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HAEMATOLOGICAL, BIOCHEMICAL AND HISTOLOGICAL TOXICITY OF THE ETHANOLIC EXTRACT OF MASSULARIA ACUMINATA (G. DON) BULLOCK EX HOLY. (RUBIACEAE) STEMS IN RAT

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ABSTRACT

Massularia acuminata is a medicinal plant used in the treatment of various ailments and particularly as an aphrodisiac. In order to verify its safety, the ethanolic extract of its stems was administered orally daily for 28 days to three groups of Wistar albino rats at 40, 80 and 160 mg/kg body weight. The control group was given distilled water. Blood samples were taken at the end of the experiments to determine the effect of the extract on haematological and biochemical parameters. After the rats were killed, the liver, kidneys and heart were removed and preserved in 10% formaldehyde for histological sections. This study showed that the repeated administration of the ethanolic extract of the stems of *Massularia acuminata* did not cause any significant disturbance of the red blood cell and haemoglobin levels compared to the control batch. However, white blood cell and platelet levels were altered in the treated groups compared to the control group. Biochemically, the ethanolic extract of *M. acuminata* stems did not alter the enzymatic activity of Sgpt and Sgot transaminases, as well as creatinine and urea. Histologically, the relative masses of the liver, kidney and heart of the treated groups were not altered compared to the control group. No lesions were observed in kidney and heart tissue in any of the experimental groups. In contrast, apoptosis was only observed in 30% of the rats in the batch treated with the extract at 160 mg/kg body weight. In sum, this study

suggests that the ethanolic extract of the stems of *Massularia acuminata* would not show harmful effects at doses of 40 and 80 mg/kg body weight in rats during subacute administration.

KEYWORDS: *Massularia acuminata,* Rats, Haematology, Blood biochemistry, Histology.

INTRODUCTION

The use of herbal medicines has been gaining momentum around the world over the last two decades as they have a good reputation among the population. It is estimated that more than 80% of the population in these countries rely on traditional medicine for their primary health care needs.[1-2] Furthermore, it is estimated that more than 50% of the 252 medicines considered essential are of plant origin.[3] In Africa, ethnobotanical studies have identified more than 5,000 medicinal species[4-5] including 761 medicinal species and 1,421 medicinal recipes in Côte d'Ivoire.[6] Among these medicinal plants is *Massularia acuminata*. The species *Massularia acuminata* (G. Don) Bullock ex Hoyl, belonging to the Rubiaceae family, is a plant used in traditional African medicine for various indications, including hernia, sterility, ovarian disorders, fevers, difficult childbirth, rheumatic pains, oral infections, kidney ailments, haematuria, poison, dysentery, coughing and especially sexual weakness.[7-8-9-10-11]

In Côte d'Ivoire, a recent pharmacological study conducted on the ethanolic extract of the stems of this plant indicated a very significant increase in the number of sexual mounts, the number of erections, the number of ejaculations and a significant decrease in the latency time between two consecutive sexual mounts in male albino Wistar rats treated with doses of 20 and 40 mg / kg body weight.[12] Despite all the benefits of its use, few scientific studies have demonstrated its safety with repeated use.

It is in this context that the present study aimed to evaluate the haematological, biochemical and histological toxicological profile of the ethanolic extract of the stems of *Massularia acuminata* (G. Don) Bullock ex Holy. (Rubiaceae) in the albino wistar rat.

MATERIALS

Plant material

The plant material collected in Bonoua, in the department of Grand- Bassam in the South-Comoé region (Côte d'Ivoire), in November 2020. The plant was identified at the National

Floristic Centre of the Félix Houphouët Boigny University where a specimen was deposited and identification number (UCJ15291) was collected.

Animals

For this study, male rats *Rattus norgevicus*, aged eight weeks, weighing an average 125 ± 23.16 g were used for the experiment. The rats were bred in the animal house of the Physiology, Pharmacology and Pharmacopoeia Laboratory of the Research Unit of Nangui Abrogoua University. A total of 24 rats were divided into four groups animals were subjected to a temperature of 25 ± 2°C and to an alternation of 12 h of light and 12 h of darkness. The diet consisted of IVOGRAIN® pellets and the rats were provided with tap water. The experimental protocol and animal handling procedures were conducted according to good laboratory practice.[13]

METHODES

Preparation of ethanolic extract

For the preparation of the extract, two hundred grams (200 g) of powder obtained from the stem of *M. acuminata* were macerated in 2.5 L of 96% (v/v) ethanol for 24 h under continuous stirring. The resulting macerate was filtered and then concentrated under reduced pressure at 40°C using a rotary evaporator. The concentrated filtrate was dried in an oven at 40°C. The dry extract obtained constituted the ethanolic extract and was kept for the experiments.[12]

Toxicity assessment

The subacute toxicity study was determined using OECD guideline 407 which involved daily oral administration of extracts in increasing doses to four batches of animals, one dose per batch for 28 days.[14] Twenty-four (24) rats were randomly divided into four groups of 6 rats, three test groups and one control group. Each group contained an equal number of female and male rats. Groups 2, 3 and 4 were given 40, 80 and 160 mg/kg body weight, respectively. Group 1 (control) received distilled water. Prior to the administration of the extracts, the animals in each group were individually marked and weighed. They received a volume of solution of 2 ml/100 g body weight once a day by gavage through a cannula for 28 days. The animals were observed individually every morning between 7 and 8 o'clock for 28 days. The influence of the different doses administered was assessed on the basis of haematological, blood biochemical and histological data. At the end of the 28 days of experimentation, all the

animals were subjected to a histological study on the liver, kidneys and heart preserved in 10% formalin. The method used was the paraffin embedding technique.[15]

Statistical processing

The statistical study was carried out using Xlstat-Pro 7.1 statistical analysis software. The results were analysed using Tukey's and Dunnett's post hoc tests combined with a one-factor Anova. Values are given as the mean followed by the standard error and on the mean.

For the study of the relative mass of the organs, we used the following ratio.[16]

$$\text{Relative weight} = \frac{\text{absolute mass of the body (g)}}{\text{body mass of the animal on the day of sacrifice (g)}} \times 100$$

These tests give us the degree of significance for $p < 0.05$.

RESULTS

Effects of the extract on haematological parameters

Daily administration of the ethanolic extract of *M. acuminata* stems resulted in a significant increase in the white blood cell count in the treated groups compared to the control group (Table 1). Red blood cell counts and haemoglobin levels were not significantly different in rats treated at 40, 80 and 160 mg/kg body weight compared to the control (Table 1). In contrast, a significant decrease in blood platelet levels was observed in the groups treated with ethanolic extract of *M. acuminata* stems at 40 and 160 mg/kg (Table 1).

Table 1: Effect of ethanolic extract on haematological parameters in rats.

Group	White blood cells (10^3 cells/µL)	Red blood cells (10^6 cells/µL)	Hemoglobin (g/dL)	Blood platelets (10^3 cells/µL)
Group 1 (Control)	4.72 ± 1.25	7.55 ± 0.50	12.68 ± 2.53	832.16 ± 458.99
Group 2 (40 mg/kg)	6.27 ± 1.88[a]	7.35 ± 1.19	13.08 ± 1.45	699.00* ± 275.82
Group 3 (80 mg/kg)	6.83 ± 2.12[a]	6.65 ± 0.82	13.96 ± 1.16	1133.67[a] ± 300.94
Group 4 (160 mg/kg)	5.73 ± 2.31[a]	7.62 ± 0.91	13.81 ± 1.28	733.00* ± 363.65

** : significant decrease ($p<0.05$) compared to the control group; a : significant increase ($p<0.05$) compared to the control group*

Effects of the extract on some biochemical blood parameters

The mean values of Sgot and Sgpt transaminases did not change significantly in the groups treated with ethanolic extract of *M. acuminata* stems compared to the control group (Table 2).

Administration of the ethanolic extract did not affect serum creatinine and urea levels in the treated groups compared to the control group (Table 2).

Table 2: Effect of ethanolic extract on some biochemical blood parameters in rats.

Groups	Sgpt (UI/L)	Sgot (UI/L)	Creat (mg/dL)	Urea (g/L)
Group 1 (Control)	15.23 ± 2.76	22.86 ± 14.28	3.43 ± 2.61	0.83 ± 0.65
Group 2 (40 mg/kg)	16.06 ± 6.76	25.52 ± 17.14	3.47 ± 1.03	0.83 ± 0.27
Group 3 (80 mg/kg)	15.00 ± 5.52	20.03 ± 10.69	3.60 ± 1.20	0.87 ± 0.30
Group 4 (160 mg/kg)	12.66 ± 5.02	24.92 ± 14.28	3.63 ± 1.17	0.88 ± 0.31

Sgpt : serum glutamic pyruvic transaminase; Sgot : serum glutamic oxaloacetic transaminase; Creat: creatinine

Relative masses of liver, Kidney and Heart

The mean values of the relative masses of the liver, kidney and heart of the experimental groups were not significantly ($p > 0.05$) different from those of the control rats.

Table 3: Effect of ethanolic extract of *M. acuminata* stems on relative masses.

Groups	Relative weights of the organs		
	Liver	Kidneys	Heart
Group 1 (Control)	3.969 ± 1.021	0.669 ± 0.044	0.359 ± 0.014
Group 2 (40 mg/kg)	4.068 ± 0.771	0.713 ± 0.062	0.365 ± 0.027
Group 3 (80 mg/Kg)	3.405 ±0.264	0.769 ± 0.268	0.346 ± 0.050
Group 4 (160 mg/Kg)	3.449 ± 0.920	0.694 ± 0.070	0.378 ± 0.044

Effects of ethanolic extract of *Massularia acuminata* stems on liver, kidney and heart histology

Examination of liver tissue revealed the presence of apoptosis in rats in group 4 compared to groups 2, 3 and control, which showed no change in the structure of the liver tissue (Figures 1A, 1B). As for the kidney and heart tissues, exposure to the ethanolic extract of *M. acuminata* stems did not induce any lesion in the treated rats and controls (Figures 1C, 1D, 1E, 1F).

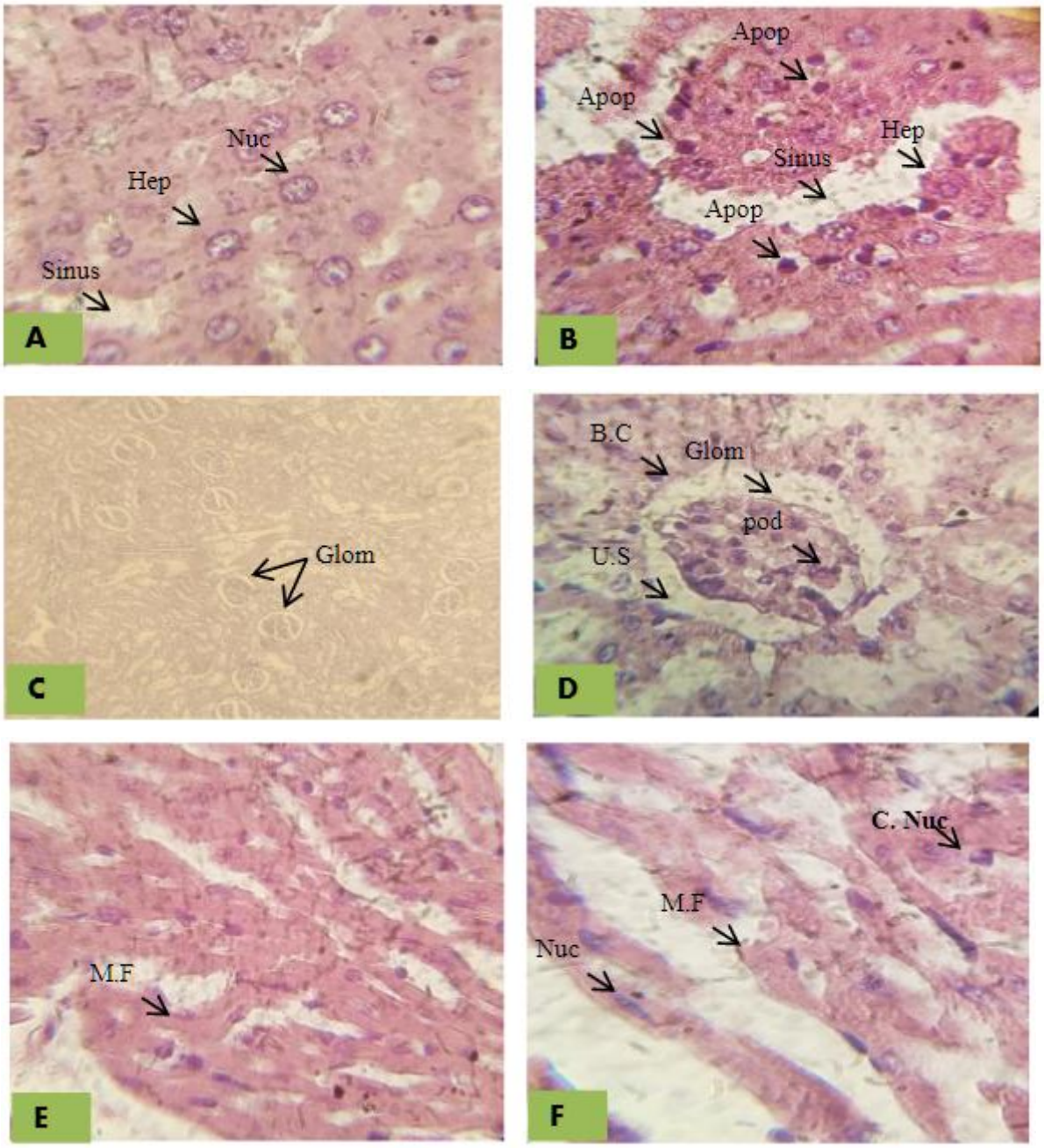

Figure 1: Microphotographs of liver, Kidney and Heart sections seen under the light microscope;

A and B: Microphotographs of liver sections from control rats (A) and rats treated with 160 mg/kg extract (B), zoom x 1000; Apop: Apoptosis; Hep: Hepatocyte; Sinus: Sinusoid; Nuc: Nucleus

C and D: Microphotographs of kidney sections from control rats (C) and rats treated with 160 mg/kg extract (D), zoom x 400 – x 1000; B. C: Bowman's capsule; Glom: Glomerula; Pod: Podocyte; U.S : Urinary space

E and F: Microphotographs of heart sections from control rats (E) and rats treated with 160 mg/kg extract (F), zoom x 1000; C.Nuc: Central nucleus; M.F: Muscle fibre; Coloration : Haematoxylin - eosin

DISCUSSION

The objective of this study was to evaluate the safety of the ethanolic extract of *M. acuminata* stems on haematological, blood biochemical and histological parameters in albino Wistar rats when administered orally daily for 28 days.

The results obtained showed a significant increase in the number of white blood cells in the treated groups compared to the control lot. Our results are similar to those obtained by Adeyemo-Salami and Ewuola[17] who observed an increase in white blood cell count in rats treated with methanolic extract of the leaves of *Paullinia pinnata* (Linn) (Sapindaceae) at doses ranging from 50 to 400 mg/kg body weight. Some authors have associated the increased leukocyte production with infection or oxidative stress or a toxic substance in the body.[18]

The results also revealed that the administration of the ethanolic extract of *M. acuminata* stems did not alter the red blood cell and haemoglobin levels. Our results show that the ethanolic extract of *M. acuminata* would not induce anaemia upon repeated administration. These results are in agreement with those of these authors[19] who state that oral administration of the hydroethanolic extract of the root barks of *Dichrostachys cinerea* (L.) Wight et Arn. (Fabaceae) does not alter red blood cell and haemoglobin levels. Effiong et al,[20] also reported that administration of ethanolic extract of the leaves of *Gongronema latifolium* Benth Hook at doses of 150 and 300 mg/kg body weight to rats for one month did not alter haemoglobin levels compared to controls.

Ethanolic extract of *M. acuminata* caused a decrease in blood platelets at 40 and 160 mg/kg bw on day 28 of the experiments in contrast to the control lot. According to some authors,[21] low platelet count is usually associated with the use of certain drugs which confuse the immune system and cause it to destroy platelets. The ethanolic extract of *M. acuminata* stems could therefore disturb the haemostatic profile during sub-acute administration at the doses studied. Our results are not similar to those obtained by Koné et al,[22] who rather observed a significant increase in blood platelet levels in rats treated with the aqueous extract of *Sacoglottis gabonensis* (Humiriaceae) barks at doses of 3.5, 35 and 350 mg/kg body weight.

Concerning the evaluation of the toxicity of the ethanolic extract of *M. acuminata* stems on some hepatic and renal blood markers, our results showed that the mean values of transaminases Sgpt and Sgot of the treated groups did not undergo a significant disturbance compared to the control group. These results are in agreement with those obtained by Gbogbo et al,[23] who did not record any disturbance of transaminases after administration in rats of a plant extract used as an aphrodisiac.

Akhtar et al,[24] further indicated that a significant increase in transaminases in the bloodstream would generally indicate liver damage, which may be reflected in intrahepatic bile duct obstruction, primary biliary cirrhosis or disorganisation of the liver architecture. Creatinine and urea are excellent markers of renal function, and their increase or decrease reflects renal dysfunction.[25] The results of our work showed that ethanolic extract did not disturb their serum levels.

Histologically, this study showed that the relative masses of the liver, kidney and heart of the treated groups showed no significant variation ($p > 0.05$) compared to the control. These results are similar to those obtained by Gbogbo et al,[26] who did not find a significant increase in relative liver and kidney mass after sub-acute administration of aqueous extract of *Spondias mombin* stem bark to rats at doses of 250, 500 and 1000 mg/kg body weight.

In contrast, histological sections of the liver showed apoptosis in rats treated with ethanolic extract of *M. acuminata* stems at 160 mg/kg body weight. While those of the kidney and heart showed no change. These results corroborate those obtained by these authors who observed liver cells in a state of apoptosis in rats treated with the aqueous extract of *Passiflora foetida* Linn. (Passifloraceae) at a dose of 1200 mg/kg body weight for 28 days.[27] According to some authors, several extra or intracellular, physiological or pathological signals can stimulate death by apoptosis, including DNA damage, oxidative stress generating free radicals[28] At high doses, the ethanolic extract of the stems of *M. acuminata* could cause damage to the DNA of hepatocytes or provoke oxidative stress generating free radicals.

CONCLUSION

The subacute toxicity evaluation of the ethanolic extract of the stems of *Massularia acuminata* (G. Don) Bullock ex Holy. (Rubiaceae) at doses of 40, 80 and 160 mg/kg body weight showed a significant increase in white blood cells and a significant decrease in blood platelets in Wistar rats compared to the control group. In contrast, red blood cell and

haemoglobin levels were not affected. Similarly, all biochemical blood parameters consisting of transaminases (Sgpt and Sgot), creatinine and urea were not altered in the treated rat groups compared to the control group. Regarding the histological evaluation, the ethanolic extract did not affect the relative mass of the liver, kidney and heart in all treated groups. In addition, no lesions were observed in the kidney and heart. However, apoptosis was observed in rats treated with the extract at 160 mg/kg body weight.

This study therefore indicates that repeated administration of the ethanolic extract of *M. acuminata* over a period of 28 days may result in haematological and hepatic toxicity.

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