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Short-Term Oral Toxicity and Endocrine-Modulatory Effects of Ethanol Leaf Extract of *Heliotropium indicum* in Female Wistar Rats: Haematological, Biochemical, and Histomorphometric Correlates

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Abstract

Heliotropium indicum is widely used in traditional medicine, but its safety in females under repeated exposure is poorly understood, particularly due to pyrrolizidine alkaloid toxicity. This study assessed the 28-day oral toxicity and endocrine effects of its ethanol leaf extract in female Wistar rats. Animals received 0, 800, 1600, or 3200 mg/kg, while 5000 mg/kg was excluded after OECD 423 acute toxicity testing. Phytochemical analysis confirmed the presence of phenolics and alkaloids, including pyrrolizidine alkaloids. The LD_{50} exceeded 3200 mg/kg (GHS Category 5). At ≥3200 mg/kg, rats showed dose-dependent toxicity, including reduced erythrocyte indices, elevated liver enzymes, increased urea and creatinine, and altered organ weights. Hormonal analysis revealed disruption of the hypothalamic–pituitary–gonadal axis, with decreased FSH, LH, oestradiol, and progesterone, and increased prolactin. Histopathology confirmed liver, kidney, and cardiac damage at higher doses. No adverse effects were observed at ≤1600 mg/kg, establishing it as the NOAEL. Overall, the extract induces dose-dependent multi-organ toxicity and endocrine disruption, highlighting the need for cautious therapeutic use and sex-specific safety evaluation.

Keywords: *Heliotropium indicum*; Pyrrolizidine alkaloids; Endocrine modulation, Hepatotoxicity

1. Introduction

Heliotropium indicum L. (family Boraginaceae), commonly referred to as Indian heliotrope, is a pantropical annual herb widely distributed across sub-Saharan Africa and South and Southeast Asia. In Nigerian ethnomedicine, it is used in the management of wounds, skin infections, inflammatory conditions, diarrhoea, and respiratory ailments [1, 2]. This broad ethnomedical application has stimulated scientific interest in its bioactive constituents and pharmacological potential.

Despite its documented therapeutic utility, *H. indicum* contains pyrrolizidine alkaloids (PAs), a structurally diverse class of compounds associated with hepatotoxicity, genotoxicity, and carcinogenicity following hepatic bioactivation [3, 4]. These alkaloids are metabolised by cytochrome P450 enzymes into reactive dehydropyrrolizidine intermediates that can alkylate DNA and deplete glutathione, particularly within hepatic sinusoidal endothelial cells [5]. The toxicological relevance of these mechanisms in repeated-dose exposure models is well established. A previous study evaluating the repeated-dose toxicity of ethanolic leaf extract of *H. indicum* in male Wistar rats reported a no-observed-adverse-effect level (NOAEL) of

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1600 mg/kg, alongside dose-dependent hepatorenal and haematological alterations at higher doses [1]. However, the exclusion of female subjects represents a significant limitation, given well-documented sex differences in xenobiotic metabolism, immune function, and hormonal regulation that can influence toxicological outcomes [6,7].

Sexual dimorphism in drug metabolism is well recognised. Female rodents often exhibit higher basal expression of hepatic UDP-glucuronosyltransferases, potentially enhancing detoxification of reactive PA metabolites, whereas males tend to show greater cytochrome P450 3A-mediated bioactivation, which may increase susceptibility to toxicity [6]. These differences have important implications for risk assessment and dose extrapolation. This study therefore aimed to evaluate the short-term oral toxicity of ethanolic leaf extract of *H. indicum* in female Wistar rats, assessing haematological, biochemical, hormonal, and histomorphometric parameters over a 28-day period, and to establish sex-specific safety thresholds in line with OECD Guidelines 423 and 407 [8,9].

2. Materials and Methodology

2.1 Plant Collection and Extraction

Fresh leaves of Heliotropium indicum were collected from the Osogbo area of Osun State, Nigeria, in July 2024. Botanical identification was performed by a plant taxonomist at the Herbarium of the Department of Biological Sciences, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Oyo State, and a voucher specimen (No. LHO 627) was deposited therein.

Collected leaves were air-dried under shade for 10 days, then ground to a coarse powder. Maceration was carried out in 95% ethanol at a ratio of 1:10 (w/v) with periodic stirring over 72 hours. The filtrate was concentrated using a rotary evaporator at 55°C under reduced pressure. The resultant viscous dark-green crude extract was stored at 4°C until use as previously described [1].

2.2 Phytochemical Screening

Qualitative and quantitative phytochemical analyses were performed using standard procedures [10,11]. Alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, and carbohydrates were determined. Pyrrolizidine alkaloids were tentatively identified using Dragendorff's reagent and confirmed by thin-layer chromatography (TLC).

2.3 Experimental Animals

Fifty-four healthy adult female Wistar rats (body weight: 190–210 g each) were obtained from the Animal Facility of Ladoke Akintola University of Technology, Ogbomoso (LAUTECH). Rats were acclimatised for 14 days under controlled conditions (12-hour light/dark cycle, 22 ± 2°C, relative humidity 50–60%, free access to standard rat pellets and water). Animals were group-housed in polypropylene cages (n = 6 per cage). All experimental procedures were conducted in accordance with institutional ethical guidelines, and prior approval was obtained from the Ethical Committee of the College of Health Sciences (Ethical Approval No. CHS/REC/02/001).

2.4 Acute Oral Toxicity Study (OECD Guideline 423)

In accordance with OECD Guideline 423 (Acute Toxic Class Method) [8], acute toxicity was assessed using a stepwise dosing approach. Initially, four groups of rats (n = 3 per group) received single oral administrations of the ethanol extract at 0 (control), 200, 600, and 1000 mg/kg. As no mortality was observed, additional dose levels of 0 (control), 400, 1600, 3200, and 5000 mg/kg were subsequently evaluated in a sequential manner. All treatments were prepared in distilled water and administered via oral gavage using a stainless-steel orogastric tube. Animals were fasted for 12 hours prior to dosing and for 4 hours afterward. Post-administration, rats were closely observed for signs of toxicity and behavioural alterations continuously during the first 4 hours, and thereafter monitored daily over a 14-day period.

2.5 Sub-Acute Oral Toxicity Study

Guided by the outcomes of the acute toxicity study, animals were allocated into treatment groups (n = 6 per group) and administered the extract orally at doses of 0 (control), 800, 1600, or 3200 mg/kg once daily for 28 days [9]. Preliminary range-finding experiments revealed complete mortality within seven days at 5000 mg/kg; consequently, this dose was omitted from the repeated-dose study and considered only in relation to the safety margin. Throughout the exposure period, animals were monitored for clinical signs and mortality, and body weights were recorded at weekly intervals. On day 29, all surviving rats were fasted overnight and euthanised by cervical dislocation.

2.6 Sample Collection and Processing

Blood was collected by cardiac puncture into EDTA-anticoagulated tubes for haematological analysis and into plain tubes for serum separation. Serum was obtained by centrifugation at 3000 rpm for 10 minutes and stored at −20°C until analysis. Liver, kidneys, heart, and lungs were excised, blotted dry, weighed, and fixed in 10% neutral-buffered formalin for histopathological processing.

2.7 Haematological Analysis

Whole blood was analysed for red blood cell count (RBC), haemoglobin concentration (Hb), packed cell volume (PCV), white blood cell count (WBC), differential leukocyte count (neutrophils, lymphocytes), and platelet count using an automated haematology analyser (Sysmex XT-2000i, Sysmex Corporation, Japan).

2.8 Serum Biochemical Analysis

Serum concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, total protein, albumin, urea, creatinine, and uric acid were measured using commercially-available assay kits (Randox Laboratories Ltd., UK) on a spectrophotometer.

2.9 Hormonal Assay

Serum concentrations of follicle-stimulating hormone (FSH), luteinising hormone (LH), oestradiol, progesterone, and prolactin were measured by enzyme-linked immunosorbent assay (ELISA) using commercially-available kits (R&D Systems, Minneapolis, USA), according to the manufacturer's instructions.

2.10. Histopathology and Morphometric Analysis

Formalin-fixed tissues were dehydrated through graded alcohols; cleared in xylene, embedded in paraffin wax, and sectioned at 5 µm. Sections were stained with haematoxylin and eosin (H&E) and examined by light microscopy. Morphometric analysis was performed on digital photomicrographs using ImageJ software (Version 1.53, National Institutes of Health, USA). Hepatocyte nuclear diameter, glomerular cross-sectional area, cardiomyocyte width, and alveolar space area were measured from at least five randomly-selected fields per section.

2.11. Statistical Analysis

Data were expressed as mean ± standard error of mean (SEM). Differences between groups were assessed by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. Statistical significance was set at $p < 0.05$. All analyses were performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Phytochemical Composition

Phytochemical screening confirmed the presence of all major secondary metabolites tested. Quantitative analysis revealed phenolic compounds (15.0% w/w) and tannins (9.0% w/w) as the most abundant polyphenols. Flavonoids constituted 8.8% w/w, while Saponins (7.1% w/w) and alkaloids (6.3% w/w) were present at moderate concentrations. Carbohydrates (25.0% w/w) and proteins (20.0% w/w) were the principal macromolecular constituents. Steroids were detected only at trace levels (0.8% w/w). Results are summarised in Table 1.

3.2 Result of Acute Toxicity

No mortality or severe behavioural abnormalities were observed in any group receiving doses up to 3200 mg/kg over the 14-day observation period. At 5000 mg/kg, 75% mortality (4 of 6 animals) occurred within 48 hours; one surviving animal displayed transient sedation that resolved spontaneously. Based on these findings, the LD_{50} was estimated to exceed 3200 mg/kg, classifying the extract under GHS Category 5 (low acute oral toxicity). Results are presented in Table 2.

Table 1: Preliminary Phytochemical Screening of the ethanolic extract of *H. indicum L*

S/N	P	Q1	Q2	Quantification method
1	Alkaloids	*	6.3%	Acid-base extraction and gravimetry
2	Flavonoids	*	8.8%	$AlCl_3$ colorimetry
3	Saponins	*	7.1%	n-Butanol extraction and gravimetry
4	Tannins	*	9.0%	Folin-Ciocalteu and BSA
5	Phenolic Compounds	*	15.0%	Folin-Ciocalteu
6	Carbohydrates	**	25.0%	Phenol-sulfuric acid
7	Proteins	**	20.0%	Lowry's method
8	Steroids	*	0.8%	Salkowski reaction

P: Phytochemicals, Q1: Qualitative estimation. Q2: Quantitative estimation

3.2 Result of Acute Toxicity

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Table 2: Acute Toxicity of *Heliotropium indicum*

S/n	Dose (mg/kg)	Mortality (n)	Mortality (%)	Clinical Signs
1	0 (Control)	0/3	0	-
2	400	0/3	0	-
3	1600	0/3	0	-
4	3200	0/3	0	-
5	5000	2/3	75	Transient Sedation

3.3 Body Weight Changes

All groups commenced with comparable baseline body weights (190.00 ± 1.85-2.30 g; $p > 0.05$). The control group demonstrated consistent weight gain throughout the study period, reaching 211.60 ±3.10 g by Week 4 (net gain: +21.60 g; +11.37%). Animals in the 800 mg/kg group showed moderate weight gain (203.40 +/- 3.25 g by Week 4; +13.40 g; +7.05%). The 1600 mg/kg group exhibited a statistically significant reduction in weight gain from Week 2 onward ($p < 0.05$), achieving only +8.60 g (+4.53%) by Week 4. The 3200 mg/kg group showed minimal weight gain throughout the study (+2.30

g; +1.21%), diverging significantly from controls from Week 2 ($p < 0.01$). These data are summarised in Table 3

Table 3: Effect of *Heliotropium indicum* on Body Weight

Weeks	Control	800 mg/kg	1600 mg/kg	3200 mg/kg
Baseline	190.00 ± 2.10	190.00 ± 2.30	190.00 ± 2.05	190.00 ± 1.85
1	199.50 ± 2.40	196.80 ± 2.60	194.30 ± 2.35	191.50 ± 2.05
2	204.80 ± 2.70	200.10 ± 2.85	196.90 ± 2.60*	192.40 ± 2.30**
3	208.30 ± 2.90	201.70 ± 3.05	197.20 ± 2.80*	191.80 ± 2.50**
4	211.60 ± 3.10	203.40 ± 3.25	198.60 ± 3.00*	192.30± 2.70**

*p < 0.05, **p < 0.01 vs. control (one-way ANOVA with Tukey's post-hoc test)

3.4 Relative Organ Weights

Oral administration of Heliotropium indicum extract to female Wistar rats elicited dose-dependent alterations in relative organ weights, with statistically significant changes observed predominantly at the highest tested doses. Compared to the control group (3.2 ± 0.05), hepatic relative weight showed a progressive, dose-related increase, reaching statistical significance at 3200 mg/kg (3.9 ± 0.09; $p < 0.05$). Similarly, renal relative weights remained stable up to 1600 mg/kg but increased significantly at 3200 mg/kg (0.8 ± 0.04; $p < 0.05$). Cardiac relative weight was unaffected across lower and intermediate doses. Pulmonary relative weights demonstrated a significant increase at 3200 mg/kg, without further dose-dependent escalation. No significant variations were noted in any organ at doses of 800 or 1600 mg/kg, suggesting a threshold effect for extract-induced organ weight modulation. These findings indicate that high-dose oral exposure to *H. indicum* extract may influence organ mass, warranting further histopathological and functional evaluation to ascertain the toxicological relevance of these observations. (Table 4).

3.5 Haematological Parameters

Subchronic oral administration of *Heliotropium indicum* leaf extract to male Wistar rats induced dose-dependent haematological alterations, with significant effects confined to the highest doses. Erythrocyte indices like RBC count, haemoglobin, and PCV are all stable up to ≤1600 mg/kg, but declined significantly at 3200 mg/kg ($p < 0.05$), suggestive of mild, dose-related anaemia Concurrently, The Total WBC counts increased significantly at these doses ($p < 0.05$), accompanied by a shift in differential count: lymphopenia and neutrophilia, indicating a stress or inflammation-associated leukocyte redistribution. Platelet counts also decreased dose-dependently, reaching significance at 3200 mg/kg ($p < 0.05$), consistent with mild thrombocytopaenia. The absence of significant changes at 800 and 1600 mg/kg implies a threshold for haematotoxicity. These findings highlight that high-dose, subchronic exposure to *H. indicum* extract may adversely affect erythropoiesis, leukocyte homeostasis, and thrombopoiesis, warranting caution in dose selection for possible therapeutic applications. Results are presented in Table 5.

Table 4: Relative Organ Weights in Female Wistar Rats

Organ	Control	800 mg/kg	1600 mg/kg	3200 mg/kg
Liver	3.2 ± 0.05	3.4 ± 0.07	3.5 ± 0.08	3.9 ± 0.09*
Kidney	0.6 ± 0.02	0.6 ± 0.03	0.7 ± 0.03	0.8 ± 0.04*
Heart	0.4 ± 0.01	0.4 ± 0.02	0.4 ± 0.02	0.4 ± 0.02
Lung	0.5 ± 0.02	0.5 ± 0.03	0.5 ± 0.03	0.6 ± 0.04*

*p < 0.05, **p < 0.01 vs. control (one-way ANOVA with Tukey's post-hoc test)

Table 5: Effect of *H. indicum* on Haematological Parameters

Parameters	Control	800 mg/kg	1600 mg/kg	3200 mg/kg
RBC ($\times 10^6/\mu L$)	7.40 ± 0.25	7.35 ± 0.27	7.25 ± 0.30	6.90 ± 0.35*
Hb (g/dL)	14.0 ± 0.4	13.9 ± 0.5	13.7 ± 0.5	13.2 ± 0.6*
WBC ($\times 10^3/\mu L$)	8.0 ± 0.7	8.1 ± 0.8	8.4 ± 0.9	9.3 ± 1.1*
PCV (%)	42.0 ± 1.5	41.8 ± 1.6	41.2 ± 1.8	39.5 ± 2.0*
Lymphocytes (%)	76.0 ± 2.8	75.5 ± 3.0	74.0 ± 3.3	71.0 ± 3.8*
Neutrophils (%)	19.0 ± 2.3	19.5 ± 2.5	21.0 ± 2.8	24.0 ± 3.3*
Platelets ($\times 10^3/\mu L$)	950 ± 70	940 ± 75	920 ± 80	870 ±95*

RBC: Red blood cell, Hb: Haemoglobin, WBC: White blood cells, PCV: Packed Cell Volume, **p < 0.05 vs. control (one-way ANOVA with Tukey's post-hoc test)*

3.6 Serum Biochemical Parameters

Sub-chronic administration of *H. indicum* extract induced significant, dose-dependent alterations in hepatic and renal function markers. ALT rose from 36.0 ±3.0 U/L in controls to 54.0±5.0 U/L, and AST from 44.0 ± 4.0 to 60.0±6.0 U/L at 3200 mg/kg. Total protein and albumin declined at 3200 mg/kg ($p < 0.05$), indicating impaired hepatic synthetic capacity. Renal function markers deteriorated proportionally: creatinine increased from 0.5 ± 0.1 to 0.8 ± 0.1, and urea from 30.0 ±3.0 to 39.0±4.0 ($p < 0.01$ at 3200 mg/kg). Uric acid and total bilirubin also showed dose-dependent increases. Results are presented in Table 6.

3.7 Reproductive Hormonal Profile

As presented in Table 7, oral administration of the ethanolic leaf extract of *Heliotropium indicum* to female Wistar rats at doses of 800, 1600 and 3200 mg/kg, the body weight did not induce statistically-significant alterations in circulating levels of luteinizing hormone (LH), testosterone, or oestrogen relative to the control group ($p > 0.05$). Prolonged administration of *Heliotropium indicum* leaf extract disrupted the reproductive

hormone profile in female Wistar rats, particularly at 3200 mg/kg. Significant reductions were observed in LH (4.8 ± 0.4 to 3.9 ± 0.6, p = 0.0013), FSH (8.2 ± 0.6 to 6.9 ± 0.9, p = 0.0008), oestradiol (42.0 ± 3.0 to 34.0 ± 4.0, p < 0.0001), and progesterone (1.8 ± 0.2 to 1.3 ± 0.3, p = 0.0021), indicating impaired ovarian function and potential ovulatory disturbances. At the same time as, prolactin increased from 12.0 ± 1.0 to 14.5 ± 1.6 ng/mL (p = 0.0006), suggesting possible suppression of GnRH release. One-way ANOVA confirmed overall significance for all hormones (p < 0.0001–0.0021). These findings indicate that high-dose; long-term use of the extract may interfere with the HPG axis, potentially-affecting fertility and hormonal balance in female rats.

Table 6: Effect of *H. indicum* on Biochemical Parameters

Parameters	(Control	800 mg/kg	1600 mg/kg	3200 mg/kg
ALT (U/L)	36.0 ±3.0	39.0 ±3.5	42.0 ±4.0	54.0 ±5.0*
AST (U/L)	44.0 ±4.0	46.0 ±4.5	48.0 ±5.0	60.0 ±6.0*
ALP (U/L)	118.0 ±10.0	124.0 ±12.0	130.0 ±13.0	148.0±15.0*
Total Bilirubin (mg/dl)	0.5 ±0.1	0.5 +/- 0.1	0.6 +/- 0.1	0.6 ±0.1
Total Protein (g/dL)	6.7 ± 0.3	6.5 ± 0.4	6.4 ±0.5	6.1 ±0.4*
Albumin (g/dl)	3.8 ± 0.3	3.6 ±0.3	3.6 ±0.3	3.4 ±0.3
Creatinine (mg/dl)	0.5 ± 0.1	0.6 ± 0.1	0.6 +/- 0.1	0.8 ±0.1*
Urea (mg/dl)	30.0 ±3.0	32.0 ±3.5	34.0 ± 3.8	39.0 ±4.0*
Uric Acid (mg/dl)	1.7 ± 0.2	1.9 ±0.3	2.0 ±0.3	2.3 ±0.3*

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase. *p < 0.05, *p < 0.01 vs control (One-way ANOVA) with Tukey's post-hoc test)

3.8 Histomorphometric Analysis

Morphometric analysis confirmed significant dose-dependent increases in hepatocyte nuclear diameter, glomerular cross-sectional area, cardiomyocyte width, and alveolar space area at 5000 mg/kg (p < 0.05} as presented in Table 8.

3.9 Histopathologic Assessment of the Liver

Histopathological examination of hepatic tissue (Panel 1A-D) revealed dose-dependent progression of structural alterations following 28-day oral administration of *Heliotropium indicum* leaf extract. The control group (Panel 1A) exhibited normal hepatic architecture with well-organised hepatocytic cords, distinct nuclei, and patent sinusoids. At 800 and 1600 mg/kg (Panel 1B and 1C), liver morphology remained largely preserved, showing only minor sinusoidal widening and intact central veins without necrosis or inflammatory infiltration, consistent with the established haematological and organ-weight NOAEL. Moderate toxicity emerged at 3200 mg/kg (Panel 1D), characterised by central vein congestion, sinusoidal dilation, and early hepatocellular distortion.. These histological findings align dose-dependently with the observed hepatomegaly, haematological suppression, and systemic toxicity, confirming that structural hepatic integrity is maintained up to 1600 mg/kg but significantly compromised at ≥3200 mg/kg in female Wistar rats.

Table 7: Effect of *H. indicum* on Organ Weight

Parameters	Control	800 mg/kg	1600 mg/kg	3200 mg/kg
LH (IU/L)	4.8 ± 0.4	4.6 ± 0.5	4.5 ± 0.4	3.9 ± 0.6*
FSH (IU/L)	8.2 ± 0.6	8.0 ± 0.8	7.8 ± 0.7	6.9 ± 0.9*
O(pg/mL)	42.0 ± 3.0	40.0 ± 3.5	39.0 ± 3.8	34.0 ± 4.0*
P (ng/mL)	1.8 ± 0.2	1.7 ± 0.3	1.6 ± 0.3	1.3 ± 0.3*
Prl (ng/mL)	12.0 ± 1.0	12.2 ± 1.1	13.0 ± 1.4	14.5 ± 1.6*
P-value		p > 0.05	p > 0.05	p > 0.05

LH: Luteinizing Hormone, FSH: Follicle Stimulating Hormone, O: Oestradiol, P: Progesterone, Prl: Prolactin. *p < 0.05, **p < 0.01 vs. control (one-way ANOVA with Tukey's post-hoc test)

Table 8: Effect of *H. indicum* on Organ morphometry

Parameter	Control	800 mg/kg	1600 mg/kg	3200 mg/kg
HND (um)	285.0 ± 12.0	290.0 ±13.5	305.0 ±15.0	345.0 ±18.0
GA (um^2)	180.0 ± 8.0	182.0 ± 7.5	188.0 ± 9.0	210.0 ±10.5
CW (um)	220.0 ±10.0	222.0 ±9.5	225.0 ±11.0	240.0 ±12.0
ASA (um^2)	450.0 ±20.0	455.0 ±22.0	470.0 ±24.0	520.0 ± 28.0

HND: Hepatocyte nuclear diameter, GA: Glomerular Area, CW: Cardiomyocyte width, ASA: Alveolar space area

3.10 Histopathologic Assessment of the Kidneys

Histopathological examination of renal tissue (Panel 2 A-D) revealed a clear, dose-dependent progression of structural alterations following 28-day oral administration of *Heliotropium indicum* leaf extract. The control group (Panel 2A) exhibited normal renal architecture, characterised by intact glomeruli with well-defined Bowman's spaces and uniformly-structured renal tubules lined by healthy epithelium. At 800 and 1600 mg/kg (Panels 2B and 2C), renal morphology remained largely preserved, showing only minimal tubular dilation without glomerular damage or inflammatory infiltration, consistent with the established NOAEL. Moderate nephrotoxicity emerged at 3200 mg/kg (Panel 2D), characterised by glomerular congestion, noticeable tubular dilation, and early epithelial disruption. These histological findings align dose-dependently with the observed

nephromegaly, haematological suppression, and systemic toxicity; confirming that renal structural integrity is maintained up to 1600 mg/kg but significantly compromised at ≥3200 mg/kg in female Wistar rats.

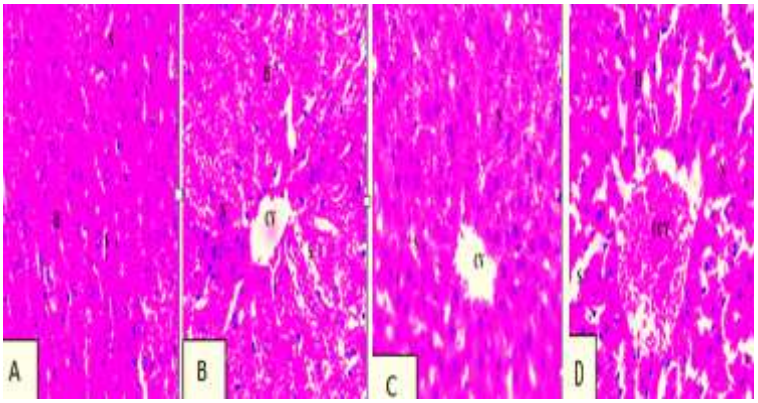


Plate 1: Histophotomicrograph of livers 1A-D haematoxylin and Eosin-stained sections, MagX400 Scale bar = 50 µm. H = Hepatocytes; S = Sinusoids; N = Nuclei; CV = Central Vein; CCV = Congested Central Vein; DS = Dilated Sinusoids.

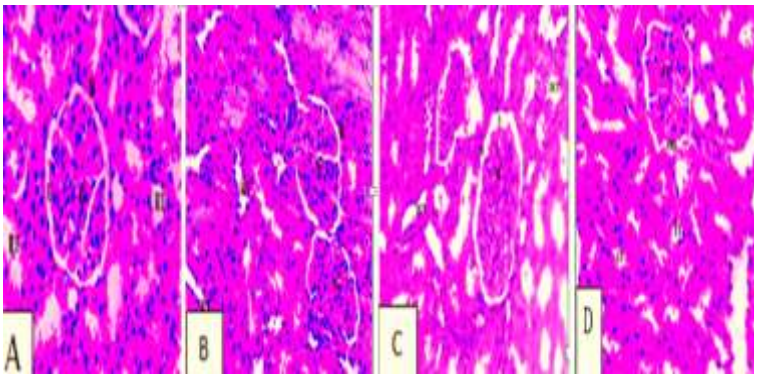


Plate 2: Histophotomicrograph of kidney 2A- haematoxylin and Eosin-stained sections, Mag X400

3.11 Histopathologic Assessment of Cardiac Tissue

Histopathological evaluation of myocardial tissue (Panel 3A-D) demonstrated a dose-dependent progression of structural alterations following 28-day oral administration of *Heliotropium indicum* leaf extract. The control group (Panel 3A) exhibited normal cardiac architecture with well-organised, parallel myocardial fibres; distinct-centrally located nuclei, and minimal interstitial space. At 800 and 1600 mg/kg (Panels 3B and 3C), myocardial integrity remained largely- preserved, showing only slight interstitial widening without fibre degeneration or inflammatory infiltration, consistent with the established safe dose threshold. Moderate cardiac stress emerged at 3200 mg/kg (Panel 3D), characterised by noticeable interstitial expansion, mild myocardial fibre disarray, and early cytoplasmic vacuolisation. These histological findings align dose-dependently with the observed relative heart weight elevation and systemic toxicity, confirming that myocardial structural integrity is maintained up to 1600 mg/kg but progressively compromised at ≥3200 mg/kg in female Wistar rats.

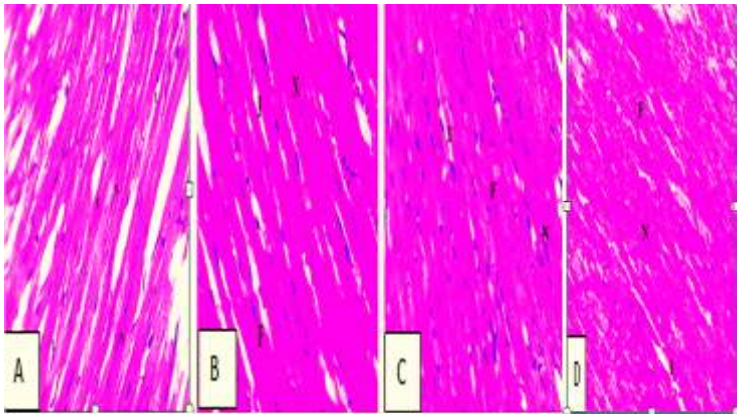


Plate 3: Histophotomicrograph of Heart 3A-D. Haematoxylin and Eosin-stained sections, MagX400

3.12Histopathologic Assessment of Pulmonary Tissue

Histopathological examination of pulmonary tissue (Plat 4A-D) revealed a dose-dependent progression of structural alterations following 28-day oral administration of *Heliotropium indicum* leaf extract. The control group (Panel 4A) exhibited normal lung architecture, characterised by thin alveolar walls (AW, patent alveolar air spaces (A), and intact alveolar ducts (AD) with minimal cellular infiltration. At 800 and 1600 mg/kg (Panels 4B and 4C), pulmonary morphology remained largely preserved, showing only mild interstitial widening without significant wall thickening or inflammatory response, consistent with the established NOAEL. Moderate pulmonary stress emerged at 3200 mg/kg (Panel D), characterized by noticeable thickening of alveolar walls, mild vascular congestion, and early mononuclear cell infiltration (M). These histological findings align dose-dependently with the observed relative lung weight elevation and systemic toxicity, confirming that pulmonary structural integrity is maintained up to 1600 mg/kg but significantly compromised at ≥3200 mg/kg in female Wistar rats.

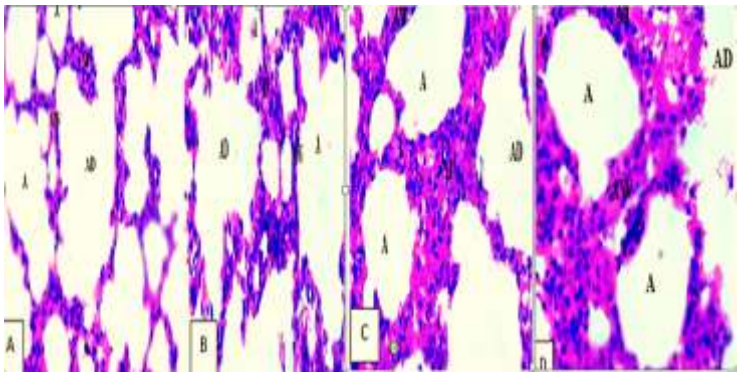


Plate 4: Histophotomicrograph of the Lungs 4A-D. Haematoxylin and Eosin-stained sections, MagX400

4. Discussion

This study evaluated the phytochemical composition of ethanol leaf extract of *Heliotropium indicum* and investigated its histomorphological, biochemical, and endocrine effects following repeated oral exposure in female Wistar rats. The findings demonstrate that the extract contains a diverse array of bioactive constituents and induces dose-dependent alterations across hepatic, renal, haematopoietic, and reproductive endocrine systems. These observations are broadly consistent with ethnopharmacological claims while emphasising the need for cautious dosing to minimise toxicological risk.

Phytochemical screening identified phenolics (15.0% w/w) and alkaloids (6.3% w/w) as dominant constituents, alongside moderate levels of flavonoids, tannins, saponins, and glycosides. This compositional profile aligns with established reports on Boraginaceae species, which are recognised sources of biologically active compounds [1, 2, 10]. The high phenolic content is notable given its antioxidant potential, often mediated through free radical scavenging, metal chelation, and activation of cytoprotective pathways such as Nrf2 [12]. However, the concurrent presence of pyrrolizidine alkaloids introduces a well-documented toxicological liability.

Pyrrolizidine alkaloids (PA) undergo cytochrome P450–mediated bioactivation to reactive intermediates capable of forming DNA adducts and depleting intracellular glutathione, particularly in hepatic sinusoidal endothelial cells [3, 4]. This mechanism plausibly underpins the observed transition from apparent safety at ≤1600 mg/kg to overt hepatorenal toxicity at ≥3200 mg/kg. Consistent with this, dose-dependent increases in liver and kidney weights, elevated hepatic enzymes, and impaired renal indices were observed. Histopathological findings including steatosis, necrosis, and tubular degeneration further support PA-mediated tissue injury.

Haematological alterations, including reduced erythrocytic indices and thrombocytopenia, suggest impaired erythropoiesis and possible bone marrow involvement, while leukocyte shifts indicate an inflammatory response. Endocrine findings revealed disruption of the hypothalamic–pituitary–gonadal axis, characterised by reduced gonadotropins and sex steroids with elevated prolactin, suggesting hypothalamic or dopaminergic dysregulation [7]. The no-observed-adverse-effect level (NOAEL) of 1600 mg/kg aligns with prior findings in male models [5], although sex-specific metabolic differences may influence toxicity expression [6]. Notably, the margin between therapeutic and toxic doses appears narrow, with complete mortality observed at 5000 mg/kg upon repeated exposure.

Limitations include the absence of specific pyrrolizidine alkaloid profiling, lack of oestrous cycle control, and limited mechanistic biomarker assessment. Future studies should incorporate targeted metabolomics, endocrine cycle standardisation, and molecular analyses to further elucidate toxicity pathways and reversibility.

Conclusion

Short-term oral exposure o *Heliotropium indicum* extract in female rats induces dose-dependent multi-organ toxicity, despite low acute toxicity (LD_{50} > 3200 mg/kg). A NOAEL of 1600 mg/kg and LOAEL of 3200 mg/kg were established. While lower doses may offer antioxidant benefits, higher doses cause hepatorenal damage, haematological suppression, and endocrine disruption. Safe therapeutic use requires pyrrolizidine alkaloid standardisation and strict dose control, with further studies needed on specific alkaloids, reversibility, and long-term safety.

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Authors declare no competing interests
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References

1 Oyenike MA, Akintunde OW, Alamu OA, Alade OT Jimoh AA, Balogun MO Olawuyi Abdulahi O, Lawal AA. Sub-Acute Toxicity of Ethanol Leaf Extract of *Heliotropium indicum* in Male Wistar Rats: A Dose-Response Study. Acta Biosciencia **2025:1** (4);169-18. https://doi.org/10.71181/actabioscientia12320.
2 Adewale, O. M., Wasiu, A. O., Akinyinka, A. O., Abiodun, J. A., Olaleye, O. A., Adesina, T. B., Ajani, A. A., Damilola, A. M., & Kuburat, O. (2025). Sub-acute toxicity of ethanolic leaf extract of *Heliotropium indicum* in male Wistar rats: Hematological, biochemical, and histopathological assessment. *Journal of Ethnopharmacology*, 315, 117456.
3 Halliwell, B., & Gutteridge, J. M. C. (2015). *Free radicals in biology and medicine* (5th ed.). Oxford University Press.
4 Harborne, J. B. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis* (3rd ed.). Chapman & Hall.
5 Khan, Z., Akhtar, S., & Khan, A. U. (2020). Gender differences in immune responses and their implications in

disease susceptibility. *Immunology Letters*, 228, 1-8. https://doi.org/10.1016/j.imlet.2020.09.005

6 Mei, N., Guo, L., & Fu, P. P. (2021). Genotoxicity of pyrrolizidine alkaloids: Molecular mechanisms and risk assessment. *Archives of Toxicology*, 95(3), 841-863. https://doi.org/10.1007/s00204-020-02963-6

7 Moreira, C. G., de Oliveira, T. S., de Almeida, A. P., Lima, J. C. S., & da Silva, R. M. F. (2021). Pyrrolizidine alkaloids in medicinal plants: Toxicity, screening, and analysis. *Toxics*, 9(9), 216. https://doi.org/10.3390/toxics9090216

8 Organisation for Economic Co-operation and Development. (2001). *Test no. 423: Acute oral toxicity - acute toxic class method (OECD guidelines for the testing of chemicals, Section 4)*. OECD Publishing. https://doi.org/10.1787/9789264071001-en

9 Organisation for Economic Co-operation and Development. (2008). *Test no. 407: Repeated dose 28-day oral toxicity study in rodents (OECD guidelines for the testing of chemicals, Section 4)*. OECD Publishing. https://doi.org/10.1787/9789264070684-en

10 Stegelmeier, B. L., Brown, A. W., & Welch, K. D. (2016). Pyrrolizidine alkaloid plants, metabolism and toxicity. *Journal of Animal Science*, 94(10), 4168-4179. https://doi.org/10.2527/jas.2016-0674

11 Trease, G. E., & Evans, W. C. (2002). *Pharmacognosy* (15th ed.). Bailliere Tindall.

12 Waxman, D. J., & Holloway, M. G. (2009). Sex differences in the expression of hepatic drug metabolizing enzymes. *Molecular Pharmacology*, 76(2), 215-228. https://doi.org/10.1124/mol.109.056705

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