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Original Article

Acute and subchronic oral toxicity assessment of leaves aqueous extract of *Triumfetta pentandra* (Tiliaceae) on mice and rats

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ABSTRACT

Triumfetta pentandra is used in African traditional medicine against diabetes mellitus, goiter and wounds. The objective of the study was to evaluate the acute and subchronic oral toxicity of leaves aqueous extract of *T. pentandra*. In acute toxicity, mice received a single administration of *T. pentandra* (0, 1250, 2500 and 5000 mg/kg) and were observed for 7 days. In subchronic study, rats were fed daily with *T. pentandra* (0, 250, 500 and 1000 mg/kg) for 28 days. Food and water intake were measured daily and relative body weigh were evaluated weekly. After 28 days, animals were sacrificed and blood samples were collected for determine, haematological and biochemical parameters. Relative organ weight and histological examinations were evaluated. There were no deaths as a result of acute toxicity, hence the LD_{50} > 5000 mg/kg. In subchronic toxicity study, decreased body weight in rats treated at 250 mg/kg was observed. The percentage of monocytes and platelets counts increased at 500 mg/kg and the percentage of granulocytes decreased at 500 mg/kg and 1000 mg/kg. Serum protein increased at doses of 250 and 500 mg/kg. Renal clearance of creatinine decreased at 1000 mg/kg. Photomicrograph of the liver revealed a congestion of the central vein at the dose 1000 mg/kg. In conclusion, leaf aqueous extracts of *T. pentandra* may be considered as relatively safe.

Key words: *Triumfetta pentandra*, leaves aqueous extract, acute and subchronic toxicity

INTRODUCTION

There is a long standing history on the fact that medicinal herbs are widely used for the prevention and treatment of diseases. They are neither only used for primary health care, not just in rural areas of developing countries, but also in developed countries where modern medicines are predominantly used [1]. In Africa, 80 % of human population use some form of traditional herbal medicine [2]. The development of resistance against modern medicine by pathogens, high costs as well non-availability of some of these drugs have, in recent times, been the causes of the special interest in traditional herbal medicine [3], reinforced by the notion that all herbal products are safe and effective. Therefore traditional medicine seems to be a major factor in the provision of health care. Studies from different ethnics groups on herbal remedies have shown that they are not only life threatening but are also having many side effects despite their reliability, affordability and availability in virtually all African villages [4,5]. In reality many plants used in phytotherapy are toxic. About 5% of intoxications are due to plants and plant preparations [6]. In some cases, adulterations, inappropriate formulations, lack of understanding of plant and drug interactions, ignorance of the dose of administration lead to adverse reactions more or less serious. In 2007, 21 deaths attributed to medicinal plants were registered in Algeria, 230 cases of intoxications with 12 deaths in Morocco [6]. In reality, plant intoxications can induce deleterious effects going from affections of all the physiological functions of the organism to irreversible lesions like renal failure, hepatic failure, hemiplegia etc… and sometimes death. These toxic effects are linked to toxic compounds (alkaloids etc…) that can be found in plants. Reports have showed that Atractylis gymnifera used in Morrocan phytomedicine for its emetic properties can induce death after 4-6 days post

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ingestion [7]. Therefore, inspite of their undeniable pharmacological importance, medicinal plants can be a source of serious health risks for the patients.

Many investigations have been carried out recently to evaluate the toxicity of medicinal plants, as such contributing to improvement of the quality of health care provision by traditional medicine. However, with regards to the diversity of the plant kingdom, new therapeutic properties are daily discovered in many plant species, leading to an expansion of their usages in traditional medicine. It is therefore imperative to carry out further investigations on the safety of medicinal plants since to the best of our knowledge. Very few studies have been done on the safety of plants like *Triumfetta pentandra*. This study is therefore designed to evaluate the effects of this plant on the biochemical and histological parameters in single and repeated administrations.

Triumfetta pentandra is an annual plant, whose leaves decoctions are used in traditional medicine, especially in the Menoua Division of the West region of Cameroon, in the treatment of diabetes mellitus. In Congo, fresh root scrapings are applied on sores and small wounds. Crushed leaves are used in treatment of goiter and deformities. However, despite all its potential therapeutic uses, no toxicity assessment has been carried out on this herbal plant. Therefore, the present study aims at assessing the acute and subchronic oral toxicity of leaves aqueous extract of *Triumfetta pentandra* on mice and rats

MATERIALS AND METHODS

Plant collection: The leaves of the plants were collected at Bamendou, a village located in Menoua Division of the West region of Cameroon during the month of March 2013. After collection, the plant material was identified in the Department of Forestry of the Faculty of Agronomy and Agricultural Science of the University of Dschang by the botanist Dr. AVANA TCHIENTCHEU M. L. Fresh leaves of *Triumfetta pentandra* were dried under shade and ground into powder.

Preparation of Aqueous Extract: Five hundreds grams (500 g) of the powder was introduced into 5 liters of distilled water, stirred and boiled for 20 minutes. The decoction, once cooled at room temperature was filtrated with a coffee filter. The filtrate was evaporated at 40 °C in a drying oven for 48 hours. The dried extract obtained weighed gave 82.37 g; corresponding to a yield of 16.41 %.

Experimental Animals: Adult mice, *Mus musculus*, aged 8-12 weeks, weighing 20-30 g, and Wistar rats aged 8-9 weeks, weighing between 115-128 g of both sexes were used for acute and subchronic toxicity respectively. These animals were raised in the animal house of the Department of Animal Biology, Faculty of Sciences at the University of Dschang, Cameroon, under standard conditions (ventilated room, 12 h under light / 12 h darkness, and temperature of 25 °). They had free access to food and water *ad libitum*. The protocol used in this study was the one used by [8].

Acute oral toxicity study: Thirty two mice of both sexes were divided into 4 groups (I-IV) of 8 mice (4 males and 4 females) each and were closely as possible, matched for weight and size per group. Group I which served as the control group received a single administration of distilled water (10 ml/kg), whereas the groups II-IV, considered as treated groups were subjected to a single administration of leaves aqueous extract of TP at respective doses of 1250, 2500 and 5000 mg/kg . Tap water and food were readily accessible to the mice throughout the study, however prior to the oral administration of a single dose of leaves *aq*ueous extract of TP; all animals were subjected to a fasting period of 12 h with free access to water. Immediately after administration, observations were methodically made for the first 4h, at 24h and thereafter daily for 6 consecutive additional days, for aggressiveness, locomotion, sensitivity to the touch, sensitivity to the pain, sensitivity to the noise, aspect of stools and mortality [9]. In every case, the dose of administration was 10 ml/kg

Subchronic oral toxicity study: Rats were also organized into 4 groups of eight rats (4 males and 4 females), and were treated with a daily oral administration of leaves aqueous extract of TP during 28 consecutive days of experiments. Group I received distilled water (10 ml/kg) and the groups II, III and IV received the extract at doses of 250, 500 and 1000 mg/kg respectively.

Behavioral changes, food and water consumption: The rats were weighed daily throughout the course of experiment. They were also closely observed daily for behavioral changes. Water and food uptake as well as the mortality were daily monitored.

Collection of urine, blood and organ samples: On day 28, rats were deprived of food and placed individually in the metabolic cages for 24 h, for urine collection. The urine collected was conserved at -20 ° and used as sample for the dosage of creatinine. On the day 29, the rats were anesthetized by sequential intra peritoneal injection of diazepam (10 mg/kg) and ketamin (50 mg/kg), for a volume of administration of 2 ml/kg and 1 ml/kg respectively. Ketamin was administered few

minutes after muscular resolution induced by the diazepam. Two volumes of blood were collected per rat and by catheterization of the abdominal artery, made possible after the opening of the abdominal cavity of rats. The first volume was introduced into EDTA (ethylenediaminetetraacetic acid) tubes for hematological analysis. The second volume was placed into tube without anticoagulant (test tube) and centrifuged at 4000 rpm for 5 minutes after blood coagulation. The serum obtained was conserved at -20 ° for biochemical analysis. The liver, the kidneys, the spleen and the lungs were carefully isolated cleaned with saline solution (0,9 %) and weighed for estimation of Relative Organ Weight. Exceptionally, the two formers (liver and kidneys) were conserved in two ways; a fragment of liver and the right kidney at -20 °C for the dosage of proteins. The remaining liver fragment and the left kidney fixed in the formalin 10 %, of a volume ten times greater than those of the organs for histopathological examinations.

Hematological parameters: The white blood cells, red blood cells and platelets counts; the percentage of lymphocytes, granulocytes and monocytes; hemoglobin and hematocrit were determined with the blood collected with EDTA containers and by using an automated hematological analyzer (Procan Electronics Inc).

Biochemical parameters: Serum alanine amino transferase (ALAT and serum aspartate amino transferase (ASAT) were determined using the colorimeric method described by [10] using kit Inmesco. Hepatic, Kidney and serum total proteins were estimated using the method of Biuret. The serum and urinary creatinine was determined according to the colorimetric enzymatic method of [11] using the Kit Inmesco. The renal clearance of creatinine was calculated using the formula below:

$C = U.V/ P$ with

C: Renal clearance of the creatinine (ml/min)
U: Urine concentration of the creatinine (mg/ml)
V: 24h urine volume (ml/min)
P: Plasma concentration of the creatinine (mg/ml)

Histological studies: Fragments of liver and left side of kidney fixed in formalin 10 % were macroscopically sectioned, dehydrated by serial increasing degree of ethanol solution, cleared with toluene, impregnated with melted paraffin(at 52 °) and hardened at -8 °C. Sections of 5 µm thickness were cut and stained with hematoxylin and eosin (H and E). The slides specimens were examined under light microscope by a histopathologist for change in the architecture and photomicrographs were taken.

Statistical Analysis: Data were analyzed using the statistical software Graphpad prism 5. All values were expressed as mean ± SEM. Data were subjected to one-way Analysis of variance (ANOVA) test, followed by Tukey's Multiple Comparison Test and two-way ANOVA test followed by Bonferroni's Test to establish the differences between the control and treated groups. Statistical differences were considered at $p < 0.05$; $p < 0.01$ and $p < 0.001$.

RESULTS

Acute oral toxicity study: During the first hours following the single administration of the leaves aqueous extract of *Triumfetta pentandra*, the reduction of locomotion and the sensitivity to noise were observed in some mice treated at of 2500 and 5000 mg/kg during 2 hours post administration. However, all behavioral changes returned to normal at the end of 24 hours. No case of death was recorded until the dose 5000 mg/kg after 7 days of observation. The LD_{50} was estimated to be higher than 5000 mg/kg.

Subchronic oral toxicity study

Relative body and organ weight, food and water intake: No significant alteration of body weight gain was recorded for the rats of groups III and IV. However, group II recorded from day 19 to 28, a significant decrease ($P < 0.05$ to $P < 0.001$) in body weight gained (Fig. 1). No significant differences ($P > 0.05$) in the relative organ weight (Figure. 2), food intake between the control and treated groups were observed. However, in groups III and IV were noticed a significant increase ($P < 0.001$) in water consumption on second week and first week respectively (Figure. 3).

Hematology results: The effects of the leaves AE of *T. pentandra* on hematological parameters of treated and control groups are presented in Table 1. The results indicate that hematological parameters (White Blood Cell, Red Blood Cell, Hemoglobin, Hematocrit and percentage of lymphocytes) remained within the physiological range throughout the treatment period (28 days). However, the percentage of granulocytes significantly decreased ($P < 0.001$) at doses of 500 ($P < 0.01$) and 1000 mg/kg while the percentage of monocytes and platelets significantly increased ($P < 0.05$) at the dose of 500 mg/kg.

Biochemical parameters: Table 2 is the summary of the results of the biochemical parameters. These results revealed no significant change ($P > 0.05$) in the serum transaminases (ALAT and ASAT), serum creatinine, renal and hepatic proteins. However, the total serum proteins in groups II and

III increased significantly ($P < 0.05$ and $P < 0.01$) as compared to the control group.

Histology results: Microscopic examination of the kidneys in all treated groups did not revealed any structural changes after 28 days of treatment (Figure 4). However, the histopathological study of the liver presented of a vascular congestion of the central vein at a dose of 1000 mg/kg (Figure 5).

DISCUSSION

In acute oral toxicity, single oral administration of the leave aqueous extract of TP did not cause any death of mice up to the highest dose (5000 mg/kg of body weight) during the 7 days of observation. According to [12], substances with LD_{50} values higher than 5000 mg/kg, ingested by oral route, are regarded as being relatively safe or practically nontoxic and does not probably contain toxic compounds. However, it should be considered as relatively toxic as noticeable changes in the behavior were observed. The reduction of the sensitivity to noise could be due to adverse effects caused by xenobiotics at the level of the central nervous system [13].

In subchronic study of the leaf aqueous extract of TP, no noticeable changes in the behavior and food consumption of rats were observed. The body weight change serves as a sensitive indication of the general health status of animals [14]. The weight gain in rats treated at 250 mg/kg was significantly lower as from day 19. Since the appetite of rats was not depressed, it could be suggested that the extract of TP would induce abnormal food assimilation.

The hematopoietic system is one of the most sensitive targets for toxic chemicals and is an important index of physiological and pathological status in human and animal [15]. The assessment of haematological parameters could be used to reveal the deleterious effects of foreign compounds including plant extracts on blood constituents of animals [16]. The percentage of granulocytes significantly decreased compared to control group while the percentage of monocytes significantly increased in rats treated at a dose of 500 mg/kg compared to the control group. It is known that Granulocyte Colony-Stimulating factor (G-CSF) and Monocyte Colony-Stimulating factor (M-CSF) stimulate the production of granulocytes and monocytes respectively [17]. This suggests that the leaves AE of *T. pentandra* would have stimulated the formation of M-CSF, depressing the production of G-CSF. Platelets play an essential role in blood clotting that takes place in plasma following the rupture of blood vessel or lesion of their epithelium. The formation of platelets is controlled by a hormone called thrombopoietin [18]. Hence, significant increase in platelet level at dosage 500 mg/kg compared to control group could be due to the fact that leaf aqueous extract of TP would stimulate the production of thrombopoeitin.

In toxicology studies, organ weight change is an important endpoint for detecting harmful effects of chemicals. Organ weight change is often associated with treatment related effects [19]. The results of this study revealed that the essential organs, such as liver, kidneys, heart, lungs and spleen, neither showed significant changes of ROW nor showed any visible signs for toxicity at the end of the treatment. This result suggests that the leaves aqueous extract of TP would not cause veritable lesion on the analyzed organs. The impact of the extract of TP on vital organs such as liver and kidney was assessed through blood enzyme activity of ASAT and ALAT. As a matter of fact, under normal circumstances, these enzymes mostly reside within the cells of the liver and kidney. But when the liver or kidney is injured for any reason or in case of diseases affecting the liver, these enzymes are spilled into the blood stream [20], and consequently their blood level rise. The results showed that the leaf AE of *T. pentandra* did not significantly affect the serum level of ALAT and ASAT suggesting that the extracts would not have injured the liver and the kidney. However, treated groups showed a slight increase (though not significant) in ALAT level compared to control group and considering that, ALAT is more specific to the liver and hence a better parameter for detecting liver injury[21], the result could suggests that the extract would have nevertheless caused a cytolysis of a certain proportion of hepatic tissue.

Tissue protein rate in treated groups were similar to the control group suggesting that the extract of TP would not have impeded on these organs, which seem to corroborate with the absence of significant variation of relative organ weight of the liver and kidney observed in the present study. However, this hypothesis seem to be contrary to the increase of serum proteins level observed since according to [22], the increase of plasma concentration of proteins is a sign of tissue injury.

Creatinine level serves as a good indicator of the renal function [23]. Any rise in the blood creatinine levels is only observed if there is marked damage of functional nephrons [24]. The leaves AE of *T. pentandra* did not change significantly the serum Creatinine in this study. The *renal clearance of a substance* is the volume of plasma that is completely cleared of the substance by the kidney per unit time [25]. The renal clearance provides a

useful way of quantifying the excretory function of the kidney and can be used to quantify the rate at which blood flows through the kidneys as well as their functions [17]. The absence of significant variation of serum creatinine would suggest that the extract of *Triumfetta pentandra* would not have affected the glomerular filtration rate even at 1000 mg/kg dosage the renal clearance of creatinine seems to indicate the reverse.

The histopathology examination of the kidney in the control and treated rats revealed no visible lesion or necrotic sign. This result suggests that the leaf aqueous extract of TP do not have toxic effect on the kidney tissue. The normal cytoarchitecture of kidney found in histological examination is consistent with the absence of the significant increased of serum creatinine mentioned before. Meanwhile, the microphotographs of the liver showed a slight vascular congestion of the central vein in groups treated with a dose of 1000 mg/kg. According to [26], histopathological condition of the liver can be characterized by a congestion and dilation of central vein filled with blood. This slight congestion and dilation of central veins could be due to a slowing down action of the extract on venous return which would have induced an accumulation of blood in the central vein and its dilation. The slight increase of ROW of the liver (though not significant) observed in group treated with the highest dosage could be explained by this congestion, since the congestion of the vessels is usually accompanied by the increase in weight of the corresponding organ [27].

CONCLUSION

This study has demonstrated that the leaves aqueous extract of T. p*entandra* is relatively safe with a LD_{50} higher than 5000 mg/kg. The lowest dose used in our study (250 mg/kg) which is slightly higher than the dose used in traditional medicine had not affected hematological and biochemical parameters. However, these parameters were relatively affected at dosages of 500 and 1000 mg/kg. These noticeable deleterious effects are confirmed by the congestion of central vein revealed in histological examinations

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Table1: Effects of daily oral administration of the leaves aqueous extract of *Triumfetta pentandra* up to 28 days on haematological parameters in rats.

Parameters	Control	250mg/kg	500mg/kg	1000 mg/kg
WBC (10^3/µl)	07.11 ± 0.53	06.93 ± 0.37	06.93 ± 1.03	05.71 ± 0.67
LYM (%)	63.86 ± 3.32	62.91 ± 3.28	69.31 ± 3.07	69.94 ± 1.40
MON (%)	08.74 ± 1.17	13.93 ± 2.17	18.93 ± 2.71*	15.55 ± 0.89
GRAN (%)	27.29 ± 3.66	23.37 ± 2.04	11.85 ± 1.33***	14.50 ± 1.60**
RBC (10^6/µl)	05.80 ± 0.16	05.65 ± 0.27	05.66 ± 0.61	05.94 ± 0.44
HGB (g/dl)	13.77 ± 0.41	13.73 ± 0.66	13.74 ± 1.13	13.51 + 1.05
HCT (%)	37.14 ± 0.97	36.60 ± 1.62	36.93 ± 3.31	38.09 ± 3.09
PLT (10^3/µl)	315.60±34.32	168.20±36.26	631.10±108.5*	519.40 ± 55.32

WBC: White Blood Cell; LYM: Lymphocytes; MON: Monocytes; GRAN: Granulocytes; RBC: Red Blood Cell; HGB: Hemoglobin; HCT: Hematocrit; PLT: Platelet. *P < 0.05; **P < 0.01; ***P < 0.001. Wistar rats (n = 8). Data are expressed as mean ± S.E.M

Table 2: Effect of the leaves aqueous extract of *Triumfetta pentandra* on biochemical parameters of the rats after 28 days

Treatment	Group I	Group II	Group III	Group IV
ALAT (IU/L)	0.033 ± 0.012	0.089 ± 0.024	0.086 ± 0.020	0.044 ± 0.012
ASAT (IU/L)	0.043 ± 0.013	0.045 ± 0.014	0.034 ± 0.005	0.018 ± 0.003
ASAT/ALAT	0.680 ± 0.105	0.394 ± 0.121	0.499 ± 0.124	0.440 ± 0.121
T P (mg/ml)	47.880 ± 2.193	66.700 ± 6.058*	70.540 ± 2.668**	61.030 ± 3.294
H P (mg/g)	29.540 ± 4.130	31.340 ± 4.089	35.670 ± 1.255	32.100 ± 3.448
RP (mg/g)	40.390 ± 2.296	37.440 ± 2.751	34.730 ± 1.646	36.890 ± 2.801
Cre (mg/ml)	0.798 ± 0.185	0.754 ± 0.195	0.967 ± 0.156	0.853 ± 0.161
RCC (ml/min)	0.033 ± 0.012	0.009 ± 0.002	0.027 ± 0.017	0.004 ± 0.002*

*P < 0.05; **P < 0.01. n = 8. Each data point represents the mean ± S.E.M

ALAT: Alanine-amino-transferase; ASAT: Aspartate-amino-transferase; TP: Total Protein; HP: Hepatic Protein; RP: Renal Protein; Cre: Creatinine; RCC: Renal Clearance Creatinine

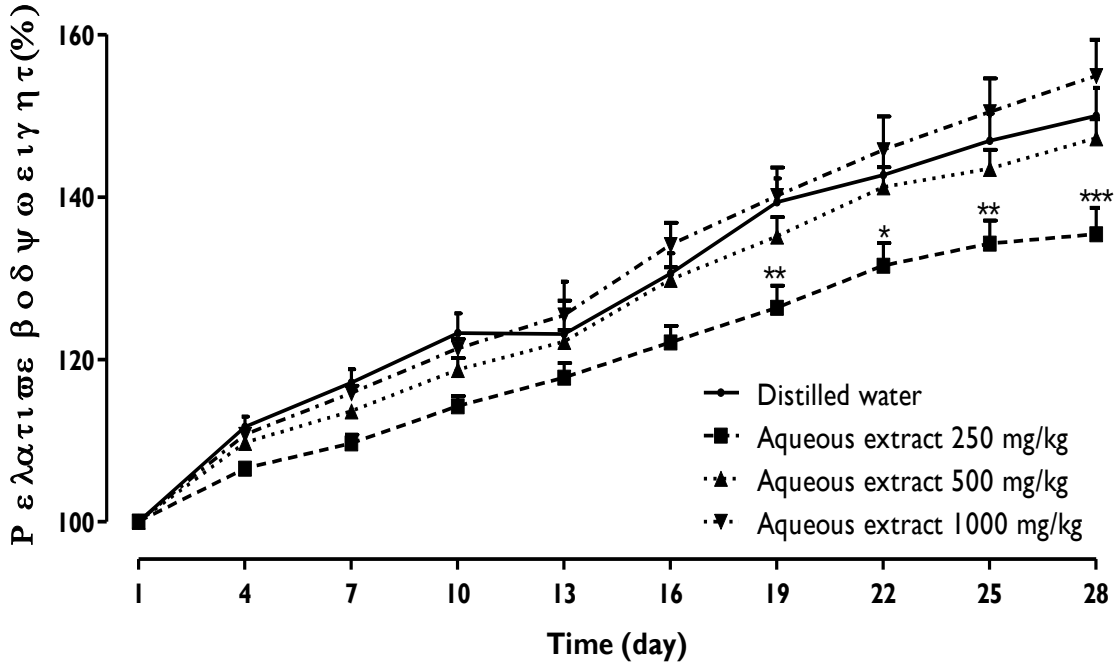

Figure 1: Effect of the leaves aqueous extract of *Triumfetta pentandra* on the relative body weight of rats after 28 days of treatment

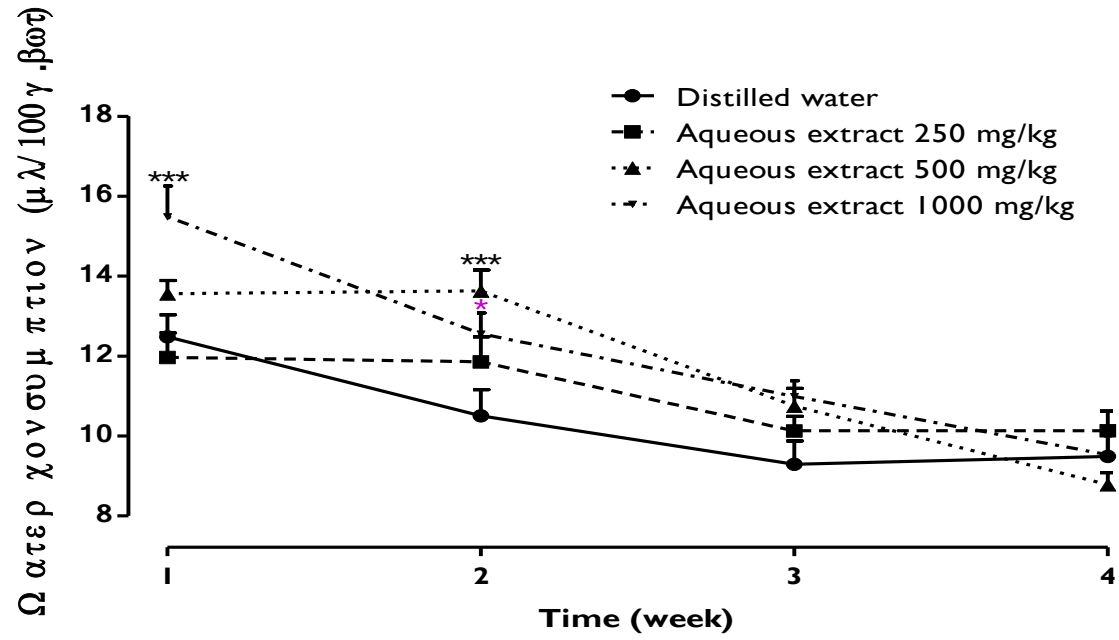

Figure 2: Effects of the leaves aqueous extract of *Triumfetta pentandra* on the water consumption of rats after 04 weeks of treatment

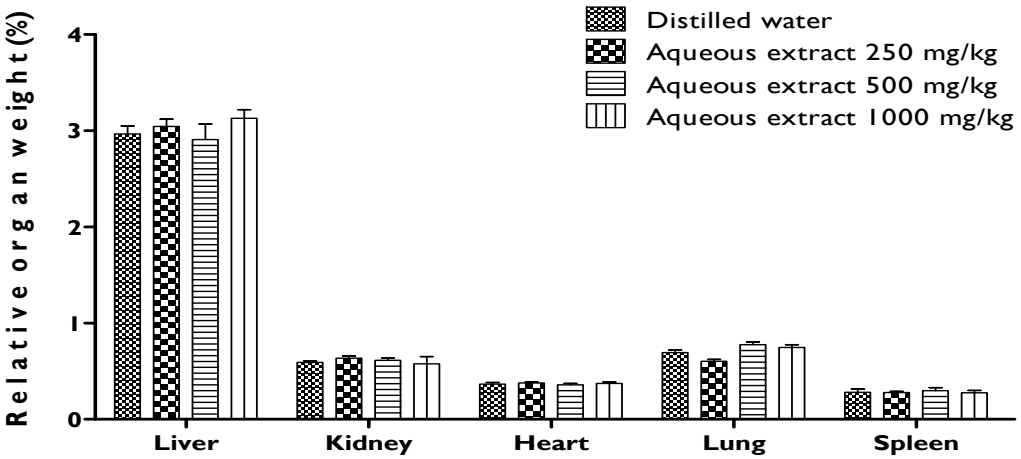

Figure 3: Effects of the leaves aqueous extract of *Triumfetta pentandra* on the relative organ weight of rats after 04 weeks of treatment

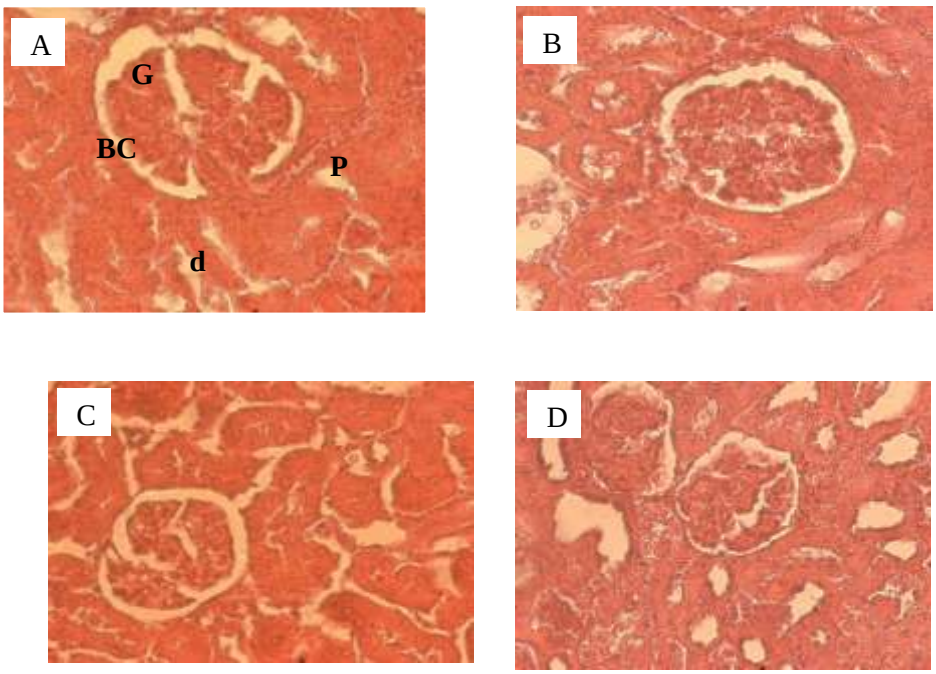

Figure 4: (40X) Micrographs of the kidney sections obtained from rats untreated and rats treated with various doses of leaves aqueous extract of *Triumfetta. pentandra*. Rats treated at the dose of 0 mg/kg (A); 250 mg/kg (B); 500 mg/kg (C) and 1000 mg/kg (D). (BC): Bowman's capsule; (BS): Bowman's space (G) Glomerulus and (d) distal tubule; (p) proximal tubule.

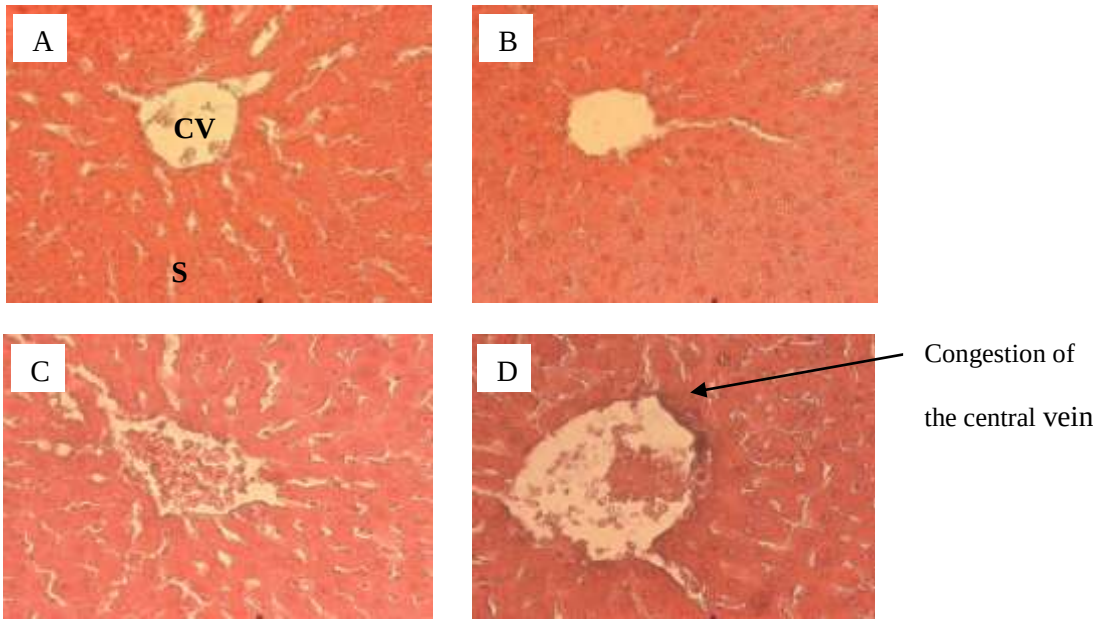

Figure 5: (40X) Micrographs of the liver sections obtained from rats untreated and rats treated with various doses of leaves aqueous extract of *Triumfetta pentandra*. Rats treated at the dose of 0 mg/kg (A); 250 mg/kg (B); 500 mg/kg (C) and 1000 mg/kg (D). Central Vein (CV); Sinusoids (S)

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