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Acute and 28-day toxicity evaluation of hydroethanolic extract of *Eragrostis tremula* (Poaceae) in Wistar rats

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Abstract
Eragrostis tremula is utilized in Nigeria and other African countries as a memory enhancer, lactation stimulant, anti-emetic, aphrodisiac and remedy for snake bites. Scientific reports revealed that this plant has anti-oxidant, anticholinesterase, cytotoxic and memory enhancing properties; despite these potentials, there is paucity of safety data to support its continued usage. This study investigated the toxicity profile of hydroethanolic extract of *E. tremula* (HEET) in Wistar rats. Acute and sub-acute (28 days at doses of 250, 500 and 1000 mg/kg *per oral*) toxicity tests were conducted using OECD 425 and 407 guidelines respectively. Limit test at 5,000 mg/kg was conducted to ascertain the midpoint lethal dose (LD_{50}). The LD_{50} of HEET was above 5000 mg/kg *per oral* and no significant alterations in haematological parameters and tissue morphology were observed. In the sub-acute study, no death was recorded. There were no changes in food intake, organ/body weight, lipid profile, liver and renal function indices. However, histology of the kidneys showed slight tubular adhesion and glomerular necrosis. HEET was practically safe after acute administration with no practical deleterious effect at 250 mg/kg after prolonged administration in rats. However, 28 days administration of high doses caused slight kidney injury.

Keywords: *Eragrostis tremula*; Acute toxicity; Sub-acute toxicity; Safety assessment, NOAEL

INTRODUCTION

Drug safety is a major standpoint that plays an important role in patients' health [1] and is characterized by the potential of drugs to cause adverse effects in relation to their usage [2]. Evaluation of drug safety is thus an indispensable aspect of the overall drug discovery and development processes and involves stepwise toxicological screening tests [3-5]. Preclinical toxicity investigations are conducted to determine the adverse effects associated with new drug molecules on vital functions before first use in humans. This includes evaluation of optimal route of administration, dosage, organs, species and age-peculiar harmful effects of an experimental drug molecule. Furthermore, the dose-reaction association as well as the no-observed adverse effect level (NOAEL) of the test substance can be determined [2]. In essence, data obtained from preclinical toxicity studies are important in assessing the safety

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and potential benefit of a candidate molecule [6].

Medicinal plants and their products are widely used globally to improve health, quality of life and to prevent and treat many human diseases [7,8]. This is because they are a rich source of compounds with diverse pharmacological activities and presumed to have less adverse effects compared to orthodox therapies [2,9]. Indeed, researches have uncovered widespread use of herbal remedies for prophylaxis and cure to many diseases over the years [10,11]. Although some of these remedies are efficacious and continuously being utilized, a great number of them are yet to be verified and their use is not adequately regulated. Above all, these herbal remedies are given without appropriate dosage monitoring or reflection of the harmful effects that may possibly arise from their long-term use [12]. The prevalent increase and incorporation of herbal products into the conventional medical practice provides justification to conduct detailed toxicological analyses of such products [11,13]. Moreover, plants also have a wide range of phyto-constituents that may be beneficial or detrimental to humans [14-16]. Toxicological studies have therefore, become essential because of the surge in their consumption and long-term use [17-21].

Eragrostis tremula Hochst. ex. Steud. is a yearly grass belonging to the family Poaceae that is utilized in African traditional medicine as a memory enhancer, lactation stimulant, anti-emetic, aphrodisiac and remedy for snake envenomation [22,23]. In addition, it is used to treat cognitive impairment and amnesia [24]. Previous investigations revealed that the whole plant extract of *E. tremula* has a median lethal dose (LD_{50}) of more than 5,000 mg/kg in mice [24,25]. The whole plant extract was also reported to possess anti-oxidant, anticholinesterase and anti-inflammatory properties [24,25]. The root extract has been reported to possess cytotoxic activity against MCF-7 human breast cancer cells [26], while the whole plant extract was reported to possess anti-amnesic activity [27]. Due to repeated and popular use of *E. tremula* extract, evaluation of its long-term toxicological profile becomes absolutely imperative. The aim of this research therefore, was to appraise the toxicity profile of hydroethanolic extract of *E. tremula* through acute and 28-days toxicity studies in rats.

EXPERIMENTAL METHODS

Laboratory animals. Non-pregnant and nulliparous female and male Wistar rats (190-200 g) were purchased from the Department of Pharmacology and Therapeutics, Ahmadu Bello University (A.B.U.), Zaria. They were housed in regular rodent cages under normal day and night rotation, with unrestricted access to use food and water. The research was conducted with the approval of A.B.U. Animal Use and Care Committee after the issuance of the ethical authorization (ABUCAUC/2020/68).

Plant collection and identification. The whole plant of *E. tremula* was collected from Batagarawa, Katsina State, Nigeria in October 2020. It was validated by a botanist at the Department of Botany, A.B.U., Zaria, and issued a voucher specimen number (900729) following comparison with a reference specimen.

Plant extraction. The fresh plant material was air-dried to a steady weight and then pulverized using pestle and mortar. The crushed plant (900 g) was put to cold maceration in a 10 L container and occasional shaking for 14 days using 5 L of ethanol (70% $^{v}/_{v}$). The macerate obtained was condensed using a vaporizer (R-111 Buchi Rotavapor, Fisher Scientific, England) and weighed. The yield of the extract was calculated and then transferred to an airtight plastic container and labeled as hydroethanolic extract of *E. tremula* (HEET). It was then deposited in a desiccator pending use.

Extract formulation. A stock solution of HEET was constituted with distilled water, then put through sequential thinning to attain the appropriate strengths needed for the experiments. The mixtures were freshly prepared daily throughout the research period to maintain stability and administered orally using a bulb tipped feeding cannula.

Phytochemical screening. The plant extract was subjected to phytochemical screening to confirm the presence of phytochemicals like alkaloids, anthraquinones, glycosides, flavonoids, saponins, triterpenes and tannins [28].

Acute toxicity study. The plant extract was subjected to oral acute toxicity study as reported by the Organization for Economic Co-operation and Development (OECD, 425) [29] using two groups of 5 female rats each. The 1st group (control) received 1 mL/kg of distilled water, whereas the 2nd group of rats (test group) received HEET (5, 000 mg/kg) orally using 18 gauge bulb tipped feeding cannula. The animals were starved over the night and weighed before the extract was administered. They were further starved of food for 3-4 hours, along with careful observation for the first 30 minutes post administration and during the first 24 hours and then daily for two weeks. The LD_{50} was subsequently estimated. Observations made included changes in behaviour pattern, eyes, mucous membrane, skin, fur, somatic and autonomic activities. Weekly body weight of the animals was taken and after 14 days' inspection, they were euthanized with mild chloroform anesthesia. Samples of blood and organs (livers, kidneys and hearts) were collected for analysis of haematological and histopathological examination respectively.

Experimental design for 28-day toxicity study. Forty rats consisting of 20 males and 20 nulliparous and non-pregnant females were used for the study. For either sex, the rats were randomly divided into four groups (n = 5 for males and n = 5 for females) and kept in separate cages. Group 1 rats served as control group and were given distilled water (1 mL/kg, orally). Rats in groups 2, 3 and 4 served as test groups and were given HEET (250, 500 and 1000 mg/kg, per oral respectively) daily for 4 weeks. These doses were selected with reference to the LD_{50} profile of HEET.

Repeated dose 28-day toxicity study. This study was done following OECD 407 guidelines for 28 days [30]. The grouped animals (5 females and 5 males at each dose level) were deprived of food over the night and administered HEET daily for 4 weeks as described in the experimental design. In the course of the experiment, the general behaviour, mortality, food and water intake of the rats were recorded. On the 29th day of the study, the rats were euthanized and blood samples and organs were harvested for further evaluation.

Evaluation of relative organ weight. The brains, lungs, kidneys, livers, hearts, stomachs, pancreases, testes and ovaries of the rats were excised after blood collection. The harvested organs were cropped to remove surrounding tissues and the relationship below was used to compute the relative organ weight:

$$\text{Relative organ weight (\%)} = \frac{\text{Weight of organ (g)}}{\text{Final body weight of rat (g)}} \times 100$$

Analysis of haematological parameters. Blood samples were collected in bottles containing ethylene diamine tetra-acetic acid (EDTA) to avoid blood clotting, and the values of white blood cell (WBC), lymphocytes, haemoglobin, haematocrit (HCT), granulocytes, red blood cells (RBC) and platelets were determined with the aid of an automated haematology machine (Cell-Dyn Emerald 22, Abbott, USA).

Analysis of biochemical parameters and electrolytes. Samples of sera were obtained and subjected to biochemical analysis [31]. The levels of alkaline phosphatase (ALP),

aspartate amino transferase (AST), alanine amino transferase (ALT), albumin, total protein, creatinine and urea were calculated. The levels of sodium, potassium, chloride, bicarbonate, triglycerides, cholesterol, low- and high-density lipoproteins were also calculated using Hitachi 902 analyzer (Roche, Germany).

Histological examination. Histopathological examination of the stomach, liver, kidney, brain, testes and ovaries were done by fixing the organs in 10% formalin solution. The organs were prepared using ethanol and paraffin wax for embedding. Thin sections (5µm) of the organs were made with the aid of a microtome and dewaxed using xylene. Thereafter, they were placed on a microscope slide and stained with haematoxylin-eosin [32].

Statistical analysis. The differences between means where two groups are involved were subjected to independent sample t-test. One-way analysis of variance (ANOVA) and Bonferroni's tests were carried out for data with more than two groups. Variables obtained over a period were analyzed using repeated measures ANOVA and Bonferroni's tests. Variation between means were regarded as statistically significant at $p \leq 0.05$, and the mean ± standard error of mean (S.E.M.) were used for presentation of the results. The analyses were done using Statistical Package for Social Sciences software (Version 20).

RESULTS

Extractive yield. The maceration of 900 g of pulverized *E. tremula* gave 93.5 g of HEET. The yield was computed as 10.4% $^{w}/_{w}$.

Phytochemical constituents. Phytochemical assessment of HEET showed the presence of tannins, steroids, triterpenes, alkaloids, saponins and flavonoids, while anthraquinones were absent.

Acute toxicity study. The LD_{50} of HEET was assessed to be >5,000 mg/kg in rats following oral administration. Acute administration of HEET (5,000 mg/kg) did not cause behavioural changes associated with toxicity or death during the two weeks' observation time. This dose did not produce notable changes in the average body weight of rats over the 14 days observation period in relation to distilled water-administered group (Figure 1) neither were there significant changes (p>0.05) in the comparative organ weights of the hearts, livers and kidneys of HEET-administered rats relative to control (Figure 2). Similarly, the extract at this dose did not cause major changes (p>0.05) in the WBC, RBC, lymphocytes, mid cells, haemoglobin, haematocrit and platelet counts relative to control (Table 1). Histological examination of the kidney and heart sections showed normal features, however, slight hypnotic nuclei of the liver sections were seen (Figure 3).

Sub-acute toxicity study

Behavioural observations and mortality. The repeated administration of HEET (250, 500 and 1000 mg/kg) to rats of both sexes did not result to noticeable behavioural changes nor indications of injury or mortality during the course of inspection. The food and water intake of both the male and female rats that received HEET at all the tested doses were not substantially different from the control (Figures 4 and 5).

The body weights of rats that received HEET did not vary appreciably in relation to control. On assessment over the study period, the mean body weights of the male and female rats increased (p<0.05) on days 7, 14, 21 and 28 relative to the day 1 (Figure 6A and 6B). The administration of HEET did not cause significant increase or decrease in the relative organ weight of both the male and female rats relative to control (Table 2).

The repeated intake of 500 mg/kg of HEET significantly increased (p<0.05) lymphocyte and granulocyte levels of the male rats relative to control. On the other hand, such variations were not seen with the rats that

received HEET at 250 and 1000 mg/kg. In the female rats, the intake of HEET at 250 and 500 mg/kg appreciably ($p<0.01$ and $p<0.05$ respectively) increased the platelet counts (Table 3).

The repeated intake of HEET did not cause major variations in ALT, ALP and albumin levels of the male rats. However, an appreciable ($p<0.05$ and $p<0.001$) decrease in AST and a corresponding increase in total protein levels respectively, were noticed with the male rats that received 250 mg/kg of HEET. In the female rats, HEET (at all dose levels) did not cause major changes ($p>0.05$) in ALT, AST, ALP, total protein and albumin levels (Table 4).

The creatinine and urea levels of both male and female rats that received HEET did not change. Similarly, there were no major changes in the serum levels of sodium, potassium, chloride and bicarbonate of both rats (Table 5).

The 28 days' daily intake of HEET did not produce prominent deviations ($p>0.05$) in lipid profile of both the male and female rats relative to control (Figure 7).

Histological examination of sections of the stomach, liver, brain and testis of the male rats showed normal features after the 28 days repeated administration of HEET. However, tubular adhesion was observed with kidney sections in male rats that received 500 mg/kg of the extract (Figure 8). Examination of the sections of stomach, liver, brain and ovary of the female rats showed normal features after administration of the extract. On the other hand, the kidney revealed slight glomerular necrosis and lymphocyte infiltration at 500 and 1000 mg/kg of HEET-administered groups respectively (Figure 9).

DISCUSSION

Medicinal plants utilized in conventional medicine are known to possess various secondary metabolites that may be responsible for their therapeutic effects [33,34]. On the same note, some of these plants and their constituents are reported to be toxic [35,36]. In this study, the phyto-constituents found present in HEET revealed important secondary metabolites which corroborates the results of previous reports [24]. These constituents are known to possess important pharmacological activities [27]. However, they are not free of adverse effects.

In the acute toxicity study, neither visible symptoms of injury nor death were observed. Also, other investigations did not reveal substantial pathological changes. These results were similar with previous findings [27] which reported that *E. tremula* extract had an oral LD_{50} of more than 5,000 mg/kg in mice, thus showing its non-toxic nature after acute administration. Based on classification of LD_{50} of chemicals substances [37], HEET was regarded to be essentially non-lethal in rats after acute oral intake.

Variation in body weight is a measure of general health state of animals and its improvement or reduction is a sign of toxic effects of drugs or chemicals [38,39]. In the sub-acute study, repeated intake of HEET did not lower the body weight of both the male and female rats, suggesting it does not exert harmful upshot on their growth. The body weight improvement as observed over time indicated that the extract did not impede food consumption. On the other hand, the extract could possibly contain phytochemicals with appetite stimulating effect. Furthermore, HEET did not produce considerable changes in the relative organ weights and could therefore be regarded as non-toxic since reduction in organ weight is an important indicator of toxicity [40].

Hematopoietic complex is a vulnerable target for harmful substances, particularly in the bone marrow where RBCs are produced. Haematological parameters are frequently used markers of toxicity due to the interplay of a toxin and or its derivative with cellular structures [41,42].

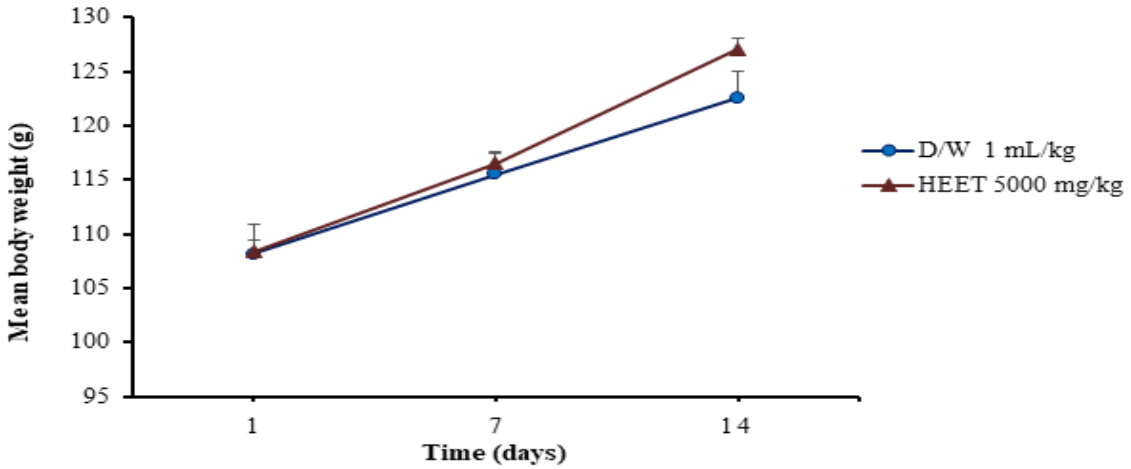

Figure 1. Effect of single dose (5000 mg/kg) administration of hydroethanolic extract of *Eragrostis tremula* on body weight. Values are Mean ± S.E.M; No significant difference as compared to D/W control - Independent sample t-test, n=5 D/W = Distilled water, HEET = Hydroethanolic extract of *Eragrostis tremula*

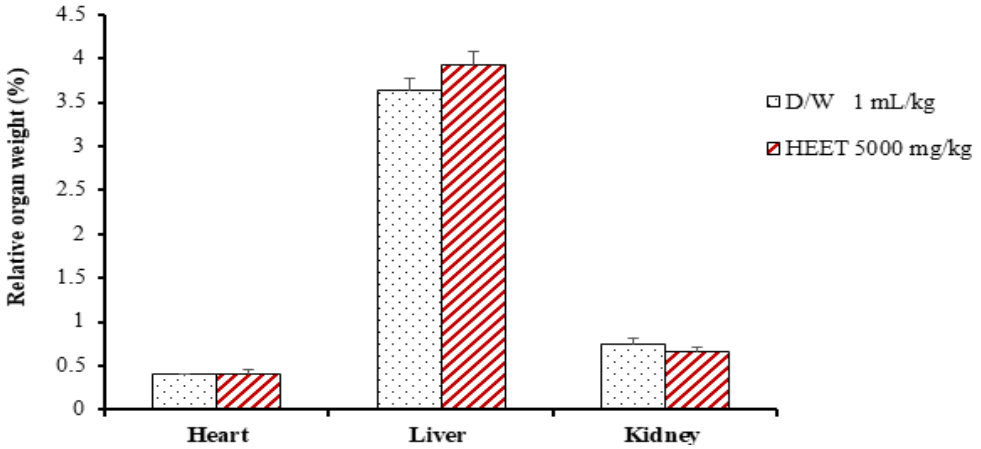

Figure 2. Effect of single dose (5000 mg/kg) administration of hydroethanolic extract of *Eragrostis tremula* on relative organ weight. Values are Mean ± S.E.M; No significant difference as compared to D/W control - Independent sample t-test, n=5 D/W = Distilled water, HEET = Hydroethanolic extract of *Eragrostis tremula*

Table 1: Effect of single dose (5000 mg/kg) administration of hydroethanolic extract of *Eragrostis tremula* on haematological parameters

Parameters	Units	Groups	
		D/W 1 mL/kg	HEET 5000 mg/kg
WBC	($\times10^3$/µL)	5.17±0.18	5.32±0.25
LYMP	(%)	49.13±1.51	50.24±6.22
GRAN	(%)	39.47±4.79	41.04±2.26
MID	(%)	11.40±1.98	8.72±1.32
RBC	($\times10^6$/µL)	4.90±0.05	4.75±0.07
HGB	(g/dL)	14.17±0.38	14.42±0.56
HCT	(%)	42.40±1.16	43.56±1.51
PLT	($\times10^3$/µL)	199.67±5.84	222.16±23.91

D/W = Distilled water, HEET = Hydroethanolic extract of *Eragrostis tremula*, WBC= White blood cells, LYMP = Lymphocytes, GRAN = Granulocytes, MID = Mid cells, RBC=Red blood cells, HGB = Haemoglobin, HCT = Haematocrit, PLT = Platelets

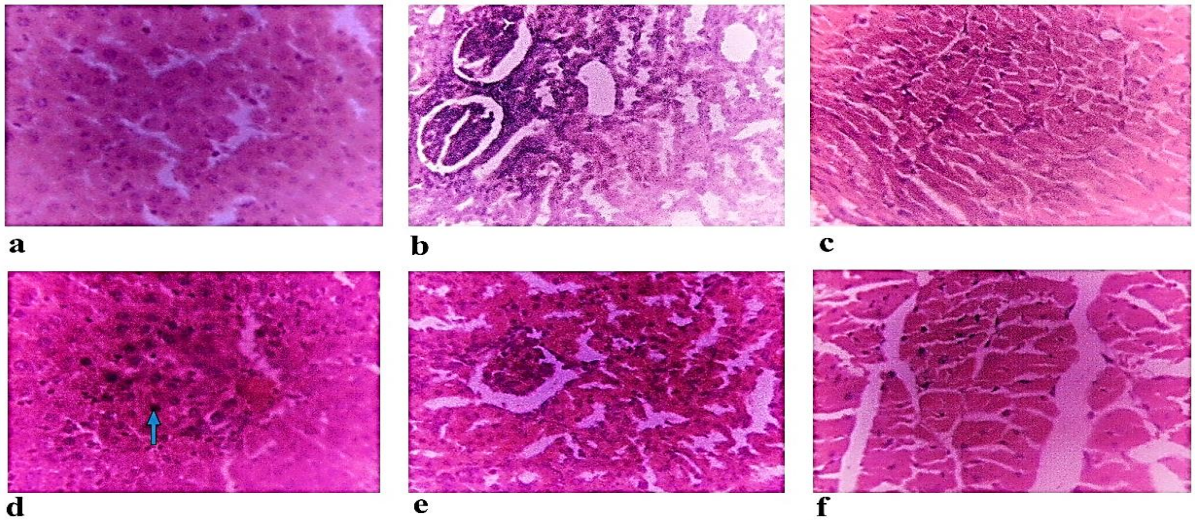

Figure 3. Photomicrographs of sections of liver, kidney and heart of rats following single dose (5000 mg/kg) administration of hydroethanolic extract of *Eragrostis tremula*. **a**: Liver section of control group showing normal hepatocytes, **b**: Kidney of control group showing normal tubules and glomerulus. **c**: Heart of control group showing normal features. **d**: HEET (5,000 mg/kg) group showing hypnotic nuclei (blue arrow), **e**: HEET (5000 mg/kg) group showing normal features of the kidney, **f**: HEET (5000 mg/kg) group showing normal cardiac cells (H&E × 250)

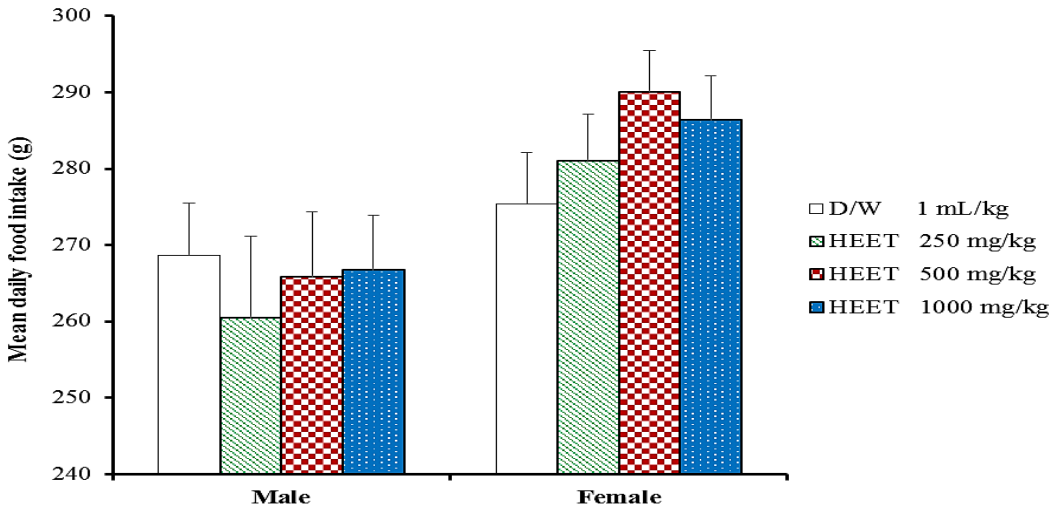

Figure 4. Effect of 28 days daily administration of hydroethanolic extract of *Eragrostis tremula* on food intake. Values are Mean ± S.E.M; No significant differences when compared to D/W group – One way ANOVA followed by Bonferroni's post hoc test, n=5 for each species D/W = Distilled water, HEET = Hydroethanolic extract of *Eragrostis tremula*

An increase in neutrophil (granulocytes) and lymphocyte counts arise under varying conditions, usually associated with stress, unprompted inflammatory reactions or drug-induced injury [43]. Neutrophils are the usual kind of WBCs that build up as a result of bacterial diseases, while lymphocytes can rise in instances of viral diseases [44]. From our findings, a remarkable rise in lymphocyte counts was seen with the male rats that received 500 mg/kg of HEET, which depicts likelihood of stress related to the group. Nonetheless, such variations were not seen with the other male groups as well as the female rats. A significant increase in platelet counts was noticed with the female rats. Considering the role of platelets in coagulation system, the rise in its quantity in certain

situations depicts a toxicological outcome via the development of thrombosis in blood vessels. On the other hand, its reduction may give rise to deleterious clinical consequences like a possibility of bleeding [45,46]. The variation in platelet counts observed with the different sexes can be interconnected hormonally, as estrogens are strong stimulators of coagulation factors synthesis in the liver [47]. The significant rise in platelet counts may also be due to infection or iron deficiency amongst other causes.

The hepatic and renal systems play important roles in drug detoxification [38]. The liver is the major organ for xenobiotic transformation and the site of formation of drug metabolites. A rise in the quantity of liver enzymes is typically a sign of liver injury [31,48].

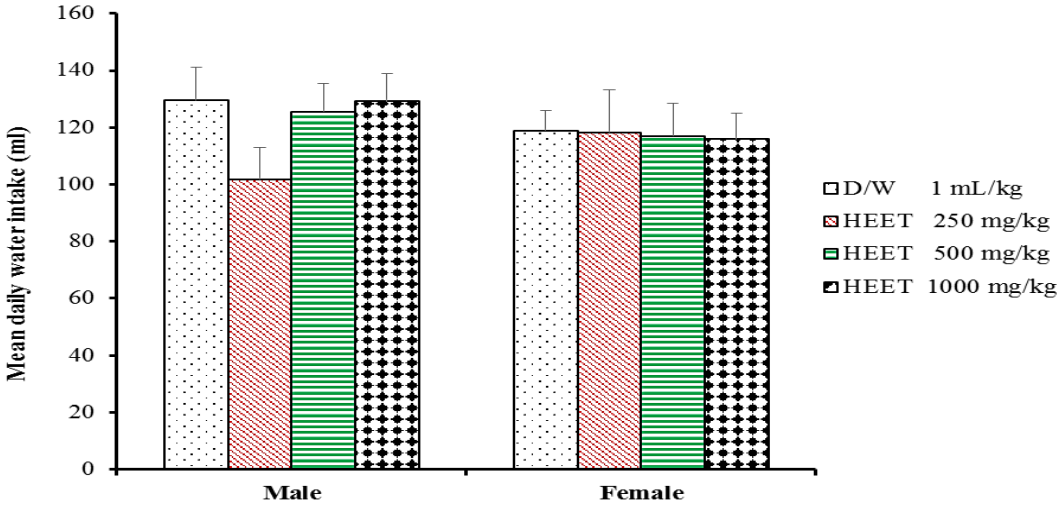

Figure 5. Effect of 28 days daily administration of hydroethanolic extract of *Eragrostis tremula* on water intake. Values are Mean ± S.E.M; No significant differences when compared to D/W group – One way ANOVA followed by Bonferroni's post hoc test, n=5 for each species D/W = Distilled water, HEET = Hydroethanolic extract of *Eragrostis tremula*

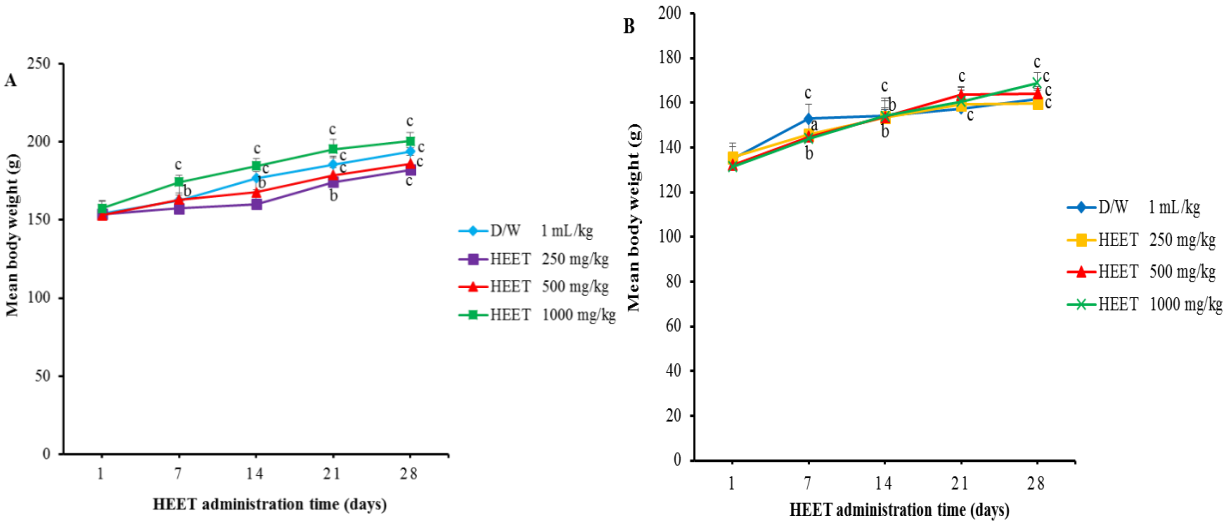

Figure 6. Effect of 28 days daily administration of hydroethanolic extract of *Eragrostis tremula* on body weights of male (A) and female (B) rats. Values are Mean ± S.E.M; [a] = $p<0.05$, [b] = $p<0.01$, [c] = $p<0.001$ when compared to Day 1- Repeated measure ANOVA followed by Bonferroni's post hoc test, n = 6 for each species D/W = Distilled water, HEET = Hydroethanolic extract of *Eragrostis tremula*

Table 2: Effect of 28 days daily administration of hydroethanolic extract of *Eragrostis tremula* on relative organ weight

Organ	Sex	Groups (mg/kg)			
		D/W 1 mL/kg	HEET 250	HEET 500	HEET 1000
Heart	Male	0.43±0.02	0.38±0.01	0.40±0.02	0.38±0.02
Liver	Male	3.64±0.19	3.55±0.15	3.40±0.13	3.66±0.12
Lungs	Male	0.88±0.09	1.03±0.10	0.93±0.14	1.09±0.18
Stomach	Male	0.72±0.01	0.65±0.04	0.74±0.05	0.62±0.01
Pancreas	Male	0.39±0.03	0.43±0.04	0.35±0.03	0.42±0.03
Kidneys	Male	0.65±0.02	0.67±0.03	0.64±0.01	0.64±0.02
Brain	Male	0.81±0.05	0.77±0.04	0.79±0.04	0.73±0.03
Testes	Male	1.26±0.04	1.21±0.03	1.34±0.09	1.15±0.05
Heart	Female	0.41±0.01	0.41±0.03	0.47±0.02	0.44±0.03
Liver	Female	3.72±0.06	3.59±0.17	3.98±0.15	3.83±0.08
Lungs	Female	1.22±0.13	1.13±0.11	1.16±0.15	1.19±0.10
Stomach	Female	0.70±0.03	0.69±0.02	0.78±0.03	0.74±0.02
Pancreas	Female	0.43±0.06	0.51±0.03	0.41±0.05	0.45±0.05
Kidneys	Female	0.65±0.01	0.66±0.03	0.66±0.02	0.67±0.03
Brain	Female	0.90±0.10	0.94±0.03	0.98±0.06	0.94±0.05
Ovaries	Female	0.08±0.00	0.08±0.01	0.09±0.01	0.08±0.00

Table 3: Effect of 28 days daily administration of hydroethanolic extract of *Eragrostis tremula* on haematological parameters

Parameters	Units	Sex	Groups (mg/kg)			
			D/W 1 mL/kg	HEET 250	HEET 500	HEET 1000
WBC	($\times10^3$/µL)	Male	6.44±0.40	6.60±0.61	4.56±0.37	6.36±0.50
LYMP	(%)	Male	35.80±1.98	41.48±2.93	59.20±5.98[b]	32.54±2.02
GRAN	(%)	Male	55.80±2.47	42.76±7.05	32.70±5.15[a]	57.18±1.79
MID	(%)	Male	8.40±0.36	15.76±1.26	8.10±1.02	10.28±0.94
RBC	($\times10^6$/µL)	Male	5.15±0.23	4.49±0.21	5.04±0.22	4.77±0.30
HGB	(g/dL)	Male	12.91±1.00	14.32±0.20	14.38±0.81	15.54±0.43
HCT	(%)	Male	40.17±3.36	43.58±1.79	42.60±2.52	47.98±2.13
PLT	($\times10^3$/µL)	Male	270.92±31.25	281.92±48.74	230.52±40.28	363.56±19.72
WBC	($\times10^3$/µL)	Female	6.52±0.37	5.42±0.54	6.78±0.71	4.64±0.93
LYMP	(%)	Female	40.28±6.71	41.42±5.91	49.84±4.60	36.96±5.07
GRAN	(%)	Female	44.64±1.52	46.94±6.42	42.30±5.22	43.50±3.06
MID	(%)	Female	15.08±0.61	11.64±0.82	7.86±0.77	19.54±1.87
RBC	($\times10^6$/µL)	Female	5.56±0.44	4.82±0.11	4.92±0.26	5.10±0.38
HGB	(g/dL)	Female	14.49±0.59	14.14±0.63	14.10±0.68	12.74±0.76
HCT	(%)	Female	43.34±1.66	44.64±1.80	40.93±1.14	38.96±2.24
PLT	($\times10^3$/µL)	Female	205.80±21.20	371.00±12.72[b]	405.40±28.28[b]	278.54±39.86

Values are Mean ± S.E.M; [a]=$p<0.05$, [b]=$p<0.01$ as compared to D/W group – One way ANOVA followed by Bonferroni's post hoc test, n=5 (Male or Female) D/W = Distilled water, HEET = Hydroethanolic extract of *Eragrostis tremula*, WBC= White blood cells, LYMP = Lymphocytes, GRAN = Granulocytes, RBC=Red blood cells, MID = Mid cells, HGB = Haemoglobin, HCT = Haematocrit, PLT = Platelets

Table 4: Effect of 28 days daily administration of hydroethanolic extract of *Eragrostis tremula* on liver function indices of rats

Groups (mg/kg)	Sex (M/F)	Parameters				
		ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Total protein (g/dL)	Albumin (g/dL)
D/W (1 mL/kg)	M	16.00±1.82	163.00±9.95	22.64±6.73	11.82±0.60	2.98±0.07
HEET (250)	M	11.20±1.59	115.20±9.39*	19.78±4.98	6.62±0.43**	2.90±0.06
HEET (500)	M	16.25±3.20	153.00±14.07	30.05±4.68	13.25±0.92	3.12±0.05
HEET (1000)	M	19.40±0.81	166.60±2.75	31.72±6.38	10.76±0.42	2.90±0.09
D/W (1 mL/kg)	F	17.20±2.15	129.20±4.52	29.90±5.39	11.06±0.60	3.00±0.12
HEET (250)	F	10.75±1.65	140.67±22.92	15.83±2.51	9.64±0.44	3.30±0.17
HEET (500)	F	13.00±1.22	129.50±16.08	17.83±0.80	10.88±0.73	3.00±0.12
HEET (1000)	F	21.00±1.58	157.00±5.31	32.73±5.90	10.70±0.40	3.02±0.08

Values are Mean ± S.E.M. * =p<0.05, ** =p<0.001 compared to D/W group – One way ANOVA followed by Bonferroni's post hoc test, n=5 (Male or female) D/W = Distilled water, HEET = Hydroethanolic extract of Eragrostis tremula, ALT= Alanine amino transferase, AST = Aspartate amino transferase, ALP = Alkaline phosphatase

Table 5: Effect of 28 days daily administration of hydroethanolic extract of *Eragrostis tremula* on renal function indices and electrolytes

Groups (mg/kg)	Sex (M/F)	Creatinine (µmol/L)	Urea (mg/dL)	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Bicarbonate (mmol/L)
D/W (1 mL/kg)	M	0.80±0.05	36.16±1.58	171.98±3.76	7.56±0.38	30.00±1.48	92.60±2.32
HEET (250)	M	0.98±0.19	42.76±2.72	178.28±2.43	7.50±0.99	35.40±1.63	89.80±5.00
HEET (500)	M	0.88±0.14	39.10±5.48	180.30±0.87	9.84±1.12	34.60±3.32	100.80±4.57
HEET (1000)	M	0.92±0.11	36.58±2.65	180.38±0.45	9.80±0.45	28.60±2.46	98.20±4.54
D/W (1 mL/kg)	F	0.78±0.05	34.66±2.08	189.32±2.46	8.58±0.83	30.00±1.48	92.40±3.68
HEET (250)	F	0.96±0.07	28.34±1.27	183.82±2.17	7.00±0.33	32.20±1.62	84.20±3.94
HEET (500)	F	0.92±0.05	36.44±1.98	184.74±1.42	11.26±0.47	30.80±2.43	78.60±3.75
HEET (1000)	F	0.86±0.05	28.68±1.46	188.96±3.39	10.62±0.98	32.00±2.02	93.40±6.27

Values are Mean ± S.E.M., No significant difference compared to D/W group – One way ANOVA followed by Bonferroni's post hoc test, n=5 (Male or female) D/W = Distilled water, HEET = Hydroethanolic extract of *Eragrostis tremula*

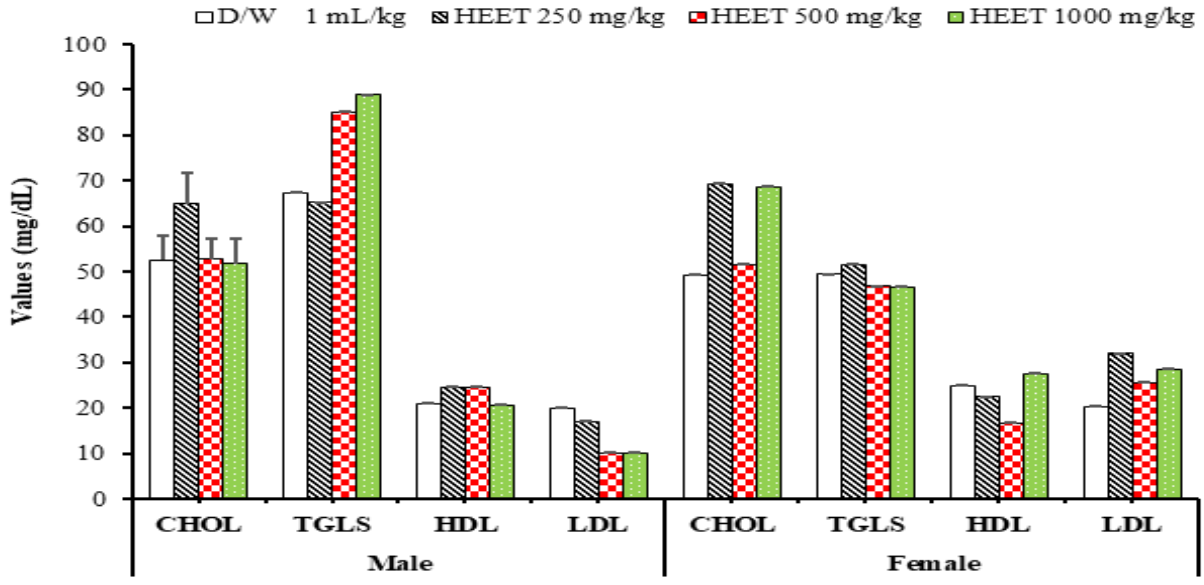

Figure 7. Effect of 28 days daily administration of hydroethanolic extract of *Eragrostis tremula* on serum lipid profile. Values are Mean ± S.E.M; No significant difference as compared to D/W group – One way ANOVA, n=5 D/W = Distilled water, HEET = Hydroethanolic extract of *Eragrostis tremula*, CHOL = Cholesterol, TGLS = Triglycerides, HDL = High density lipoprotein, LDL = Low density lipoprotein

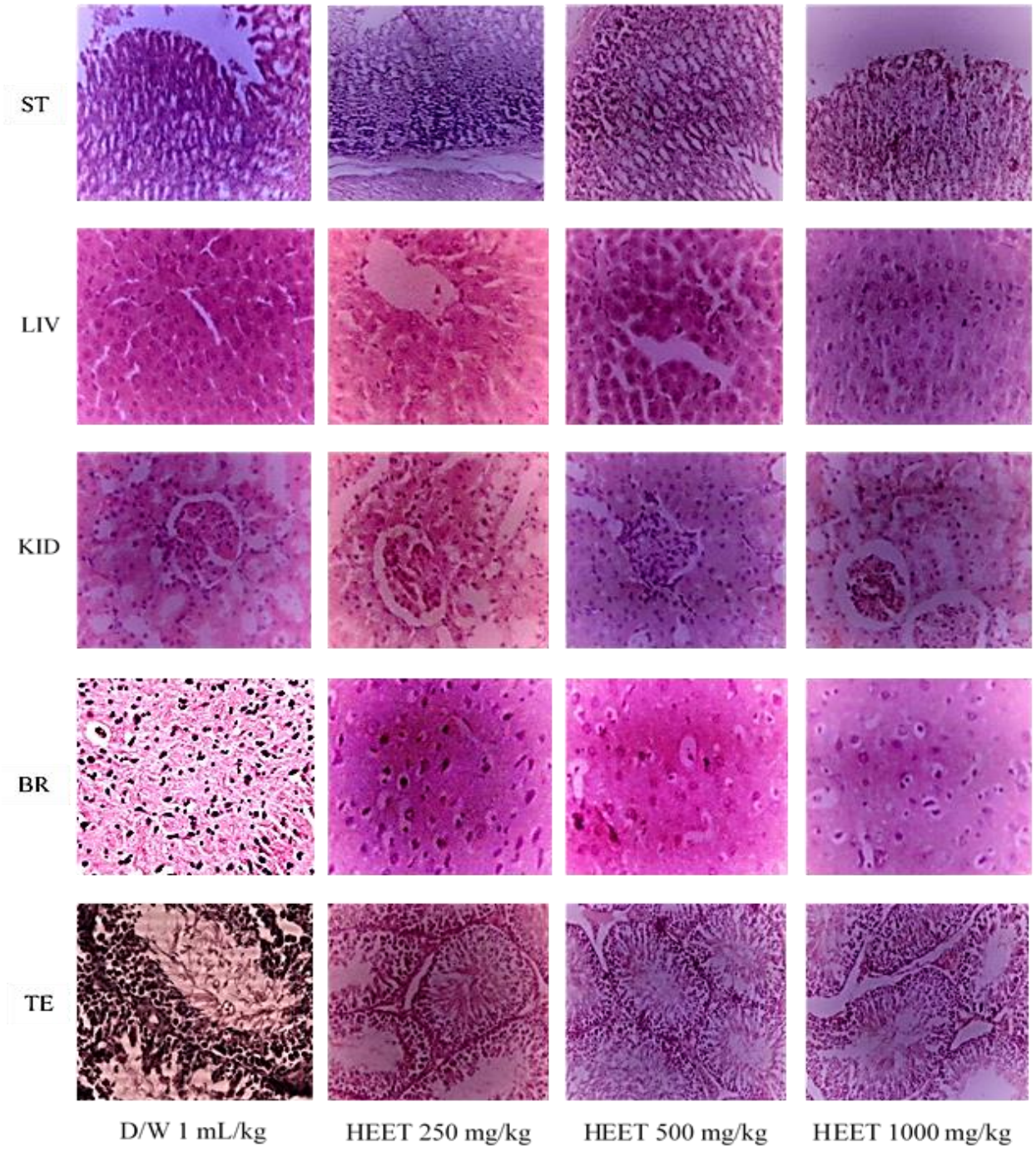

Figure 8. Photomicrographs of sections of stomach, liver, kidney, brain and testis of male rats following 28 days daily oral administration of hydroethanolic extract of *Eragrostis tremula*. ST = Stomach showing normal mucosal cells; LIV = liver showing normal hepatocytes; KID = kidney showing tubular adhesion (white arrow); BR = brain showing normal features; TE = testes showing normal spermatogenic cells (H & E × 250)

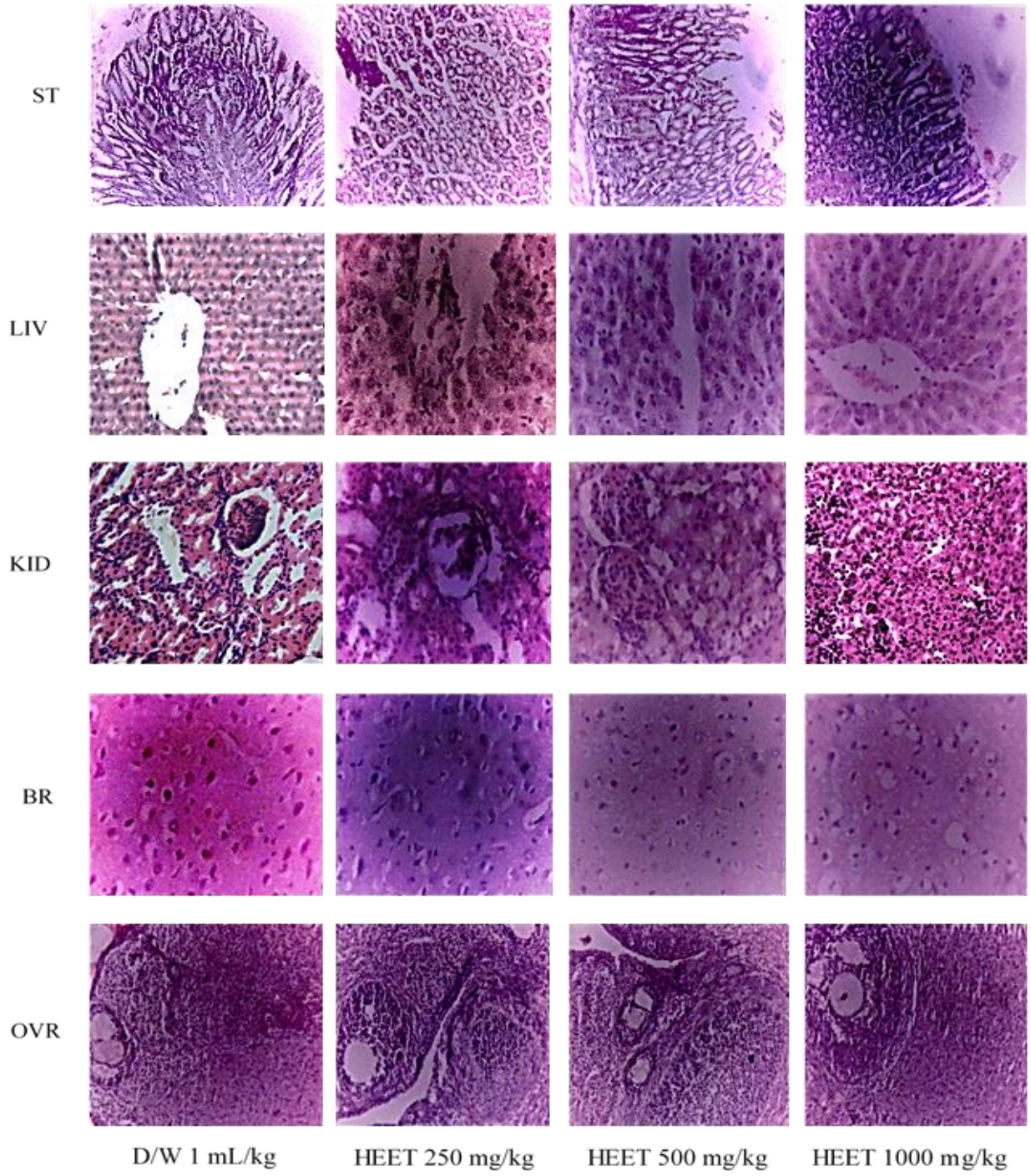

Figure 9. Photomicrographs of sections of stomach, liver, kidney, brain and ovary of female rats following 28 days daily oral administration of hydroethanolic extract of *Eragrostis tremula*. ST = stomach showing normal mucosal cells, LIV = liver showing normal hepatocytes; KID = kidney showing slight glomerular necrosis (red arrow), and lymphocyte infiltration (blue arrow); BR= brain showing normal features; OVR = ovary showing normal cells (H & E ×250)

The administration of HEET to rats of both sexes did not cause significant deviations in the levels of liver enzymes. This showed that the liver functions were not affected by HEET after 28 days daily administration. Histological examination of the liver also upholds the above findings as there were no lesions or significant changes to the liver histo-architecture in all the HEET-administered groups and in both sexes.

Creatinine and urea are key indicators of kidney function that are utilized in the diagnosis of kidney disease or examination of drug treatment outcomes [49-51]. Creatinine is a component of urine and the final product of creatine phosphate catabolism in muscles. High level of creatinine may be a sign of malfunction of nephrons, which implies renal failure [50,52]. In this study, the creatinine levels in both the male and female rats were not altered, which suggests the administration of HEET did not affect their renal function. Urea on the other hand is the major nitrogenous waste product produced from protein catabolism and excreted from the body more or less completely by the kidneys. It is utilized in the diagnosis of pre-renal cases where urea nitrogen to creatinine fraction is raised especially in acute kidney malfunction. However, it is not an efficient indicator of glomerular filtration rate because its excessive production is influenced by a number of factors including high protein diet, dehydration and urea cycle enzymes which are not directly related to renal function [53]. Histological assessment of the kidneys showed minor tubular adhesion in the male rats while slight glomerular necrosis and lymphocyte infiltration were seen in the female rats. In line with this observation, the administration of higher doses of HEET over a long period might pose slight adverse effects to the kidneys.

Results obtained from the lipid profile tests did not show significant changes in the quantities of triglycerides, high density lipoprotein, cholesterol, as well as low density lipoprotein after the 28 days daily administration of HEET. These parameters are essential markers for metabolic diseases like diabetes mellitus and coronary artery disease [54,55]. The absence of significant alterations on all the investigated lipid parameters suggests that the extract may not alter lipid metabolism or cause associated cardiovascular problems.

The sub-acute toxicity studies evaluated the effect of HEET on possible targets of toxicity such as the immune and endocrine systems. Generally, the extract was considered relatively safe in rats following the 28 days' repeated daily administration as there were no death or major pathological changes in most of the parameters examined. The NOAEL of the extract after sub-acute administration was thus resolved to be 250 mg/kg in rats.

Conclusions. The acute toxicity profile of HEET revealed that it is practically non-toxic in rats after single oral administration. Toxicity profile of the extract following 28 days daily oral intake revealed a no-observed adverse effect level at 250 mg/kg. Nevertheless, sub-acute administration of large doses of the extract caused slight histopathological lesions in the kidneys.

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REFERENCES

1. Alshammari TM. Drug safety: The concept, inception and its importance in patients' health. Saudi Pharm J. 2016;24(4):405–412. https://doi.org/10.1016/j.jsps.2014.04.008
2. Jeon WY, Seo CS, Ha H, Shin HK, Cho JW, Kim DH, Lee MY. Subchronic toxicological evaluation of *Bojungikki-tang* water extract: 13-Week oral repeated-dose toxicity study in Crl:CD (SD) rats. J

Ethnopharmacol. 2020;252:112551. https://doi.org/10.1016/j.jep.2020.112551.

3. Horii I. The principle of safety evaluation in medicinal drug - how can toxicology contribute to drug discovery and development as multidisciplinary science? J Toxicol Sci. 2016;41(Special): SP49–SP67. https://doi.org/10.2131/jts.41.SP49

4. Colerangle JB. Preclinical development of nononcogenic drugs (small and large molecules), in: Faqi, A.S., (Ed.). A comprehensive guide to toxicology in nonclinical drug development. Academic Press, U.S. 2017; pp659–683. https://doi.org/10.1016/B978-0-12-803620-4.00025-6

5. Pognan F, Beilmann M, Boonen HCM, Czich A, Dear G, Hewitt P, Mow T, Oinonen T, Roth A, Steger-Hartmann T, Valentin JP, Van Goethem F, Weaver RJ, Newham P. The evolving role of investigative toxicology in the pharmaceutical industry. Nat Rev Drug Discov. 2023;22(4):317–335. https://doi.org/10.1038/s41573-022-00633-x

6. Abubakar A, Nazifi AB, Abdulazeez J, Abubakar AZ, Maje IM, Tanko Y, Anuka JA, Abdurahman EM. Repeated dose 13-week oral toxicity evaluation of *Chlorophytum alismifolium* Baker methanol extract in Wistar rats. Comp Clin Pathol. 2023; https://doi.org/10.1007/s00580-023-03451-8

7. Akram M, Nawaz A. Effects of medicinal plants on Alzheimer's disease and memory deficits. Neural Regen Res. 2017;12(4):660–670. https://doi.org/10.4103/1673-5374.205108

8. Shaito A, Thuan DTB, Phu HT, Nguyen THD, Hasan H, Halabi S, Abdelhady S, Nasrallah GK, Eid AH, Pintus G. Herbal medicine for cardiovascular diseases: Efficacy, mechanism, and safety. Front Pharmacol. 2020;11:422. https://doi.org/10.3389/fphar.2020.0042

9. Chen Q, Zhu L, Tang Y, Zhao Z, Yi T, Chen H. Preparation-related structural diversity and medical potential in the treatment of diabetes mellitus with ginseng pectins. Ann N Y Acad Sci. 2017;1401(1): 75–89. https://doi.org/10.1111/nyas.13424

10. Aiello E, Russo R, Cristiano C, Calignano A. The safety assessment of herbals with a new and ethical approach. Nat Product Res. 2018;32(15): 1838–1848. https://doi.org/10.1080/14786419.2017.1402316

11. Kalantari A, Salimi A, Kalantari H, Broojeni JE, Rashidi I, Vanani AR, Bácskay I. The hepatoprotective effect of livergol microemulsion preparation (nanoparticle) against bromobenzene induced toxicity in mice. Toxicol Rep. 2019; 6: 444–448. https://doi.org/10.1016/j.toxrep.2019.05.005

12. Jeon WY, Jin SE, Seo CS, Lee MY, Shin HK, Han SC, Ha H. Safety assessment of *Gyejibokryeong-hwan* water extract: study of acute and subacute toxicity, and influence on drug metabolizing enzymes. J Ethnopharmacol. 2019;240:111913. https://doi.org/10.1016/j.jep.2019.111913

13. Jităreanu A, Trifan A, Vieriu M, Caba I-C, Mârțu I, Agoroaei L. Current trends in toxicity assessment of herbal medicines: A narrative review Processes. 2023;11(1):83. https://doi.org/10.3390/pr11010083

14. Denny KH, Stewart CW. Acute, subacute, subchronic, and chronic general toxicity testing for preclinical drug development. In: Faqi, A.S. (Ed.). A comprehensive guide to toxicology in nonclinical drug development. Academic Press, Michigan 2017; pp 109–127. https://doi.org/10.1016/B978-0-12-803620-4.00005-0

15. Kale OE, Awodele O, Akindele AJ. Subacute and subchronic oral toxicity assessments of *Acridocarpus smeathmannii* (DC.) Guill. & Perr. root in Wistar rats. Toxicol Rep. 2019;6:161–175. https://doi.org/10.1016/j.toxrep.2019.01.00

16. Bazzaz, BSF, Fork SD, Ahmadi R, Khameneh D. Deep insights into urinary tract infections and effective natural remedies. Afr J Urol. 2021;27:6. https://doi.org/10.1186/s12301-020-00111-z

17. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. 2014;4:177. https://doi.org/10.3389/fphar.2013.00177

18. Aouachria S, Boumerfeg S, Benslama A, Benbacha F, Guemmez T, Khennouf S, Arrar L, Baghiani A. Acute, sub-acute toxicity and antioxidant activities (in vitro and in vivo) of *Reichardia picroide* crude extract. J Ethnopharmacol. 2017;17(208):105–116. https://doi.org/10.1016/j.jep.2017.06.028

19. Erhirhie EO, Ihekwereme CP, Ilodigwe EE. Advances in acute toxicity testing: strengths, weaknesses and regulatory acceptance. Interdiscip Toxicol. 2018;11(1):5–12. https://doi.org/10.2478/intox-2018-0001

20. Li Y, Kandhare AD, Mukherjee AA, Bodhankar SL. Acute and sub-chronic oral toxicity studies of hesperidin isolated from orange peel extract in Sprague Dawley rats. Regul Toxicol Pharmacol. 2019;105:77–85. https://doi.org/10.1016/j.yrtph.2019.04.001

21. Borgert CJ, Fuentes C, Burgoon LD. Principles of dose-setting in toxicology studies: the importance of kinetics for ensuring human safety. Arch Toxicol. 2021;95:3651–3664. https://doi.org/10.1007/s00204-021-03155-4

22. Burkill HM. The useful plants of west tropical Africa, Vol 2. Royal Botanic Gardens, Kew 1985.

23. Soladoye MO, Osipitan AA, Sonibare MA, Chukwuma EC. From 'vagabonds' to ethnobotanical relevance: Weeds of the campus sites of Olabisi Onabanjo University, Ago-Iwoye, Nigeria. Ethnobot Leaflets. 2010;14:546–558.

24. Nazifi AB, Magaji MG, Aliyu M, Danjuma NM. Memory-enhancing potentials of hydroalcoholic extract of *Eragrostis tremula* Hochst. ex Steud. (Poaceae) in mice. J Herb Drugs. 2019;10(1):11–21.

25. Nazifi AB, Magaji MG, Aliyu M, Danjuma NM. *Eragrostis tremula* Hochst. ex Steud. attenuates scopolamine-induced cognitive deficit through inhibition of oxidative stress, acetylcholinesterase, amyloid beta and pro-inflammatory cytokines. In: Neuroscience Next 2020 Abstracts. Alzheimers Dement 2020;16Suppl12: e12278. https://doi.org/10.1002/alz.12278

26. Marafa HA. *Ex vivo* cytotoxic activity of *Eragrostis tremula* on MCF-7 human breast cancer cells via induction of apoptosis. Cancer Biol Ther Oncol 2020;4:3. https://cancer-oncology.cancersummit.org/abstract/2020/ex-vivo-cytotoxic-activity-of-eragrostis-tremula-on-mcf-7-human-breast-cancer-cells-via-induction-of-apoptosis

27. Nazifi AB, Abubakar A, Magaji MG, Aliyu M, Danjuma NM. Ameliorative effect of ethanol extract of *Eragrostis tremula* Hochst. ex Steud. against diazepam-induced amnesia in mice. Bull Natl Res Cent. 2022;46:113. https://doi.org/10.1186/s42269-022-00800-5

28. Evans WC. Trease and Evans' Pharmacognosy Sixteenth ed. Elsevier Health Sciences, London, U.K. 2009.

29. OECD. Acute oral toxicity: up-and-down procedure. OECD guidelines for the testing of chemicals, 2001;425:1–26. https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecdtg425.pdf.

30. OECD. Test No. 407: Repeated dose 28-day oral toxicity study in rodents, OECD guidelines for the testing of chemicals, Section 4, OECD publishing, Paris 2008. https://doi.org/10.1787/9789264070684-en

31. Abubakar A, Nazifi AB, Hassan FI, Duke KA, Edoh TD. Safety assessment of *Chlorophytum alismifolium* tuber extract (Liliaceae): Acute and sub-acute toxicity studies in Wistar rats. J Acute Dis. 2019; 8(1):21–27. https://doi.org/10.4103/2221-6189.250374

32. Rolls G. Microtomy and paraffin section preparation. Leica Biosystem, Wetzlar, Germany 2011.

33. Prasathkumar M, Anisha S, Dhrisya C, Becky R, Sadhasivam S. Therapeutic and pharmacological efficacy of selective Indian medicinal plants – A review. Phytomed Plus. 2021;1(2):100029. https://doi.org//10.1016/j.phyplu.2021.10009

34. Agidew MG. Phytochemical analysis of some selected traditional medicinal plants in Ethiopia. Bull Natl Res Cent. 2022;46:87. https://doi.org/10.1186/s42269-022-00770-8

35. Anywar G, Kakudidi E, Byamukama R, Mukonzo J, Schubert A, Oryem-Origa H, Jassoy CA. Review of the toxicity and phytochemistry of medicinal plant species used by herbalists in treating people living with HIV/AIDS in Uganda. Front Pharmacol. 2021;12:615147. https://doi.org/10.3389/fphar.2021.615147

36. Bose S, Datta R, Kirlin WG. Toxicity studies related to medicinal plants. In: Mandal SC, Chakraborty R, Sen S (eds) evidence based validation of traditional medicines. Springer, Singapore 2021. https://doi.org/10.1007/978-981-15-8127-4_30

37. Loomis TA, Hayes AW. Loomis's essentials of toxicology. 4th edition, Academic press, California 1996; pp 17–32. https://doi.org/10.1016/B978-012455625-6/50002-7

38. Dongmo OLM, Epoh NJ, Tadjoua HT, Yousuf S, Telefo PB, Tapondjou LA, Choudhary MI. Acute and sub-acute toxicity of the aqueous extract from the stem bark of *Tetrapleura tetrapteura* Taub. (Fabaceae) in mice and rats, J. Ethnopharmacol. 2019; 236: 42–49. https://doi.org/10.1016/j.jep.2019.02.026

39. Vaibhav V, Meshram R, Shukla PK, Kalonia T, Bhute AR. A preliminary study of organ weight after histological exclusion of abnormality during autopsy in the adult population of Uttarakhand, India. Cureus 2022;14(7):e27044. https://doi.org/10.7759/cureus.27044

40. Waller DG, Sampson AP. Medical pharmacology and therapeutics. Elsevier Philadelphia 2018; pp. 64–71.

41. Arika WM, Nyamai DW, Musila MN, Ngugi MP, Njagi ENM. Hematological markers of *in vivo*

toxicity. J Hematol Thrombo Dis. 2016;4:236. https://doi.org/10.4172/2329-8790.1000236

42. Khalil AM, Rehan IF, Zigo F, Halás S, Farkašová Z, Diab HM. Selected hematological parameters and oxidative stress in red blood cells (RBCs) after accidental cases of nitrate toxicity in the goat fed cabbage. J Appl Anim Res. 2023;51(1):300–307. https://doi.org/10.1080/09712119.2023.2194360

43. Rosales C. Neutrophil: A cell with many roles in inflammation or several cell types?. Front Physiol. 2018;9:113 https://doi.org/10.3389/fphys.2018.0011

44. Kabak M, Çil B, Hocanlı I. Relationship between leukocyte, neutrophil, lymphocyte, platelet counts, and neutrophil to lymphocyte ratio and polymerase chain reaction positivity. Int Immunopharmacol. 2021;93:107390. https://doi.org/10.1016/j.intimp.2021.107390

45. Traesel GK, Menegati SE, Dos Santos AC, Souza RI, Villas Boas GR, Justi PN, Kassuya CA, Sanjinez Argandoña EJ, Oesterreich SA. Oral acute and subchronic toxicity studies of the oil extracted from pequi (*Caryocar brasiliense*, Camb.) pulp in rats. Food Chem Toxicol. 2016;97:224–231. https://doi.org/10.1016/j.fct.2016.09.018

46. Piel-Julian M-L, Mahevas M, Germain J, Languille L, Comont T, Lapeyre-Mestre M, Payrastre B, Beyne-Rauzy O, Michel M, Godeau B, Adoue D, Moulis G, CARMEN investigators group. Risk factors for bleeding, including platelet count threshold in newly diagnosed immune thrombocytopenia adults. J Thromb Haemost. 2018;16(9):1830–1842. https://doi.org/10.1111/jth.14227

47. Dupuis M, Severin S, Noirrit-Esclassan E, Arnal J, Payrastre B, Valera M. Effects of estrogen on platelets and megakaryocytes. Int J Mol Sci 2019;20: 3111. https://doi.org/10.3390/ijms20123111.

48. Lu JY, Ho SL, Buczek A, Fleysher R, Hou W, Chacko K, Duong TQ. Clinical predictors of recovery of COVID-19 associated-abnormal liver function test 2 months after hospital discharge. Sci Rep. 2022; 12: 17972. https://doi.org/10.1038/s41598-022-22741-9

49. Sharma A, Mucino MJ, Ronco C. Renal functional reserve and renal recovery after acute kidney injury. Nephron Clin Pract. 2014;127(1-4):94–100. https://doi.org/10.1159/000363721

50. den Bakker E, Gemke RJBJ, Bökenkamp A. Endogenous markers for kidney function in children: a review. Crit Rev Clin Lab Sci. 2018;55(3):163–183. doi:10.1080/10408363.2018.1427041

51. Kellum JA, Romagnani P, Ashuntantang G, Ronco C, Zarbock A, Anders H. Acute kidney injury. Nat Rev Dis Primers. 2021;7:52. https://doi.org/10.1038/s41572-021-00284-z

52. Mariappan G, Saha BP, Sutharson L, Singh A, Garg S, Pandey L, Kumar D. Analgesic, anti-inflammatory, antipyretic and toxicological evaluation of some newer 3-methyl pyrazolone derivatives. Saudi Pharm J. 2011;19(2):115–122. https://doi.org/10.1016/j.jsos.2011.01.003

53. Gowda S, Desai PB, Kulkarni SS, Hull VV, Math AA, Vernekar SN. Markers of renal function tests. N Am J Med Sci. 2010;2:170–173. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3354405/

54. Palazhy S, Viswanathan V. Lipid abnormalities in type 2 diabetes mellitus patients with overt nephropathy. Diabetes Metab J. 2017; 41(2):128–134. https://doi.org/10.4093/dmj.2017.41.2.128

55. Wang L, Yan N, Zhang M, Pan R, Dang Y, Niu Y. The association between blood glucose levels and lipids or lipid ratios in type 2 diabetes patients: A cross-sectional study. Front Endocrinol. 2022;13: 969080. https://doi.org/10.3389/fendo.2022.969080