



Acute and Subchronic Toxicity Assessment of *Eragrostis cilianensis* All. Lut. Ethanol Extract: Effects on Renal, Hepatic, and Hematological Parameters

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Abstract

Introduction: *Eragrostis cilianensis* has been used widely for many purposes, however, its safety profile has not yet been well documented. This study assessed the impact of single and repeated dosing of *Eragrostis cilianensis* ethanol extract on nephron, hepatic and hematological parameters.

Methodology: The plant was extracted by cold maceration using 70% v/v ethanol based on the standard protocol. The acute and subchronic toxicity studies were evaluated using OECD 425 and 407 guidelines respectively. Effects of the extract on biochemical, hematological and histopathological markers were assessed.

Results: The oral median lethal dose (LD50) of the extract in rats, was found to be greater than 5000 mg/kg. The sub chronic toxicity study of the extract following 28 days daily administration revealed no statistically significant differences ($p > 0.05$) in the renal, hepatic, and haematological function biomarkers. However, the kidney photomicrographs revealed a slight distortion in the histology. In conclusion, the extract appears to be relatively safe at the tested doses; however, caution is advised in individuals with underlying kidney disease.

Keywords: *Eragrostis cilianensis*; Toxicity Studies; Renals; Hepatic; And Hematological Parameters

Abbreviations

Eragrostis cilianensis is a loosely clump forming annual grass with erect or ascending branches that can grow up to 15-90 cm tall [1]. It is widely distributed in Southern Europe, Africa, Eastern Mediterranean, Central and East Asia, Indonesia and Philippines and usually grows in deciduous woodlands, reverine and pan margin thickets, wooded grassland and black alluvia turf and clay soils (Flora-Zambesiaca). *E. cilinensis* All. Lut. has a common name as gray love grass and is locally called “Komayya”, “AkoYay’ang’an” and “Ahihia ihunanya isi awo” in Hausa, Yaroba and Igbo respectively [1].

With the ever-increasing use of herbal medicines worldwide and the rapid expansion of the global market for these products, the safety and quality of medicinal plant materials and finished herbal medicinal products have become a major important area for health authorities, pharmaceutical industries and the public due to continued rise in herbal medicine related toxicities and chronic illnesses [2].

Toxicological evaluations of medicinal plants are critical in drug development. These toxicities are usually dependent on the plant part and the amount consumed, the species and stage of development, as well as the susceptibility of the subject [3]. Noting that the cumulative effects of plants ingested over time are not well understood in traditional medicine, toxicological evaluations such as acute, sub-chronic and chronic toxicity studies are commonly employed in ascertaining the safety of medicinal plants [4]. *Eragrostis cilianensis* being used widely for numerous purposes, there is need to evaluate its safety especially on kidneys, liver and blood.

Experimental procedures

Animals

Wistar rats (120–160 g) were purchased from the Animal House, Department of Pharmacology and Therapeutics, Bayero University, Kano, Nigeria. The rats were housed in cages with no more than five per cage, provided with unrestricted access to food and water, and maintained under a 12-hour light/dark cycle (07:00–19:00). Prior to experimentation, all animals were ac-

climatized for at least seven days in a temperature and humidity controlled environment. The study protocols were approved (BUK/ACUREC/CAP/PG15) by the Animal Care and Use Research Ethics Committee (ACUREC) under the Directorate of Research, Innovation, and Partnership (DRIP), Bayero University, Kano, Nigeria.

Plant material

The plant *Eragrostis cilianensis* was collected from Darazo town along Basurka road of Darazo Local Government Area of Bauchi state. It was identified at the Department of Plant Biology, Herbarium Unit, Bayero University Kano, Kano state Nigeria, where Herbarium Accession Number BUKHAN 0320 was issued. The plant material was shade dried to a constant weight and size reduced using pestle and mortar.

Extraction of the plant material

The plant material was extracted using cold maceration technique with ethanol, following the standard protocol outlined by [5]. A total of 897 g of powdered *Eragrostis cilianensis* was soaked in 5L of 70% ethanol for four days. After maceration, the mixture was decanted, filtered, and evaporated to dryness in an oven until a brownish-black residue was obtained. The dried extract was then stored in an airtight container for future use.

Acute toxicity study

The Organization for Economic Cooperation and Development (OECD) 425 protocol [6] was employed using rats. The animals were divided into three groups, each consisting of three subjects ($n = 3$), and administered a single oral dose of 5000 mg/kg of the *Eragrostis cilianensis* ethanol extract (ECE). They were then monitored for 24 hours for immediate signs of toxicity, including mortality, and further observed for up to 14 days to assess changes in feeding behavior and body weight.

Sub-chronic toxicity study

The sub chronic toxicity evaluation was carried out using OECD 407 protocol [7]. Total of 24 rats were divided into four groups and six rats were allocated to each group ($n = 6$). The ECE was dissolved in distilled water and administered orally daily for 28 consecutive

days at graded doses of 125, 250 and 500 mg/kg p.o. to group 2, 3 and 4 respectively while group 1 was administered distilled water (1 ml/kg) only, which served as a control. The animals were observed daily during the experimental period for the sign of toxicity such as mortality or morbidity, changes in posture, changes in the fur of the skin, eyes, mucous membranes and behaviors. At the end of the 28 days of administration, the animals were fasted overnight, but with free access to water. On the 29th day, they were anesthetized with ether and humanely sacrificed. Blood samples were collected for biochemical and hematological studies while liver and kidneys were collected for histopathological examinations.

Statistical analysis

The normality of data distribution was checked using Shapiro-Wilk normality test. The data collected were analyzed using the Statistical Package for Social Sciences (SPSS), version 22. Mean differences were assessed through one-way Analysis of Variance

(ANOVA), followed by Dunnett's *post hoc* test. A p-value of less than 0.05 was considered statistically significant. Results were expressed as mean $\pm$ standard error of the mean (SEM).

Results

Acute toxicity study

The median lethal dose (LD_{50}) of ECE in rats were found to be greater than 5000 mg/kg p.o. Signs and symptoms observed in the test animals following single exposure to ECE included decreased locomotor activity and CNS depression.

Subchronic toxicity study

Effect of *E. cilianensis* ethanol extract on liver markers in rats

Liver function markers remain unchanged following 28 days daily oral administration of ECE in rats. There was no significant ($p = 0.7914$) difference in the liver parameters following 28 days administration of ECE except for total protein which showed significant ($F_{3, 35} = 424.4$, $P = 0.0001$). increase at a dose of 125 mg/kg when compared with control group (Table 1).

Treatment (mg/kg)	ALT (IU/L)	AST (IU/ml)	ALP (IU/ml)	TP (g/dl)	ALB (g/dl)
D/W1ml/kg	19.50 $\pm$ 2.10	19.75 $\pm$ 4.09	25.83 $\pm$ 7.07	4.03 $\pm$ 0.41	2.53 $\pm$ 0.19
ECE (125)	22.75 $\pm$ 2.50	39.25 $\pm$ 6.75	37.05 $\pm$ 2.35	6.35 $\pm$ 0.37*	3.33 $\pm$ 0.36
ECE (250)	28.25 $\pm$ 8.50	35.00 $\pm$ 10.73	29.30 $\pm$ 5.47	4.65 $\pm$ 0.79	2.48 $\pm$ 0.09
ECE (500)	14.50 $\pm$ 1.89	40.25 $\pm$ 12.25	27.40 $\pm$ 6.79	4.40 $\pm$ 0.63	3.05 $\pm$ 0.45

Table 1: Effect of 28 days Daily Oral Administration of ECE on Liver Parameters in Rats.

Values are expressed as Mean $\pm$ S.E.M., * $P = 0.0001$ as compared to W/D group – One way ANOVA followed by Dunnett's *post hoc* test, $n = 6$, D/W = Distilled water, ECE = *Eragrostis cilianensis* Ethanol Extract, ALT = Alanine transaminase, AST = Aspartate transaminase, ALP = Alkaline phosphatase, TP = Total Protein, ALB = Albumin.

Effect of ECE on Renal Function in rats

Renals function markers remain unaffected following 28 days daily oral administration of ECE in rats. There was no significant ($p = 0.2345$) difference in the renal function parameters following 28 days oral administration of ECE as compared with control group (Table 2).

Effects of ECE on Hematological Parameters

Hematological parameters remain unaffected following 28 days daily oral administration of ECE in rats. The result of the daily administration of ECE for 28 days revealed no significant ($p > 0.05$) difference in the haematological parameters as compared with control group (Table 3).

Treatment (mg/kg)	Urea (mg/dl)	Creatinine (mEq/L)	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mg/dl)	Bicarbonate (mg/dl)
D/W 1ml/kg	56.85 ± 9.65	0.75 ± 0.06	74.03 ± 10.39	10.10 ± 1.49	30.50 ± 1.71	88.25 ± 9.38
ECE (125)	37.60 ± 10.50	1.13 ± 0.05	94.30 ± 3.86	13.28 ± 1.55	36.00 ± 3.08	86.50 ± 3.23
ECE (250)	33.35 ± 8.94	0.88 ± 0.22	73.38 ± 5.23	11.30 ± 1.45	38.00 ± 1.73	100.50 ± 7.14
ECE (500)	40.28 ± 6.93	1.05 ± 0.09	86.20 ± 5.49	11.43 ± 1.51	29.50 ± 5.12	95.75 ± 3.45

Table 2: Effects of 28 Days Daily Oral Administration of ECE on Renal Function Parameters in Rats.

Values are expressed as Mean ± S.E.M., No significant differences as compared to D/W group – One way ANOVA followed by Dunnett’s *post hoc* test, n = 6, D/W = Distilled water; ECE = *Eragrostis cilianensis* Ethanol Extract.

Treatment (mg/kg)	WBC (×10 ³ /μl)	LYMP(%)	MID(%)	GRAN(%)	RBC (×10 ⁶ /μl)	HGB (g/dl)	HCT (%)	PLT (×10 ⁶ /μl)
D/W 1ml/kg	5.08 ± 0.28	64.18 ± 1.82	4.38 ± 0.36	31.73 ± 1.49	5.75 ± 0.23	12.60 ± 0.53	38.00 ± 1.29	201.53 ± 32.50
ECE (125)	4.93 ± 0.27	60.48 ± 1.16	6.20 ± 1.22	33.65 ± 2.21	6.03 ± 0.06	16.60 ± 2.52	47.50 ± 3.95	192.00 ± 9.11
ECE (250)	5.18 ± 0.29	58.93 ± 2.87	4.95 ± 0.39	36.05 ± 2.57	6.08 ± 0.09	12.80 ± 0.43	39.25 ± 1.84	171.25 ± 9.06
ECE (500)	4.75 ± 0.12	61.08 ± 2.32	5.68 ± 0.45	32.95 ± 2.65	6.08 ± 0.09	12.98 ± 0.48	40.00 ± 1.89	200.00 ± 15.59

Table 3: Effect of 28 Days Daily Oral Administration of ECE on Haematological Parameters In Rats.

Values are expressed as Mean ± S.E.M., No significant differences as compared to D/W group – One way ANOVA followed by Dunnett’s *post hoc* test, n = 6, D/W = Distilled water; ECE = *Eragrostis cilianensis* extract, WBC = White blood cells, LYMP = Lymphocytes, MID = , GRAN = Granulocytes, RBC = Red blood cells, HGB = Haemoglobin, HCT = Haematocrit, PLT = Platele.

Effect of ECE on Rats Liver Tissue

Lower doses of ECE revealed no distortion on the liver histology. The photomicrograph of the transverse section of the liver tis-

sues showed no distortion in the hepatocytes at 125 and 250 mg/kg doses of ECE. However at a dose of 500 mg/kg, a periportal necrosis was observed (Figure 1).

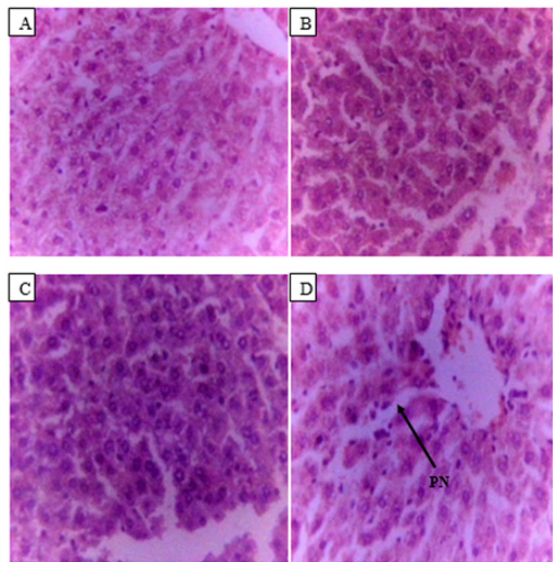


Figure 1: Photomicrograph of Transverse Section of the Liver of Rats Treated with ECE, arrow showing Periportal necrosis (PN)Following 28 days Daily Oral Administration (H and E Stain × 100), A=Distilled water 1 ml/kg; B = ECE 125 mg/kg; C= ECE 250 mg/kg; D= ECE 500 mg/kg. ECE = *Eragrostis cilianensis* extract.

Effect of ECE on Rats Kidney Tissue

Mild distortion on the kidneys histology was induced by ECE. The transverse section of the kidney tissues exhibited slight dis-

tortions in the photomicrograph ranging as Slight Tubular Necrosis and Hyperplasia of inflammatory cells at doses of 125, 250 and 500 mg/kg of ECE respectively (Figure 2).

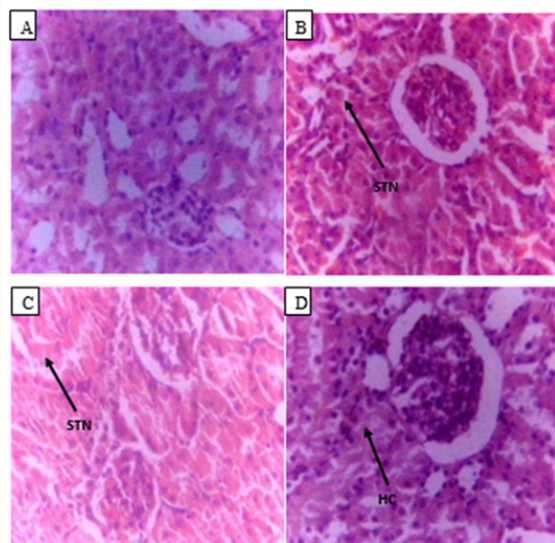


Figure 2: Photomicrograph of Transverse Section of the Kidney of Rats Treated with ECE, arrow showing Hyperplasia of inflammatory cells (HC) and Slight Tubular Necrosis (STN) Following 28 days Daily Oral Administration (Hand E Stain $\times 100$), A = Distilled water 1 ml/kg; B = ECE 125 mg/kg; C= ECE 250 mg/kg; D= ECE 500 mg/kg. ECE = *Eragrostis cilianensis* extract.

Discussion

Medicinal plants had continued to gain popularity worldwide and raise concerns among policy-makers, health professionals and the public regarding its safety, effectiveness, quality, availability, preservation and regulation [8]. Despite the availability of most medicinal plant products, very few have been investigated scientifically to establish the safety profile and therefore the importance of safety assessment of medicinal plants with relevant therapeutic applications cannot be over emphasized [9].

Toxicity studies are usually carried out to establish safety profile of substances and are very important stages in drug discovery. Acute toxicity study is the initial stage in toxicological investigation recommended for new and unknown substances. However, that should not be considered as biological constant because of irreproducibility [10]. In the current study, the acute toxicity of ECE to determine the median lethal dose (LD_{50}) was evaluated using

the protocol described by the Organization of Economic Cooperation and Development (OECD 425) oral administration [6]. The median lethal dose (LD_{50}) following acute oral administration of ECE was found to be greater than 5000 mg/kg body weight. Therefore, the acute oral administration of ECE at a dose of 5000 mg/kg did not result to lethality or behavioral signs of toxicity and should be considered practically nontoxic in rats. This is according to [10], assumptions that LD_{50} of 1 mg/kg or less considered as highly toxic, with greater than 5000 mg/kg considered as practically nontoxic.

It has been a common belief that natural products are less associated with adverse effects however, traditional usage of plants as seen as evidence based in terms of safety does not always hold true, hence, the need to assess plant products for safety over time [11]. In this study, the subchronic evaluation of ECE was conducted to assess its effect on structural, hematological and biochemical parameters with reference to some selected essential organs and medium.

The ECE does not demonstrate statistical significant changes in the liver function parameters even though exhibited some variations in a non-dose dependent manner; therefore the extract could be said to have little or no effect on the liver biochemical parameters and the result was consistent with the photomicrographs of the transverse section of the liver where no structural distortion was observed except at the higher dose of the extract where slight periportal necrosis was observed [12]. Again the extract exhibited no statistical significant changes in the kidney function parameters, however slight structural distortions were observed in the photomicrograph of the transverse sections of the kidney which should serve as the call for caution in an underlying kidney diseases at the doses used in this study [12]. Furthermore, the effect of ECE on hematological parameters revealed insignificant difference which suggested that at the doses used in this study may not be associated with significant changes in hematological parameters.

Conclusion

This research revealed that, *Eragrostis cilianensis* ethanol extract is relatively safe after acute and sub-chronic administration in rats. However, caution should be exercised for use in individuals with kidneys associated problems.

Conflict of Interest Statement

The authors reported no conflicts of interest regarding the research, authorship, or publication of this study.

Contributions by the Authors

IM conceptualized the study idea and designed the research. IM conducted the experiment and analyzed the data. SY wrote the first draft of the manuscript. While MAH and SYM edited the manuscript. All authors read and approved the final version of the manuscript.

Data Availability Statements

The datasets will be made available from the corresponding author on request.

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