INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com ISSN 2230 – 8407

Research Article

TOXICOLOGICAL STUDIES OF THE AQUEOUS EXTRACT FROM *BRILLANTAISIA* VOGELIANA (NÉES) BENTH. (ACANTHACEAE)

Aimé Valère SOH OUMBE[1]*, Mathieu NDOMOU[2], Amadou FEWOU[3], Anatole Constant PIEME[4], Jeanne NGOGANG YONKEU[4]

[1]*Department of Biochemistry, Faculty of Sciences University of Yaoundé I, P.O.Box 812 Yaoundé-Cameroon

[2]Department of Biochemistry, Faculty of Sciences University of Douala, P.O.Box 24157 Douala-Cameroon

[3]Anatomopathology laboratory service, Laquintinie Hospital Douala, P.O.Box 4035 Douala-Cameroon, Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, P.O.Box: 1634 Yaoundé-Cameroon

[4]Department of Physiological and Biochemical Sciences, Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, P.O.Box: 1634 Yaoundé-Cameroon

Article Received on: 03/05/12 Revised on: 20/06/12 Approved for publication: 11/07/12

ABSTRACT

Brillantaisia vogeliana (Acanthaceae) is used in folk medicine in the West region of Cameroon to manage obesity. In this study, we investigated the toxic effect of the aqueous extract of B.v. in rats.

Three doses of aqueous extract (250, 500, 1000 mg/kg) were administrated orally once per 2 days during a period of 28 days and different hematology, biochemistry and histopatology parameters were determined. The results showed that there were no mortality, no significant differences in the body and relative organ weight between control and treated animals, except for the kidney. Hematological analysis showed no significant difference in any of the parameters examined (WBC count, platelet, RBC count, hematocrit, total leucocyte count and hemoglobin estimation) between control and treated groups. There were also no significant change in blood chemistry parameters, including creatinine, urea nitrogen (UN), alkaline phosphatase, aspartate amino transferase (ASAT), calcium, alpha amylase, total protein and phosphorus, except alanine aminotransferase (ALAT) between control and treated groups. Histopathological abnormalities changes were detected in organs of animals treated with various doses of product.

Keywords: *Brillantaisia vogeliana*, acanthaceae, aqueous, extract, subacute, toxicity

INTRODUCTION

Use of plant for healing purposes is getting increasingly popular as they are believed as being beneficial and free of side effects. However, information about several available medicinal plants to the consumer do not have any scientific data supports. The use of medicinal herbs as phytomedicine is based simply on a traditional folk utilization that has been perpetuated along several generations (DACOSTA et al., 2000). An example of a very poorly studied medicinal plant is *Brillantaisia vogeliana*, a plant of the Acanthaceae family. It is usually a shrub about 1.5 m (5ft) with branches and green bark. It is widely spray in Tropical Africa and it has been found in Ghana, Nigeria, Sao-Thome, Gabon, Congo and Cameroon (HEINE, 1938). In Cameroon it is found in West region precisely in Bandjoun village, known as "*bùbù koup*".

This species have been used to facilitate deliverance and delivery (ADJANOHOUN *et al*., 1985), treat stomach ailment (BURKILL, 1985). In vivo studies showed that an aqueous extract of *Brillantaisia vogeliana* (Bv) has anti-hyperlipidemic activity. It reduces the risk of coronary disease in human by lowering serum triglycerides, total cholesterol and LDL cholesterol and increasing the level of HDL cholesterol and consequently it has weight loss properties (NOUBISSI, 2000). Its anti-diabetic property has been also demonstrated (KAMGANG *et al*., 2005). However no studies have so far been performed on the toxicological potential of BV to human. Due to the importance of this plant, the present study aimed to determine the subacute oral toxicity of Bv leaves extract.

MATERIAL AND METHODS

Preparation of extract: Leaves of *Brillantaisia vogeliana (Bv)* were kindly collected from Pr. NGOGANG's garden at Emana quarter of Yaoundé Cameroon. The plant has been already identified and authentified in the National *Herbarium*. The collected material was washed and dried for a week at the room temperature and then pulverized. Powder of *B. vogeliana (50 g)* was prepared in 500 ml of boiled water. The mixture was cooled at the room temperature, filtered and dried at 40°C in an oven (Air concept FRILABO; model AC 60) until dryness. The crude extract obtain was stored in the fridge (Sharp corporation; model Nice Crystal Gold) at 4°C for future use.

Animals and animals' husbandry: All animals used in this study were purchased from the Faculty of Medicine and Biomedical Sciences of the University of Yaounde I-Cameroon. The rats were 8 weeks old and weighing 150-195g at the onset of dosing. Animals were conditioned to the housing facilities for 5 days prior for testing. They were fed with appropriated rodent meal and water *ad-libitum* during the study. The body weight was measured weekly.

Subacute oral toxicity: After a sighting study, a range dose of 250, 500 and 1000 mg/kg of B.v was selected to purchase subacute toxicity studies. Twenty four female albinos rats were randomly distributed into four groups of six animals each (control and tested). The control group received distilled while the treated groups received 250, 500 and 1000 mg/kg of body weight by oral gavages once per 2 days during 28 days.

Clinical parameters: At the end of the experiment, all rats were fasted 16h prior for blood collection. Blood samples were collected in EDTA and dry tubes on day 29 of the test period. EDTA samples were used for the determination of hematological parameters (hematocrit, hemoglobin concentration, erythrocyte, total leucocytes and platelet and lymphocyte). Another aliquot of blood collected in dry tube was centrifuged at 3000 rpm for 5 min with a centrifuge (Rotifix 32A; Hettich Zentrifugen; model Werk-NK). The serum obtained was used for the determination of biochemical markers such as calcium, phosphorus, alanine aminotransferase (ALAT), aspartate aminotransferase

(ASAT), creatinine, total protein, alpha amylase, urea nitrogen and alkaline phosphatase (ALP).

Histopathology: Organs (liver, kidneys, lungs, spleen and heart) were quickly removed, washed with cold saline solution and weighed immediately. These organs were preserved in 10% formaldehyde solution. Fixed tissues were sectioned using microtome (Microtom HM 315), stained with hematoxylin and eosin, and examined microscopically for toxicant-induced changes.

Statistical analysis: All values are expressed as mean ± standard deviation. Analysis of variance (ANOVA) and student's t-test (Sigma-Stat version 3.5) were used for analysis. Statistical comparison was carried out using Student Newman Keuls Multiple Range Test. $P<0.05$ was considered statistically significant.

RESULTS

No death was recorded during the period of treatment in either control or treated groups. Organs of both control and treated groups were physically identical. The animals show no changes in general behavior. The weight of organs and the ration of the body/organ weights of the rats treated once with an aqueous extract of *Brillantaisia vogeliana* at 250, 500 or 1000 mg/kg dose for two days during 28 days are given in table 1. There were not significant differences between control and treated groups for the body and organ weight ($p>0.05$). Significant difference ($p<0.05$) was observed with kidney weight between animals which received 250 and 1000 mg/kg dose compared to control.

The figure 1 which represents the variation of the body weights of rats during the toxicity studies shows an increased weight gain in all the groups. In spite of this extensive increasing weight gain observed in groups, there were not significant differences between them ($P>0.05$).

No significant changes were observed in the weights organs of animals treated (Table 1). Although no significant difference in the kidney weight has not been detected between all tested groups, significant augmentation was observed with relative kidney weight in group 1 when compared to control and group 3 (Table 1). Marker of kidney functionality like urea and creatinine plasma analysis did not show expressive alteration (Table 2). Likewise, no gross alterations were observed in plasma levels of biochemical parameters except in ASAT which showed a significant augmentation in treated groups. Compared with control, we observed a significant decreased ($P<0.005$) concentration of phosphorus in treated groups.

The table 3 shows results of hematological parameters after administration of *B. vogeliana* leaves extract. When compared to control, the values of platelet, lymphocytes increased and while those of WBC decreased.

Pathological examinations of tissues on a gross and microscope indicated some detectable abnormalities. The figure 2 shows photomicrographs of liver section. Animals of control group show normal liver architecture with portal triad (fig 2A). Rats treated with 250 mg/kg body weight show differentiated liver cells and slight modification of architecture. There is a transformation of cells to red homogeneous mass known as "Mallory corps" generally met in alcoholic hepatitis. Nucleuses have various size and some of them show elementary lesion (fig 2B). Rats treated with 500 mg/kg body weight show dark liver nucleus cells with varying size but cytoplasm color is conserved; some of them are retracted in the shape (fig 2C). Liver section from rat treated with 1000 mg/kg body weight showed a slight modification of portal triad. There is no distinction between nucleus and cytoplasm membrane. Some architectural alterations are found including modification of size, shape and orientation of liver cells i.e change of polygonal shape of cells. The centre of nucleus is clear stimulating sandy liver cells usually found in a chronic viral hepatitis. Inflammatory filtrate is also observed but enclosed in the portal triad which can be described as peace-meal necrosis (fig 2D).

The figure 3A shows photomicrographs of kidney section from normal rat with normal kidney architecture. The rats treated with 250 mg/kg body weight show normal cells with inflammatory tube. Some of these nucleuses are found in tube aperture (fig 3B) while those treated with 500 mg/kg body weight show tube architectural disturbance or disorganization including reduction of their size, thinning of their thickness with central nucleus rejecting cytoplasm to the extremity (fig 3C). Rats which received 1000 mg/kg body weight show slight homogeneous tubes not well recognized as well as glomerules. There is an infiltration in the aperture of some tube with the same color characteristic which could be due to the destruction of some tubes. These tubes are more atrophied with a thin coating. There are also several optic empty gaps found inside and outside the tubes (fig 3D).

Figure 4A shows photomicrographs of the heart section from normal rat showing normal architecture. The animals treated with 250 mg/kg body weight show some architectural alterations with scattered nucleus, destruction or hemolysis of myocardial fiber lead to "ghost wave" (fig 4B). The group of rats treated with 500 mg/kg body weight shows an increase of muscular fiber volume with normal nucleus. The architecture is completely modified with cylindrical structures (fig 4C). The section from rats treated with 1000 mg/kg body weight shows the variation of fiber size with dissociation of the muscular fiber more elongated with several central and peripheral nucleuses (fig 4D).

Figure 5A shows photomicrographs of lung section from normal rat with lung architecture. The rats treated with 250 mg/kg body weight show mononuclear inflammatory infiltrate with predominant lymphocytes or a massive development of lung cells which could be reactional (fig 5B). Concerning the animals treated with 500 mg/kg body weight, the histopathological picture of lung shows cells gather to small isolated islets afterwards confluent progressively associated with rare nodular formation (fig 5C). Group of rat treated with 1000 mg/kg body weight show a complex architecture, cells regroup to islets presenting various confluence size or not, reshuffled alveolus and inclusions appearing as optical empty vacuole (fig 5D).

Figure 6 shows photomicrographs of spleen section of rat stained with heamatoxylin and eosin. No important structural modification was observed with different groups of rats except those treated with 1000 mg/kg body weight which exhibited destroyed white spleen cells only (fig 6D).

DISCUSSION

This research aimed to study the toxicity of the aqueous extract of *Brillantaisia vogeliana* leaves. The measurement of enzyme activities in tissues and body fluid plays a significant and well defined role in investigation and diagnosis of diseases (Malomo, 2000). Such measurement can also give an insight to the site of cellular tissues damage as a result of assault by sub-acute or chronic use of plant extract. Tissues enzyme assay can indicate the cellular damage long time before the structure damage can be proved by conventional histological techniques. Alkaline

phosphatase is a marker for the plasma membrane and endoplasmic reticulum functionality (Wright and Plummer, 1974). A significant augmentation of ASAT level was noted in all groups of rats treated with extract compare to the control (Table 2). The increment of the enzyme activity after 28 days of extract administration may be attributed to enhancement of the membrane component including ALP into intra-cellular fluids or the stimulation of the enzyme molecule by the phosphate groups used for the phosphorylation of ethanolamine and choline. These phosphate groups are needed to synthesize two major membrane components such phospholipidylethanolamine and posphatidylcholine which are important in the membrane fluidity and the permeability of the epithelial cells (Malbica et Hart, 1971). Alkaline phosphatase activity is often employed to assess integrity of plasma membrane and endoplasmic reticulum (Akanji et *al*., 1993). In addition, the estimation of the activities of ALAT, ASAT and ALP can make assessment of liver function. When liver cell plasma membrane is damaged by hepatotoxic substances, a variety of enzymes normally located in the cytosol are released into the blood stream trough injured liver cell wall (Lim et *al*., 2000). Their estimation in the serum is a useful quantitative marker of the extent and type of hepatocellular damage (Miltra et *al*., 1998). The reduced activities of ASAT and ALP observed in treated rats in our study might be related to the extensive liver protection induced by the extract. Tendency of these enzymes to return towards a normal level could be a manifestation of anti-hepatotoxic effect of aqueous extract of *B*. *vogeliana*.

Weight gain has been observed in all treated rats compared with net weight gain of control animals. Since food intake was measured in this study, we can rule out that the reported increase of body weight could be a consequence of appetite stimulation at high dose used, because in our previous study, we found that administration of aqueous extract at 42 mg/kg body weight showed rather a reduction of body weight (data not shown) due to a diuretic effect of the herb as shown with *E. macrophyllus b*y DACOSTA et al. (2000). In fact, this effect can not be merely a consequence of a toxic effect of herb but it might be due to the presence of pharmacological substances either active in very low doses and/or whose activities are counteracted by other substances present at smaller amounts. In addition, this effect could be related to the mechanism by which the extract could participate in the insulin signaling process (Ribnicky et *al*., 2004).

Result of this study indicated that the whole aqueous extract of *B. vogeliana* did not significantly increase the concentration of lymphocytes, platelet and decreased the concentration of WBC. Changes occurred in the blood indices showed highest effect in the group of rats which received 250 mg/kg body weight. It could be due to the presence of some vitamin and mineral constituent of the leaf which appear to be most likely as the active ingredient responsible for the haematinic effect of acanthacaea family (Akah et *al*., 2009).

In conclusion, the aqueous extract of *B. vogeliana* shows no toxic effects in spite of different variations noted in the parameters tested.

ACKNOWLEDGEMENTS

We thank the chief of service of Laquintinie Hospital laboratory, for providing necessary facilities. We acknowledge the head, Anatomopathology laboratory service of Douala Laquintinie Hospital for financial support during histopathology studies.

REFERENCES

1. DA Costa, L.L., Albano, F., Laranja, G.A.T., Alves, L.M., Silva, L.F.M., DE Souza, G.P., Araujo, I.M., Neto, J.F.N., Felzenszwalb, I., Kovary, K. Toxicological evaluation by in vitro and in vivo assay of an aqueous extract prepared from *Echinodorus macrophyllus* leaves. *Toxicology letters* 2000; 116: 189-98.
2 .Heine, H. Flore du Gabon. Publié sous les auspices du gouvernement de la république du Gabon et sous la direction de A. Aubreville, 1938 : 83-95.
3. Adjanohoun, E.J., Ake, A.L., Chibon, P., Devechy, H., Duboze, E., Eyme, J. Medicine traditionnelle et pharmacopée. Contribution aux études ethnobotaniques au Gabon. *Agence de cooperation culturelle et technique*, 1985: 18-19.
4. Burkill, H.M. The useful plants of West Tropical Africa. 2nd Ed: Royal Botanic Gardens.KEW; 1985: 7-8.
5. Noubissi, K.F. Etude des effets de *Brillantaisia vogeliana* (Nées) Benth. (Acanthacées) sur le taux des lipides sériques chez les rats. Thèse de Doctorat 3ème cycle Université de Yaoundé I, 2000
6. Kamgang, R., Youmbe, M.R., N'dille, M.P. and Ngogang, Y.J. Cameroon local diet-induced glucose intolerance and dyslipidemia in adults wistar rats. *Diabetes research and clinical prat*. 2005; 69 Supl 31: 224-230.
7. Malomo, S.O. Toxicological implication of Ceftriaxone administration in rats. *Nigeria J. biochem. Molecular biol.* 2000; 15: 33-8.
8. Wright, P.J. and Plummer, D.T. The use of urinary enzyme measurement of direct renal damaged caused by nephrotoxic compound. *Biochem. Pharmacol*. 1974; 23: 65-73.
9. Malbika, J.O. and Hart, L.G. Effect of Adenosine triphosphate (ATP) and anti-inflammatory agents on purified lysosomal having high acid phosphatase and labile glucuronidase activity. *Biochem. pharm.* 1971, 20: 2017-22.
10. Akanji, M.A., Olangoke, O.A. and Oloyede, O.B. Effect of chronical consumption of metabisulfite on the integrity of the kidney cellular system. *Toxicol*. 1993, 81: 173-179.
11. Lim, H.K., Kim, H.S., Chung, M.W. and Kim, Y.C. Protective effects of bergenin, the major constituent of *Mallotus japonicum*, on galactsamine-intoxicated rat hepatocytes. *Journ. of ethnopharma*. 2000; 70: 69-72.
12. Miltra, S.K., Venkataranganna, M.V., Sundaram, R., Gopumadhavan, S. Protective effect of HD-03, a herbal formulation, against various hepatotoxic agents in rats. *J. ethnopharmacol*, 1998; 63: 181-186.
13. Rinicky, D.M., Poulev, A., O'Neal, J., Wnorowski, G., Malek, D.E., Jäger, R. and Raskin, I. Toxicological evaluation of the ethanolic extract of *Artemisia dracunculus* L. for use as a dietary supplement and in functional foods. *Food and chemical tox*. 2004; 42: 585-98.
14. Akah, P.A., Okolo, C.E. and Ezike, A.C. The haematinic activity of the methanol leaf extract of *Brillantaisia nitens Lindau* (Acanthaceae) in rat. *Afr. Jour. of Biotech*. 2009; 8 Supl 10: 2389-93.

TABLE 1: MEAN OF ORGANS WEIGHT AND ORGAN TO BODY WEIGHT RATIO (IN PARENTHESIS) OF B. *VOGELIANA* TREATED RATS.

	Groups of rats			
Organs	Control *(Distilled water)*	Group1 *(250 mg/kg)*	Group 2 *(500 mg/kg)*	Group 3 *(1000 mg/kg)*
Liver (g)	6.13 ± 0.75 (3.01 ± 0.07)	6.38 ± 0.90 (3.30 ± 0.54)	6.04 ± 0.36 (3.01 ± 0.17)	5.76 ± 0.51 (2.77 ± 0.26)
Kidney (g)	1.17 ± 0.14 (0.57 ± 0.04)	1.38 ± 0.17 (0.71 ± 0.03)[a, d]	1.29 ± 0.29 (0.64 ± 0.07)	1.29 ± 0.07 (0.62 ± 0.03)
Lungs (g)	1.98 ± 0.09 (0.98 ± 0.15)	1.90 ± 0.32 (0.98 ± 0.18)	1.86 ± 0.33 (0.93 ± 0.19)	1.75 ± 0.33 (0.84 ± 0.14)
Heart (g)	0.63 ± 0.07 (0.31 ± 0.05)	0.64 ± 0.05 (0.33 ± 0.01)	0.60 ± 0.07 (0.30 ± 0.02)	0.61 ± 0.06 (0.29 ± 0.02)
Spleen (g)	0.65 ± 0.15 (0.33 ± 0.11)	0.60 ± 0.06 (0.31 ±0.01)	0.63 ± 0.19 (0.26 ± 0.17)	0.66 ± 0.10 (0.32 ± 0.04)

Data are expressed as mean ± SEM, n=6. Superscripted letters indicates significant differences between means

TABLE 2: BIOCHEMICAL PARAMETERS OF AQUEOUS EXTRACT AFTER 28 DAYS

Groups of rats				
Parameters	Control *(Distilled water)*	Group1 *(250 mg/kg)*	Group 2 *(500 mg/kg)*	Group 3 *(1000 mg/kg)*
Urea nitrogen (mg/dl)	0.373 ± 0.006	0.330 ± 0.039	0.330 ± 0.035	0.376 ± 0.045
Creatinine (mg/dl)	8.10 ± 0.87	8.14 ± 1.33	8. 36 ± 0.75	7.58 ± 0.61
Alk. phosphatase (U/I)	22.53 ± 6.12	22.10 ± 6.28	18.28 ± 3.76	18.38 ± 6.87
ALAT (U/I)	107.00 ± 15.62	102.60±14.81	103.40±27.71	86.00±13.29
ASAT (U/I)	43.00 ± 1.00	48.40 ± 3.13[a]	49.80 ± 1.64[a]	48.6 ± 2.88[a]
Calcium (mg/dl)	15.17 ± 1.80	13.10 ± 1.76	13.94 ± 2.19	14.86 ± 3.08
Alpha amylase (U/I)	29.27 ± 2.36	33.82 ± 5.31	35.60 ± 5.91	29.46 ± 4.32
Total protein (g/dl)	48.8 ± 0.9	49.8 ± 3.24	49.72 ± 2.73	48.06 ± 1.72
Phosphorus (mg/dl)	35.73 ± 8.60	22.64 ± 6.56[a]	15.66 ± 2.99[a]	15.50 ± 0.79[a]

Data are expressed as mean ± SEM, n=6. Superscripted letter indicates significant difference between means

TABLE 3: HEMATOLOGICAL PARAMETERS AFTER 28 DAYS TREATMENT WITH THE *B. VOGELIANA* AQUEOUS EXTRACTS.

Groups of rats				
Parameters	Control *(Distilled water)*	Group1 *(250 mg/kg)*	Group 2 *(500 mg/kg)*	Group 3 *(1000 mg/kg)*
WBC ($\times 10^6$/ml)	7.83 ± 1.95	6.40 ± 0.95	6.50 ± 1.18	7.30 ± 2.40
Lymphocytes (%)	4.03 ± 1.36	4.27 ± 1.20	4.46 ± 0.99	4.23 ± 1.91
RBC ($\times 10^3$/µl)	7.54 ± 0.63	8.27 ± 0.96	7.13 ± 1.56	7.16 ± 0.71
HGB (g/dl)	12.40 ± 0.89	13.27 ± 0.85	11.78 ± 2.16	10.98 ± 2.36
HCT (%)	40.80 ± 4.03	44.77 ± 3.74	38.12 ± 7.89	39.28 ± 4.10
PLT ($\times 10^3$/ml)	332.67±122.40	580.33±102.03	369.40±168.65	417.50±247.85

Data are expressed or not as mean ± SEM, n=6.

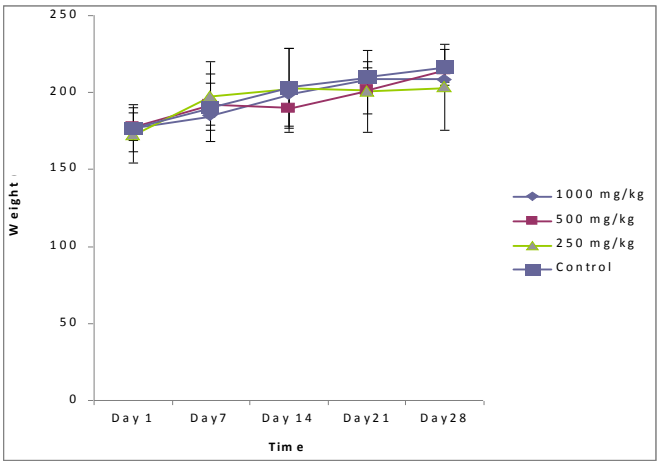
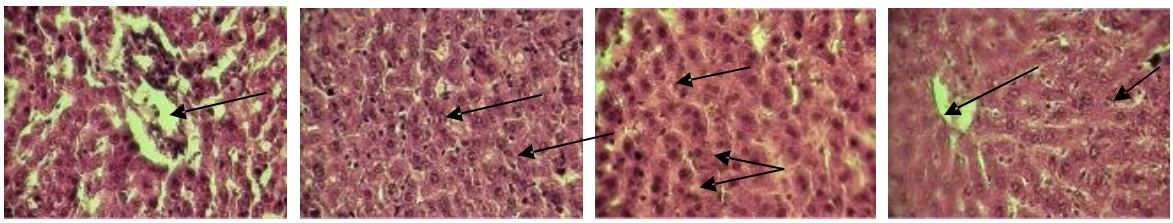
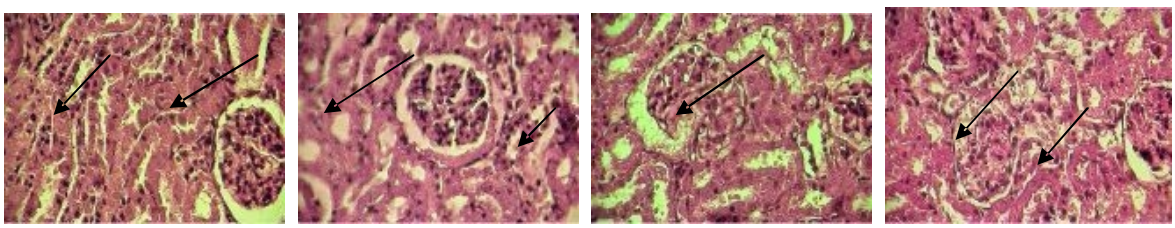


Figure 1: body weight trend for rats dosed with aqueous extract of Brillantaisia vogeliana at 250, 500 or 1000 mg/kg body weight.



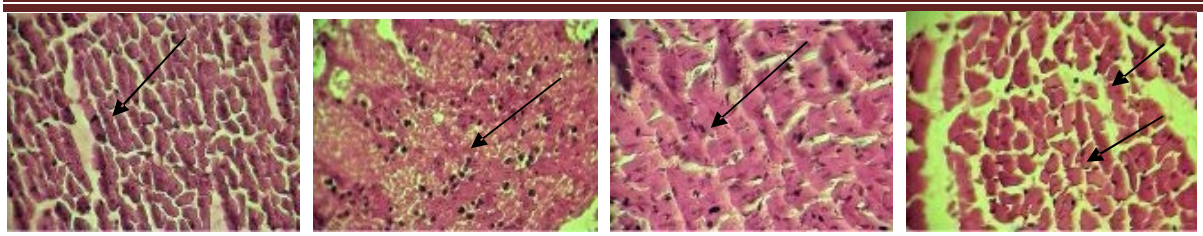
A B C D

Figure 2: Photomicrographs of liver section of rat stained with heamatoxylin and eosin (×400). A: control or normal rat; B: rat treated with 250mg/kg; C: rat treated with 500mg/kg; D: rat treated with 1000mg/kg



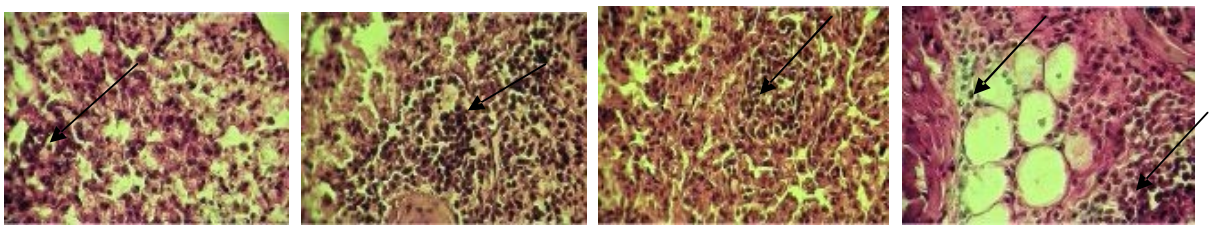
A B C D

Figure 3: Photomicrographs of kidney section of rat stained with heamatoxylin and eosin (×400). A: control or normal rat; B: rat treated with 250mg/kg; C: rat treated with 500mg/kg; D: rat treated with 1000mg/kg



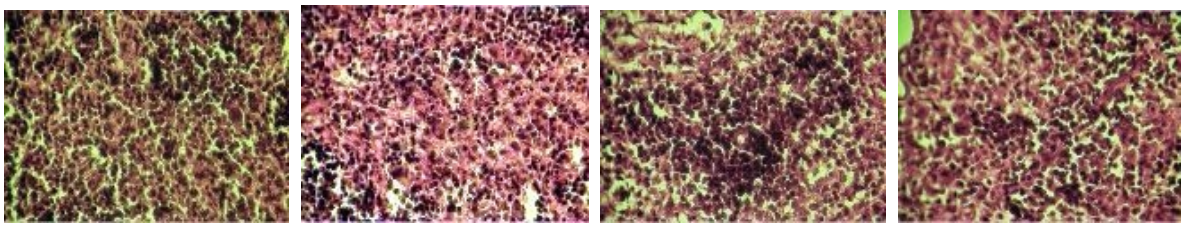
A B C D

Figure 4: Photomicrographs of heart section of rat stained with heamatoxylin and eosin (×400). A: control or normal rat; B: rat treated with 250mg/kg; C: rat treated with 500mg/kg; D: rat treated with 1000mg/kg



A B C D

Figure 5: Photomicrographs of lung section of rat stained with heamatoxylin and eosin (×400). A: control or normal rat; B: rat treated with 250mg/kg; C: rat treated with 500mg/kg; D: rat treated with 1000mg/kg



A B C D

Figure 6: Photomicrographs of spleen section of rat stained with heamatoxylin and eosin (×400). A: control or normal rat; B: rat treated with 250mg/kg; C: rat treated with 500mg/kg; D: rat treated with 1000mg/kg

Source of support: Nil, Conflict of interest: None Declared