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Original Article

Toxicological profile of the aqueous extract of *Mangifera indica* Linn (Anarcadiaceae)

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ABSTRACT

Mangifera indica is a plant used in traditional medicine in the West region of Cameroon. This study aimed to assess the toxicity profile of the aqueous extract of *Mangifera indica* (AEMI) stem bark. The extract was tested on larvae of *Artemia salina* for cytotoxicity study and in rats for acute and sub-chronic oral toxicity studies. For acute evaluation, AEMI was given to rats at single dose of 5000 mg/kg and they were observed for 14 days. For sub-chronic study, AEMI was administered at doses of 300, 400 and 500 mg/kg for 28 days; body weight, food and water intake, hematological, biochemical and histological parameters were evaluated. The results showed that AEMI has no cytotoxic effect and the mean lethal concentration (LC_{50}) was 4168 µg/mL. No mortality or signs of toxicity were observed in the acute study. In the subchronic study, water consumption, serum AST, triglycerides, total and LDL cholesterol significantly decreased ($p<0.05$) whereas platelets count, urinary volume, serum creatinine and HDL cholesterol significantly increased ($p<0.05$). Histopathological analysis revealed some abnormalities on the livers and kidneys of rats treated at dose of 500 mg/kg. The results suggest that AEMI may have deleterious effects at high doses following long term administration

Keywords: *Mangifera indica*; cytotoxicity; acute toxicity; sub-chronic toxicity.

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INTRODUCTION

Focus on plant research has recently increased all over the world, where much evidence was collected to show the immense potentials of medicinal plants used in various traditional systems [1,2]. The World Health Organization (WHO) estimates that 70 to 80% of the people in developing countries use traditional medicine as a major source of health care [2]. However, many people underestimate the toxicity of natural products and do not realize that these agents could be as toxic as/ or more than synthetic drugs [3]. So far, many plants have been reported to be toxic to both human and animals [2,3]. So, the traditional use plants for medicinal purposes, does not guarantee their safety. Plants in folk medicine should therefore, be evaluated for safety or toxicity and necessary recommendations made on their use.

Mangifera indica is a tropical tree shrub and belong to the family of Anacardiaceae. It consists of about sixty gender and six hundred species; it is widely used as a source of food, medicines and timber [4]. In Cameroon, *M. indica* is well known for its medicinal purpose. The stem bark is widely used especially in West Cameroon to treat diarrhea, malaria, tooth ache and dysentery. In spite of the use of *M. indica* in traditional medicine, scientific data on the plant is limited. Also, systematic evaluation of its toxic effects is lacking. Therefore, this study was designed to assess the toxicological profile of the aqueous extract of *M. indica* (AEMI) stem bark in order to determine its limits of use.

MATERIAL AND METHODS

Plant material: The stem barks of *Mangifera indica* (Anacardiaceae) were collected in Bandounga (West Cameroon) in November 2017 and a voucher specimen was authenticated by comparison to specimen registered under the reference number HNC N°18646/HNC at the National Herbarium of Cameroon.

Aqueous extract preparation: The stem barks of *M. indica* were cleaned with water to remove adhering dirt, air-dried for two weeks and then ground to a fine powder using an electric blender. The extraction was carried out by maceration of 1000 g of powder with 5 L of distilled water for 72 h, with constant stirring. The resultant mixture was filtered using Whatman filter paper (No.1) and the filtrate was concentrated in an oven at 45°C. This extract was dissolved in distilled water upon administration.

Animal material

Artemia salina: Brine shrimp (*Artemia salina)* larvae were used as animals' indicator for the preliminary cytotoxicity test. Brine shrimp (*Artemia salina*) eggs obtained locally were hatched in artificial salt water. These eggs were incubated for 24 h in a hatching tank with water. These hatching conditions correspond to the natural environment (shallow salt water) [5]. After an average day from hatching, the shrimp larvae were used for the experimental bioassay. At this time the larvae still live on their own yolk sac and received no further food during the experimental time. Live larvae have a high mobility [5]. Nauplii were collected with a Pasteur pipette and kept for an additional 24 h under the same conditions to reach the metanauplii stage.

Rats: Young male and female Wistar albino rats weighing 150-200g were used in this study. These animals were raised in the animal house of the Laboratory of Animal Physiology and Phytopharmacology of the University of Dschang-Cameroon, under standard natural conditions and had free access to water and food. All experimental procedures used in the present study followed the "Principles of Laboratory Animal Care" from NIH publication Nos. 85-23 and were approved by the ethic committee of the Cameroon Ministry of Scientific Research and Technology which has adopted the guidelines established by the European Union on Animal Care and Experimentation (EEC Council 86/609).

Cytotoxicity assay: The sample to be assayed was dissolved in artificial sea water. Five milliliters (5 mL) of test solution with different concentrations 10, 100 and 1000 µg/ml of AEMI were taken in five different beakers of 25 mL for each sample. About 70 Nauplii were added to each set of beakers containing the samples. Control containing distilled water in artificial sea water was included in each experimental set. Twenty-four hours later, the number of survivors was counted, recorded and the mean lethal concentration (LC_{50} value) was calculated [6,7].

Mortality (%) = (Number of dead Nauplii/ Initial number of live Nauplii) x 100

Acute toxicity study: The acute toxicity test was carried out following the method described by the Organisation for Economic Co-operation and Development (OECD) guideline 425 [8] with small modifications. Sixteen (16) rats of both sexes were deprived of food for 24 hours prior to extract administration and randomly divided into two groups of 8 animals each. Group 1 was considered as control group and received only distilled water (10 mL/kg) by gavage. Group 2 orally received a single dose of 5000 mg/kg (bw) of AEMI. The general behavior of the animals was monitored for 3h after treatment; after this period, the animals received food and water *ad libitum*. The number of

dead animals in each group was noticed within 72h following the administration of the extract. The surviving animals were monitored daily for 14 days. During this period, body weight, food and water consumptions were recorded. The mean lethal dose (LD_{50}) was also estimated.

Sub-chronic toxicity: Thirty-two (32) albinos' rats of both sexes were used. They were divided into four groups of 8 animals each. Group 1 was considered as control and orally received distilled water. The tests group (2, 3 and 4) received the plant extract at doses of 300, 400 and 500 mg/kg bw respectively once a day, for 28 consecutive days. During this period, body weight, food and water consumptions were recorded each week.

Sample collection: On the 28th day of experiment, animals were subjected to overnight fasting during which their urine was collected (the rats were individually placed in metabolic cage for 24h to collect urine). After this, animals were anaesthesia by intraperitoneal injection of diazepan (10 mg/kg) and ketamine (50 mg/kg). The blood samples were collected by catheterization of abdominal artery into ethylen diamine tetra acetic acid (EDTA) tubes for hematological analysis and in the tubes without anticoagulant for biochemical analysis. Animals were then dissected. After macroscopic pathological examinations, the organs (kidney, liver, lung, spleen, stomach and heart) were weighed and some of them were kept for histopathological study.

Hematological analysis: Hematological parameters including red blood cells (RBC), haemoglobin (Hgb), hematocrit (HTC), mean globular volume (MGV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelets (PLT), concentration corpuscular tenor (CPT), mean platelets volume (MPV), white blood cells (WBC), monocytes (MO), granulocytes (GR) and lymphocytes (LYM), were determined using fully automated hematology analyser (PCE-210N).

Biochemical analysis: Blood samples collected in dry tubes were centrifuged at 3000 rpm for 15 min. The serum was separated and stored at -20 °C for the determination of aspartate amino transferase (AST) activity, alanine amino transferase (ALT) activity, total protein (TP), total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) and creatinine using commercial kits (SIGMA GmbH, Germany). Urine was collected in dry tubes and was used for creatinine assay using the same commercial kits. The urine volume and renal clearance of creatinine were determined for each dose of extract.

Histopathological study: The animals were sacrificed after blood collection and small pieces of stomach, kidneys and liver were collected, weighted and stored in 10% formaldehyde solution for histopathological study. The fixed organs were mascroscopically sectioned, dehydrated by serial increasing degree of ethanol (80°, 95°, and 100°) solution and embedded in paraffin for histological analysis. The micrometer sections were cut by a microtome in 3–5 µm slices and stained with haematoxylin and eosin. Slides were examined under a light microscope (100x and 200x). Any alterations compared to the normal structures were registered.

Statistical analysis: The values were expressed as mean ± standard error of the mean (SEM). The statistical analysis of data was carried out using one-way ANOVA followed by Turkey's or a two way ANOVA followed by Bonferroni tests using Graph Pad Prism Version 5.03. The p values $p< 0.05$ were considered significant.

RESULTS

Cytotoxicity: In cytotoxicity study all doses of extract resulted in mortality of less than 50%. The LC_{50} was 4168 µg/mL (Table 1).

Acute oral toxicity: No mortality and no behavioral changes were recorded in rats during the 14 days of observation after administration of a single oral dose of 5000 mg/kg bw of AEMI. Furthermore, no significant change in body weight (Figure 1), food intake (Figure 2) and water consumption (Figure 3) was recorded in the treated animals compared to the control group.

Sub-chronic toxicity: No deaths and no significant changes in general behavior were observed during the treatment period neither in the control group nor in the treated groups.

Effects of AEMI on body weight, food intake, water consumption and relative organ weight: Daily administration of AEMI at doses of 300, 400 and 500 mg/kg for 28 days did not cause a significant ($p\geq0.05$) variation in body weight, food intake and relative organ weight of treated rats compared to control rats (Figures 4, 5, 7). However, the dose of 400 mg/kg bw significantly ($p<0.05$) reduced the water consumption of rats at the first week of treatment (Figure 6).

Effects of AEMI on hematological parameters and biochemical parameters: Table 2 shows the effects of sub-chronic oral administration of AEMI on hematological parameters assessed on Wistar rats. It appears from this table that, the extract did not cause significant variation in hematological

parameters, except of platelets count (PLT) which significantly ($p<0.05$) increased in rats treated at dose of 500 mg/kg compared to the control group.

Effects of AEMI on biochemical parameters: AEMI did not cause any significant ($p\geq0.05$) variation in serum ALT activity, total bilirubin, conjugated bilirubin, total protein, urinary creatinine and renal clearance. However, serum AST activity, TC, TG and LDL cholesterol levels were significantly ($p<0.05$ to $p<0.001$ respectively) decreased, while HDL cholesterol, creatinine and urinary volume were significantly ($p<0.01$) increased in treated rats compared to control (Table 3).

Histopathology analysis: Histopathological analysis revealed no abnormalities in the structure and morphology of the stomach in all treated groups as compared to the control (Figure 8). However, the AEMI at 500 mg/kg caused vascular congestion of portal vein, capillary sinusoid dilation, peri-vacsular portal vein fibrose on the livers (Figure 9 D) and infiltration of inflammatory cells on the kidney as compared to the control (Figure 10 D).

DISCUSSION

The evaluation of the toxic action of plant extracts is indispensable in order to consider a treatment safe; it helps define the intrinsic toxicity of the plant and the effects of an acute overdose [9]. The brine shrimp lethality test was considered as a convenient probe for primary toxicity assessment, detection of fungal toxins, heavy metals, and pesticides, and cytotoxicity testing of dental materials (10]. Brine shrimp bioassay is considered as a rapid preliminary screening for the presence or absence of bioactivity and also is used to determine the cytotoxicity of crude extracts [11]. Extracts from natural products whose LC_{50} value is less than 100 µg/ml are considered to have toxic effects; also extract is generally considered as non-toxic if its LC_{50} value is greater than 100 µg/mL in the brine shrimp lethality assay [5,12]. The AEMI was assessed for its cytotoxicity using a sensitive *in vitro* brine shrimp lethality bioassay. LC_{50} value of the AEMI was greater than100 µg/mL; therefore this extract can be considered as non-toxic.

Although significant progress have been made in the development and application of *in vitro* toxicity assays, *in vivo* safety evaluation remains the most useful tool for identifying target organ toxicity [13]. The rat has been the specie of choice for the vast majority of preclinical toxicology studies performed in the evaluation of pharmaceutical candidates [14]. Therefore, the rats were use for *in vivo* study.

The oral administration of a single dose of AEMI (5000 mg/kg) in acute toxicity study induced no mortality. There are various studies on LD_{50} determination of plant extract which report that substances with LD_{50} higher than 5000 mg/kg via oral route may be considered practically non-toxic and safe [8,15,16]. Therefore, the observations from acute toxicity studies suggest that AEMI is relatively non toxic.

Sub-chronic toxicity treatment showed that 28 days administration of AEMI did not produce any death. There was no significant variation in body weight and food intake of treated rats compared to control rats. Body weight and relative organ mass are used in toxicity study as important indicators to assess the response therapy and adverse effects of drugs and the physio-pathological status of animals [17]. The liver, stomach and kidneys are the most affected organs after drugs consumption and absorption. From this study, no significant variation in body weight and relative organs mass was obtained in all treated animals compared to control. These results showed that AEMI is devoid of toxic effects within the administrated doses. However, water consumption significantly decreased in animals treated with AEMI at 400 mg/kg the first week of treatment. It is known that, the reduction of water consumption is due to the decrease of the activity of hypothalamic osmorecptors which are known to regulate thirst [18]. These results showed that, the repeated administration of AEMI at 400 mg/kg would inhibit the hypothalamic osmoreceptors activity of thirst, which conducted to the reduction of water consumption.

Assessement of blood parameters in animal studies is relevant to evaluate the risk of alterations of the hematopoietic system in toxicity studies, for necessary application to humans [19]. In this study, the hematological profile of treated rats showed no significant difference with control group, except for the platelets which significantly increased in the group treated with AEMI at dose of 500 mg/kg. It is well known that platelets play an important role in blood coagulation. This result therefore indicates that the extract could promote the coagulation process.

Administration of the EAMI did not cause any significant changes in the ALT activity, total bilirubin, conjugated bilirubin, total protein. However at 500 mg/kg, it caused a significant decrease in the AST activity. ALT and AST are considered marker for liver function [20]. The reduction of AST activity could suggest possible hepatoprotective effects of extracts. This hypothesis was not corroborated with histolopathological analysis which showed

alteration in the liver. The high level of cholesterol particularly LDL-cholesterol, are mainly responsible for hypercholesterolemia, a risk factor for cardiovascular diseases such as atherosclerosis and myocardial infarction, which are common causes of mortality and morbidity [21]. In this study, a significant increase in HDL-cholesterol levels and a reduction in TC, TG and LDL-cholesterol levels were observed in the treated animals, suggesting that the extract may have hypolipidemic effects.

Kidneys function was assessed by determining serum and urine creatinine. Creatinine is chemical substance produced by muscle metabolism and transported through the bloodstream to the kidneys for excretion [22]. Its level increases in the serum when the cortex and/or glomeruli are damaged [23]. In this study, AEMI did not affected urine creatinine and clearance in rats while serum creatinine levels increased at 500 mg/kg bw. These results could indicate a probable nephrotoxic effect of AEMI at high doses. This hypothesis was confirmed by histopathological analysis of the kidneys which showed inflammation with infiltration of leucocytes in rats treated at dose of 500 mg/kg.

Histopathological analysis revealed no abnormalities in the structure and morphology of the stomach in all treated groups. The structure of hepatic tissue of rats treated at dose of 500 mg/kg revealed vascular congestion of portal vein, capillary sinusoid dilation, peri-vacsular portal vein fibrose. Signs of liver damage are usually manifest as a result of architectural disarray, vascular congestion, hepatocytes necrosis, apoptosis, or inflammatory cell infiltration in either acute or chronic conditions [24]. The architectural appearance of the kidneys from the rats in the control group, presented a normal histological appearance, as against the kidneys from the rats in the test groups, which presented cells inflammation. Any damages of the kidney caused either by chemical agents, drugs or plant extract could be manifested as vascular congestion (glomerulus), inflammatory cell infiltration and hyaline globule in collecting tubules [25]. All these features were clearly observed in rats treated with extracts at dose of 500 mg/kg. These results suggest that AEMI could cause damages to liver and kidney at high doses.

CONCLUSION

These finding of toxicological profile of aqueous extract of *Mangifera indica* stem barks showed that, AEMI was practically nontoxic in rats after acute toxicity study. However, this plant extract may have deleterious effects on the liver and kidney at high doses following long term administration. This confirms its safe ethno medicinal use at low doses.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this article.

Table 1: cytotoxic effect of AEMI on the viability of brine shrimp nauplii.

Extract	Concentration (µg/mL)	% of mortality	LC_{50}
AEMI	10	22.85	4168 µg/mL
	100	25.71	
	1000	45.71	

All values were the mean of three replicates.

Table 2: Hematological parameters of rats in sub-chronic toxicity of AEMI

Parameters	**Control**	**AEMI (mg/kg)**		
		300	**400**	**500**
RBC (x10^{6}cellules/µL)	5,12 ± 7,85	4,00 ± 1,04	5,33 ± 1,05	7,01 ± 5,39
Hgb (g/dL)	11,13 ± 1,33	12,43 ± 1,09	12,45 ± 4,54	13,78 ± 0,72
HTC (%)	27,63 ± 3,22	29,56 ± 2,64	30,19 ± 3,38	33,88 ± 2,01
MGV (fl)	56,24 ± 2,31	52,94 ± 1,47	55,68 ± 2,60	49,18 ± 1,58
MCH (p/L)	22,59 ± 0,93	22,29 ± 0,86	23,31± 1,55	20,14 ± 0,91
MCHC (g/dL)	37,21 ± 2,80	42,08 ± 0,81	41,70± 1,10	36,34 ± 4,54
PLT (x 10^3 cellules/µL)	309,18 ±57,84	437,50 ± 20,29	440,37 ± 27,21	469,50 ± 25,36*
CPT (%)	0,23 ± 0,02	0,27 ± 0,01	0,28 ± 0,01	0,29 ± 0,01
MPV (fl)	6,37 ± 0,13	6,25 ± 0,10	6,67 ± 0,23	6,20 ± 0,08

WBC (x 10^3 cellules/µl)	6,46 ± 0,39	3,57 ± 0,58	2,17 ± 0,34	3,34 ± 0,53
MO (x 10^3 cellules/µL)	0,32 ± 0,15	0,53 ± 0,24	0,16 ± 0,13	0,32 ± 0,21
GR (x 10^3 cellules/µL)	0,41 ± 0,18	0,56 ± 0,26	0,20 ± 0,12	0,47 ± 0,27
LYM (x 10^3 cellules/µl)	0,18 ± 0,38	0,24± 0,17	0,18 ± 0,29	0,25 ± 0,34

Values are mean ± ESM of 8 animals. *p < 0,05 : significantly different from the control.
RBC: red blood cells, Hgb: haemoglobin, HTC: hematocrit, MGV: mean globular volume, MCH:mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, PLT: platelets, CPT: concentration corpuscular tenor, MPV: mean plateletsvolume, WBC:white blood cells, MO: monocytes, GR: granulocytes, LYM : lymphocytes.

Table 3: Biochemical parameters of rats in sub-chronic toxicity of AEMI

Parameters	**Control**	**AEMI (mg/kg)**		
		300	**400**	**500**
ALT (U/L)	42.06 ± 2.20	41.59 ± 5.16	44.37 ± 4.76	48.52 ± 5.29
AST (U/L)	141.5 ± 6.3	140.6 ± 8.5	131.3 ± 6.8	77.95 ± 7.02***
Total bilirubin (mg/dL)	0.056 ± 0.015	0.054 ± 0.009	0.033 ± 0.009	0.034± 0.012
Conjugated bilirubin (mg/gL)	0.024 ± 0.007	0.023 ± 0.003	0.018 ± 0.003	0.020 ± 0.007
Total protein (g/dL)	70.08 ± 7.46	52.00 ± 6.58	49.92 ± 8.56	55.68 ± 6.85
Total cholesterol (mg/dL)	200.00 ± 48.79	49.99 ± 9.62**	44.44 ± 9.39**	46.66 ± 6.45**
HDL cholesterol (mg/dL)	25.05 ± 2.50	31.32 ± 2.26	37.18 ± 1.55**	36.21 ± 1.87**
LDL cholesterol (mg/dL)	305.6 ± 7.34	242.3 ± 18.71*	66.60 ± 10.5***	179.5 ± 19.39***
Triglycerides (mg/dL)	539.1 ± 43.60	430.3 ± 50.83	184.3 ± 20.65***	406.00 ± 33.43
Urinary creatinine (mg/dL)	58.74 ± 5.08	42.62 ± 1.98	45.50 ± 5.09	54.21 ± 7.03
Serum creatinine (mg/dL)	0.21 ± 0.04	0.14 ± 0.01	0.37 ± 0.06	0.57 ± 0.09*
Urinary volume (mL/100g)	3.60 ± 0.56	5.47 ± 0.71	5.00 ± 0.68	7.50 ± 0.77**
Renal clearance (mL/min)	0.61 ± 0.16	0.91 ± 0.18	0.57 ± 0.25	0.51 ± 0.11

Values are mean ± ESM of 8 animals. *p < 0,05 ; **p < 0,01 ; ***p < 0,001 : significantly different from the control.

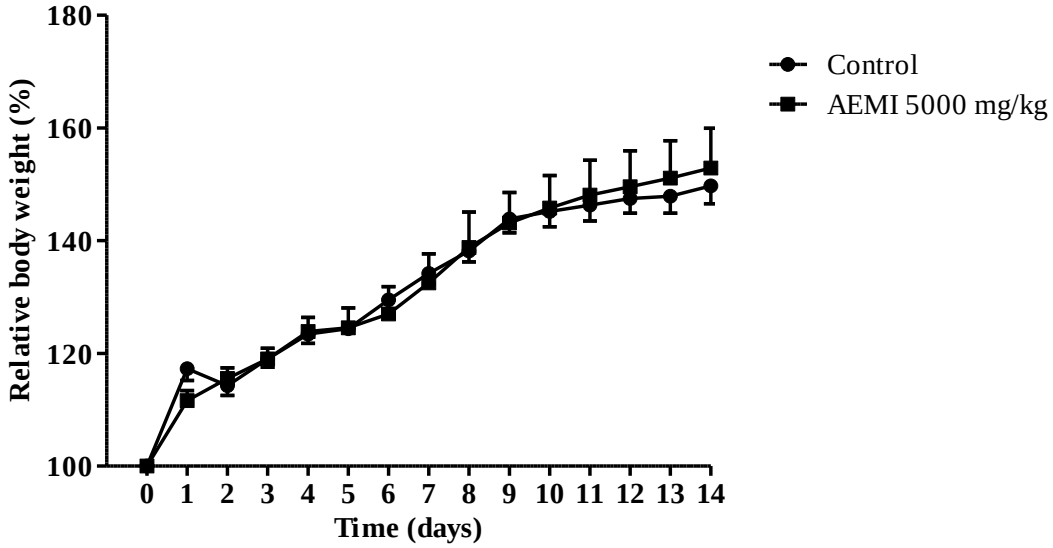

Figure 1: Body weight evolution of rats in acute toxicity study of aqeuous extract of *Mangifera indica*. n=8.

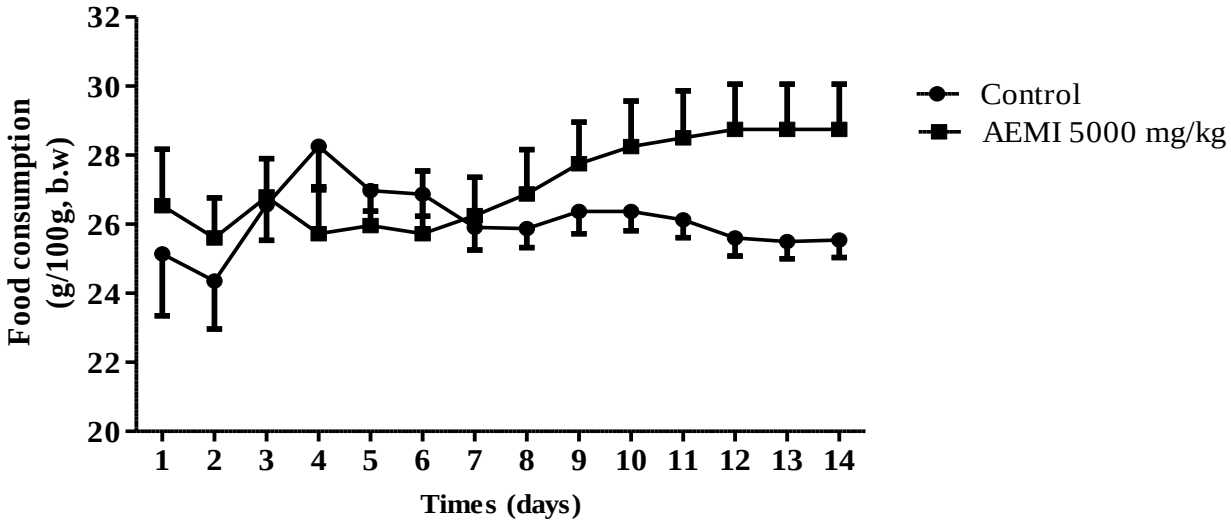

Figure 2: Food consumption of rats in acute toxicity study of aqueous extract of *Mangifera indica*. n=8

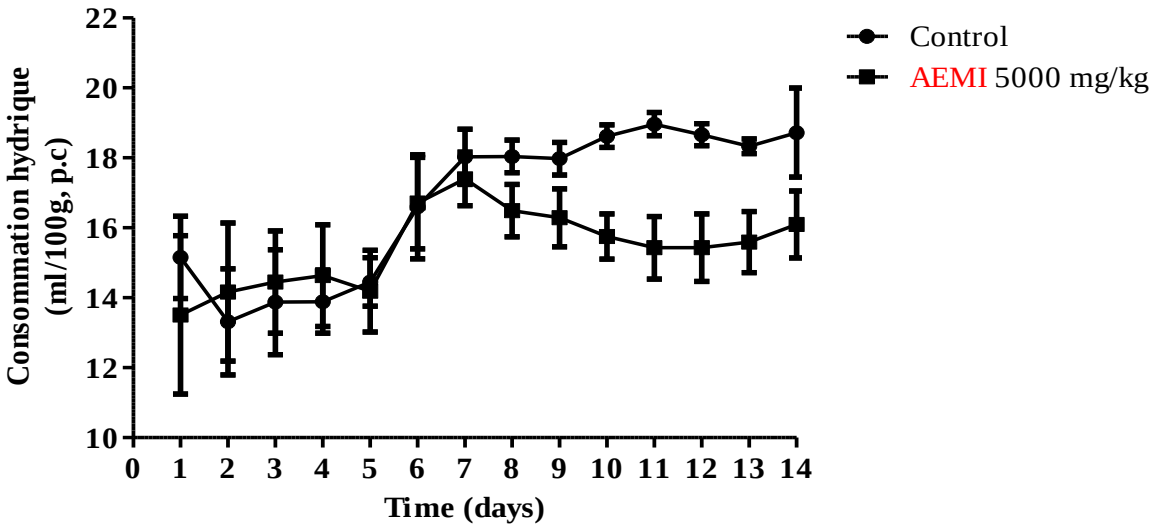

Figure 3: Water consumption of rats in acute toxicity study of aqueous extract of *Manaifera indica*. n=8

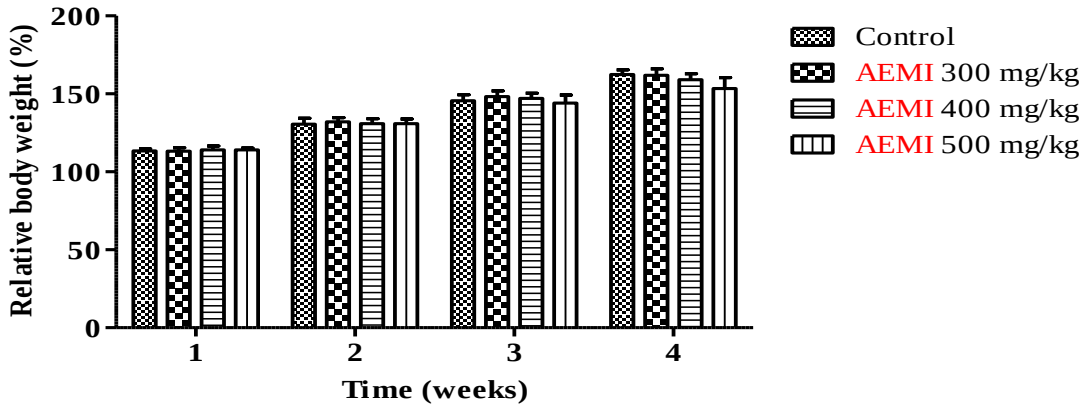

Figure 4: Body weight evolution of rats in subchronic toxicity study of aqueous extract *Mangifera indica*. n=8

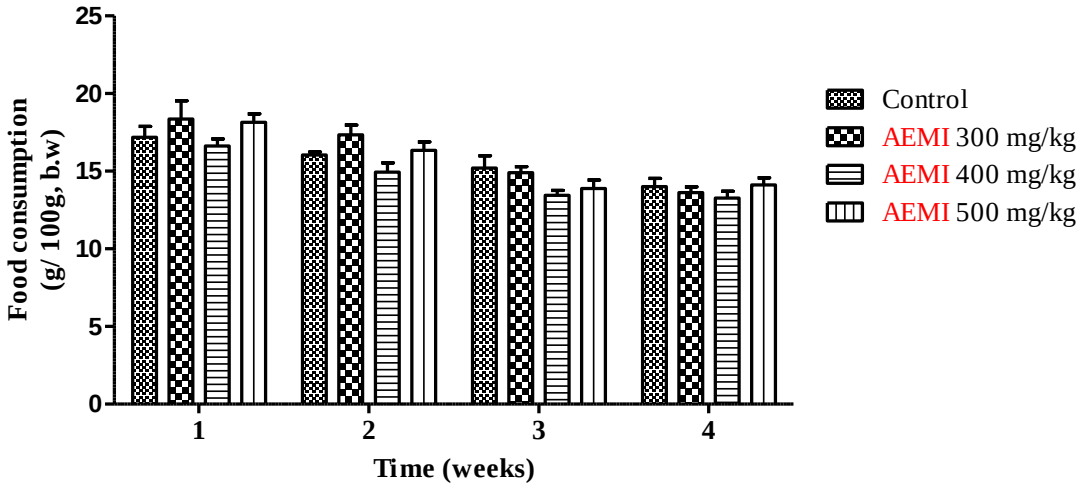

Figure 5: Food consumption evolution of rats in sub-chronic toxicity study of aqueous extract of *Mangifera indica*. n=8

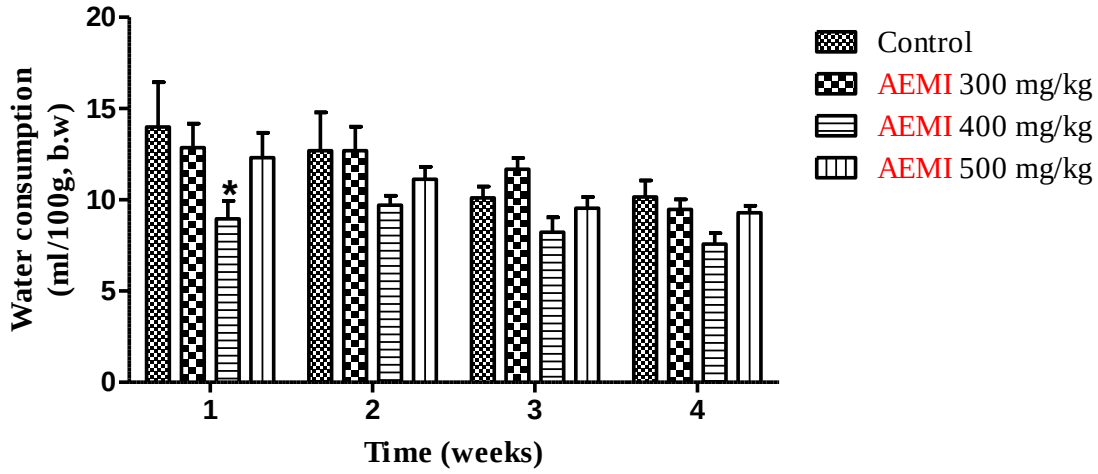

Figure 6: Water consumption evolution of rats in sub-chronic toxicity study of aqueous extract of *Mangifera indica*. n=8.*p<0.05 significantly differend compared to control.

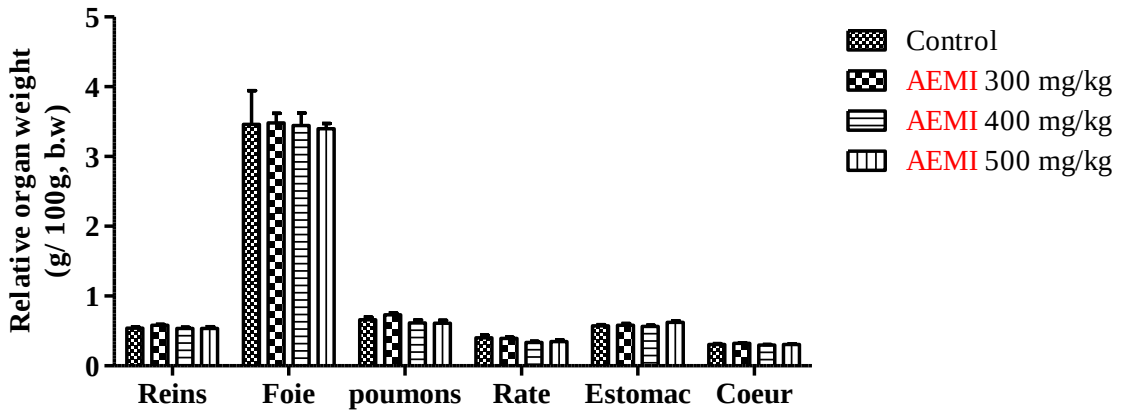

Figure 7: Effects of aqueous extract of *Mangifera indica* on relative organ weight of rats after 4 weeks of sub-chronic toxicity study. n=8

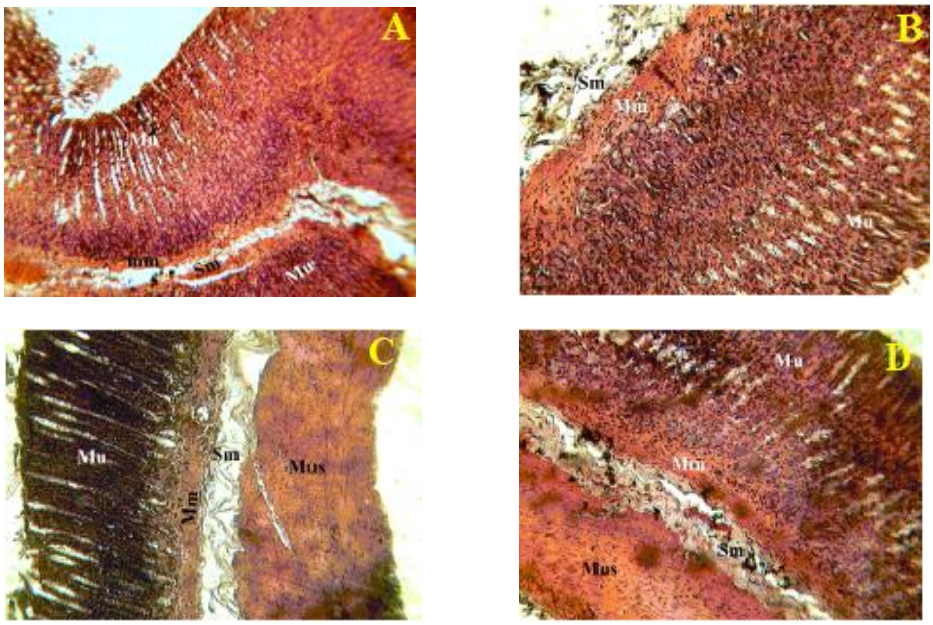

Figure 8: Histology of stomach sections following 28 days treatment (200X). Mu-Mucosae, Sm- Submucosa, Mm- Muscularis mucosae, Mus - Muscularis. No change in all the treated groups compared to control. A - Control, B- Rats treated with AEMI at 300 mg/kg bw, C - Rats treated with AEMI at 400 mg/kg bw, D - Rats treated with AEMI at 500 mg/kg bw.

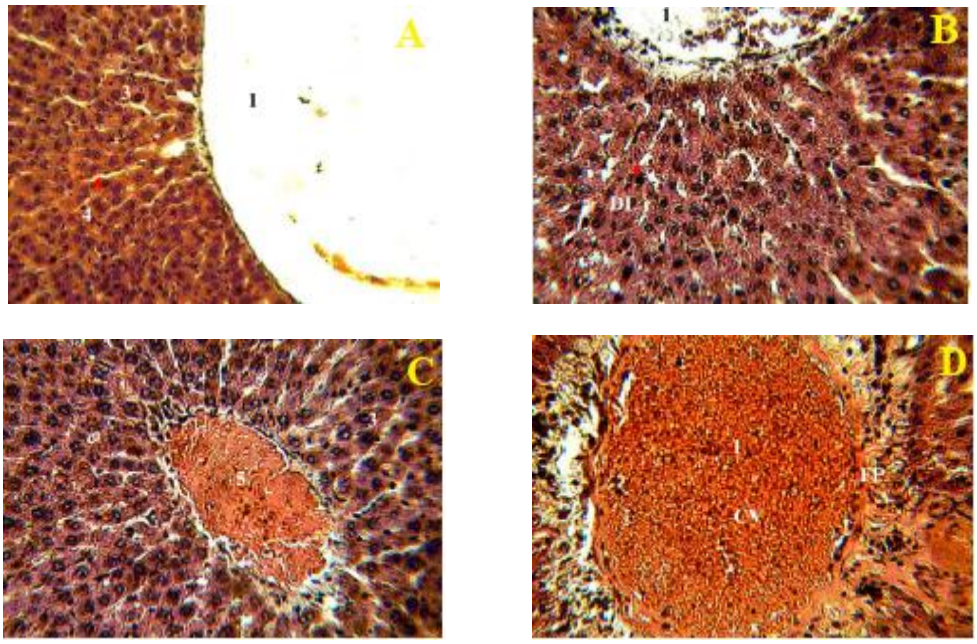

Figure 9: Histology of liver sections following à 28-day treatment (200X). 1- Hepatic portal vein, 3- hepathocyte, 4- Sinusoid capillary, 5- centro-lobular vein, CV : vascular congestion, DL : sinusoid capillary dilation, FP : peri-vascular fibrose. Sinusoid capillary dilation at 300 mg/kg, vascular congestion and peri-vascular fibrose at 500 mg/kg. A- Control, B- Rats treated with AEMI at 300 mg/kg bw, C- Rats treated with AEMI at 400 mg/kg bw, D- Rats treated with AEMI at 500 mg/kg bw.

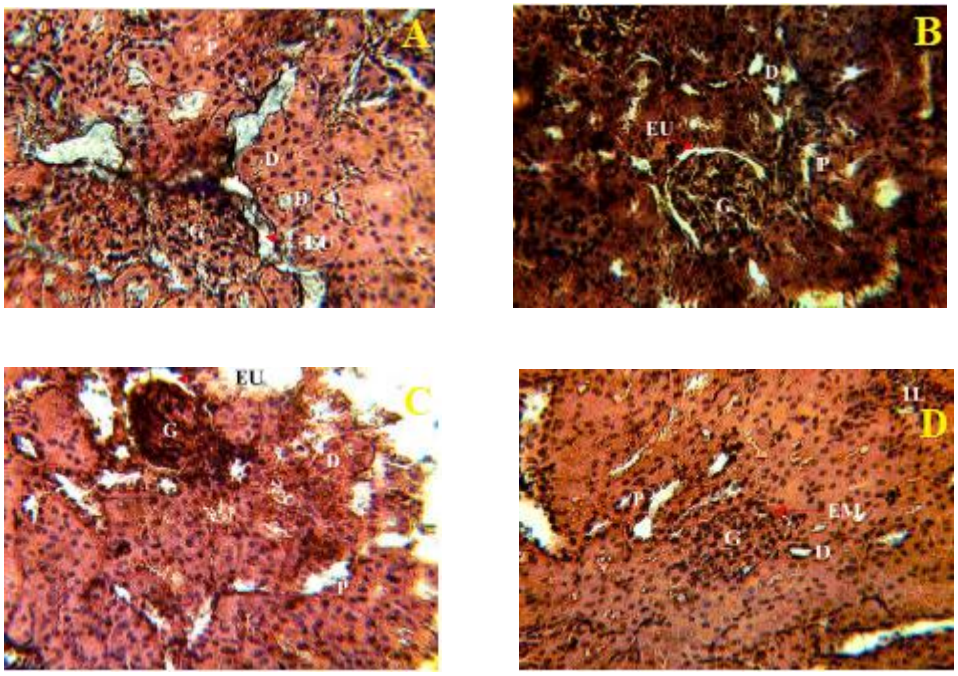

Figure 10: Histology of kidney sections following à 28-day treatment (200X). IL : leucocytes infiltration, G : glomerul, EM : mesangial expansion, EU : urinary space, D : distal tube, P : proximal tube. Leucocytes infiltration at 500 mg/kg. A- Control, B- Rats treated with AEMI at 300 mg/kg bw, C- Rats treated with AEMI at 400 mg/kg bw, D- Rats treated with AEMI at 500 mg/kg bw.

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