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Toxicological Evaluations of the Shoot Extracts of *Achyranthes aspera Linn*

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Abstract
Achyranthes aspera Linn, a medicinal plant from the Amaranthaceae family, is commonly used in Nigeria to treat various illnesses, including malaria. Many people use medicinal plants as an alternative to traditional medicine. However, some plants may contain harmful chemicals that can have serious side effects on the body. This study investigated the effects of an aqueous shoot extract of Achyranthes aspera Linn on healthy female albino mice in terms of acute toxicity. The methods employed in this study encompassed various procedures including plant collection, authentication of Achyranthes aspera shoots and plant extraction using analytical grade chemicals. Swiss albino mice were acquired and grouped per protocol as experimental animals. Acute toxicity assessments, blood biochemical/haematological assays, histological examination, and statistical analysis were performed. The results showed that the LD50 of the extract was found to be greater than 5000mg/kg body weight, and no deaths occurred during the study. Additionally, there were no abnormalities in the mice's haematological parameters. Interestingly, the mice treated with the extract showed statistically significant lower levels of serum Alanine transaminase compared to the control group with a p-value less than 0.05. The plant's crude extract has been shown to induce some minor liver and kidney damage. The study indicates that Achyranthes aspera Linn could be a safe herbal medicine with minimal adverse effects on both liver and kidney tissues.

Keywords: Achyranthes aspera Linn, Amaranthaceae, Acute toxicity assessment, Haematological assays, Liver and kidney histological examination

INTRODUCTION

Diseases and infections continue to increase globally, even though conventional medicines are available. The high costs, side effects, and low success recorded in treating different illnesses have contributed to the rapid spread of diseases in Nigeria (Rishton, 2008; Ukoha *et al*., 2017). Although many use herbal medicine as an alternative to treat various illnesses, some herbs

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may have harmful components, while others are relatively safe (Pangale, 2011). These herbal medicines often come from medicinal plants that may contain beneficial chemicals or phytochemicals needed to cure disease (Rota *et al*., 2008), however, some of these chemicals are often unknown but are considered secondary metabolite components which are directly responsible for therapeutic activities. Screening these chemicals in medicinal plants to determine their presence and availability could provide new useful information to the scientific community and support claims of their therapeutic benefits.

The study of the harmful actions of chemicals on biological tissues is referred to as toxicology. This may include an understanding of chemical reactions, their interactions and mechanisms, and consequential structural changes in the tissues (Olejniczak *et al*., 2001). The literature surveyed showed that it is of tremendous significance in the sense that potential health hazards in man as well as the safety of the chemical or drug is obtained (Ukwuani *et al*., 2012). This is done using a range of concentrations of the chemical or drug which would give a graded effect somewhere between the two extremes that is a minimal concentration which produces no effect and a maximal concentration that causes death. The use of animals (mice) has long served as a preferred specimen for medical research in animal models due to their anatomical, physiological and genetic similarities to humans (Bryda, 2013).

Achyranthes aspera L. is a perennial shrub, popularly known as the "pricky chaff flower" belongs to the family *Amaranthaceae.* It is widely spread in the world, in Nigeria, it is known as "*kiban Katangare*" (Hausa). The dietary Achyranthes aspera extract has been shown to improve shrimp health and disease resistance (Ko *et al*., 2023), possesses antimicrobial, antidiabetic, and anti-inflammatory properties useful for treating diseases (Samdershi *et al*., 2023), and has various pharmacological activities including diuretic, laxative, and anti-allergic effects (Pal *et al*., 2023). Reports had it that ethanolic extract of the roots of *A. aspera* showed post-coital anti-fertility in female albino rats (Vasudeva and Sharma, 2006). Likewise, Alcoholic extract of the root of *A. aspera* was found to exhibit anti-inflammatory activity in Wister rats. The shoot aqueous extract of A. aspera has also been reported to exhibit antiplasmodial activity against *P. berghei* in albino mice (Mankilik, Longdet and Luka, 2021). *Achyranthes aspera* was reported to contain numerous active constituents and possessed various medicinal properties. Literature shows that extracts from *Achyranthes aspera* leave, seeds and roots possess chemicals like e*cdysterone, achyranthine, betaine, pentatriaontane, 6-pentatriacontanone, hexatriacontane* and *tritriacontane* which possess various pharmacological activities (Goyal *et al*., 2007) but, the plant shoot has not been fully authenticated. This study therefore aimed at evaluating the impact of the shoot extract of *Achyranthes aspera* safety in healthy albino mice.

MATERIALS AND METHODS

Plant collection and identification

Mature shoots of *Achyranthes aspera* were collected from "Nchiya" in Mangu LGA, Plateau State, North Central Nigeria. It was authenticated by a taxonomist at the Herbarium unit of the Forestry Research Institute (FRIN), Jos, Plateau State Nigeria, voucher number 31820 was deposited in the herbarium for future reference.

Chemicals

All chemicals and reagents used were of analytical grade procured from Sigma Aldrich, USA.

Experimental Animals

A total of fifteen Swiss female albino mice with an average weight of 16-21g were purchased

from the Animal House Unit of the Pharmacology Department, Faculty of Pharmaceutical Sciences, University of Jos, Plateau State, Nigeria. The animals were grouped into experimental and control groups, in standard clean cages containing saw dust inside with five animals per group (n=25) and were kept at room temperature. For feeding, ECWA– Vital feeds, Jos was used with unlimited clean tap water *ad-libitum*. The study was approved by the Institute of Animal Ethical Committee as regulated by the board for control and supervision of experimental animals. Ethics and consent approval to participate was obtained with approval number (UJ/FPS/F17-00379). The mice were quarantined for two weeks before the experiment to alleviate any nonspecific stress. Animals were handled humanely throughout the study period (OECD, 2008).

Plants Extraction

The plant shoots were washed with tap water, air-dried under shade for 7 days, and then pulverized using mortar and pestle into powdered form. The powdered shoot was extracted with distilled water as described by Yared *et al* (2012). A 50g plant shoot powder was mixed with 200 ml of water and kept at 25^{o}C for 3 days, then filtered through a cheesecloth and evaporated at 40^{0}C to semi-solid and finally air dried. The aqueous crude extract obtained (2.32 g) was put in an air-tight sterilized container and kept at 4^{o}C in a refrigerator until used.

Acute toxicity study

The lethal dose of fifty per cent (LD_{50}) for the aqueous shoot extract of *Achyranthes aspera* was determined using female albino mice according to the Organization of Economic Cooperation and Development, 425 protocol (OECD, 2008). The aqueous extract was administered in a single dose orally through gavage, using an oral intubation tube or catheter. Mice were deprived of food for 3 hours before dosing. After each extract dose administration, observation was done at 30-minute intervals for 4 hours, then after 24 hours for any behavioural change or death. The doses given were 2000 mg/kg and 5000 mg/kg body weight of extracts. All animals were observed for 14 days (Ahmed, 2015; Alelign et al., 2020; Teshome et al., 2021).

Animal Grouping and Treatment

The acute toxicity study test was carried out based on the OECD, 425 guidelines. A total of fifteen Swiss albino mice of 5 each (n=5) were randomly allocated into 3 groups each. The animals were kept under observation for up to 14 days after the extract administration to find any delayed mortality. The aqueous extract was administered in a single dose using an oral catheter tube as follows:

i. Group 1 (n=5) (Normal control): Mice in this group were given feeds and clean water *ad libitum* only
ii. Group 2 (n=5) Mice in this group were administered with crude aqueous extract of the shoot of *Achyranthes aspera* at 2000 mg/kg body weight
iii. Group 3 (n=5) Mice in this group were administered with crude aqueous extract of the shoot of *Achyranthes aspera* at 5000 mg/kg body weight

At the end of the 14-day study, mice were lightly anaesthetized with chloroform dapped in cotton wool in a desiccator. Mice were sacrificed, and blood was collected and used for haematological and biochemical examination. The liver and kidney were excised for histopathological examination.

Measurement of animal body weight

The body weight of all the experimental mice was taken by using a weighing pan (JJ 200 G&G® Deutschland Electronic scale) before commencing the first oral administration and then weekly (every morning) before mice were fed, by properly placing the mice in the weight

pan and weight recorded. This is done up to 14 days.

Blood Collection
At the end of the 14 days, all the experimental mice were fasted overnight, cervically dislocated after slightly anaesthetized using cotton wool dipped in chloroform in a desiccator and blood samples were collected by cardiac puncture in a tube with anticoagulant ethylene diamine tetra acetic acid (EDTA) for Haematology and into a tube without anticoagulant for blood chemistry.

Biochemical Analysis
For biochemical analysis, the blood samples in the plain test tubes were allowed to stand for 3 hours for complete clothing and then centrifuged at 5000 rpm for 15 mins using bench to centrifuge (Bench Centrifuge MSE minor). The plasma was withdrawn and transferred into other clean vials. The sera were kept at -20^{0}C until analysis for biochemical indices. The concentration of alanine amino transaminases (ALT), albumin (ALB) and creatinine (CR) were automatically determined using a COBAS C111 clinical chemical analyzer (Roche diagnostic, GMBh).

Haematological assay
Blood samples in the test tubes containing EDTA were immediately processed for haematology using an Automated Haematological Analyzer (Mindray BC 5300, Mindray Medical Japan). White blood cell count (WBC), Red blood cell count (RBC), Haemoglobin Concentration (HGB), Haematocrit (HCT), Mean Corpsular Volume (MCV), Mean Corpsular Haemoglobin (MCH), Mean Corpsular Haemoglobin Concentration (MCHC) and Platelets Concentration (PLT) were determined.

Histological examination
Conventional Haemtoxylin-eosin technique was used for histopathological examination of the liver and kidney tissue as described (Drury and Wallington, 1980). After the 14-day experiment, mice were lightly anaesthetized with chloroform soaked in cotton wool in a desiccator and euthanized. The liver and kidney tissue of the treated and control mice were cleaned and fixed in 10% neutral buffer formation at room temperature. After 24 hours of fixation, the tissues were washed in ascending grades of alcohol (80%, 95%, 100%) cleaned in xylene and embedded in molten paraffin (60^{0}C melting points) blocks. The blocks were sectioned at a thickness of 5-6 µm using Leica Rotary Microtone (LIECA RM 2125 RT China, checked in Germany). The deparaffinization was done by heating the section in the oven at 80^{0}C (60 µm) cleaned with xylene after dewaxing, tissue sections were treated with descending grades of ethyl alcohol (100%, 90%, 80% & 70%) washed and stained in haematoxylin, cleaned in 1% acid alcohol. Sections were stained using eosin and dehydrated in the oven at 80^{0}C cleaned in xylene and mounted with drops of distern plasticizer xylene (DPX). These slides were then examined under a light microscope (Olympus CH), and tissue sections from the treated groups were examined for any evidence of histopathological changes concerning those of the control. Following evaluation, microscopy images of the selected slides from both the treated and control groups were taken at x40 magnification using an automatic built-in digital photo camera.

Statistical analysis
Statistical analyses on the experiment data were performed and expressed as mean ± Standard Error of Mean (SEM); data were analyzed by one-way analysis of variance (ANOVA) and compared using Duncan's multiple range test (DMRT), with a significant difference accepted at $p<0.05$.

RESULTS AND DISCUSSION

Acute toxicity of crude aqueous shoot extracts *Achyranthes aspera*

The administration of the aqueous shoot extract of *A. aspera* via intragastric route at different doses of 2000 mg/kg and 5000 mg/kg bwt did not produce any sign of morbidity and did not lead to mortality in the female albino mice during the period of acute toxicity study. This result indicated that the LD_{50} was beyond 5000 mg/kg bwt for the aqueous shoot extract of *A. aspera*. Our previous study confirmed the aqueous shoot extract of *A. aspera* to possess vital phytochemicals like flavonoids, and phenols and possess biological activity such as antimalarial activity and is said to have little or no adverse effects (Mankilik, Longdet and Luka, 2021). In the body's metabolic process, the blood profile usually provides important information on the response of the body to injury or lesion deprivation and stress (Bosco *et al*., 2014). Therefore, the extent of the toxic effect of drugs and or plants can be determined by the assessment of haematological parameters (Raza, Al-Shahanah, El-Hayidah and Al-Majed, 2002). Although, some herbal preparations of plant extracts are widely used as alternatives in treating various ailments, however, some have been reported to possess some harmful components (Pangale, 2011). Therefore, the study on the toxicity of aqueous extract of *A. aspera* showed that it is safe at a lower dose.

Table 1 Effect of crude aqueous extract of *Achyranthes aspera* shoot on body weight of mice

	Post Sampling Period (Days)				
	Day 0 ($\bar{x}$± SEM)	Day 3 ($\bar{x}$± SEM)	Day 6 ($\bar{x}$± SEM)	Day 9 ($\bar{x}$± SEM)	Day 14 ($\bar{x}$± SEM)
Mice (Normal Control)	21.14±1.01^{a}	24.16±1.16^{a}	26.22±1.08^{b}	27.36±1.11^{b}	27.96±0.66^{b}
Mice+2000mg/kg body wt. Extract	28.74±1.73^{b}	28.68±1.53^{b}	27.90±1.40^{b}	27.08±1.50^{b}	26.60±1.77^{b}
Mice+5000mg/kg body wt. Extract	24.54±1.18^{a}	20.48±5.21^{a}	20.88±5.28^{a}	20.70±5.21^{a}	20.32±5.13^{a}

Where btw, body weight; SEM, standard error of means; n, number of mice in each group; and a, and b, significant different ($p<0.05$).

Table 1 above shows the effect of Achyranthes aspera extract on body weight in mice over 14 days. The changes in body weights of mice following administration of the crude aqueous extract of *Achyranthes aspera* showed a gradual loss in body weight of mice treated with the plant extract, while an increase in weight was observed in normal control mice. However, the loss in weight by mice that received the extract was not significantly different ($p>0.05$) between day 0 and day 14 of extract administration. Sadashur and Krishna (2011) reported the LD50 of *A. aspera* leaves to be greater than 5000 mg/kg body weight, adding that such values are considered a vital factor in monitoring the health of an animal, and loss in body weight is frequently the first indicator of the onset of an adverse effect. Therefore, this study did not show any significant ($p<0.05$) reduction in body weight compared with the zero-day value. This means the aqueous shoot extract of *A. aspera* at both doses did not cause weight gain in mice. However, the weight loss during the experiment could be attributed to some changes in the metabolic process.

Table 2 Effect of crude aqueous extract of *Achyranthes aspera* shoot on some biochemical indices in mice

Parameters	Animal Grouping (n=5) Normal Control Mice (x̄± SEM)	Mice +2000 mg/kg Btw Extracts (x̄± SEM)	Mice +5000 mg/kg Btw Extracts (x̄± SEM)
ALT (μ/L)	58.77 ±5.37^{bc}	48.75± 2.19^{b}	31.48 ±2.45^{a}
ALB (g/L)	30.46± 0.35^{a}	63.3 ±3.60^{a}	28.86 ±0.74^{a}
Creatinine (mol/L)	57.13 ±5.04^{a}	64.59 ±12.30^{ab}	92.02 ±5.82^{b}

Where btw, body weight; SEM, standard error of means; n, number of mice in each group; a, b and c, statistically significant difference ($p<0.05$).

The results of the administration of the crude aqueous extract of *Achyranthes aspera* shoot on biochemical indices in mice are presented in Table 2 above. The findings showed a gradual reduction in hepatic parameters, including ALT and serum alanine transaminase, while albumin and creatinine levels were elevated. The alterations in the biochemical indices were dose-dependent and significantly different ($p < 0.05$) compared to the normal control. Alanine transaminase (ALT), one of the major intracellular enzymes of the liver, is released into the bloodstream when liver cells (hepatocytes) are damaged, leading to elevated levels in the blood (Thapa and Walia, 2007). However, in this study, ALT levels at both doses were significantly reduced ($p < 0.05$) compared to the control. This result contrasts with the findings of Ketema (2015). The significant ($p < 0.05$) decrease in ALT levels at both doses may indicate the non-destructive effect of the extract, suggesting a hepatoprotective property (Li *et al.*, 2014). ALT plays a role in preventing liver damage by inhibiting the elevation of serum ALT. Being largely localized in the liver, ALT is commonly used as a biomarker for liver function (Giboney, 2005). Additionally, ALT is purely cytosolic and highly specific to hepatocytes, further underscoring its reliability as a liver function indicator.

Table 3 Effects of Crude Aqueous Extracts *of Achyranthes aspera* (shoot) at 2000 mg/kg and 5000 mg/kg Body Weight on Haematological Parameters in Mice

Animal Grouping (n=5)	Mice+2000mg/kg Btw Extracts (x̄± SEM)	Mice+5000mg/kg Btw Extracts (x̄± SEM)	Mice+Distilled Water (x̄± SEM)
WBC (x10^{9}/L)	12.06±2.09^{c}	9.77±2.12^{b}	5.12±1.19^{a}
RBC (x10^{12}/L)	8.90±0.61^{ab}	8.10±0.44^{ab}	7.93±0.70^{a}
HGB (g/dL)	14.96±0.93^{b}	13.18±0.98^{a}	14.03±1.05^{ab}
PCV (%)	42.96±3.24^{b}	37.73±2.60^{a}	38.30±2.87^{a}
PLT (x10^{9}/L)	952.00±91.73^{c}	711.00±113.95^{a}	730.00±44.61^{b}
Neu (%)	55.72±5.80^{b}	27.03±10.01^{a}	28.50±9.37^{a}
LYM (%)	38.69±6.93^{a}	57.50±10.13^{b}	61.87±3.93^{b}
Mon (%)	3.20±1.01^{a}	27.68±15.48^{c}	8.20±5.89^{b}
Eos (%)	2.50±0.91^{b}	1.58±1.28^{ab}	1.13±0.53^{a}
Bas (%)	0.46±0.03^{a}	10.28±0.91^{b}	0.35±0.10^{a}
Lym (x10^{9}/L)	4.48±1.01^{b}	2.95±1.52a	3.30±0.99^{a}
Mon (x10^{9}/L)	0.35±0.12^{a}	5.56±1.30c	0.60±0.48^{b}
Eos (x10^{9}/L)	0.27±0.10^{b}	1.03±0.81c	0.04±0.02^{a}
Bas (x10^{9}/L)	0.06±0.01^{b}	0.33±0.21c	0.01±0.00^{a}

MCV (fL)	47.62±0.40[b]	0.04±0.02a	48.47±0.76[b]
MCH (pg)	16.84±0.17[a]	46.45±0.79b	17.77±0.30[a]
MCHC (g/dL	35.40±0.54[b]	16.23±0.35a	36.63±0.23[b]
MPV (fL)	6.14±0.17[a]	29.90±5.14b	5.97±0.13[a]

Where btw, body weight; $\bar{x}$, mean; SEM, standard error of means; n, number of mice in each group; a, b and c, statistically significant difference ($p<0.05$).

The results of changes in haematological parameters in mice following the oral administration of the crude aqueous extract of *Achyranthes aspera* shoot are presented in Table 3. No abnormalities were observed in any of the haematological parameters assessed. However, an increase in white blood cell count (WBC), packed cell volume (PCV), and platelet count was observed in mice treated with a lower extract dose (2000 mg/kg body weight).

Haematological parameters, such as red blood cell (RBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC) are valuable in diagnosing anaemia (Sarma, 1990; Tietze, 2011). In this study, the aqueous shoot extract of *A. aspera* caused no significant changes in MCV or MCH compared to the control group. However, there was a significant ($p < 0.05$) decrease in MCHC at the higher dose (5000 mg/kg).

The observed increase in WBC and RBC counts suggests that the extract contains phytochemicals capable of enhancing immunity, as previously reported (Chakrabarty and Vasudeva, 2009; Matthew *et al*., 2020). In botanical toxicity studies, an increase in WBC often indicates an immune-stimulating effect of the plant extract (Tousson *et al*., 2011). Most importantly, the increase in WBC was not abnormally high, suggesting that the *A. aspera* extract does not induce leukocytosis or suppress normal WBC production (Weingand *et al*., 1996). These findings align with earlier studies reporting no significant changes in WBC levels across different groups compared to controls (Cleveland Clinic, 2017; Reddy and Kanole, 2014).

Regarding platelet count, thrombocytopenia characterized by abnormally low platelet levels can result from decreased production or increased destruction of platelets (Tousson *et al*., 2011). Conversely, thrombocytosis involves an abnormal increase in platelet count (Cleveland Clinic, 2017). In this study, platelet count changes were not significant ($p > 0.05$) at the higher dose but showed a significant ($p < 0.05$) increase at the lower dose (2000 mg/kg body weight). This modulation of platelet count suggests that the extract may enhance clotting and prevent haemorrhage without causing coagulation issues (Yakubu *et al*., 2017). Previous studies support the haemostatic potential of *A. aspera* by demonstrating its ability to reduce bleeding and clotting times (Okon *et al*., 2015). Therefore, the observed haemostatic activity could be attributed to phytochemicals such as tannins and alkaloids present in the extract (Trease and Evans, 1989). The insignificant changes in blood parameters at higher doses align with findings by Reddy and Kamble (2014), where only minor, non-significant alterations in blood components were noted.

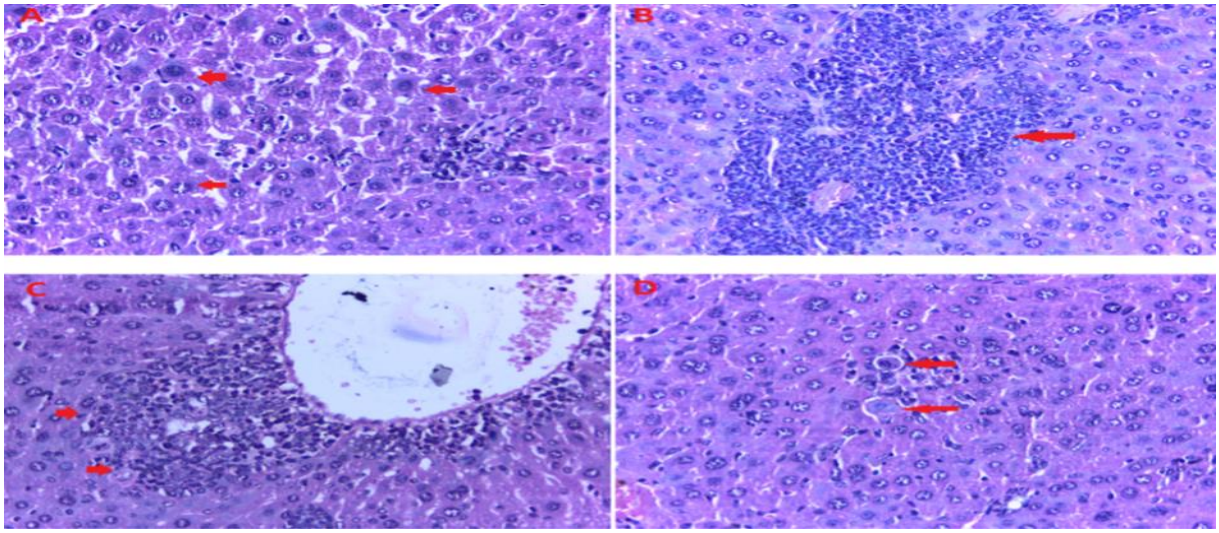

Figure 1. Photomicrograph of Liver section treated with 2000 mg/kg body weight crude aqueous extracts of *A. aspera* (H&E stain, magnification x40)

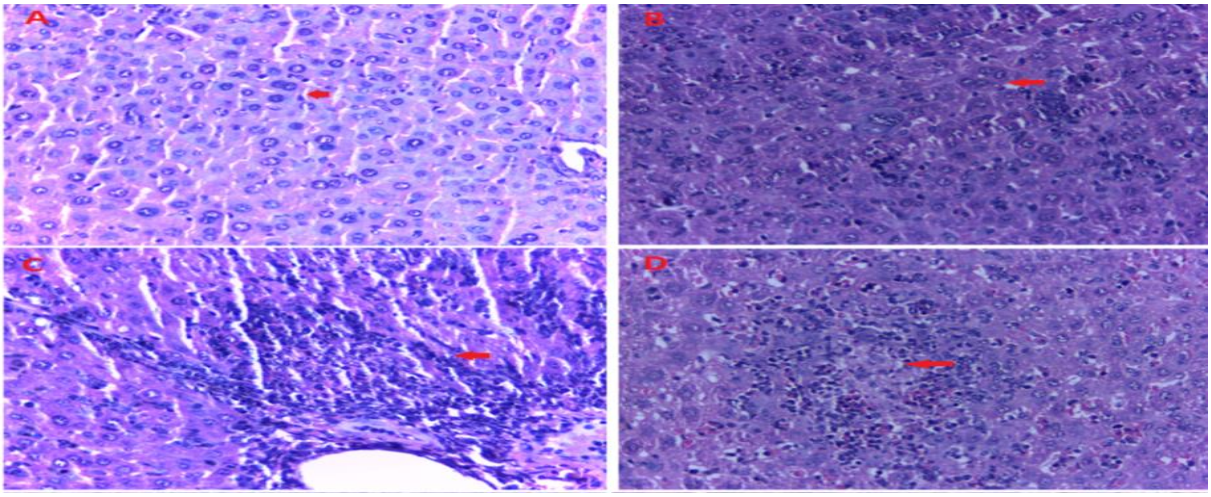

Figure 2. Photomicrograph of Liver section treated with, 5000 mg/kg body weight crude aqueous extracts of *A. aspera* (H&E stain, magnification x40)

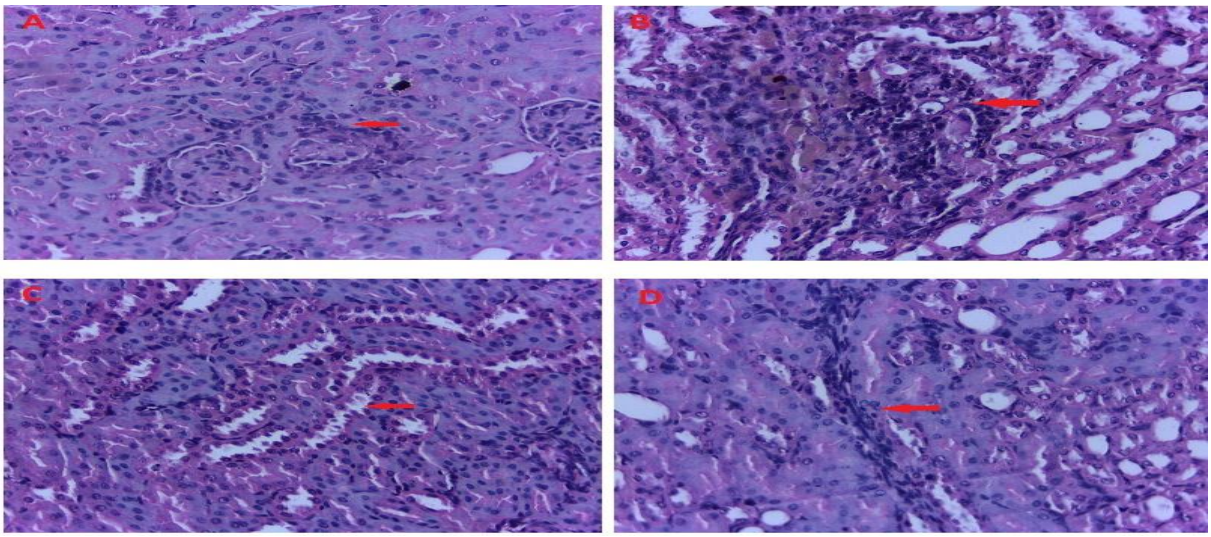

Figure 3. Photomicrograph of Kidney section treated with 2000 mg/kg body wt. crude aqueous extracts of *A. aspera* (H&E stain, magnification x40)

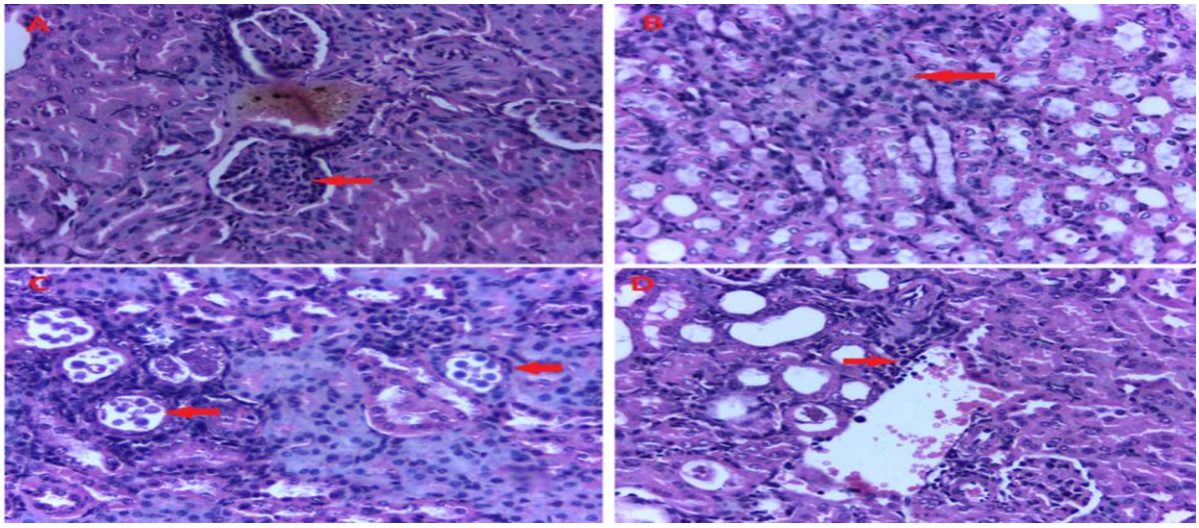

Figure 4. Photomicrograph of Kidney section treated with 5000 mg/kg body wt. crude aqueous extracts of *A. aspera* (H&E stain, magnification x40)

The results of the histological examination of the liver and kidney are presented in Figures 1, 2, 3 and 4. Occasional slight nuclear enlargement and coarse granulation, several groups of inflammatory cells and massive influx around the portal area and parenchyma and apoptotic cells were observed in the liver tissue of mice administered both extract doses (Figure 1 and 2). Inflammatory cell infiltrate at both the cortex and medulla and some tubules shows a slight level of degeneration (Figure 3 & 4). Therefore, the inflammations and other related alterations in hepatic and renal issues could be due to the impurity of the plant extract (Plaskova and Mlcek, 2023).

CONCLUSION

In conclusion, the aqueous shoot extract of *Achyranthes aspera* demonstrated significant immunomodulatory and hepatoprotective effects in mice, particularly at lower doses, without causing notable toxicity. The extract enhanced white blood cell and platelet counts, suggesting immune-boosting and haemostatic potentials, while maintaining liver integrity as indicated by reduced ALT levels. These findings support the safety and therapeutic potential of *A. aspera* shoots, although further studies are needed to fully understand its long-term effects and mechanisms of action.

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LIST OF ABBREVIATIONS

ALB, Albumin; ALT, Alanine transaminase; CR, Creatinine; H&E, Haemoglobin and Eosine stain; HB, Haemoglobin; LD50, Median Lethal Dose; MCH, Mean Corpsular Haemoglobin; MCHV, Mean Corpsular Haemoglobin Volume; MCV, Mean Corpsular Volume; RBC, Red Blood Cells; WBC, White Blood Cells

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