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Original Research Article

Acute and subacute toxicity of an aqueous extract of *Piper umbellatum* (Piperaceae) leaves in rats

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Piper umbellatum **is widely used for treatment of various ailments in traditional medicine. The aim of this study was to investigate potential toxicity effect of aqueous extract of *Piper umbellatum* (Piperaceae) leaves in rats. During acute toxicity study, female rats were orally administered with EAPU at single doses of 1000, 2000 and 5000 mg/kg according to OECD Guidelines 423. For the sub-acute toxicity EAPU were so orally administered doses of 400, 800 and 1000 mg/Kg daily for 28 days of both sexes except for the control group which received NaCl 0.9 %. Animals were sacrificed and blood samples were collected for hematological and biochemical analyses. Kidney, liver and heart were harvested and evaluated for histopathological changes.The acute toxicity study revealed no lethal effects and behavioural signs of toxicity at the tested doses indicating that LD_{50} is greater than 5000 mg/kg. In sub-acute study, EAPU induced significant ($p < 0.05$) reduction of the body weight only in female and no significant change relative organe weigth, food and water intake in comparaison to the control group on the both sexes. There is significant increases in the levels of WBC at 1000 mg/kg of the extract only in the female. In respect of liver function parameters, significant reductions in gamma glutamyl transferase and bllirubin levels respectively at doses of 400 and 1000 mg/kg relative to control is observed. Compared to control, the extract significantly decreases dose dependently the level of triglyceride only in the female ($p < 0.05$; $p < 0.01$; $p < 0.001$). There is significant decreases in the levels of urea in both sexes and creatinin only in the male rat. Histological study shows normal structure of liver, kidneys and heart of control and treated rats. Results indicate that oral doses of aqueous extract of *Piper umbellatum* is relatively safe in rats.**

Key words*: Piper umbellatum,* acute and subacute toxicity, haematological and biochemical parameters, histopathological examination.

INTRODUCTION

Herbal plant is use for treatment of various ailments in traditional medicine, about eighty percent of the world's population depend on traditional medicine for primary health care (Ugwah et al., 2013; Ekor, 2014). The medicinal plants contain active molecules that are at the origin of the therapy. But this molecules can lead to vitals organ functions impairment (Cristavao et al., 2007). *Piper umbellatum* plant of the family Piperaceae have various pharmacological property including anti-inflammatory (Perazzo et al., 2005.), nephroprotective, anti-oxidant (Domis and Oyen, 2008 ; Agbor et al., 2012 ; Bagatela et al., 2013) immunomodulatory, wound healing and antimicrobial properties. It used as diuretic and for treatement of cardiovascular deseases (Agbor et al., 2012 ;

Nwauzoma et al., 2013). There are few studies evaluating the safety of *Piper umbellatum* while numerous studies have examined its pharmacological actions (Roersch, 2010 ; Da Silva et al., 2016).

Our study aim to evaluate, single dose toxicity and 28-day subacute by oral route, effects of an aqueous extract of *Piper umbellatum* on hematological, heart, liver and kidney biochimical parameters in Wistar rats. Theremore histopathological examination will be done.

MATERIAL AND METHODS

Plant material and extraction method

The *Piper umbellatum* was collected in Yakassé-mé town to Côte d'Ivoire. This plant is authenticated the July 10 1980, by an expert in Botany (Professor Ake-Assi Laurent) of the *Centre National de Floristique* (UFR-Biosciences, Félix Houphouët-Boigny University, Abidjan, Côte d'Ivoire).

The fresh leaves of *Piper umbelatum* are dried in ambient air, away from the sun. They are then milled in a micro mill (IKA LABORTECHNIK TYPE A 10). One hundred grams (100 g) of powder are added to one liter distilled water and boilled during 15 minutes.

The solution obtained is carefully filtered on hydrophilic cotton and "Wattman" filter paper. The filtrate collected in a flask is then evaporated under vacum at 60° C., using a rotary evaporator of "Büchi" type and oven-dried at 50 ± 5° C. A perfectly water-soluble fine powder it represents an aqueous extract of *Piper umbellatum* (EAPU).

Animals

Rats (*Rattus norvegicus*, Muridae, L.1753) of Wistar strain were used to carry out this work. They are reproduced at the vivarium of the *Ecole Normale Supérieur* (ENS, Abidjan). The resulting litters are fed and watered *ad libitum* to reach a weight between 160 and 180 g under standard environmental conditions, temperature 25° C, with a light-dark cycle of 12 hours.

Experimental design

The sub-acute toxicity study was carried out according to Organisation for Economic Co-operation and Development, test guidelines 407 for testing chemicals (OECD, 2008). A total of 48 male and female Wister rats weighing between 160 and 180 g were randomly divided into four groups (n = 6 males and 6 females /group). Rats in treatment groups orally received *Piper umbellatum* aqueous extract at doses of 400, 8 00 and 1000 mg/kg every day. The extract was administered *per os* on a daily basis for 28 days. Rats in control groups were administrated NaCl 0.9 % (vehicle).

During the experimental period, the body weights of all groups were measured twice a week. Animals were also visually observed for mortality, changes in behavioral patterns.

Hematological, biochemical analyzer and histopathological examination

At the end of the treatment period, all rats fasted all night (12h). They are anesthetized (Thiopentan sodium) and blood samples are collected for measurement of hematological parameters (EDTA-2K coated tubes) and biochemical (dry tubes). Hematological analyses were performed using an automated hematological analyzer (MYDRAY BC 30S). The following parameters such as red blood cell (RBC), erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume (MCV), white blood cell (WBC), lymphocytes, eosinophils, neutrophils, monocytes and platelet counts were determined. Blood samples were collected and centrifuged at 3000 rpm for 10 minutes. Serum samples were removed, kept in Eppendorf tubes and stored at -20°C. Serums were further analysed using Semi-automatic biochemical analyzer (spectrophotometer Rayto RT-9200) to determine the level of alanine amino transferase, aspartate amino transferase, gamma glutamyl transferase, total bilirubin, conjugated bilirubin, triglycerides, total cholesterol, HDL, and LDL. After euthanasia, the rats were sacrificed and the organs were removed for autopsy, measurement of organ weight and histopathological examination.

Statistical analysis

The values are expressed as mean ± standard error of mean of six experiment (mean ±. SEM). GraphPad Prism 7 software, (Microsoft, San Diego California, USA) is used for statistical analysis of data and graphical representations. The significance differences between treatments is determine using to the variance analysis (ANOVA) of the Tukey-Kramer multiple comparison test. Difference is considered as statistically significant when $P < 0.05$.

RESULTS

Acute toxicity study

Aqueous extract of *Piper umbellatum* at a dose of 1000 ; 2000 and 5000 mg/kg produced no significant weight variation in treated group compare to control ($P > 0.05$; Figure 1).

In addition, treatements induced no abnormal signs of toxicity in behavioral patterns in treated group compare to control.

Sub-acute toxicity studies

Food and water intake

There was no variations in food consumption and water intake (Figure 2) during the period of experiment in male and female compare to control group ($p > 0.05$).

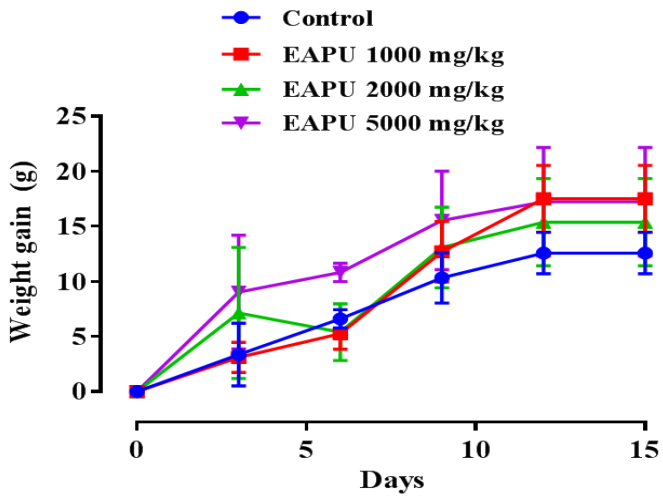

Figure 1 : Changes in the body weights in females rat adminstered *Piper umbellatum* leaf extract in the acute toxicity study. Values are expressed as mean ± SEM, n=6. (* p < 0.05 ; ** p < 0.01 ; *** p < 0.001 as compared to the control group).

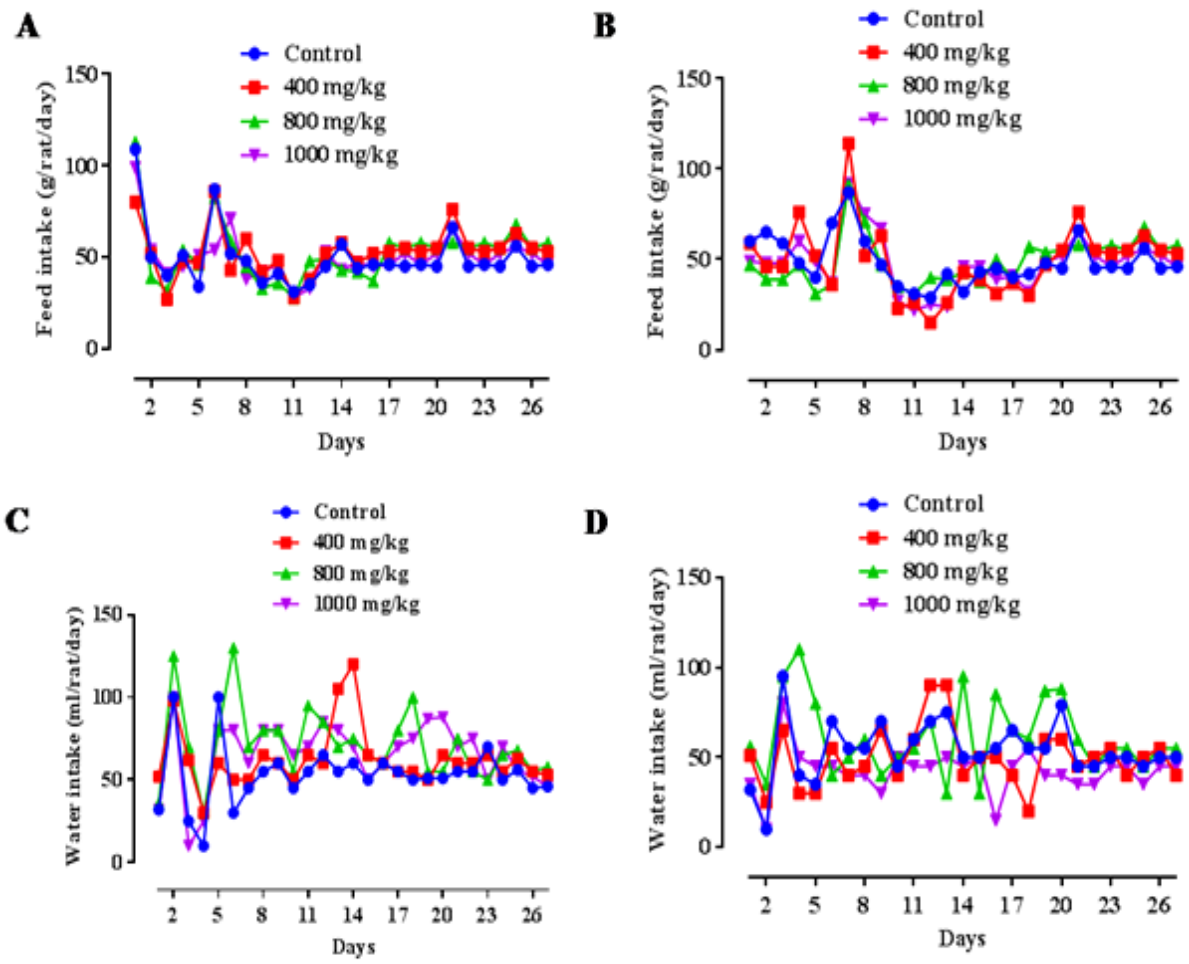

Figure 2: Effects of the aqueous extract of *Piper umbellatum* leaf on the food and water consumption in male (A and C) and female (B and D) rats. Values are expressed as mean ± SEM, n=6. (* p <0.05 ; ** p < 0.01 ; *** p < 0.001 as compared to the control group)

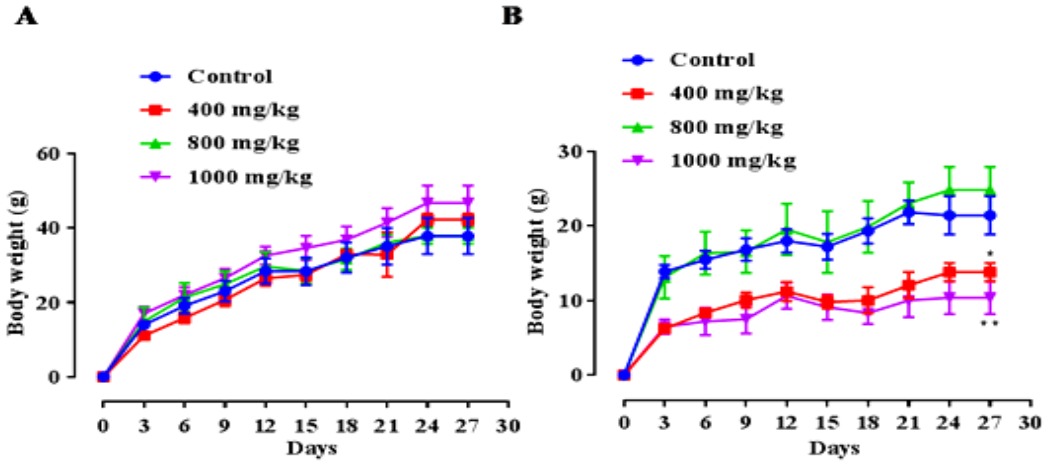

Figure 3 : Changes in the body weights in males (A) and females (B) rat treated with *Piper umbellatum* leaf extract in the subacute toxicity study. Values are expressed as mean ± SEM, n=6. (* p < 0,05, ** p < 0,01, *** p < 0,001 as compared to the control group).

Table 1. Relative organs weight of males and females rats after 28 days of treatment with *Piper umbellatum* leaf extract in the subacute toxicity study

		Doses (EAPU mg/Kg B. W)		
(%)	**Control**	**400**	**800**	**1000**
Males				
Heart	0.29 ± 0.03	0.34 ± 0.05	0.33 ± 0.03	0.35 ± 0.05
Kidney	0.44 ± 0.06	0.49 ± 0.03	0.45 ± 0.02	0.49 ± 0.03
Liver	2.53 ± 0.63	2.51 ± 0.12	2.77 ± 0.62	2.75 ± 0.43
Adipose tissue	1.57 ± 0.53	2.26 ± 0.95	1.49 ± 0.58	2.07 ± 0.51
Lung	0.64 ± 0.10	0.68 ± 0.13	0.62 ± 0.06	0.80 ± 0.37
Females				
Heart	0.36 ± 0.01	0.35 ± 0.053	0.34 ± 0.02	0.40 ± 0.13
Kidney	0.46 ± 0.03	0.47 ± 0.028	0.46 ± 0.02	0.49 ± 0.10
Liver	2.21 ± 0.10	2.39 ± 0.089	2.68 ± 026	2.62 ± 0.98
Adipose tissue	3.93 ± 0.34	2.16 ± 0.40	3.47 ± 1.44	3.33 ± 0.85
Lung	0.69 ± 0.18	0.81 ± 0.14	0.74 ± 0.13	0.83 ± 0.22

Each value represents the mean ± Standard deviation ; (n = 6); values are statistically no different from control at ( ) p > 0.05. One way analysis of variance (ANOVA) and Tukey-Kramer multiple comparisons test.

Body weight of rats

Piper umbellatum extract at a doses of 400 ; 800 and 1000 mg/kg produced no change in the body weight of the treated rats for 28 days. Indeed, as summarized in Figure 3, the body weight of the rats increases relatively during the study. When compared with the control groups, EAPU treatments at 400, 800 and 1000 mg/kg doses, did not cause significant change in the body weight of of male rats ($p > 0.05$) but at 400 and 1000 mg/kg dose at day 28, they cause significant reduction of body weight in female rats ($p < 0.05$; $p < 0.01$ respectively).

Relative organ weights

Data related to relative organ weights for both male and female rats treated with EAPU extract are summarized in Table 1. Similarly, gross examination of internal organs of both the control and treated groups including, heart, liver, and kidneys, did not reveal any abnormal findings related to the administration of the extract ($p > 0.05$).

Hematological study

Administration of leaf aqueous extract of *Piper umbellatum* induced no changes in total and differential WBC counts in male compare to untreated group ($p > 0.05$; Table 2).

Furthermore, the levels of RBC increased significantly to 5 % and 7 % respectively at 400, and 800 mg/kg and decrease to 6 % at 1000 mg/kg on the male rats compare to untreated group ($p < 0.01$; $p < 0.05$; Table 2).

In female rat, EAPU at 1000 mg/ kg caused significant

Table 2. Hematological parameters of males rats after 28 days of treatment with *Piper umbellatum* leaf extract in the subacute toxicity study

Parametres	Control	Doses (EAPU mg/Kg B. W)		
		400	800	1000
WBC (10/mm³)	15.40 ± 0.2	15.63 ± 0.47	12.20 ± 0.20	17.25 ± 0.05
RBC (10/mm³)	6.59 ± 0.21	6.93 ± 0.11**	7.07 ± 0.15**	6.2 ± 0.02*
Hemoglobin (g/dL)	13 ± 0.10	12.53 ± 0.55	13.45 ± 0.05	12.39 ± 0.50
Hematocrit (%)	40.10 ± 0.30	38.73 ± 2.03	40.25 ± 3.85	37.35 ± 2.45
MCV (µm³/red cell)	62.40 ± 1.60	58.40 ± 0.40	60.70 ± 0.50	59.70 ± 3.70
Platelets (103cells/mm3)	459.50 ± 0.50	372.67 ± 2.52	501 ± 1	387 ± 2
Neutrophils (%	1369 ± 1	2338 ± 2	913 ± 1	1263 ± 3
Eosinophils (%)	154 ± 1	156.33 ± 1.53	244 ± 1	288 ± 2
Monocytes (%)	1015 ± 2	1556.6 ± 2.31	998 ± 2	1267.5 ± 2.5
Lymphocytes (%)	12852 ± 1.73	11748 ± 2.89	10122 ± 1	14434.5 ± 0.5

*Each value represents the mean ± Standard deviation; (n = 6); values are statistically different from control at * p < 0.05 and ** p < 0. 01. One way analysis of variance (ANOVA) and Tukey-Kramer multiple comparisons test.*

Table 3 . Hematological parameters of females rats after 28 days of treatment with *Piper umbellatum* leaf extract in the subacute toxicity study

Parametres	Control	Doses (EAPU mg/Kg B. W)		
		400	800	1000
WBC (10/mm³)	10.33 ± 0.49	17 ± 0.4	9.90 ± 0.10	14.87 ± 1.8****
RBC (10/mm³)	6.36 ± 0.77	6.02 ± 0.06	6.6 ± 0.02	6.06 ± 0.51
Hemoglobin (g/dL)	12.80 ± 0.17	11.80 ± 0.06	12.20 ± 0.10	13.07 ± 0.38
Hematocrit (%)	39 ± 0.60	37.70 ± 0.3	38.55 ± 0.15	38.73 ± 0.97
MCV (µm³/red cell)	62.63 ± 0.60	69.19 ± 0.82	65.41 ± 0.49	62.07 ± 2.54
Platelets (10³cells/mm³)	237.3 ± 3.79	210 ± 1	246 ± 1	267.67 ± 1.53
Neutrophils (%)	597.6 ± 4.04	548.5 ± 3.5	595 ± 3	1090 ± 1.73****
Eosinophils (%)	179.6 ± 1.53	229 ± 3	194 ± 1	192.33 ± 2.52
Monocytes (%)	810 ± 3	1018.5 ± 1.5	995 ± 2.01	985.67 ± 2.19
Lymphocytes (%)	8712.6 ± 2.5	9594 ± 6	8627.5 ± 3.5	12598 ± 2****.

*Each value represents the mean ± Standard deviation; (n = 6); values are statistically different from control at **** P < 0.0001. One way analysis of variance (ANOVA) and Tukey-Kramer multiple comparisons test.*

increase to 44 % in the levels of WBC, lymphocytes and monocytes compare to control (p < 0.0001; Table 3).

Biochemistry analysis

Tables 4 and 5 summarize the levels or activities of biochemical parameters in male and female rats.

No significant differences among both sexe were noted in the level of AST, ALT, total and direct bilirubin (p > 0,05 ; Table 4 and 5).. However, EAPU extract induced significant reduction of GGT at all tested doses (p < 0,05 ; p < 0,01 ; p < 0,001; Table 5).

All tested doses of EAPU extract induced no significant modification of total cholesterol, triglycerides, LDL, and HDL in male groups of rats compared to the control groups (p > 0,05 ; Table 4). Hovewer administration of the extract at these doses, caused significant decrease, in dose-dependent manner, only on female rat, in the levels of triglycerides (p < 0,05 ; p < 0,01 ; p < 0,001; Table 5), and the LDL level only at 1000 mg/kg dose (p < 0,05).

Moreover, a significant decrease (p < 0,05) in the activity of LDH was noted only in male rats treated with 1000 mg/kg of EAPU extract compared to the control group. (p < 0,001 ; Table 4).

Furthermore, there were significant reduction observed in urea of EAPU extract treated groups of male rat (p < 0,001 ; p < 0,01 ; p < 0,01; Table 4) and female rat (p < 0,001; Table 5), respectevly at 400, 800 and 1000 mg/kg doses, when compared to the control groups. It also, observed on the male rats at the dose levels of 800 and 1000 mg/kg an significant diminution of creatinemia (p < 0,01 ; p < 0,001; Table 4).

Histopathology analysis

At the end of treatment, vital organs, including heart, liver, kidneys, were subjected to histopathological examination. The microscopic observation showed no remarkable

Table 4. Biochimical parameters of males rats after 28 days of treatment with *Piper umbellatum* leaf extract in the subacute toxicity study

Parametres	Control	Doses (EAPU mg/Kg B. W)		
		400	800	1000
Triglycerides (g/L)	1.69±0.13	2.01 ± 0.22	2.06 ±0.17	1.97±0.15
HDL cholesterol (g/L	0.20 ± 0.09	0.23 ± 0.08	0.28 ± 0.05	0.21 ± 0.04
Total cholesterol (g/L)	0.36 ± 0.09	0.32 ± 0.05	0.35 ± 0.10	0.25 ± 0.04
LDL cholesterol (g/L)	0.05 ± 0.04	0.1 ± 0.05	0.19 ± 0.08	0.24 ± 0.05
CK-MB (UI/L)	1644 ± 0.9	1139 ± 1.8	1401 ± 1.2	1272 ± 2.3
LDH (UI/L)	3579 ± 8.8	3762 ± 9.1	3564 ± 3.5	1757 ± 6.4***
AST (UI/L)	192 ± 18.36	206.6 ± 18.30	233.6 ± 21.82	230.8 ± 11.31
ALT (UI/L)	63.93 ± 1.09	57.5 ± 14.98	60.8 ± 7.93	81.61 ± 3.09
GGT (UI/L)	2.67 ± 0.77	2.09 ± 0.28	3.78 ± 0.6	3.63 ± 0.20
Total bilirubin (mg/L)	1.56 ± 0.08	1.29 ± 0.18	1.43 ± 0.19	1.48 ± 0.08
Conjugated bilirubin(mg/L)	0.56 ± 0.06	1.15 ± 0.11	1.04 ± 0.15	1.25 ± 0.27
Urea (g/L)	0.47 ± 0.06	0.27 ± 0.03***	0.30 ± 0.03**	0.35 ± 0.05**
Creatinine (mg/L)	14.78 ± 0.36	13.63 ± 0.47	13.36 ± 0.3**	11.43 ± 0.4***
Na+(mmol/L)	143.7 ± 0.14	144.7 ± 1.30	144.3 ± 1.43	144.7 ± 2.06
K+(mmol/L)	5.33 ± 0.36	5.96 ± 0.13	5.13 ± 0.1	5.53 ± 0.13
Cl-(mmol/L)	105 ± 0.85	104.7 ± 0.84	106.3 ± 1.20	104.3 ± 1.18

*Each value represents the mean ± Standard deviation; (n = 6); values are statistically different from control at ** p<0. 01 and *** P < 0.001. One way analysis of variance (ANOVA) and Tukey-Kramer multiple comparisons test*.

Table 5 . Biochimical parameters of females rats after 28 days of treatment with *Piper umbellatum* leaf extract in the subacute toxicity study

Parametres	Control	Doses (EAPU mg/Kg B. W)		
		400	800	1000
Triglycerides (g/L)	2.88 ± 0.10	1.91 ± 0.43*	1.54 ± 0.17**	0.86 ± 0.05***
HDL cholesterol (g/L	0.40 ± 0.04	0.41 ± 0.13	0.41 ± 0.13	0.41 ± 0.08
Total cholesterol (g/L)	0.63 ± 0.17	0.64 ± 0.19	0.51 ± 0.18	0.58 ± 0.09
LDL cholesterol (g/L)	0.03 ± 0.4	0.09 ± 0.15	0.07 ± 0.31	0.06 ± 0.8*
CK-MB (UI/L)	1075 ± 3.6	1013 ± 1.6	1039 ± 1.01	1047 ± 1.22
LDH (UI/L)	3529 ± 1.3	3534 ± 5.1	3187 ± 1.4	3514 ± 5
AST (UI/L)	181.8 ± 6.64	178.8 ± 9.41	186.3 ± 2.09	180.1 ± 6.53
ALT (UI/L)	82.79 ± 1.85	92.1 ± 0.87	90.03 ± 2.61	89.92 ± 1.69
GGT (UI/L)	2.79 ± 0.50	0.83 ± 0.22**	1.86 ± 0.25*	0.20± 0.02****
Total bilirubin (mg/L)	1.88 ± 0.20	1.58 ± 0.20	1.57 ± 0.06	1.16 ± 0.10*
Conjugated bilirubin(mg/L)	1.21 ± 0.10	1.35 ± 0.20	1.28 ± 0.31	1.21 ± 0.40
Urea (g/L)	0.52 ± 0.05	0.50 ± 0.03	0.64 ± 0.48	0.28 ± 0.05***
Creatinine (mg/L)	13.44 ± 0.14	12.64 ± 0.44	11.78 ± 0.29	11.2 ± 0.28
Na^+(mmol/L)	147.3 ± 1.28	146.3 ± 0.88	147 ± 1.31	145.3 ± 1.20
K^+(mmol/L)	6.16 ± 0.33	5.93 ± 0.17	6.31 ± 0.25	5.86 ± 0.12
Cl^-(mmol/L)	105.3 ± 0.88	105.5 ± 0.84	105.7 ± 0.71	107 ± 0.77

*Each value represents the mean ± Standard deviation ; (n = 5); values are statistically different from control at *p<0.05. ** p<0. 01 and ***P<0.001. ****P<0. 0001.One way analysis of variance (ANOVA) and Tukey-Kramer multiple comparisons test.*

pathological changes for all organs in EAPU treated groups compared to the control groups (Figure 4).

DISCUSSION

The principal aim of evaluating the safety of any medicinal plant is to identify the nature and significance of adverse effect and to establish the exposure level at which this effect is observed. Hence, the present study was conducted to assess its toxicological profile by performing acute oral toxicity in female rats for 14 days and sub-acute oral toxicity in rats of both sexe for 28 days. In this study, no significant adverse effect was observed in the acute toxicity study after administration of a single dose of the EAPU up to 5000 mg/kg. All animals treated with the EAPU survived beyond the 14 days observation period. Administered through oral route to rats at 1000, 2000 and 5000 mg/kg a long acute toxicity testing did not produce any sign of toxicity and death in the animals.

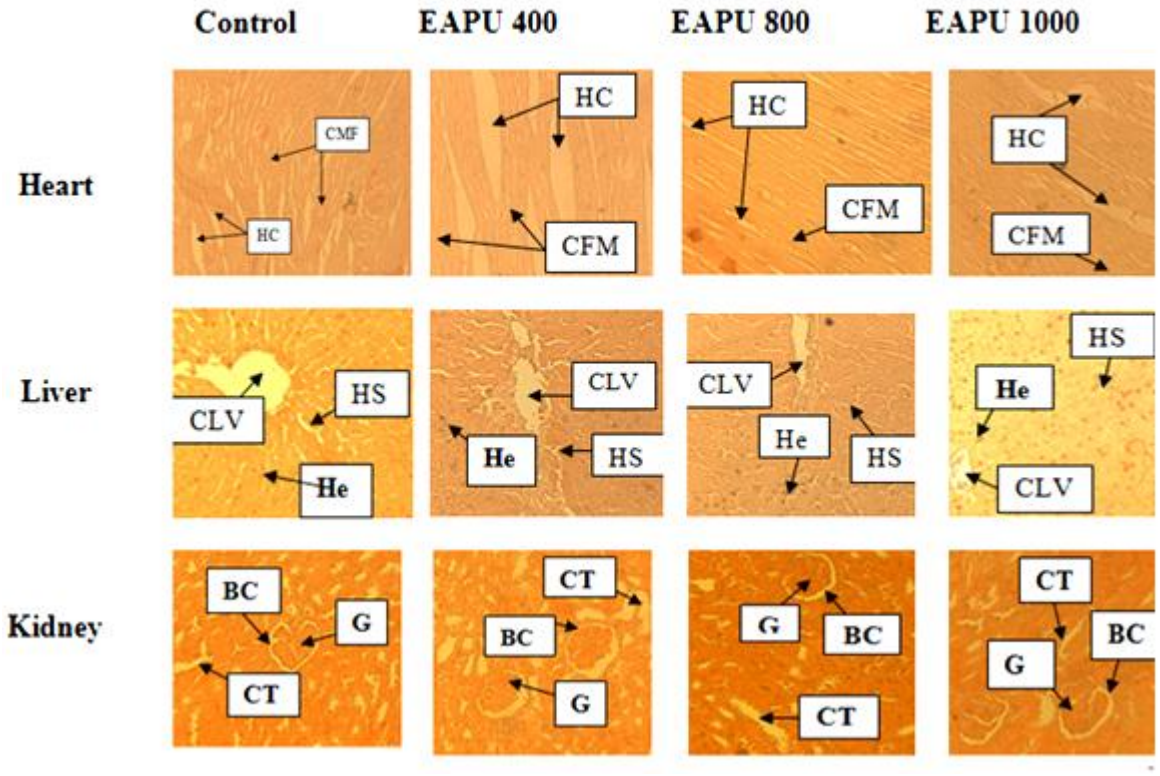

Figure 4: Effects of the aqueous extract of *Piper umbellatum* leaf on heart. liver and kidney histology in Wistar rats. Treatement group are compared to the control group

HC : Heart Cavity**; CFM :** Cardiac Muscle Fiber **; He:** Hepatocyte **; HS :** Hepatocyte spacer**; VCL:** Veine Centrolobulaire ; **G:** glomerule **; CT:** Convoluted Tubule**; BC:** Bowman's Capsule
Hematoxylin and eosin (under ×100 magnification power)

According to OECD criteria under its Globally Harmonised Classification System (GHS) for chemical substances and mixtures with LD50 > 5000 mg/kg are categorised as category 5 (OECD, 2001). This suggests that the oral LD50 of the plant being greater than 5000 mg/kg day be safe. These findings are not different those of Barros et al. (2005) who showed, single oral dose of 1000, 2000 and 5000 mg/kg of dried ethanolic root extract of *Pothomorphe umbellata* L. Miq. was unable to induce mortality or toxic effects in rats and mice.

In sub-acute study , aqueous extract of *Piper umbellatum* provokes in rats, no variations in food and water intake in both sex but affect the body weight in rats after daily administration during 28 days only on female. In toxicological study, the changes in body weight are one of the first critical parameters and serve as a sensitive indication of the general health status of animals (Raza et al., 2002 ; Teo et al., 2002). The significant decrease in body weight gain observed in female rats in the 400 and 1000 mg/kg group had no toxicological significance because it not appear at those of 800 mg/kg.

These results may sugerate possible weigth management by aqueous extract of *Piper umbellatum.* Tom et al. (2018) in previous study had showed that stem bark aqueous extract of *Harungana madagascariensis* significantly decrease body weight gain observed in female rats in the 600 mg/kg group.

Assessment of hematological parameters can be used to explain hematological functions of a chemical compound or plant extracts in an organism (Yakubu et al., 2007) Blood act as a pathological reflector of the status of exposed animals to toxicants and other conditions and/or agents (Olafedehan et al., 2010). The increase in WBC count may have been due to enhancement in the rate of entry of leucocytes into the blood from the bone marrow and a diminished rate of removal from circulation. Granulocyte-macrophage colony stimulating factor, macrophage colony stimulating factor, interleukins (IL-2, IL-4 and IL-5) regulate the proliferation, differentiation and maturation of committed stem cells responsible for the production of WBCs (Ganong, 2001).

Therefore, such increase in WBC counts may be due to

over-production of these haematopoietic regulatory elements by the stromal cells and macrophages in the bone marrow (Son et al., 2003). These stimulant effects could be associated with the adjuvant activity of some phytochemicals found in the extracts. Alkaloids, tannins, phenolic compounds and flavonoids have generally been reported as immunostimulants (Lakshmi et al., 2003 ; Dashputre and Naikwade, 2010). The observed increases in RBC, levels aqueous extract of *Piper umbellatum* suggests that the extract could have stimulated erythropoietin release in the kidney, which is the humoral regulators of RBC production (Degruchy, 1976 ; Jorum et al., 2016). Thus imply that though the extract may stimulate the production of red blood cells. These effects can must attribuate to presences of phytochemicals like flavonoids, tannins and terpenes may be responsible for the haemopoietic stimulating effects (Ohlsson and Aher, 2006). These effects are similar to those of obtain with several plant extract from pharmacopoeias and deemed increases RBC and immunostimulants activity (Jorum et al., 2016). These authors showed that dichlorométhane-Methanol leaf extract of *Carpobrotus edulis* induced general increase in the levels of red blood cells, Hemoglobin and WBC.

Biochemical parameters have significant roles as a marker in toxicological evaluation, because of their response to clinical signs and symptoms produced by toxicants. Evaluation of hepatic and renal function is of prime importance to assess the toxic properties of extracts and drugs (Rahman et al., 2001 ; Loha et al., 2019). Administration of *Piper umbellatum* aqueous extract caused significant decrease, in dose-dependent manner, on female rat serum triglycerides and the LDL level at 1000 mg/kg dose. Moreover, it provokes a significant decrease of LDH in male rats treated with same dose. Serum triglycerides, LDL cholesterol changes could give information on the cardiovascular diseases. So, theses observation in the present investigation apparently indicate possible cardiovascular desease protective effect of aqueous extract of *Piper umbellatum* in rat for comparaison, a study perfomed by Qi et al. (2013) *Ulva pertusa* (Chlorophyta) at 600, 1200 and 3000 mg/kg doses decreased significantly triglycerides and total cholesterol concentrations on female rats.

The kidneys are a vital organ highly vulnerable to toxic compounds due to the high volume of blood flows throughout it. It filters large types of toxins, which can accumulate in the kidney tubules (Akindele et al., 2014). Urea and creatinine are considered as sensitive biomarkers of renal damage (Gowda et al., 2010). In this study, EAPU induced significant decrease in the levels of creatinemia and urea, in treated groups in comparison to the controls groups. These findings may indicate that the extract at the doses tested did not induce alterations in renal function or kidney damage but can protect it. Thereby, this study suggests a further assessment of the nephroprotective potential of *Piper umbellatum* leaves extract (Iteire et al., 2019). Chromatographic analysis of *Piper umbellatum* by Da Silva et al. (2014) revealed the presence of appreciable amounts of quercetin and rutin, two flavonoids well known for their antioxidant properties (Suzuki et al., 1998). One cannot rule out the participation of other compounds in this action, given that they are mentioned in the literature the presence of terpenes, steroids and other secondary metabolites in *P. umbellatum* (Roersch, 2010). These metabolites in *Piper umbellatum* may works in synergy or individually for induced this effects.

Conclusion

The oral LD50 of aqueous extract of *Piper umbellatum* has been shown to be greater than 5000 mg/kg and is generally considered safe. *Piper umbellatum* has also been shown to cause reduction of body weight hence can be used as an anti-obesity agent. Prolonged administration revealed that it may cause reduction of triglycerides, LDL cholesterol and kidney markers. These observations suggest that *Piper umbellatum* may be hypolipidemic and nephroprotective agent.

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Conflicts of interest

The authors declare they have no conflicts of interest.

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