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# ASSESSMENT OF INTRAPERITONEAL TOXICITY OF $X_{42}$ FRACTION OF *TERMINALIA IVORENSIS* (COMBRETACEAE) IN RAT

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**Keywords:**

*Terminalia ivorensis*, Acute and sub acute toxicity, Biochemical markers, Hematological parameters

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**ABSTRACT:** The present work focuses to check the acute and sub-acute toxicity studies of $X_{42}$ fraction of *Terminalia ivorensis* in rats. **Materials and methods:** The acute toxicity of the extract was evaluated in rats. The rats were administered intraperitoneally the fraction at the dose of 50, 100, 200 and 300 mg/kg bw and recorded general symptoms and mortality. For sub acute toxicity, the rats received intraperitoneally, normal saline (control), 2.5; 5.0 and 10.0 mg/kg of the extract for 28 days. 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. **Results:** Acute toxicity study produced dose dependent mortality with median Lethal Dose ($LD_{50}$) of 200 mg/kg. Repeated administration of fraction caused no mortality. There were no significant changes ($p>0.05$) observed in serum biochemical markers, hematological markers, body weight changed, relative organs weight when compared to control group. **Conclusion:** The results show that the extract of *T. ivorensis* is moderately toxic when given intraperitoneally.

**INTRODUCTION:** The use of herbal medicines in the treatment of various disease conditions has expanded rapidly, globally. This is attributable to the affordability, accessibility and efficacy herbal remedies. 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 diseases [1].

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The use of herbs in treatment of disease has declined in the west, but it continues to exist throughout the developing countries [2]. The increasing use of herbs therefore makes it pertinent that pre-clinical toxicological studies be carried out on these natural products.

However abusive use of these medicinal plants exposed people to various accidents such as renal failure, heart disease and various intoxications. In order to help these people to get real benefit without any risk from the use of medicinal plants, our laboratory began for over a decade research work to extract actives principles of many medicinal plants by checking their therapeutic virtues in order to give them scientific basis [3]. Among the most used plants by traditional healers

include *Terminalia ivorensis* (Combretaceae) which is used for its antidiarrhea, antidiabetics, antihypertensive, antiparasitic and anticough virtues. The *in-vitro* anti-microbial properties of this fraction have already been assessed by some members of our laboratory.

Thus, *Candida albicans* and *Aspergillus fumigatus* two of the most pathogenic fungi species to human are sensitive to this fraction with the 50% of inhibition concentration ($IC_{50}$) are 2.64 and 4.54 µg/ml respectively [3]. Several antibiotics are use by intraperitonealé route to increase their biodisponibility.

The aim of this study is to evaluate acute and sub acute toxicity of $X_{42}$ fraction of *T. ivorensis* by intra peritoneal route in rats.

## MATERIALS AND METHODS:

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

**Experimental Animals:** Adult male and female Wistar rats (*Rattus norvegicus*), aged 2 to 3 months, weighing 95-110 g, were obtained from vivarium of Superior Normal School Abidjan. The animals were maintained in standard conditions (normal temperature; 12:12 h dark/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.

**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 fine powder with an electric crusher (IKA-MAG). One hundred (100) grams of this powder were extracted in a mixture of solvent with 700 ml of ethanol 96° and 300 ml of water by homogenization in blender. After six (6) cycles of homogenization, the homogenate obtained was dried in a white fabric and filtered successively twice with cotton and once with whatman filter (3mm).The filtrate was concentrated with a rotary evaporator BUCHI at 60°C [4]. It gave hydro alcoholic extract. Then, a portion of this extract had been delipided by hexane with the soxhlet. A residue non hexane-soluble called $X_{42}$ is obtained [3].

**Acute Toxicity Study:** The acute toxicity by intra peritoneal route (IP) of $X_{42}$ fraction of *Terminalia ivorensis* was performed on Adult females and males rats. A total of 30 animals were divided into 05 groups of 06 rats each (03 males and 03 females). Animals were kept overnight for fasting before treatment. Four (04) groups received the dose of 50; 100; 200 and 300 mg/kg body weight respectively. Control animals were administered with normal saline. The rats were observed in detail for any indications of toxicity effect within the first six hours after the treatment period, and daily further for a period of 14 days and mortality recorded within 24 hours. The extract was administered to rat at a volume of 0.5 mL.

**Sub-Acute Toxicity Study:** Sub-acute toxicity studies (28-days repeated toxicity study by IP route) was carried out as per OECD guidelines [6] according to [7]. The rats of either sex weighing 90-110g were assigned to 4 groups (5 males and 5 females each), Group-I received normal saline as vehicle control group, Group-II, Group-III and Group-IV received $X_{42}$ fraction at 2.5; 5.0 and 10.0 mg/kg body weight (bw) at volume of 0.5 mL.

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

**Biochemical and Hematological Analysis:** For biochemical analysis, blood was centrifuged at 3000 rpm for 15 min and serum was also obtained

and stored - 40 °C. The serum was analyzed for various parameters such as Aspartase amino Transferase (ASAT), Alanine amino transferase (ALAT), Glucose, total protein, serum urea, creatinine. Total cholesterol (CT), Triglycerids (TG), and the blood electrolytes. Dosages were made using an automated biochemistry analyzer (Cobas integras, Abott ®) with Biolabo biochemical kits. For hematological study, the EDTA tube blood was used and red blood cell (RBC), white blood cell (WBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV) mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and percentage of total lymphocytes (LYMP) were assessed with an automatic hematological analyzer.

**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 Graph Pad Software.

## RESULTS:

**Extraction:** The hydoalcoholic extract was obtained with an average percentage of 32.2 %. The $X_{42}$ fraction was obtained with a percentage of 90 % from hydroalcoholic extract by delipidation. The powder is brown.

**Acute Toxicity:** After the extract was administered, animals were observed 24 hours. During the six (06) first hours, animals treated with the dose of 300 mg/kg were died. No mortality was observed at 50mg/kg during the 24 hours after administration and the 14 days of observation.

However, somnolence, lack of appetite, convulsions and immobility were observed during 6 hours compared to the control group. The $LD_{50}$ value was calculated by using Miller and Tainter method. $LD_{50}$ of $X_{42}$ fraction was found is 200 mg/kg bw.

**TABLE 1: EFFECT OF DOSE OF $X_{42}$ ON THE MORTALLY OF RATS**

| Doses (mg/kg de Pc) | Control | 50 | 100 | 200 | 300 |
|---|---|---|---|---|---|
| Number of rats | 06 | 06 | 06 | 06 | 06 |
| Mortality (%) | 00 | 00 | 16.66 | 50 | 100 |
| Mortality (probits) | 3.3 | 3.3 | 4.03 | 5.0 | 6.70 |

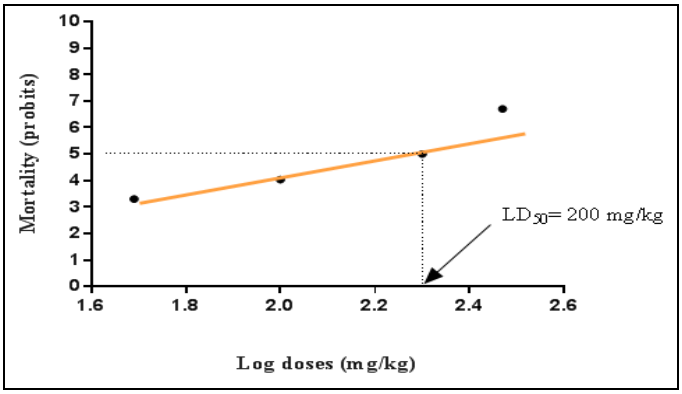


**FIG. 1: GRAPHICAL ESTIMATION OF MEAN LETHAL DOSE**

### Sub-Acute Toxicity:

**Effect of $X_{42}$ on Body and Organs Weights:** The body and organs weights and organ weight/body weight of rats are given in **Table 2**.

**TABLE 2: BODY AND ORGANS WEIGHTS OF RATS IN SUB ACUTE TOXICITY STUDY IN CONTROL AND GROUPS TREATED WITH $X_{42}$ FRACTION**

| Parameters | Control | 2.5 mg/kg | 5.0 mg/kg | 10.0mg/kg |
|---|---|---|---|---|
| **Body weight(g)** | | | | |
| Week0 | 99.91±3.31 | 94.5±1.16 | 98.56±2.2 | 108.2±1.03 |
| Week1 | 103.3±2.64 | 100.0±1.46 | 103.2±2.72 | 111.7±.53 |
| Week2 | 106.1±2.63 | 103.1±1.41 | 106.8±1.2 | 115.5±1.42 |
| Week3 | 111.5±2.69 | 107.4±1.31 | 111.0±1.23 | 121.5±1.14 |
| Week4 | 116.0±2.66 | 112.6±1.5 | 116.1±1.15 | 126.4±1.0 |
| **Absolute weight (g)** | | | | |
| Liver | 3.62±0.31 | 3.63±0.11 | 3.34±0.24 | 3.71±0.07 |
| Heart | 0.54±0.03 | 0.49±0.00 | 0.50±0.02 | 0.51±0.02 |
| Kidney | 0.62±0.03 | 0.65±0.00 | 0.70±0.01 | 0.69±0.03 |
| **Relative weight (%)** | | | | |
| Liver | 3.34±1.4 | 3.22±0.88 | 2.9±0.70 | 3.0±0.83 |
| Heart | 0.46±0.10 | 0.43±0.09 | 0.43±0.08 | 0.40±0.09 |
| Kidney | 0.53±0.11 | 0.57±0.10 | 0.60±0.12 | 0.54±0.09 |

Data are expressed as mean ± SEM, n=10 for each group (5males and 5females). No statistical difference was found between the control and treated groups ($P>0.05$).

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 control.

**Effect of $X_{42}$ on Hematological Parameters:** Hematology rats were measured after 28 days of treatment with $X_{42}$ fraction. Blood is collected in EDTA tubes by amputation of tail and analyzed using an Automatic hematology analyzer (Sysmex KX-21). The effect of $X_{42}$ on Hematological parameters in rats treated is presented in **Table 3**.

**TABLE 3: HEMATOLOGICAL VALUES OF RATS IN SUB-ACUTE TOXICITY STUDY IN CONTROL AND GROUPS TREATED WITH $X_{42}$ FRACTION**

| Parameters | Control | 2.5 mg/kg | 5.0 mg/kg | 10.0 mg/kg |
|---|---|---|---|---|
| WBC ($10^9$/L) | 16.78±0.43 | 17.15±1.52 | 17.65±1.9 | 13.67±0.8 |
| RBC ($10^{12}$/L) | 8.04±0.17 | 7.50±0.14* | 7.80±0.15 | 7.45±0.07* |
| HGB (g/dl) | 13.83±0.13 | 12.92±0.27 | 13.77±0.32 | 13.27±0.14 |
| HCT (%) | 39.2±0.59 | 37.35±0.88 | 39.75±0.86 | 38.55±0.39 |
| MCV (fl) | 51.08±0.6 | 50.17±0.7 | 51.05±0.63 | 51.78±0.21 |
| MCH (pg) | 17.31±0.26 | 16.77±0.2 | 17.65±0.2 | 17.83±0.05 |
| MCHC (g/dl) | 34.37±0.18 | 33.48±0.46 | 34.63±0.33 | 34.37±0.12 |
| PLT ($10^9$/L) | 1048±21 | 1176±77 | 1132±122 | 1047±65 |
| LYMP (%) | 72.7±2.4 | 70.5±3.9 | 67.2±0.8 | 71.9±2.5 |

Values are expressed as mean ± SEM, n= 10 for each groups (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).

The values of RBC decrease significantly at 10.0 mg/kg. The results show that globally hematologic parameters have not evolved significantly compared to the control group.

**Effect of $X_{42}$ on Biochemical Parameters: Table 4** shows the effect of $X_{42}$ on biochemical parameters in rats. The results show that on the 16 biochemical's parameters measured during the period (28 days), no parameters have changed significantly. At doses of 2.5, 5, 10 mg/kg, fraction resulted in no significant (p>0.05) change settings.

**TABLE 4: BIOCHEMICAL'S PARAMETERS OF RATS IN SUB-ACUTE TOXICITY STUDY IN CONTROL AND GROUPS TREATED WITH $X_{42}$ FRACTION**

| Parameters | Control | 2.5 mg/kg | 5.0 mg/kg | 10.0mg/kg |
|---|---|---|---|---|
| ALAT (UI/L) | 49.0±0.85 | 51.5±0.88 | 51.17±1.5 | 53.33±1.45 |
| ASAT (UI/L) | 196.8±4.8 | 215.3±6.0 | 194.8±9.7 | 218.8±6.4 |
| CPK (UI/L) | 1473±55 | 1263±67* | 1471±49 | 1408±24 |
| LDH(UI/L) | 1630±97 | 1321±111* | 1619±82 | 1595±69 |
| ALP (UI/L) | 299.8±26.6 | 306.7±19.3 | 309.7±21.7 | 273.0±9.3 |
| Glucose (g/L) | 0.79±0.03 | 0.86±0.03 | 0.87±0.03 | 0.78±0.02 |
| Urea (g/L) | 0.29±0.01 | 0.28±0.01 | 0.29±0.00 | 0.26±0.00 |
| Creatinine (mg/L) | 4.67±0.49 | 5.34±0.49 | 4.84±0.30 | 5.00±0.36 |
| Total protein(g/L) | 61.28±0.78 | 62.27±1.93 | 63.76±0.15 | 64.85±0.98 |
| Triglycerids(g/L) | 0.37±0.01 | 0.44±0.01* | 0.39±0.00 | 0.38±0.01 |
| Total Cholesterol(g/L) | 0.70±0.05 | 0.70±0.01 | 0.77±0.05 | 0.67±0.02 |
| HDL-Cholesterol(g/L) | 0.40±0.01 | 0.37±0.02 | 0.35±0.01 | 0.36±0.01 |
| Sodium (meq/L) | 140.3±0.76 | 138.7±0.71 | 139.2±0.90 | 139.2±0.40 |
| Potassium(meq/L) | 10.58±0.24 | 10.88±0.36 | 10.78±0.49 | 10.77±0.37 |
| Chore (meq/L) | 99.58±0.54 | 98.57±0.54 | 98.81±0.64 | 98.82±0.28 |
| Calcium (mg/L) | 103.3±2.0 | 106.8±1.4 | 107.0±2.2 | 103.3±3.2 |

Values are expressed as mean±SEM, n=10 (5 males and 5 females).

∗Statistically significant (p<0.05, control group vs treated groups). ASAT: Aspartate aminotransferase ALAT: Alanine aminotransferase; LDH: Lactate dehydrogenase; CK: Creatine kinase; ALP: Alkaline Phosphatas.

**DISCUSSION:** Worldwide, various medicinal plants and botanical drugs have been widely adapted as primary therapeutic agents or supplements for treating various human ailments [7]. Based on the findings that herbal medicines are abused, there is a great need to look into their acute and chronic toxicity effects.

The present study has given the toxicological profile of *T. ivorensis* by acute and repeated toxicity studies. After administration of $X_{42}$ fraction by IP route with doses ranging from 50 mg/kg to 300 mg/kg, we observed 00 % and 100% of mortality respectively at the first 24 hours and during 14 days of observation. As against of the control group, convulsions, agitation, low appetite and drowsiness were observed.

According to Miller and Tainter [8] method, the lethal dose 50 ($LD_{50}$) is 200 mg/kg bw. The $LD_{50}$ indicating that the fraction is moderately toxic [9]. The fraction can be dangerous for vulnerable populations. The similar results were obtained by [10] who indicated that the $LD_{50}$ of methanol extract of *Achyranthus aspera* is 200 mg/kg.

The administration of $X_{42}$ at doses of 2.5; 5.0; 10.0 mg/kg bw had no significant effect ($p>0.05$) on weight parameters of animals compared to control group, which means that this fraction has a negligible effect on the growth of animals. In general, body weight gain and mouse internal organs changes reflect the toxicity after exposure to toxic substances [11].

Body weight changes are indicators of adverse effects of chemical drugs and products, and it will be important if the loss of body weight is more than 10 % from the initial weight [12]. However, since the eating pattern and average weekly weight gain were comparable between treated and untreated animals, the extract could be claimed to be non-toxic to the animals.

In sub acute toxicity study, the gross examination of internal organs revealed no detectable inflammation or changes in color compared with the control. The organs weights showed no significant difference between control and treated groups. It had been reported that reduction in body and also internal organ weights are considered sensitive indices of toxicity after exposure to toxic substance [13]**.** Liver and kidney weights were considered useful in toxicity studies because of these sensitivity to predict toxicity and correlates well with histopathological changes. There is little interanimal variability and thus, it is frequently a target organ of toxicity. In addition, liver is known as primary detoxification organ [14].

In the present study, organ weights such as kidney, heart and liver in all treated groups were not significantly different from those of control group. These results could mean that the integrity of all the above organs was not tampered with by the extract.

However, this deduction can only be possibly true if the results of the effects of the extract on relative organ weight, serum biochemical indices and histopathology of these organs are considered together. Decrease or increase in cell counts and depletion of plasma constituents or their elevation beyond reference range could equally demonstrate haematoxicity [15].

Moreover, anemia following administration of an agent can be as a result of lysis of blood cells and/or inhibition of blood cell synthesis by the active constituents of the extract, and decrease in hematological parameters in experimental animals has been associated with anemia [16]. It was found that, the plant extract did not affect the haematograms of the rats in a manner that would suggest adverse effects on their bone marrow, which is a source of recticulocytes.

The hematologic parameters are important ally of the studies toxicity, therefore the hematopoietic system is extremely sensible the activities of toxic agents, mainly those with mutagenic or cytotoxic potential, resulting in qualitative or quantitative alterations, transitory or permanent and that they can limit the use of medicines [17]. Treatment of rats with $X_{42}$ fraction at repeated doses for 28 days, caused no significant change ($p>0.05$) in hematological parameters in rats treated compared to the control group.

This implies that fraction did not possess any potential of inducing anaemia throughout the 28 days period of administration. The results are in line with [18] with the methanolic extract of *Ceiba pendantra.* Several biochemical parameters in the

blood of the animals which were treated with *T. ivorensis* were also examined. The biochemical parameters are important because they allow the evaluation of all organs and the general status of the animal's body, especially with regard to renal and liver functions [16].

A study of hepatic tissue integrity and metabolism may be useful in the evaluation of toxic effects of medicinal plants on the liver. Our result shows that the activities of enzymes such as the ALAT, ASAT, LDH and ALP were not disturbed by $X_{42}$. These enzymes are key enzymes used in order to assess the hepatic tissue integrity [19].

Therefore, fraction $X_{42}$ would not have harmed hepatic cells. The values of the hepatic metabolites such as Total proteins, total cholesterol, HDL-cholesterol, triglycerides and glucose are also normal range compared to control group. Liver plays a role in the metabolism and regulation of glucose, lipids and proteins and any damage to the liver would lead to an increase in levels of these metabolites [20]. The present study did not change the level of these metabolites. This result would not influence the hepatic metabolism. The same results were obtained by [21] *et al.*, with the hydro alcoholic extract of *Terminalia mantaly*.

Indeed, this fraction content flavonoids, molecules known as being hepatoprotectrice [16]. The effect of this plant on the liver would be due to the presence of flavonoids. Creatinine and urea are substantially removed from the blood by glomerular filtration. It means that, the concentrations of these metabolites in urine are regulated by the kidney which has a real role of blood filter. Thus, metabolites concentrations which are not correctly eliminated increase in the blood. This is one of the leading causes of kidney failure [22].

This markers of renal function are not seen their rates varied by $X_{42}$. This indicates that, there is no adverse effect on kidney functions after 28 days of administration by IP route. Similarly, renal function is well preserved by the administration of stem bark from *Trichilia emetica* extract by oral route in rats [23]. A cardiac injury induces, in the bloodstream, the release of enzymes which are present in cardiac cells followed by the increase of their activities in the serum [24]. ASAT, ALAT, LDH and CPK are 4 biomarkers commonly used to assess myocardial integrity [25].

Generally, the increase of CPK activity in serum is associated the attacks of cardiac tissues [26]. The data showed no change in the level of these biomarkers in control and all treated groups.

These results indicate that fraction $X_{42}$ at the above mentioned doses would not have harmed cardiac tissue. This result is in line with [27] *et al.*, who reported that Dichloromethane-ethanol extract of *Morinda morindoides* preserves the integrity of heart.

Cardiac function is also under the control of electrolytes. Sodium, Potassium, Calcium, Magnesium and Chloride homeostasis are involved in cardiac function [24]. Calcium is important for heart concentrations. In this study, the concentrations obtained were in normal range reported by the previous authors [28]. The normal range of calcium would be explained by the intracellular calcium flow is not disturbed. This shows that, $X_{42}$ fraction do not deteriorate the cardiac contractility.

**CONCLUSION:** The results show that the $X_{42}$ fraction of *T. ivorensis* bark is moderately toxic by intraperitoneale route. But daily administration of $X_{42}$ fraction of *T. ivorensis* for 28 days did not cause mortality, changes in body weight at the doses tested. Also, no significant changes in hematological and biochemical parameters globally were observed at the end of the experiment. However, extremely high doses may not be advisable. Further investigation is needed to evaluate the long-term safety, cells toxicity and chemical composition of this extract.

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**CONFLICT OF INTEREST:** The authors do not have conflict of interest over this research work.

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