



Acute and Sub-Acute Toxicological Evaluation of the Hydromethanol Seed Extract of *Phaseolus lunatus* (Lima Beans) in Wistar Rats

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Abstract

Psychosis remains a chronic global mental health challenge. Although several orthodox drugs are available, many people especially in developing countries rely on herbal medicines for its management., many people especially, in developing countries rely on herbal medicines for its management. In Esan communities of Edo State, Nigeria, the seeds of *Phaseolus lunatus* are used in the management of psychotic illness. This study evaluated the acute and sub-acute toxicity of the hydromethanol seed extract of the plant. The dried seeds were pulverized and extracted using a 1:1 mixture of water and methanol. Following phytochemical screening, the oral LD₅₀ of the extract was estimated, and sub-acute toxicological evaluations were carried out in adult Wistar rats given daily oral doses of 400, 800, or 1600 mg/kg for 28 consecutive days. Parameters assessed included body and organ weights, hematological indices, serum electrolytes, urea, creatinine, liver enzymes, proteins, lipids, glucose, and histopathology of major organs. The extract contained carbohydrates, alkaloids, phenolic compounds, flavonoids, steroids, oils, and anthraquinones. The oral LD₅₀ was greater than 5000 mg/kg. No significant changes were observed in hematological indices or relative organ weights. At 1600 mg/kg, urea and bicarbonate levels were significantly altered, alkaline phosphatase was elevated, and globulin was reduced. The extract lowered blood glucose and increased low-density lipoproteins in a dose-dependent manner. Organ histology was largely normal, with mild vascular ulcerations in the heart and liver at the highest dose. These findings suggest that the hydromethanol seed extract of *P. lunatus* is relatively safe in acute exposure but may produce adverse hepatic, renal, and cardiovascular effects following repeated administration at high doses.

Keywords: *P. lunatus*; liver enzymes; lipid profile; hematological parameters; blood glucose, histopathology

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Introduction

Alchornea laxiflora [Benth.] Pax and K.Hoffm The use of herbal medicines has increased worldwide and spans all social classes (Mahady 2001; Wachtel-Galor and Benzie 2011; Zahn *et al.* 2019). In many resource-poor countries, communities rely heavily on traditional remedies because of economic limitations, inadequate access to modern healthcare, and the high cost of orthodox medicines. Herbal preparations are often employed for the management of mild to moderate illnesses (Welz *et al.* 2018; Zahn *et al.* 2019). The pharmaceutical industry continues to depend on plants as important sources of novel therapeutic molecules (Wachtel-Galor and Benzie 2011; WHO 2023). Herbal remedies are widely perceived as being more affordable and associated with fewer adverse effects than synthetic drugs, which has contributed to the growing interest in their therapeutic applications (Karimi *et al.* 2015; Basaran *et al.* 2022). Despite this, they are often not standardized, and their adverse safety profiles remain under-investigated (Cohen and Ernst 2010; Kunle *et al.* 2012; Ekor 2014).

Phaseolus lunatus L. (Fabaceae), commonly known as lima bean, is cultivated in tropical and subtropical regions for both food and medicinal purposes. Its potential food applications have been extensively reviewed (Adebo 2023). Traditionally, ground seeds are applied to cuts and abscesses to promote healing in some African communities (Temegne *et al.* 2021). Pharmacological studies have reported its trypsin and chymotrypsin inhibition (Haynes 1967), hypocholesterolemic effects (Oboh and Omofoma 2008), antidiabetic properties (Johnson *et al.* 2013), and cysteine proteinase inhibition (Lawrence and Nielsen 2001). Extracts of Chinese lima beans have also demonstrated antiviral, antibacterial, and anticancer activities (Wong and Ng 2005). More recently, its anti-inflammatory effects have been observed in rat models (Maliza *et al.* 2024).

While herbal medicines are widely used, acute consumption of high doses or prolonged use for chronic conditions such as psychosis may lead to toxic effects. Given the increasing promotion of herbal products and their possible integration with orthodox medicine, systematic toxicological evaluations are essential (Balkrishna *et al.* 2024). Although *P. lunatus* seeds are traditionally used to manage psychosis, there is little or no evidence regarding their safety profile. This study therefore investigated the acute and sub-acute toxicological effects of the hydromethanol seed extract of *P. lunatus* in Wistar rats.

Materials and Methods

Animals

Adult Wistar rats (170–250 g) and Swiss mice (20–25 g) of both sexes were obtained from a private breeding facility in Benin City, Nigeria. They were housed in the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, under natural light, humidity, and

temperature, with free access to food and water. Animals were acclimatized for two weeks prior to experimentation. Care and use complied with the NIH Guide for the Care and Use of Laboratory Animals (NIH, 2002). Ethical approval was obtained from the Faculty of Pharmacy Ethics Committee (Ref: EC/FP/024/04).

Collection and Extraction of plant material

Dried, uninfected lima bean (*P. lunatus*) seeds were purchased from Irrua market, Edo State, Nigeria. Identification and authentication were done by Professor Henry A. Akinnibosun of the Department of Plant Biology and Biotechnology, University of Benin. A voucher specimen (UBH-M337) was deposited at the departmental herbarium. Seeds were pulverized and extracted in 1:1 methanol: water (v/v) for 48 h, filtered through muslin cloth, and concentrated in a water bath at 75 °C. The concentrate was dried in an oven at 45 °C to yield 8.7% w/w of hydromethanol extract (HSE), which was stored in an amber container at 4 °C until use.

Phytochemical screening

Preliminary phytochemical analysis of the extract was carried out according to Sofowora (1993).

Acute toxicity study

Oral acute toxicity was evaluated using a modified Lorke (1983) two-stage method. Phase 1 involved three groups (n=3) receiving 10, 100, and 1000 mg/kg of HSE orally. In the absence of mortality, Phase 2 involved administration of 1600, 2900, and 5000 mg/kg to single mice. Mortality and signs of toxicity were monitored over 24 h.

Sub-acute (28-day) oral toxicity study

Wistar rats were randomized into four groups (n=6, equal sexes). Group A received distilled water (10 mL/kg), while Groups B, C, and D received 400, 800, and 1600 mg/kg/day HSE, respectively, for 28 days. On day 29, the overnight-fasted rats were sacrificed by cervical dislocation. Blood was collected through the abdominal aorta for hematological and biochemical analyses.

Effect on body and relative organ weights

Body weight was recorded every three days. After sacrifice, organs (kidney, spleen, lungs, liver, brain, and heart) were excised, blotted, and weighed. Relative organ weight (ROW) was calculated as:

$$\text{ROW} = \frac{\text{Organ weight}}{\text{Body weight}} \times 100$$

Hematological and biochemical analyses

Hematological parameters were measured using an automated analyzer (Sysmex KX-21N, Sysmex Inc., Kobe, Japan). Serum biochemistry assay of urea (Fawcett and Scott, 1960), creatinine (Delanghe and Speeckaert, 2011), ALT and AST (Reitman and Frankel, 1957), ALP (Walter and Schütt, 1974), albumin (Doumas *et al.*, 1971), total protein and globulin (Lubran, 1978), and bilirubin (Jendrassik and Grof, 1938) were done. Blood glucose was measured using Accu-Chek® glucometer strips.

Histopathology

The organs were fixed in 10% buffered formalin, processed, embedded in paraffin, and sectioned at 4 μm (Adedayo, 2011). Sections were stained with hematoxylin-eosin and examined microscopically at $\times 400$ magnification and micrographs were taken using Olympus digital microscope.

Statistical analysis

Data are expressed as mean \pm SEM (n=6). One-way ANOVA followed by Dunnett's multiple comparison test was performed using GraphPad Prism 6.0. A p-value of less than 0.05 was considered significant.

RESULTS

Phytochemical composition of *P. lunatus* hydromethanol seed extract

Carbohydrates, alkaloids, phenolic compounds, flavonoids, steroids, oils and anthraquinones were detected in the extract (Table 1).

Table 1: Phytochemical constituents of the extract

Classes of Secondary Metabolites	Inference
Carbohydrate	+
Reducing sugars	+
Alkaloids	+
Phenolic compounds	+
Flavonoids	+
Steroids	+
Oils	+
Saponins	-
Anthraquinones	+

+ = present, - = absent

Oral acute toxicity of *P. lunatus* hydromethanol seed extract

Neither death nor obvious toxic symptoms were observed and the estimated LD₅₀ of the extract was greater than 5000 mg/kg.

Effect of the extract (HSE) on body weight

There was a significant ($P < 0.05$) reduction in the whole body of rats treated with the extract. The weights of the various organs relative to the whole-body weight did not change significantly compared with the control (Table 2).

Effect of the extract (HSE) on hematological parameters

The red blood cell indices comprising the red cell counts (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were not significantly altered after 28 days of administration (Table 3). Similarly, platelet count, white blood cell count, and white blood cell differentials were not significantly altered by the treatment (Table 4).

Effect of the extract (HSE) on serum electrolytes, urea, and creatinine

Serum concentration of Na⁺, K⁺, Cl⁻, HCO₃⁻ and creatinine were not significantly changed following 28 days of treatment with the extract except for increase in the levels of urea at the dose of 1600 mg/kg ($P < 0.01$) and a reduction in the concentration of bicarbonate at the doses of 400 ($P < 0.05$) and 1600 mg/kg ($P < 0.01$) (Table 5).

Table 2. Effect of the extract on body and relative organ weights after 28 days

Group	Body wt. change (g)	Spleen	Kidney	Lung	Heart	Brain	Liver
Control	+46.6 ± 6.7	0.5±0.0	1.2±0.1	1.5±0.1	0.6±0.0	1.6±0.1	5.5±0.4
HSE 400 mg/kg	-21.5 ± 7.3*	0.5±0.1	1.4±0.1	1.4±0.1	0.7±0.0	1.7±0.1	5.7±0.4
HSE 800 mg/kg	-26.6 ± 5.2*	0.6±0.1	1.3±0.1	1.4±0.1	0.6±0.0	1.5±0.1	6.0±0.4
HSE 1600 mg/kg	-31.7 ± 5.5*	0.6±0.1	1.1±0.1	1.3±0.1	0.6±0.0	1.7±0.0	5.7±0.3

*P < 0.05 vs. control, n = 6.

Table 3. Effect of the extract on red blood cell indices after 28 days

Group	RBC (10 ⁶ /μL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)
Control	7.5 ± 0.3	15.0±0.5	51.8±2.4	67.6±3.6	20.0±0.2	29.1±1.2
HSE 400 mg/kg	8.5 ± 0.2	17.0±0.7	57.0±2.2	66.9±2.1	19.9±0.5	29.8±0.3
HSE 800 mg/kg	8.6 ± 0.4	17.3±0.9	59.0±3.0	69.0±2.4	20.2±0.6	29.3±0.3
HSE 1600 mg/kg	7.5 ± 0.1	14.9±0.1	50.5±2.0	69.0±1.9	19.8±0.5	29.5±0.9

n = 6

Table 4. Effect of the extract on platelets and white blood cell indices after 28 days

Group	Platelet (10 ⁶ /μL)	WBC (10 ³ /μL)	GRAN (10 ³ /μL)	LYM (10 ³ /μL)	MID (10 ³ /μL)
Control	449.7±33.7	4.8±0.6	0.1±0.0	4.5±0.6	0.2±0.1
HSE 400 mg/kg	394.4±21.0	6.0±0.3	0.2±0.0	5.5±0.3	0.3±0.1
HSE 800 mg/kg	429.0±20.4	6.0±0.3	0.2±0.0	5.1±0.4	0.2±0.1
HSE 1600 mg/kg	445.8±37.8	5.3±0.2	0.2±0.0	5.0±0.4	0.2±0.1

n = 6

Table 5. Effect of the extract on serum electrolytes and renal function after 28 days

Group	Urea (mg/mL)	Creatinine (mg/dL)	Na ⁺ (mmol/L)	K ⁺ (mmol/L)	HCO ₃ ⁻ (mmol/L)	Cl ⁻ (mmol/L)
Control	28.8±2.1	0.7±0.0	139.8±4.6	8.0±0.7	18.3±1.5	111.7±3.2
HSE 400 mg/kg	32.4±2.5	0.8±0.0	139.8±4.6	7.8±0.7	12.6±0.8*	106.6±2.1
HSE 800 mg/kg	35.0±0.8	0.7±0.0	134.4±0.6	7.8±0.5	15.6±1.6	107.0±1.7
HSE 1600 mg/kg	38.0±1.7**	0.8±0.1	135.8±0.9	6.8±0.5	12.5±0.7**	108.0±1.3

*P < 0.05, **P < 0.01 versus control, n= 6.

Effect of the extract (HSE) on fasting blood sugar

Administration of the extract for 28 days significantly reduced their fasting blood sugar. The significant was higher at the 800 and 1600 mg/kg (P < 0.01) dose than in the 400 mg/kg dose (P < 0.05) (Figure 1).

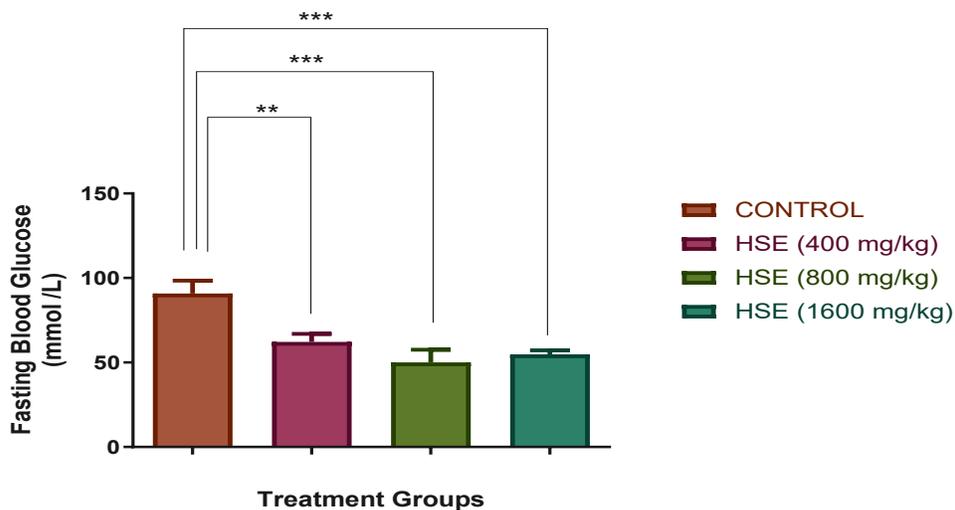


Figure 1. Effect of the extract on fasting blood glucose after 28 days. **P < 0.01, ***P < 0.001 versus control. n=6.

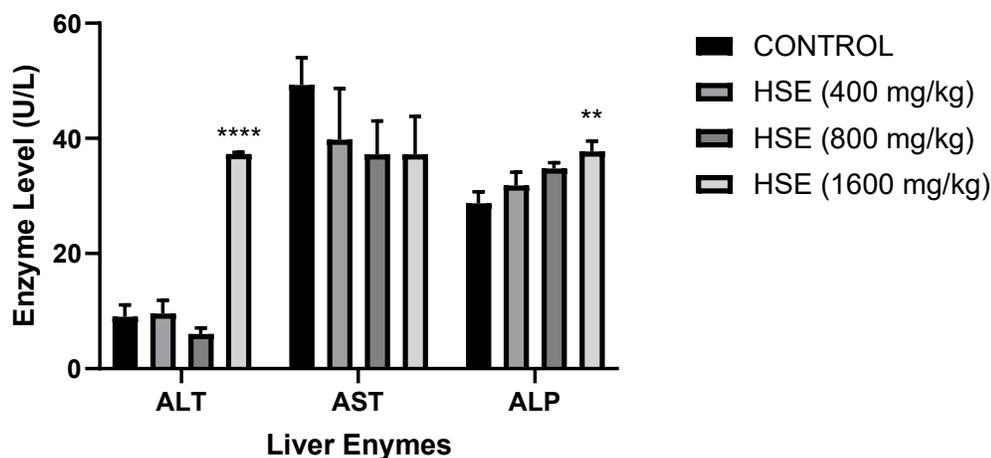


Figure 2. Effect of the extract on liver function enzymes. ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase. ** $P < 0.01$, **** $P < 0.0001$ vs. control, $n=6$

Effect of the extract (HSE) on liver proteins

The liver protein indices: total protein (TP), albumin (ALB.), globulin (GB), total bilirubin (TB) and conjugated bilirubin (CB) were all not significantly different from the control values after 28 days of treatment with *P. lunatus* hydromethanol seed extract (HSE) (Table 6).

Effect of the extract (HSE) on lipid indices

The total cholesterol was significantly elevated above control values at the dose of 1600 mg/kg/day. There were no significant alterations in the values of triglycerides (TG) and high-density lipoproteins (HDL), but the level of low-density lipoprotein (LDL) was significantly ($P < 0.05$, $P < 0.01$, $P < 0.001$) elevated at the three doses of the extract (Table 7).

Effect of the extract on the histology of some vital organs

The photomicrographs of the heart, liver, lungs, brain, kidney, and spleen of rats given oral doses of the extract are presented as Figures 3,4,5,6,7, and 8 respectively. In the heart (Figure 3), focal ulceration of the coronary arteries occurred at the doses of 800 and 1600 mg/kg/day. In the liver (Figure 4), activated Kupffer cells and focal vascular ulcerations were seen in the extract-treated groups. Figure 5 shows that in the lungs, there were patchy alveolar dilatation, bronchial ulceration in the extract-treated groups, and interstitial haemorrhage was evident at the 1600 mg/kg dose. The brains (Figure 6) and kidneys (Figure 7) of all the extract-treated rats were essentially normal. In the spleen (Figure 8), there was increased red blood cell sequestration in all extract treated groups with activation of histiocytes at the highest doses of 1600 mg/kg.

Table 6. Effect of the extract on liver protein indices after 28 days

Group	TP (g/dL)	ALB (g/dL)	GB (g/dL)	TB (mg/dL)	CB (mg/dL)
Control	6.0±0.1	3.5±0.1	2.5±0.1	0.3±0.1	0.1±0.0
HSE 400 mg/kg	5.8±0.1	3.7±0.1	2.1±0.1	0.3±0.1	0.1±0.0
HSE 800 mg/kg	6.0±0.1	3.9±0.1	2.1±0.1	0.3±0.1	0.1±0.0
HSE 1600 mg/kg	5.8±0.1	3.8±0.1	2.1±0.1	0.3±0.1	0.1±0.0

n=6. TP: total protein, ALB: albumin, GB: globulin, TB: total bilirubin, CB: conjugated bilirubin.

Table 7. Effect of the extract on lipid profile

Group	T. CHOL (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
Control	61.2±2.8	85.5±3.0	32.0±1.2	12.2±2.2
HSE 400 mg/kg	74.4±9.3	88.6±5.2	31.4±5.8	25.4±3.1*
HSE 800 mg/kg	75.2±5.7	78.0±8.7	31.8±3.0	28.0±3.4**
HSE 1600 mg/kg	88.0±5.1*	97.7±7.1	36.2±3.4	32.3±3.9***

*P < 0.05, **P < 0.01, ***P < 0.001 versus control, n=6. T. CHOL: total cholesterol, TG: triglyceride, HDL: high-density lipoprotein, LDL: low-density lipoprotein.

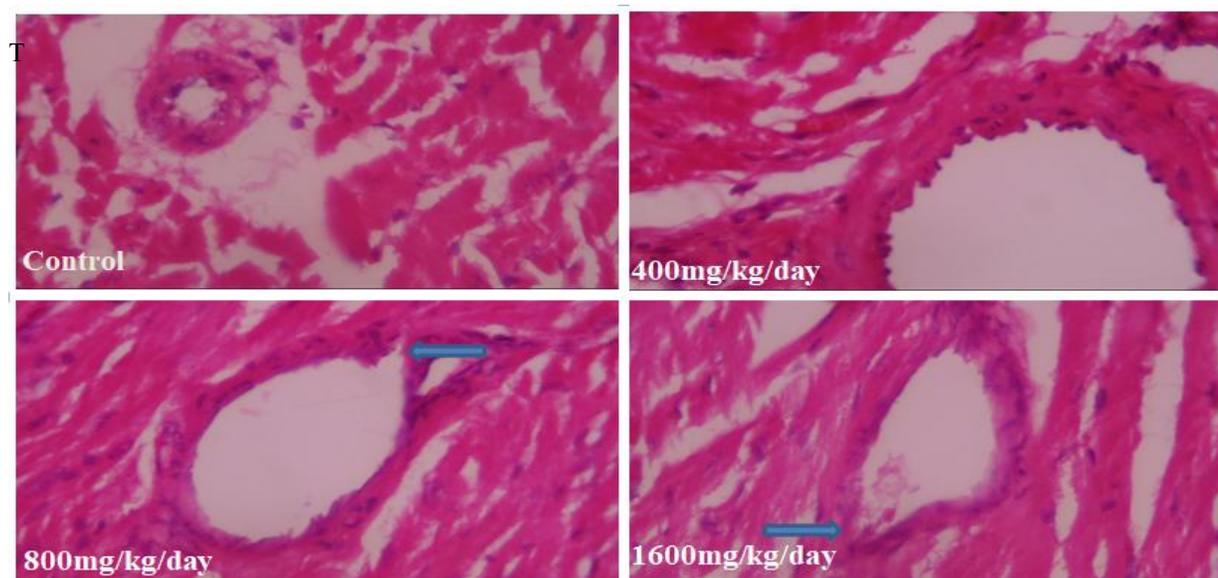


Figure 3: Representative photomicrographs of the heart (H&E, ×400), blue arrow= focal ulceration in the coronary artery.

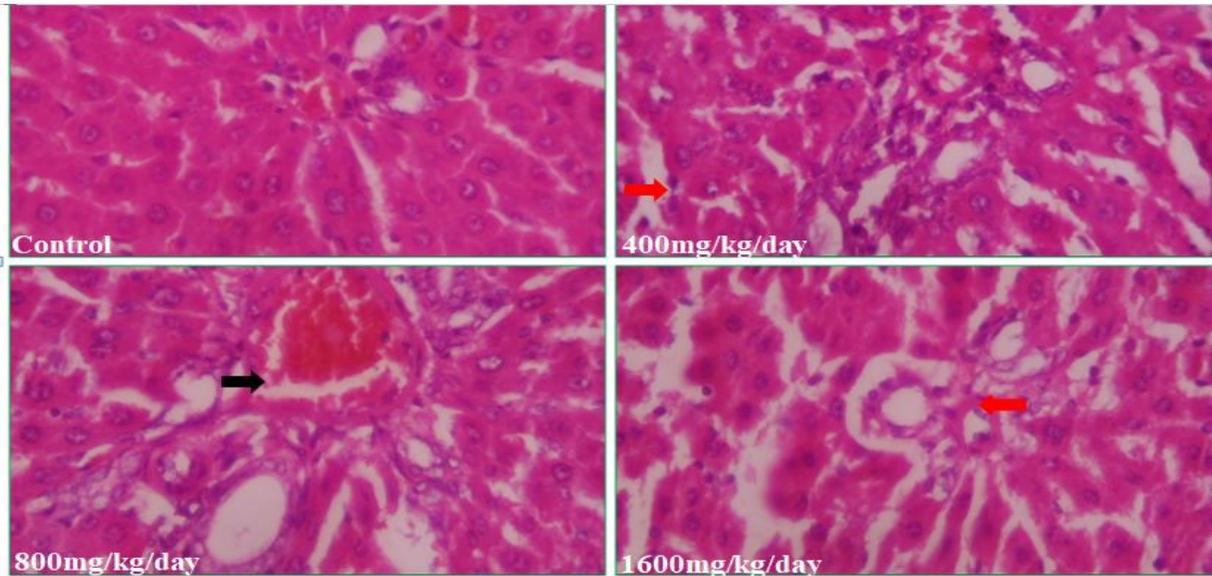


Figure 4: Representative photomicrographs of the liver (H&E, ×400), red arrow=Kupffer cell activation, black arrow = focal vascular ulceration.

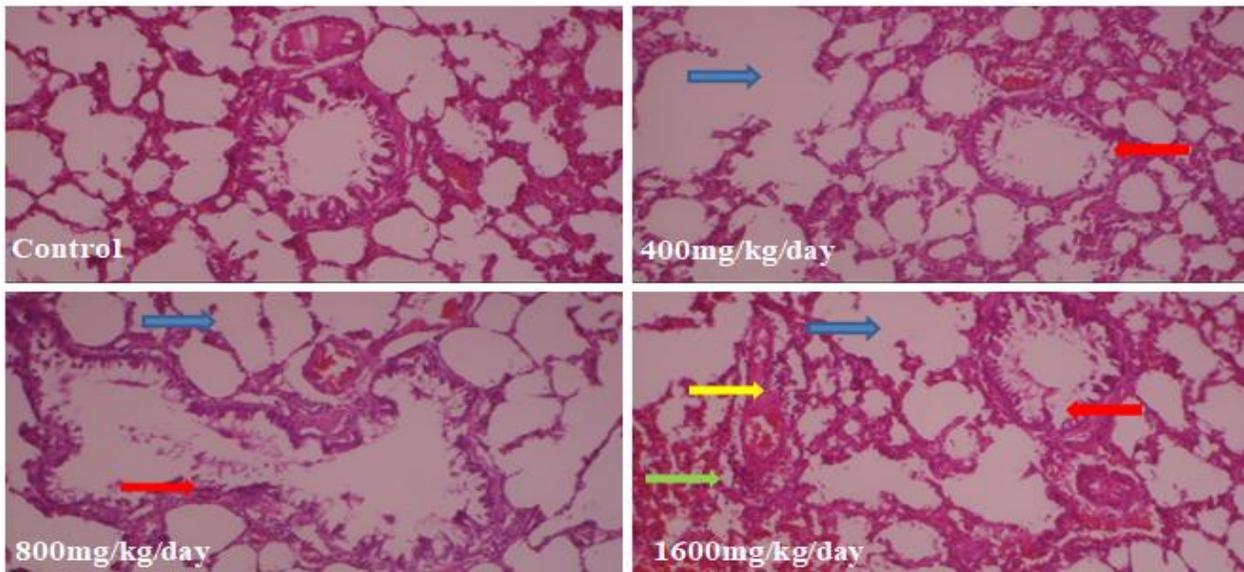


Figure 5: Representative photomicrographs of the lung (H&E, ×400), blue arrow=patchy alveolar dilation, red arrow=focal bronchial ulceration, yellow arrow=vascular distortion, lemon arrow=interstitial hemorrhage.

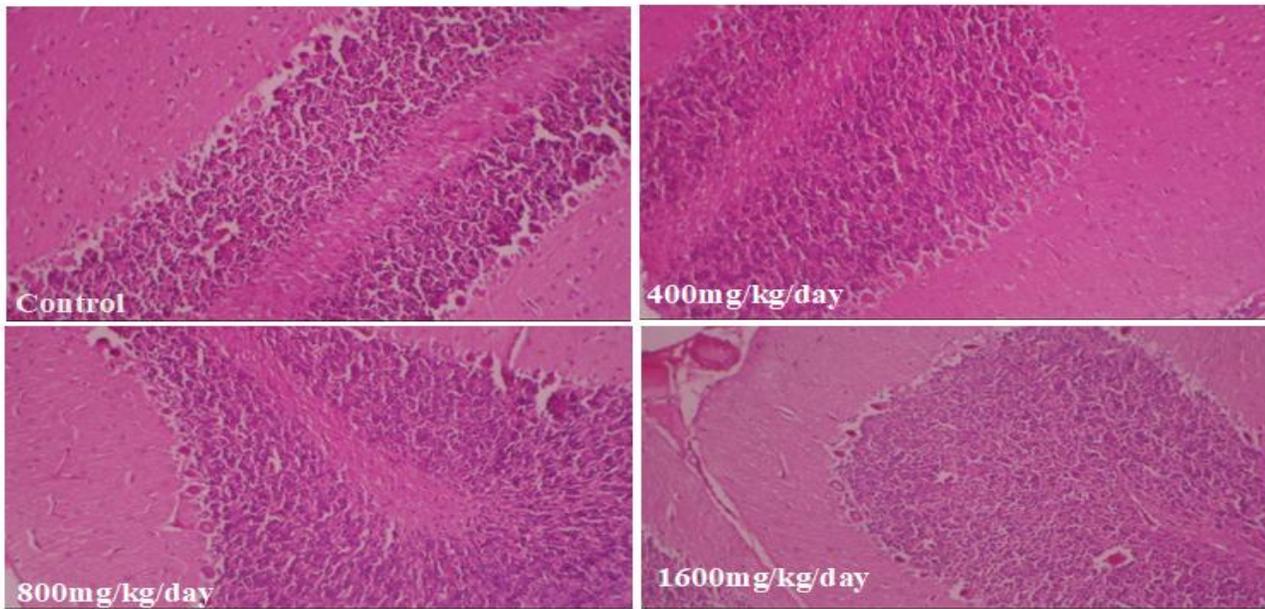


Figure 6: Representative photomicrographs of the brain (H&E, ×400). Histology is normal at all doses.

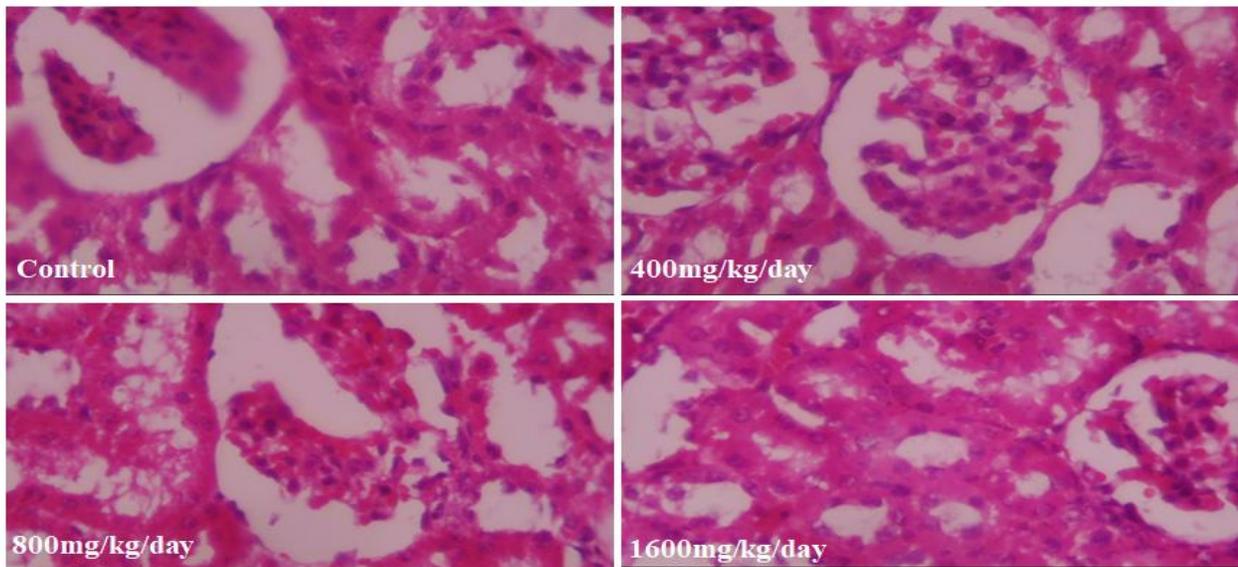


Figure 7: Representative photomicrographs of the kidney (H&E, ×400). Histology is normal at all doses.

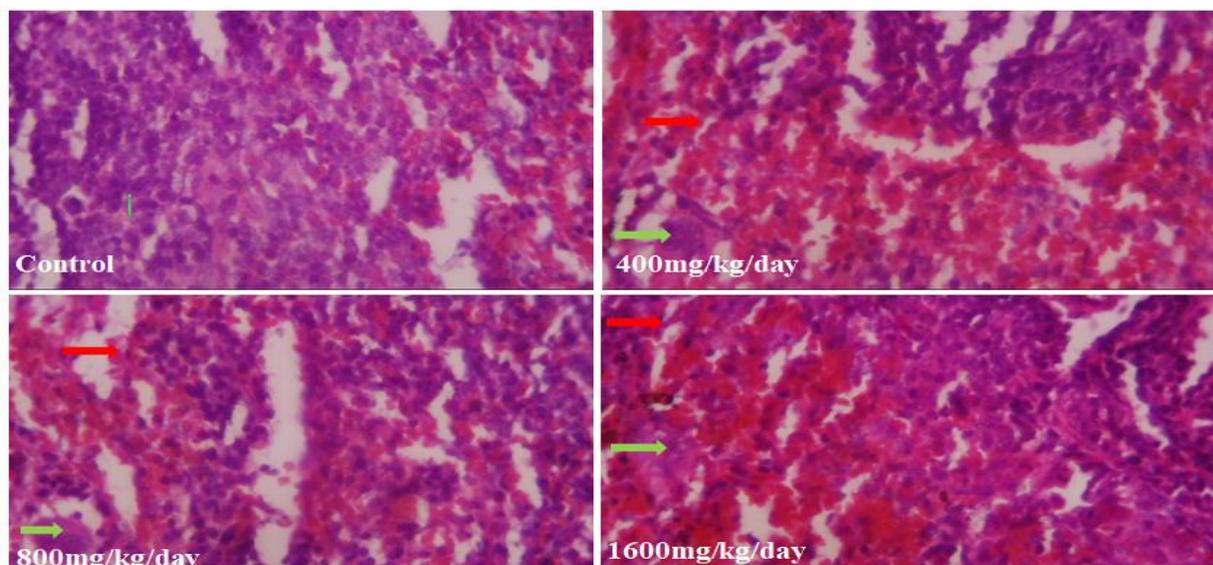


Figure 8: Representative photomicrographs of the Spleen (H&E $\times 400$), red arrow=increased red cell sequestration, lemon arrow=activation of histiocyte

Discussion

Plants contain diverse bioactive compounds that confer both therapeutic benefits and potential toxic risks (Lai and Roy, 2004). The presence of alkaloids, phenolics, flavonoids, and steroids in the *P. lunatus* extract aligns with the nutritional and pharmacological richness of legumes. However, such phytochemicals can exert harmful effects at high concentrations, emphasizing the importance of toxicological evaluation (Cohen and Ernst, 2010; Kunle *et al.*, 2012; Ekor, 2014). This is particularly relevant to substances used in chronic disorders such as psychosis, where long-term accumulation may lead to organotoxicity (Clemenson *et al.*, 2000; Ekor, 2014).

The LD₅₀ informed the dose selection for the sub-acute toxicity study (Ozolua *et al.*, 2010; Saganuwan, 2017). Sedation observed at high doses suggests possible central nervous system depressant or sleep-enhancing properties requiring further pharmacological investigation. Despite the significant decrease in body weight, relative organ weights were unchanged, suggesting that weight loss did not stem from organ atrophy. Organ weights are sensitive toxicity indicators (Teo *et al.*, 2002; Michael *et al.*, 2007), and their stability supports the absence of severe gross organ damage. Hematological parameters remained within normal limits, indicating that erythropoiesis, platelet turnover, and immune status were not compromised. This is consistent with findings that legume extracts often show minimal hematotoxicity (Adeneye *et al.*, 2008). Renal indices, however, showed notable alterations. While sodium, potassium, and chloride remained stable, bicarbonate levels were significantly reduced, reflecting a tendency toward metabolic acidosis, elevated urea at 1600 mg/kg suggests

impaired renal clearance or altered nitrogen metabolism (Gowda, 2010). The normal protein levels imply that the hyperuricemia was not driven by increased purine turnover from proteins but may have been secondary to reduced bicarbonate and associated acid-base imbalance (Meert *et al.*, 2007; Kim, 2021). The elevation of ALT and ALP at 1600 mg/kg indicates hepatocellular and possible cholestatic injury (Ozer *et al.*, 2008; Moriles *et al.*, 2024). The pattern of high ALT with normal AST mirrors what has been reported in non-alcoholic fatty liver disease and herb-induced liver injury (Dufour, 2009). Increased ALP also raises concern for possible bone involvement (Lowe *et al.*, 2023; Makris *et al.*, 2023). Preservation of protein and bilirubin values suggests that synthetic function of the liver remained intact despite enzymatic disturbances.

The extract demonstrated strong hypoglycemic activity, consistent with previous findings that *P. lunatus* peptides enhanced insulin secretion, inhibited carbohydrate absorption, and increased glucose uptake (Jenkins *et al.*, 1980; Thompson *et al.*, 2009; Miguel *et al.*, 2016; Erukainure *et al.*, 2018). While this highlights potential antidiabetic utility, the glucose-lowering effect raises the risk of hypoglycemia, especially in individuals using antidiabetic medicines. Dyslipidemia involved elevated LDL at all doses and increased total cholesterol at 1600 mg/kg; indicating a shift toward an atherogenic profile. LDL is a major biomarker for cardiovascular disease, and elevated levels increase the risk of atherosclerosis and arteriosclerosis (Lee *et al.*, 2021; Wang *et al.*, 2023). These results contrast with previous reports of lipid-lowering effects of heat-treated *P. lunatus* (Oboh and Omofoma,

2008), likely due to differences in extraction method, phytochemical concentration, and mode of administration. Histopathological alterations corroborated the biochemical findings. Vascular ulceration in the heart and liver, alveolar dilation in the lungs, and splenic RBC sequestration suggest tissue-specific toxicity at high doses (Gupta and Gupta, 2020). Interestingly, some organs showed microscopic changes without matching biochemical derangements. Normal renal histology despite hyperuricemia, and normal RBC indices despite splenic sequestration, indicate that early pathological changes may precede measurable systemic abnormalities. Overall, the extract appears safe at lower doses but demonstrates organ-specific toxicity, metabolic disturbances, and cardiovascular risks at high or prolonged dosing. These findings support the need for caution in chronic use and justify further investigation into dose optimization, mechanism of toxicity, and safer therapeutic windows.

Conclusion

The hydromethanol seed extract of *Phaseolus lunatus* appears safe following acute oral exposure, with an LD₅₀ above 5000 mg/kg. However, repeated administration at doses above 400 mg/kg/day for 28 days induced alterations in liver enzymes, urea, bicarbonate, lipids, and histological changes in some organs. The risk of hypoglycemia and dyslipidemia may limit its therapeutic use in psychosis or other chronic conditions.

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Conflict of interest

The authors declare that they have no conflicting interests.

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