

The Green Bitter Weed (*Hymenoxys odorato*) Plant Extract Is Toxic to the Liver and Kidney of Wistar Rats

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ABSTRACT

Background: Medicinal plants are of great importance to health of individual and communities. About 80% of the population in Uganda relies on traditional medicine because western-trained medical personnel are limited especially in villages. Most Ugandans use *Hymenoxys odorato* for medicinal purposes e.g. to treat colds, fever, coughs, anti-helminthes, locally used as tea, anti-allergy and also as an anti-venom to relieve snake bites. **Method:** A group of 25 male wistar rats of 150 g–210 g were kept for 14days while being fed and treated with the extract. At 14th day, anesthesia was given and blood samples collected by cardiac puncture for hematological and biochemical investigations. Serum was analyzed for Alkaline Phosphatase, Aspartate Transaminase and Alanine Transaminase while whole blood was used for complete blood count. The liver and kidney were removed and placed in 10% formalin to prepare for histology staining using haematoxylin and eosin technique. **Results:** The extract elevated hepatic biomarker enzymes i.e. ALP, ALT and AST. The increase was found to be significantly different ($P > 0.05$) at 400 and 500 mg/kg doses as compared to the control group. Histological sections of the liver showed distortion of liver cytoarchitecture, steatosis, necrosis of hepatocytes and congestion of the sinusoids at high doses 300, 400 and 500 mg/kg body weight. In the sections of the kidney, there was mild distortion of the integrity of the kidney with glomerular hypercellularity at high doses (400 and 500 mg/kg per body weight). **Conclusion:** *Hymenoxys odorato* aqueous extract has toxic effects on the liver and kidney of wistar rats. The effects were observed to be in a dose dependent manner.

Key words: Green bitter weed; *Hymenoxys odorato*; Liver and Kidney toxicity

INTRODUCTION

Medicinal plants are of great importance to health of individual and communities. About 80% of the population in Uganda relies on traditional medicine because western-trained medical personnel are limited or not really accepted by the community, and traditional healers are easily consulted with in the same community [1] [2]. Herbs and spices are generally considered safe and effective for the treatment of ailments [3] [4]. Uganda imports most of its drugs from abroad and often experiences serious shortages. That points to the demand for traditional medical practitioners for medical plants and the fact that the majority of people, in rural and urban alike, depend largely on herbal medicines for treating a variety of diseases [5]. This reliance is mainly due to the high cost of conventional medicine and inaccessibility of modern health care facilities in most areas. Most Ugandan folk-users tend to be for medicinal purposes e.g. colds, fever, coughs, to build strength or as antiworm concoctions [6]. This research work studies another plant, *Hymenoxys odorato* commonly known as green bitter weed. Most Ugandans use *Hymenoxys odorato* plant extract for medicinal purposes such as treatment of colds, fever, coughs, anti-helminthes, locally used as tea, anti-allergy and also as an anti-venom to relieve snake bites. This study was to assess effects of the plant aqueous extract on the cytoarchitecture of the liver and the kidney, the biochemical parameters as well as the hematological parameters on wistar rats. This is important because

little information is available in literature to justify the claim that the plant has toxic and hemorrhagic effects. This area of biomedical research has not only provided lasting solutions to some deadly diseases but also revealed the possible adverse effects of abuse of these plants extract by herbal medicine [7]. The medicinal values of this plant lie in some chemical substances that produce a definite physiological action on the human body [8]. The most important of these chemical bioactive compounds are: flavonoids, alkaloids, tannins and phenolic compounds [9]. Despite the advances made in orthodox medicine there has been an increasing interest in complementary and alternative medical systems particularly by; those who have not benefited from previous treatment, having apprehension concerning the toxicity and safety of modern drugs and by those who benefit from the holistic approach offered by traditional medicine. Despite the toxicity and hemorrhagic claim of green bitter weed leaf in folklore medicine of Uganda, there is no published scientific evidence that has either substantiated or refuted this claim. This study is set to provide scientific evidence to the acclaimed toxicity potentials of the leaf extract of green bitter weed leaf in wistar rats.

MATERIALS AND METHODS

Preparation of Extract

The fresh leaves of *Hymenoxys odorato* were obtained from the banks of river Rwizi in Mbarara district of western Uganda. Samples of the leaves were taken for botanical identification at the department of Botany in Mbarara University of Science and Technology. The fresh leaves were air dried at about $24\pm3^{\circ}\text{C}$ temperature for 72 hours to prevent alteration of its vital constituents. The dried leaves were then pulverized and the resultant powder was weighed to give 110g. The ground plant material was transferred into a glass beaker that contained 1000mL of sterile distilled water and allowed to soak for 48 hours, to allow dissolution and extraction. The homogenate was then filtered through Whitman's No. 1 filter paper to obtain a filtrate. The filtrate obtained was evaporated to near dryness using a soxhlet apparatus (Buchi™, BOOBT40HJK, SWISS) at 120 rpm and temperature of 40°C for 16 hours. After evaporation to dryness, 85g of the dried extract was obtained and stored at 6°C in the refrigerator. Four doses of aqueous *Hymenoxys odorato* extract were prepared. These included 200mg/kg, 300mg/kg, 400mg/kg and 500mg/kg which were administered according to animal body weight.

Study Animals

We used 25 healthy male adult wistar rats which were randomly selected. The animals were maintained in wooden cages with iron netting at the experimental animal house unit of the Department of Pharmacology of Kampala International university-Western Campus. They were allowed to acclimatize for a period of two weeks while being fed on commercial rat feed and water ad libitum under controlled environmental conditions of 12-hour light/dark cycle. After acclimatization, the rats were divided into five groups (n=5).

Administration of Extract

Each group was given respective doses of 200 mg/kg, 300 mg/kg, 400mg/kg and 500 mg/kg of the extract. The control group received 8ml/kg of saline. The treatment groups were given the plant aqueous extract by oral intubation using a calibrated syringe with a rubber cannula attached. The control group received quantities of saline equivalent to the volume of extract given to experimental groups. The extract was administered at 8:00 am daily for 14 days. The body weight of the rats was determined using a digital weighing scale.

Animal Sacrifice and Tissue Processing

Fourteen days after administration of the extract, all the rats were sacrificed by anaesthetizing them with chloroform. Blood samples were collected by cardiac puncture and the blood was collected in heparinized and non-heparinized plastic tubes for hematological and biochemical investigations respectively. Hematological studies were analysed by an automated hematology analyzer (Sysmex). The blood in non-heparinized tubes was allowed to clot; serum separated from the clot and centrifuged into clean tubes for biochemical analysis. Laparotomy and evisceration of the liver and kidney was done; each organ was removed, weighed and placed in 10% formalin to prevent putrefaction and to prepare tissues for histology staining using haematoxylin and eosin technique.

Assay of serum marker enzymes

Serum obtained from clotted blood samples of rats was analyzed for Alkaline Phosphatase (ALP), Aspartate Transaminase (AST), Alanine Transaminase (ALT) using kits procured from Span Diagnostics Limited, Sachin, India.

Statistical analysis of data

Data obtained was analyzed by one-way Analysis of Variance (ANOVA) and student's *t*-test to determine the significance of differences in groups. Values of probability less than 5% was considered statistically significant. Graph prism software version 6 was used in the analysis.

RESULTS

Table 1: Effects of *Hymenoxys odorato* plant extract on blood parameters

	Control	200mg/kg ±SD	300mg/kg ±SD	400mg/kg ±SD	500mg/kg ±SD
WBC 10 ⁹ /L	4.08±1.75	4.37±1.38	4.942±0.53	4.76±0.42	4.31±1.27
LYM 10 ⁹ /L	2.41±1.38	2.09±0.75	2.756±0.29	3.16±0.43	2.692±0.98
MON 10 ⁹ /L	0.52±0.32	0.67±0.27	0.916±0.12	0.6±0.15	0.52±0.15
RBC 10 ⁹ /L	7.95±0.68	8.88±0.48	9.01±0.37	7.7±0.37	7.75±0.17
HGB (g/dl)	15.52±1.00	15.22±0.43	15.22±0.44	15.85±0.95	16.02±0.44
HCT %	47.8±2.88	46.98±2.44	47.41±1.89	49.38±2.31	49.26±1.6
MCV (fl)	60.6±6.35	53.6±1.52	52.6±1.94	63.75±1.5	63.6±2.9
MCH (pg)	19.62±1.99	17.12±0.47	16.9±0.31	20.4±0.37	20.64±0.9
MCHC (g/dl)	32.46±0.51	32±0.81	32.14±0.78	32.13±0.41	32.5±1.03
PLT 10 ⁹ /L	773.8±297.6	815.6±562.7	673.8±115.2	1069.5±367.3	793±95.7

The table above shows Mean±SD of blood cellular elements of experimental rats and how they varied from the control group. There was no significant difference between the values from the test group as compared to those from the control group (*P*>0.05).

Biochemical Changes in Experimental Rats

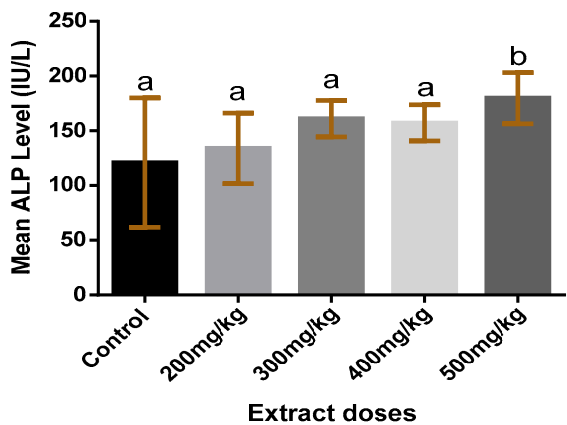


Fig 1: Graphical illustration of changes in the level of ALP in the liver as doses increase. The graph above shows mean ALP against different extract doses. ALP levels increased with increase in the extract dose. At 200mg/kg, 300 mg/kg and 400 mg/kg there was no statistical difference as compared to the control group ($p>0.05$). At 500 mg/kg there was statistical difference in ALP levels as compared to the control group ($p=0.03$).

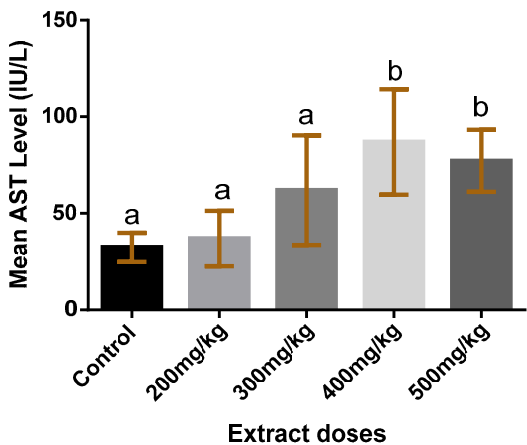


Fig 2: Graphical illustration of changes in the level of AST in the liver as doses increase. The graph above shows means serum AST levels against different extract doses. At 200mg/kg and 300mg/kg, AST levels there was no statistical difference as compared to the control group. At 400mg/kg and 500mg/kg there was a statistical difference in comparison to the control group ($p<0.05$).

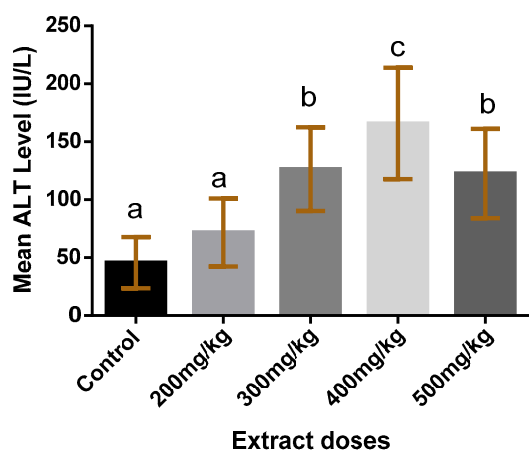


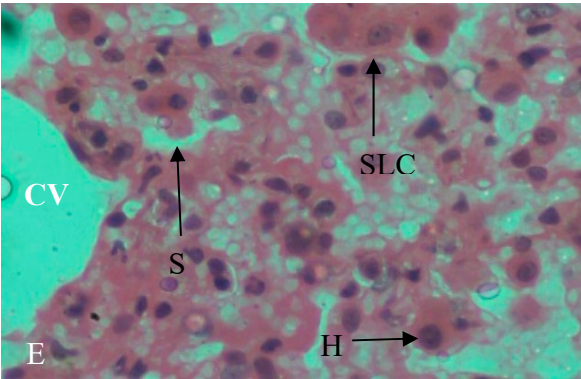
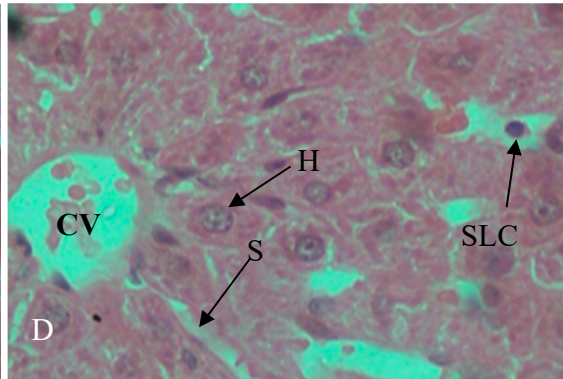
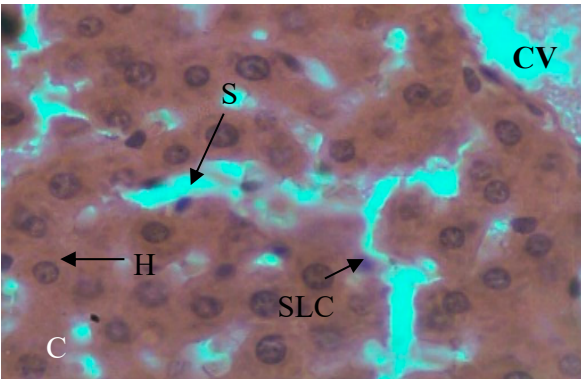
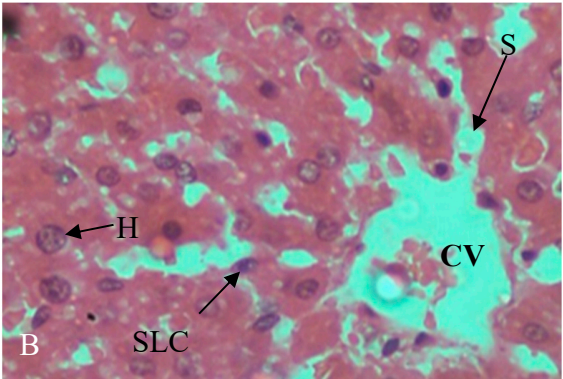
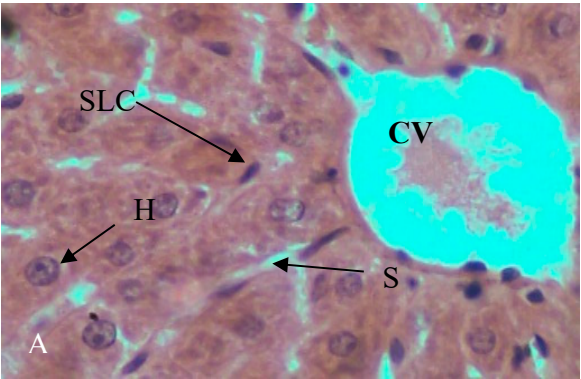
Fig 3: Graphical illustration of changes in the level of ALT in the liver as doses increase. The mean level of ALT activity in the treated rats increased at doses of 200 mg/kg, 300 mg/kg, 400 mg/kg and 500 mg/kg compared to the control group. There was statistical significance ($P < 0.05$) in all the treated groups as compared to the control group.

Physical and morphological Changes in Experimental Rats

In the kidneys, the histopathologic changes were that of acute tubular necrosis with diffused interstitial and glomerular haemorrhage. This suggests that irreversible cellular injury affecting the epithelial parenchyma and endothelial cells occurred. Similar changes were observed in the liver in which, the hepatocytes exhibited severe ballooning degeneration with early steatohepatitis in some foci. In addition, massive hepatocyte necrosis with mallory body formation and extensive hemorrhage was also evident.

Rats receiving the aqueous extract of the plant *Hymanoxys odorato* at the doses 200 mg/kg, 300 mg/kg, 400 mg/kg and 500 mg/kg/day did not die within the two weeks of treatment but were depressed throughout the period of the study. There were no obvious signs observed in rats treated with water.No significant difference was detected in body weights between the control and the treated rats.

Histological Observation of the Liver



Plates of liver sections stained with H and E X400.

Plate A; shows photomicrographs of control group with normal hepatic cytoarchitecture.

Plate B; is the plate of animal administered with 200 mg/kg bw of ELHO with distortion of liver architecture, mild congestion of sinusoids and necrosis.

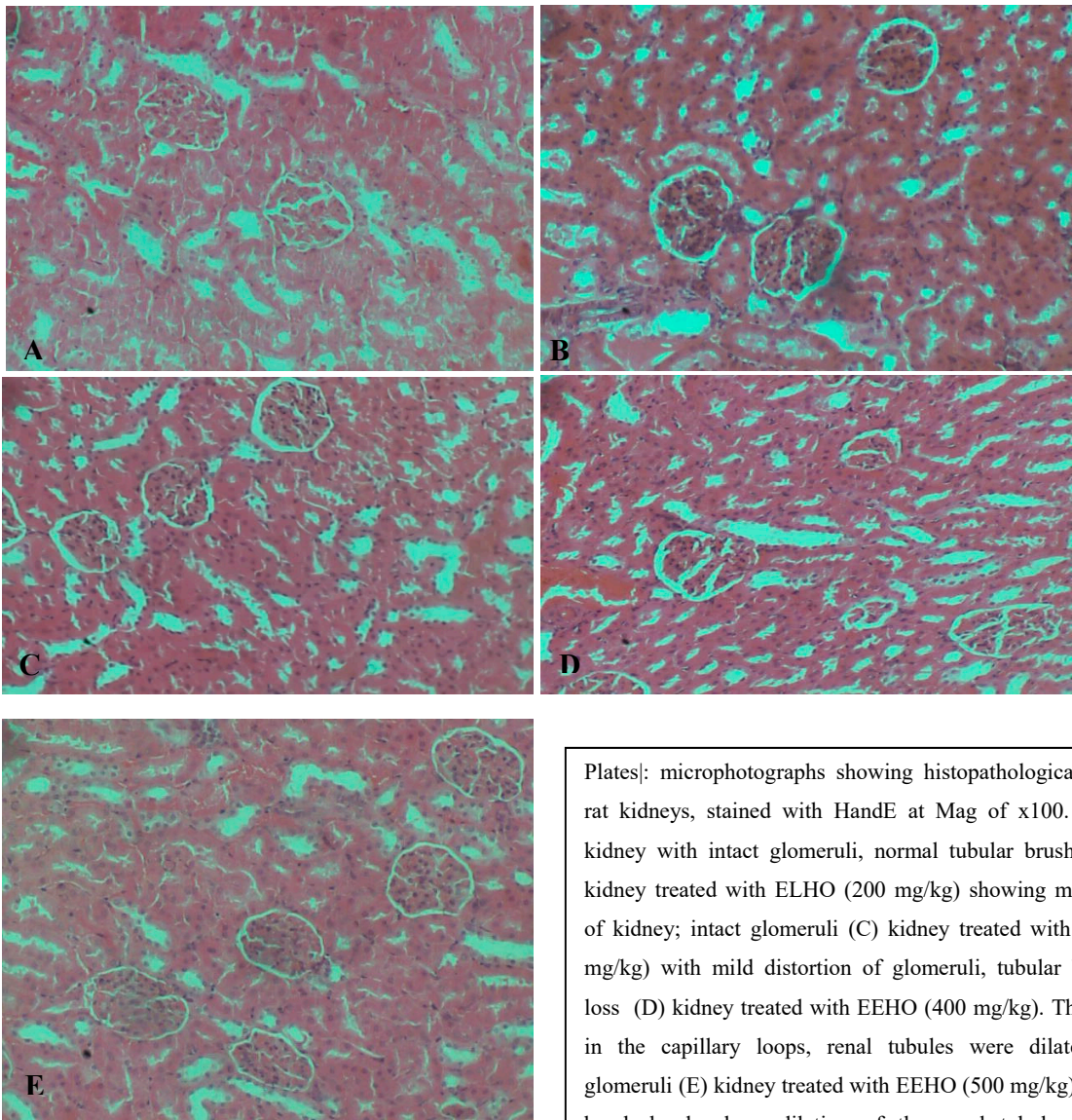
Plate C; shows plate of rats with 300 mg/kg bw with disorganized hepatocyte arrangement sinusoid congestion, necrotic hepatocytes.

Plate D; showing plate of rat administered with 400 mg/kg bw with hypertrophy of the hepatocytes and congestion of the sinusoids.

Plate E; administered with 500 mg/kg body weight with dilatation of central vein, hepatocytes necrosis, steatosis.

- S= sinusoid
- CV= central vein
- SLC= sinusoidal lining cells
- H= Hepatocyte

Histological Observation of the Kidney



Plates|: microphotographs showing histopathological changes in rat kidneys, stained with HandE at Mag of x100. (A) Control kidney with intact glomeruli, normal tubular brush border; (B) kidney treated with ELHO (200 mg/kg) showing mild distortion of kidney; intact glomeruli (C) kidney treated with EEHO (300 mg/kg) with mild distortion of glomeruli, tubular brush border loss (D) kidney treated with EEHO (400 mg/kg). The congestion in the capillary loops, renal tubules were dilated, distorted glomeruli (E) kidney treated with EEHO (500 mg/kg) with tubular brush border loss, dilation of the renal tubules, glomerular hypercellularity.

DISCUSSION

The present study showed that *Hymenoxys odorato* leaf extract did not induce obvious toxic changes in the RBC, Hb, PCV hematocrit and MCHC of rats. The absence of significant changes on these indices may suggest that the extract does not possess toxic substances that can cause an anemic condition in rats. There was an insignificant increase in the total WBCs, neutrophils and monocytes at 200 mg/kg, 300 mg/kg, 400 mg/kg and 500 mg/kg; toxic plants do not produce a direct effect on WBC and its functional indices. The reductions observed in the platelet count may impair the repair of minute breaks in capillaries and other small vessels. Therefore, continued administration of the extract at high doses may result in widespread hemorrhages as seen at histology due to coagulation deficiency because platelets play a crucial role in reducing blood loss and repairing vascular injury. Kidney histology of treated rats showed features consistent with renal epithelial injury from toxins. Many herbal preparations have been found to exhibit renal tubular necrosis showing extensive interstitial fibrosis and severe tubular loss most prominent in the outer cortex [10].

Cellular damage exhibits good correlation with enzyme leakage [11]. Serum AST, ALT, and ALP are the most sensitive markers employed in the diagnosis of hepatic damage [11]. However, the escalation in the activities of these enzymes in serum could be attributed to leakage of these cytosolic enzymes into the circulatory system resulting from hepatocellular damage [12]. This is indicative of the onset of hepatocellular damage due to liver dysfunction and disturbance of the biosynthesis of these enzymes, with alteration in the permeability of liver membrane. The determination of different enzyme activities such as AST, ALT and ALP have been found to be of ultimate importance in the assessment of liver damage or necrosis, thereby releasing the enzymes into the circulation thus increased their activities in the serum [13].

The significant increase in serum AST activity revealed that liver cells necrosis which leads to the leakage of the enzyme from the liver to the serum. Serum ALT is also known to increase in liver disease and it has been used as a tool for measuring hepatic necrosis [13]. The significant increase in serum ALT activity may indicate that the extract has remarkable effect on the integrity of the liver at the concentration used. It may also suggest tissue damage which caused the enzyme to leak out of the liver in to the blood. The significant increase in serum ALP activity may be due to the leakage of the enzyme from mass of damage liver tissue and destroyed cell membrane. Extract of *Hymenoxys odorato* significantly increased the levels of serum AST, ALP and ALT. This is diagnostic of hepatocellular damage, as seen in disorders that cause the death of numerous liver cells (extensive hepatic necrosis) such as acute viral hepatitis A or B. In addition, fatty liver observed in the treated rats is similar to what is obtained in alcoholic cirrhosis. The administration of *Hymenoxys odorato* leaf extract appears to be relatively non-toxic to animals at low dosages. This is because there was no apparent damage to the physiology and biochemistry of the blood of rats in this study. However, at high dosages, the alterations observed on rat leucocytes, platelets, AST, ALT and ALP suggest dose selective toxicity of *hymenoxys odorato* extract when repeatedly consumed on a daily basis for a prolonged time.

In the kidney, there are histopathological changes in the groups treated with the extract ranging from mild to severe damage to the integrity of the kidney. These include hypercellularity of the glomerulus, tubular brush border loss and necrosis of the epithelium. The control administered with normal saline shows normal cytoarchitecture of the kidney with no evidence of glomerular hypercellularity or necrosis of the epithelium.

In the liver, histopathological changes were observed in the treated groups severity of which is in a dose dependent manner. The changes include distortion of the cellular arrangement, dilation of the central vein, necrosis of the hepatocytes and congestion along the sinusoids. These might be because of the toxic phytochemicals such as anthraquinones present in the extract.

Conclusion

Hymenoxys odorato caused liver and kidney toxicity in wistar rats. In the kidneys, there was acute tubular necrosis with diffused interstitial and glomerular haemorrhage suggestive of irreversible cellular injury affecting the epithelial parenchyma and endothelial cells. In the liver, hepatocytes exhibited severe ballooning degeneration with early steatohepatitis in some foci. There was also massive hepatocyte necrosis with mallory body formation and extensive hemorrhage. The severity of the damage increased as the dose was increased.

Recommendation

Considering the potential toxicity of *Hymenoxys odorato* herbal practitioners should be educated on this especially when they recommend this plant as part of a complex regimen in the long term management of chronic illnesses. Further studies would be required to isolate the specific component(s) of the plant responsible for the toxicity in order to standardize the plant preparation for maximum therapeutic benefit.

Ethical approval

Approval to conduct this study was sought from Kampala International University, Faculty of Biomedical Sciences Research and Ethics Committee before commencement of the study. All other ethical issues pertaining to maintaining of plant and animal rights were strictly adhered to and observed during the study.

Conflicts of Interest

The authors declare that there exist no conflicts of interest

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