as mean±SEM, n=5 males and 5 females

Effect of X42 on mortality of the rats (Acute toxicity)

In the toxicity study, oral administration of the X42 fraction of *T. ivorensis* at 5000mg/kg did not produce any deaths and clinical signs of toxicity in rats (table1). Toxicological studies are the platform for hazard identification stage of safety assessment[33]. Acute exposure to X42 fraction of *T. ivorensis* at dose of 5000mg/kg to female rats did not cause any mortality and adverse effects 24h post first dose treatment and during 14 days of observation. This could suggest that the oral LD50 value in rats was greater than 5000mg/kg. The limit test is primarily used in situations where the investigator has information indicating that the test material is likely to be non-toxic or low toxicity[20]. This finding, therefore, suggests that the extract at the limit dose tested is essentially non-toxic and safe in oral formulation. According to the chemical labeling and classification of acute systemic toxicity recommended by OECD, the X42 fraction of *T.ivorensis* was assigned class 5 status (LD50>5000mg/kg) which was the lowest toxicity class. The results were in line with Kamo et al[15], who indicated that the LD50 of the hydroalcoholic extract of *Terminalia mantaly* is more than 5000 mg/kg and Mama et al[17] with the extract of *Sacoglottis gabonensis.*

Sub-Acute toxicity

The sub-Acute dose was selected based on the rats LD50 value which kept rats alive,1/5, 1/10 and 1/20 of 5000mg/kg. In the repeated dose 28-days oral toxicity study, there were no deaths and treatment-related signs in all groups of animals.

Effect of X42 on body and organs weight.

No significant differences in body weight and organs weight were observed between control and treated groups during this period (Table 2 and Table 3). In general, increases and decreases in body weights of animals can be used as an indicator of adverse effects of drugs and chemicals[32]. However, Harizal et *al.*[11] reported that increases in the body weights of animals are more closely related to body fat accumulation rather than to the toxic effects of drugs or chemicals. Rhiouani et *al.*[27] suggested that reductions in the body weights of animals in toxicity studies may be associated with normal physiological adaptation responses to the plant extracts or compounds, which lead to low appetite and hence, lower caloric intake by the animals. High doses of plant extracts or compounds might also induce stress in the animals, thereby reducing their food intake, which may lead to reductions in their body weights[13].

In the present toxicity study, X42 fraction could not affect the body weights of the rats at any dose throughout the treatment periods. There were no significant differences in body weight gain of both control and treated groups.

Organ weight also is an important indicator of physiological and pathological status of animals. The relative organ weight is fundamental to confirm whether the organ was exposed to the injury or not. The heart, liver, kidney, spleen and lungs are the primary organs affected by metabolic reaction caused by toxicant[10].

In the present study, organ weights in all the treated groups of both sexes were not significantly different from those of control groups. The macroscopic examinations of the organs did not show any changes in color and texture compared to the control. Hence it can be suggested that X42 fraction of *T. ivorensis* is almost non-toxic.

Effect of X42 on hematological parameters

The hematological profile of treated and control group were summarized in table4. Treatment of rats with X42 fraction at repeated doses for 28 days, caused no

Table 4: Hematological parameters of rats after 28 days of treatment with X42

Parameters	Sex	Control	250mg/kg	500mg/kg	1000mg/kg
WBC (10^9/L)	M	14.67±0.72	13.57±0.14	15.43±0.85	14.60±1.90
	F	14.83±0.61	14.15±1.01	15.03±0.48	15.38±1.14
RBC (10^{12}/L)	M	7.10±0.16	6.32±0.16∗	7.08±0.10	7.16±0.13
	F	6.56±0.11	6.99±0.14	6.3±0.20	6.71±0.20
Hemoglobin (g/dl)	M	12.20±0.36	12.00±0.11	12.95±0.23	12.85±0.12
	F	12.10±0.17	12.58±0.08	11.9±0.27	11.98±0.13
Hematocrit (%)	M	41.37±0.81	38.93±1.54∗	40.00±0.39	40.75±0.44
	F	37.90±1.10	40.08±0.77	37.35±0.90	37.33±1.04
MCV (fL)	M	59.20±0.70	55.63±1.88	54.03±0.43∗	56.93±1.07
	F	57.87±2.24	57.30±0.10	54.88±1.09	54.80±0.46
MCH (pg)	M	17.47±0.29	18.07±0.55	18.48±0.46	18.03±0.33
	F	18.50±0.37	18.03±0.30	19.00±0.21	17.78±0.32
MCHC (g/dl)	M	31.20±0.66	33.73±1.78	33.25±0.42	31.68±0.42
	F	31.97±0.58	31.45±0.52	33.73±1.21	32.23±0.57
Platelet (10^9/L)	M	795±37.4	664±11.7	708±29.9	610±82.9
	F	720±24.2	792±75.4	531±36.9	718±64.8
Neutrophils (%)	M	10.33±0.33	9.66±1.20	10.67±1.33	9.00±0.57
	F	8.33±0.88	9.00±0.57	10.67±0.66	9.33±0.33
Eosinophils (%)	M	2.00±0.00	2.33±0.66	2.00±0.81	2.00±0.00
	F	2.33±0.88	2.00±0.00	2.25±0.47	2.00±0.00
Lymphocytes (%)	M	85.33±0.66	83.33±1.76	83.67±2.60	87.00±1.29
	F	85.33±2.90	88.50±0.95	84.00±2.16	87.75±0.85
Monocytes (%)	M	3.50±0.50	4.00±0.57	4.00±0.70	3.75±0.85
	F	4.00±1.15	3.75±0.25	4.33±0.33	3.00±0.00

Values are expressed as mean±SEM, n=5 males and 5 females. ∗ Statistically significant ($p<0.05$, control group vs treated groups)White blood cells (WBC); Red blood cells (RBC);Hemoglobin (Hgb); Mean corpuscular hemoglobin concentration (MCHC); Platelets (PLT); Lymphocyte (Lym) ; Hematocrit (HCT); Mean corpuscular volume (MCV);Mean corpuscular hemoglobin (MCH).

significant change ($p>0.05$) in hematological parameters in rats treated compared to the control group. Red blood cells (RBC), White blood cells (WBC), Platelets (PLT), Lymphocytes (LYM), Hemoglobin (Hb) and other hepatocytes are the most sensitive target of toxic compounds and is an important index of pathological and physiological state of human and animal[18]. In our study, there were no significant changes in these parameters. Or a modification of these parameters provides information on the human toxicity when these data are derived from studies in animals[21]. Changing these parameters also determine the safety of a substance subject to a toxicological study. RBC, WBC Hemoglobin, platelet, LYM and others parameters are in the range of normal values[9]. However, the normal range of this parameter can be altered by the intake of toxic plants which was not observed in this study**.** These results are similar with that of Boga[5] which showed that the Total Dichloromethane-Ethanol Extract of *Morinda morindoides* did not changes hematological parameters in rats.

Effect of X42 on biochemical parameters

The data of biochemical parameters in treated and control rats were presented in table 5. There were no significant differences between control and X42 fraction treated groups in the biochemical parameters measured. In addition, most of biochemical parameters were not also altered by the fraction. The lack of significant alterations in the levels of ALAT, ASAT, alkaline phosphates, glucose, δGT and total protein which are good indicators of liver function, suggests that sub-chronic administration of extract neither altered hepatocytes of rats nor the normal metabolism of the animals. This implies that the extract at the doses tested had no effects on the liver. Transaminases such as ASAT and ALAT are used as biomarkers to conclude the probable toxicity of drugs and xenobiotics[26]. Normally, destruction to the liver parenchymal cells will result in an increase of both these enzymes in the blood. Their concentration in serum informs about a hepatocyte injury[16]. Urea, creatinine and uric acid are markers of renal function have not seen their rates varied by X42 fraction. The normal values of kidney parameters suggest that sub-acute administration of X42 did not cause any damage to the kidney.

On the level of the cardiac function, creatinine kinase, ASAT, ALAT, triglycerids and total cholesterol are the biological parameters which variation of the enzymatic activity and concentrations, testifies to vitality or not to Cardiac muscle[6]. Our results showed that X42 fraction at the doses used, could not provoked significantly change ($p>0.05$) of Serum biochemical values of these parameters compared with rats in the Control group,

suggesting that this fraction did not affect kidney, heart or the liver. Blood electrolytes, such as chlore (Cl^-), potassium (K+), magnesium (Mg2+) and sodium (Na+),

Table 5: Biochemical parameters of rats after 28 days of treatment with X42

Parameters	sex	Control	250mg/kg	500mg/kg	1000mg/kg
Glucose (g/l)	M	0.96±0.08	0.97±0.06	0.84±0.01	0.82±0.04
	F	0.88±0.06	0.91±0.07	0.83±0.01	0.82±0.03
ASAT (U/L)	M	149.7±11.2	167±1.00	173±18.1	166.3±13.3
	F	149.3±20.8	147.1±7.63	147.7±4.9	167.3±22.1
ALAT (U/L)	M	37.33±2.6	30.0±0.57	35.33±3.2	39.75±11.8
	F	35.0±1.52	32.33±1.2	37.0±2.3	32.75±1.44
δGT (U/L)	M	3.66±0.33	3.00±0.0	2.33±0.3	3.00±0.0
	F	3.00±0.0	2.75±0.25	2.50±0.50	2.75±0.25
PAL (U/L)	M	449±19.4	387±80.7	429±57.3	389±33.4
	F	418±57.1	358±30.7	485±14.0	354±8.9
CPK (U/L)	M	985±101	983±62	988±227	952±40
	F	1000±109	1048±268	1004±65	1085±287
Urea (g/l)	M	0.36±0.0	0.32±0.04	0.34±0.02	0.32±0.0
	F	0.33±0.03	0.34±0.01	0.33±0.02	0.35±0.01
Creatinine (mg/l)	M	6.06±1.04	6.50±0.28	6.00±0.17	6.63±0.31
	F	6.33±0.66	6.66±0.33	6.70±0.26	7.00±0.0
Uric acid (g/ml)	M	2.86±0.33	3.12±0.12	2.36±0.14	2.70±0.10
	F	2.60±0.36	2.92±0.39	2.25±0.12	2.80±0.11
Total protein (g/l)	M	37.7±1.85	38.4±0.88	41.0±8.5	39.0±2.04
	F	37.7±11.7	38.4±9.33	38.25±6.1	39.7±5.78
Total cholesterol (g/l)	M	0.60±0.01	0.56±0.03	0.62±0.15	0.62±0.09
	F	0.64±0.15	0.52±0.02	0.49±0.08	0.92±0.03
Triglycerids (g/l)	M	0.71±0.09	0.75±0.02	0.68±0.06	0.66±0.02
	F	0.73±0.27	0.70±0.25	0.69±0.11	0.67±0.01
Soduim (Na) (mEq/l)	M	145±3.38	146±3.18	144±1.45	140±2.16
	F	147±1.00	150±0.88	147±2.05	140±2.86
Chlore(Cl) (mEq/l)	M	104±2.18	105±2.64	103±0.5	100±1.85
	F	105±0.5	110±0.5	104±1.44	101±1.31
Potassium (K) (mEq/l)	M	10.3±0.58	8.93±0.56	7.90±0.45	7.95±0.21
	F	11.15±1.85	10.75±0.81	8.32±0.12	10.75±3.16
Magnesium (Mg) (mg/l)	M	22.3±1.2	19.0±4.61	22.67±0.8	21.0±3.85
	F	19.0±2.51	19.0±2.3	20.0±0.57	21.75±0.79

Values are expressed as mean±SEM, n=5 males and 5 females. ASAT: Aspartate aminotransferase (U/l); ALAT: Alanine aminotransferase (U/l); Urea (mg/l); Creatinine (mg/l), LDH: Lactate dehydrogenase (U/l); CK: Creatine kinase (U/l); PAL: Alkaline Phosphatase (U/L); Total proteins (g/l); Cholesterol (g/l), Triglycerides (g/l); δGT: Gamma glutamyltranspeptidase (U/I).

no significant variation ($p>0.05$) were noted with respect to the control group. That suggests that X42 fraction could not influence the hemodynamic balance. It is clear from our study that at doses used, X42 is not toxic and could be used without causing damage to the body. Our results are consistent with those of Prasanth et al[25] which showed that the administration of the ethanolic extract of *Celtis timorensis leaves* is not harmful to rats at the same doses. The study of sub acute toxicity showed that X42 fraction preserves the integrity of vital organs.

CONCLUSION

Treatment with single oral dose of 5000 mg/kg did not result in any toxic signs or mortality in the acute toxicity studies. Daily oral administration of X42 fraction of *T. ivorensis* for 28 days did not cause mortality, changes in body weight and body weight gain. Also, no significant changes in hematological and biochemical parameters were observed at the end of the experiment. This extract does not cause damage to the toxic targen organs such as the heart, liver and kidney. The X42 fraction of *T. ivorensis* can be used without risk of intoxication. It's would be necessary to carry out feature studies including histological investigations and cells toxicity studies.

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interests regarding the publication of this paper.

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