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Research Article

Assessment of Acute and Chronic Toxicity of the Active Fraction of *Lavandula stoechas* in Albino Mice: A Comprehensive Study

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Abstract

This study builds upon prior research where the principal components, phenethylamine, and α-tocopherol, of *Lavandula stoechas* (*L. stoechas*) were identified as responsible for its nootropic potential. The current investigation aimed to determine the acute and chronic toxicity profiles of the active fraction of *L. stoechas* (AfL.s) in albino mice (ethical approval vide letter no; GCU-AEC-682/24). Acute toxicity assessment involved oral administration of AfL.s at doses of 350 mg/Kg and 300 mg/Kg, with toxicity signs monitored for 72 hours. For chronic toxicity evaluation, two groups of mice were administered either 10 ml/kg P.O. of normal saline or 18 mg/Kg P.O. of AfL.s daily for three consecutive months. At the end of the 90 days, blood sampling was done via cardiac puncture to perform hematological and biochemical analyses. Additionally, the brain and liver were harvested for histopathological examination. The acute toxicity study revealed that AfL.s induced hyperexcitation, convulsions, ataxia, muscle spasms, and blanching in mice. Chronic toxicity evaluation indicated that a dose of 18 mg/Kg P.O. of AfL.s led to non-significant ($P \geq 0.05$) alterations in hematological parameters; RBC count, platelet count, hemoglobin and serum biochemistry; cholesterol, creatinine, glucose, AST (aspartate transaminase), ALT (alanine aminotransferase), and TB (total bilirubin). However, WBC count, hematocrit, and alkaline phosphatase (ALP) levels exhibited slight increases, accompanied by a significant ($P \leq 0.05$) reduction in body weight in the treated mice. Histopathological assessments did not reveal any signs of toxicity. Consequently, AfL.s at an oral dose of 18 mg/Kg P.O. is deemed safe for long-term use in mice.

Keywords: *L. stoechas*; Acute Toxicity; Chronic Toxicity; AST; ALT; Hematology.

1. Introduction

Lavandula stoechas (Lamiaceae) is predominantly found in Mediterranean regions and is commonly referred to as French or Spanish Lavender. In the Indo-Pak region, it is locally recognized as Ustukhuddus (Khan et al. 2024). It has been extensively studied for its diverse therapeutic applications in traditional medicine, exhibiting analgesic, antimicrobial, antiseptic, antidiabetic, antispasmodic, and antiepileptic properties (Gilani et al. 2000). Research has demonstrated that the essential oils of *L. stoechas* (LESO) mitigate hyperglycemia in alloxan-induced hyperglycemic mice by inhibiting oxidative stress (Sebai et al. 2013). Additionally, LESO has been employed to alleviate postpartum discomfort; its poultices are

applied to the back and abdomen of women to relax back muscles and facilitate the swift expulsion of the placenta, respectively (Cornwell and Dale 1995). Furthermore, LESO is topically applied to treat eczema and allergies (Kim and Cho 1999) and its inhalation and body massage have been utilized to alleviate mental stress and sleep disorders (Dunn, Sleep, and Collett 1995, Fismer and Pilkington 2012). Traditionally, *L. stoechas* has been employed in aromatherapy for the rapid relief of migraine headaches, body aches (Prashar, Locke, and Evans 2004, Cavanagh and Wilkinson 2002), as well as anxiety, depression, and stress-related issues (Dunn, Sleep, and Collett 1995, Fismer and Pilkington 2012). Commercially, the plant's essential oils are utilized as flavoring agents in cosmetics (Prashar, Locke, and Evans 2004) and are employed as fumigants to eradicate insects in storage areas (Bown 1995, Ebadollahi, Safaralizadeh, and Pourmirza 2010).

Extensive research has been conducted on *L. stoechas,* leading to the identification of numerous chemical constituents from various parts of the plant. A comprehensive list of these constituents includes several significant compounds such as camphor, fenchone, erythrodiol, lavanol, eucalyptol, lupeol, longipene-2-ene-monoacetate, luteolin, oleanolic acid, pinocarvyl acetate, myrtenol, terpinol, vitexin, β-sitosterol, ursolic acid, and vegatic acid (Bouyahya et al. 2017, Ulubelen and Olcay 1989). These chemical entities are accountable for the plant's diverse biological and pharmacological activities. For instance, the sedative properties of *L. stoechas* are attributed to linalool (Re et al. 2000). Linalool binds to various receptors within the brain, inducing a state of calmness and enhancing overall well-being and human behavior (Cavanagh and Wilkinson 2002). Moreover, studies have demonstrated that camphor present in *L. stoechas* is responsible for brain stimulation, and the essential oils derived from the plant are widely employed in aromatherapy (Prashar, Locke, and Evans 2004).

Studies have demonstrated that the essential oils extracted from *L. stoechas* can serve as an insecticide against the cigarette beetle (Ebadollahi et al. 2010). However, overall, these oils exhibit low toxicity and are frequently employed either as a dietary supplement or as a crucial pharmacological ingredient in numerous pharmaceutical preparations (Arantes et al. 2016).

Considering the historical use of *L. stoechas* in traditional medicine, where it was extensively utilized as a brain tonic (Gilani et al. 2000, Hakeem, Siddiqui, and Khan 1991), a project was devised to investigate the biomolecules present in the aerial parts of *L. stoechas* responsible for memory enhancement. In a previous study, we reported that the active fraction of *L. stoechas* (AfL.s) contained two principal components, phenethylamine and α-tocopherol, which are accountable for the anti-amnesic activity observed in albino mice (Mushtaq et al. 2021). Consequently, the existing study was intended to evaluate the chronic effects of a therapeutic dose of AfL.s (18 mg/Kg P.O) in albino mice.

2. Materials and Methods

2.1. Extraction, Purification and Anti-amnesic Activity

The aerial parts (stems, leaves, and flowers) of *L. stoechas* were procured from a local market in Lahore, Pakistan, and authenticated by a botanist from Government College University (GCU), Lahore, under herbarium number GC.Herb.Bot.3386. Fractional extraction was employed to obtain various fractions of *L. stoechas*. The primary mother fraction was prepared in methanol using the simple maceration technique. Subsequently, secondary fractions, including n-hexane, chloroform, ethyl acetate, n-butanol, and aqueous fractions, were derived from the mother fraction. The *in vitro* and *in vivo* (anti-amnesic) activities were assessed in mice through behavioral and biochemical studies, as detailed in prior studies of this project (Mushtaq, Anwar, and Ahmad 2018a, b). The findings revealed that the aqueous fraction of *L. stoechas* exhibited the most potent anti-amnesic activity. Consequently, this fraction was selected for further investigation to

identify the biomolecules responsible for the anti-amnesic potential of *L. stoechas*. The aqueous fraction was subsequently purified into various sub-fractions using column chromatography. These sub-fractions were subjected to *in vitro* tests to evaluate their anticholinesterase activity. The most active sub-fraction, which exhibited the highest inhibition of acetylcholinesterase, was chosen for GC-MS analysis. This active sub-fraction was designated as AfL.s (Active fraction of *L. stoechas*) and was administered to Swiss albino mice to assess behavioral and biochemical responses. The detailed methodologies for fractionation, column chromatography, GC-MS analysis, *in vitro* studies, and *in vivo* behavioral and biochemical assessments, along with their outcomes, are elucidated in our previous study (Mushtaq et al. 2021). In the current study, AfL.s was evaluated for acute and chronic effects in albino mice.

2.2. Animals

Toxicity studies were conducted on female Swiss albino mice weighing 20 ± 5 g. The animals were housed in the animal facility at Government College University, Lahore, Pakistan, and were maintained under standard laboratory conditions throughout the study duration. They were provided with a standard pellet diet and water *ad libitum*. The ambient temperature was maintained at 25 ± 5°C with 50% relative humidity, and a 12-hour light-dark cycle was maintained. Bedding for the animals was changed daily to ensure cleanliness and hygiene. The permission regarding the use of the animals was obtained from the institutional animal ethics committee vide letter no; GCU-AEC-682/24.

2.3. Study Design for Acute Toxicity

Acute toxicity studies were conducted under the guidelines of the Organization for Economic Co-operation and Development (OECD, 423, 2001). The median effective dose (ED_{50}) and median lethal dose (LD_{50}) of AfL.s were determined to be 18 mg/Kg P.O and 325 mg/Kg P.O, respectively, in mice, as detailed in our previous study (Mushtaq et al. 2021). Based on the established LD_{50} of AfL.s, acute toxicity studies were performed at two dose levels. One group of animals (n=6) received a single oral dose of AfL.s at 350 mg/Kg, while the other group (n=6) received a single oral dose of AfL.s at 300 mg/Kg. Following the administration of the doses, the animals were monitored for a minimum of 72 hours for signs of toxicity, including ataxia, convulsions, blanching, cyanosis, hyperactivity, depression, hypnosis, jumping, irritability, pilo erection, salivation, loss of traction, muscle spasm, sedation, ptosis, stimulation, rigidity, Strabo tail, redness, and secretion (El Hilaly, Israili, and Lyoussi 2004).

2.4. Study Design for Chronic Toxicity

The study aimed to investigate the chronic effects of the therapeutic dose of AfL.s (18 mg/Kg P.O) on the brain, liver, and kidneys of albino mice. The animals were divided into two groups: Group I (Control) and Group II (Treatment). Each group consisted of six animals. The control group received an oral administration of 10 ml/kg of normal saline daily for three months. In contrast, the treatment group was orally administered AfL.s at a dose of 18 mg/Kg daily in the morning for consecutive three months. The dose of AfL.s at 18 mg/Kg P.O was determined as the therapeutic dose for anti-amnesic activity based on previous studies, which demonstrated that this dose exhibited maximum therapeutic effects (Mushtaq et al. 2021). The body weight of the animals was recorded daily before the administration of the dose. To evaluate the chronic effect of the AfL.s dose of 18 mg/Kg P.O on the body weight of the animals, weights recorded on the 1^{st}, 30^{th}, 60^{th}, and 90^{th} days of the treatment were included in the study. At the end of the 3rd month of treatment, blood samples were collected via cardiac puncture and stored in two types of tubes: one containing EDTA for hematological analysis and the other without EDTA for serological studies. Hematological parameters were assessed immediately using fresh blood samples. Serum was obtained by centrifuging the blood samples without EDTA to evaluate the biochemical

Table 1: Effect of acute toxic dose of AfL.s (350 mg/Kg P.O) in mice.

Behavioral Changes	Before Dosing	Observation After Dose Administration						
		1 hr	2 hr	6 hr	12 hr	24 hr	48 hr	72 hr
Ataxia	–	+	+	+	+	+	+	+
Convulsions	–	+	+	+	+	+	+	–
Blanching	–	+	+	+	+	+	+	–
Cyanosis	–	–	–	–	–	–	–	–
Hyperactivity	–	–	+	+	+	+	+	+
Depression	–	–	–	–	–	–	–	–
Hypnosis	–	–	–	–	–	–	–	–
Jumping	–	+	+	+	+	+	+	–
Irritability	–	+	+	+	+	+	+	+
Pilo erection	–	–	+	+	+	+	–	–
Salivation	–	–	+	+	+	–	–	–
Loss of Traction	–	–	–	–	–	–	–	–
Muscle Spasm	–	–	–	+	+	+	+	+
Sedation	–	–	–	–	–	–	–	–
Ptosis	–	–	–	–	–	–	–	–
Stimulation	–	–	+	+	+	+	+	+
Rigidity	–	–	+	+	+	–	–	–
Strabo Tail	–	–	+	+	+	+	+	–
Redness	–	–	–	–	–	–	–	–
Secretions	–	–	+	+	+	+	+	–

Presence of characteristic: +, Absence of characteristics: –

markers (El Hilaly, Israili, and Lyoussi 2004, Mushtaq et al. 2017).

2.5. Blood Analysis

Hematological parameters, including red blood cells (RBCs), white blood cells (WBCs), platelets, hematocrit, and hemoglobin levels, were determined using the Merck Micro Lab 300 auto analyzer. Biochemical parameters such as serum cholesterol, glucose, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (TB), and creatinine were assessed using diagnostic kits from Human Germany. The Merck Micro Lab 300 auto-analyzer was employed to analyze the serum biochemical markers following standard procedures.

2.6. Histopathological Examinations

Chloroform was employed for euthanizing the animals. Subsequently, the animals were dissected, and their brains (mid-brain), livers (left lobe), and kidneys (corticomedullary junction) were isolated. These tissues were then fixed in 10 % formalin after being thoroughly washed with normal saline. Tissue slides were prepared by obtaining small tissue samples, which were embedded in paraffin. After freezing in a refrigerator, the tissues were sectioned into thin slices with a thickness of 5 µm using a microtome to ensure uniformity. The sections were initially washed with ethanol and then rehydrated with distilled water. Hematoxylin and eosin staining were performed on the slides, and xylene was used for the final slide fixation (Ren et al. 2018).

Microscopic examination of the stained slides was conducted at resolutions of 10X and 40X using a microscope equipped with a top-mounted camera. The photomicrographs of the tissue slides were examined to assess the presence of necrosis, fibrosis, degeneration, inflammation, and edema. The scoring for each parameter was done by assigning grades to each i.e. (normal: 0, mild: 1, moderate: 2, and marked: 3). The scores were then

Table 2. Effect of acute toxic dose of AfL.s (300 mg/Kg P.O) in mice

Behavioral Changes	Before Dosing	Observation After Dose Administration						
		1 hr	2 hr	6 hr	12 hr	24 hr	48 hr	72 hr
Ataxia	−	+	+	+	+	+	+	+
Convulsions	−	+	+	+	+	+	−	−
Blanching	−	+	+	+	+	−	−	−
Cyanosis	−	−	−	−	−	−	−	−
Hyperactivity	−	−	−	+	+	+	+	+
Depression	−	−	−	−	−	−	−	−
Hypnosis	−	−	−	−	−	−	−	−
Jumping	−	+	+	+	+	+	+	−
Irritability	−	+	+	+	+	+	+	+
Pilo erection	−	−	+	+	−	−	−	−
Salivation	−	−	+	+	+	−	−	−
Loss of Traction	−	−	−	−	−	−	−	−
Muscle Spasm	−	−	−	+	+	+	−	−
Sedation	−	−	−	−	−	−	−	−
Ptosis	−	−	−	−	−	−	−	−
Stimulation	−	−	+	+	+	+	+	−
Rigidity	−	−	+	−	−	−	−	−
Strabo Tail	−	−	+	+	+	+	+	−
Redness	−	−	−	−	−	−	−	−
Secretions	−	−	+	+	+	−	−	−

Presence of characteristic: +, Absence of characteristics: −

added up for the calculation of the Histology Activity Index (HAI) for the mouse brain, liver and kidney. The greater the HAI more tissue damage (Knodell et al. 1981).

2.7. Statistical Analysis

Data are presented as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test was applied to the dataset. The values of the treated group were compared with those of the normal control group. A P-value ≥ 0.05 was considered non-significant and denoted by "ns", while P-values ≤ 0.05, ≤ 0.01, and ≤ 0.001 were considered significant, more significant, and highly significant, respectively, and denoted by "a", "b", and "c".

3. Results

3.1. Acute Toxicity

Following the administration of acute toxic doses (350 mg/Kg P.O) of AfL.s, symptoms such as ataxia, hyperactivity, jumping, blanching, and convulsions were observed within 1 hour. Additional effects, including hyperactivity, piloerection, hypersalivation, muscle spasm, Strabo tail, and rigidity, manifested within two hours post-administration of AfL.s at 350 mg/Kg P.O. The animals exhibited behavioral changes for up to two weeks; however, the most pronounced effects were observed within the initial 72 hours, as depicted in Tables 1-2. After 72 hours, the stimulatory effects in the mice began to diminish.

3.2. Chronic Toxicity

It was observed that the chronic treatment with AfL.s at a dose of 18 mg/Kg P.O significantly reduced the body weight of the mice compared to the control group, as depicted in Table 3. The administration of chronic doses of AfL.s to animals did not significantly alter the hematological parameters, including red blood cell count (RBCs), white blood cell count (WBCs), hemoglobin levels, platelet count, and hematocrit

Table 3: Effect of active fraction of *L. stoechas* (AfL.s) on body weight of mice

Days	Body Weight of Mice (g)	
	Group-1 (Normal saline 10 ml/Kg P.O)	**Group-II (AfL.s 18 mg/Kg P.O)**
1st Day	20.83 ± 0.60	20.16 ± 0.30^{ns}
30th Day	30.66 ± 1.05	31.33 ± 0.91^{ns}
60th Day	41.66 ± 1.02	40.83 ± 0.94^{ns}
90th Day	55.67 ± 1.54	50.58 ± 1.33^{a}

ns: non-significant ($P > 0.05$), a: significant ($P < 0.05$)

Table 4: Effect of active fraction of *L. stoechas* (AfL.s) on hematological and biochemical serum markers

Tests		Group-1 (Normal saline 10 ml/Kg P.O)	Group-II (AfL.s 18 mg/Kg P.O)
Hematological Parameters	RBCs (10^6/µL)	8.78±0.21	8.51±0.41^{ns}
	WBCs (10^3/µL)	6.48±0.23	8.01±0.27^{b}
	Platelets (10^4/µL)	9.65±0.30	8.97±0.54^{ns}
	Hemoglobin (g/dL)	13.51±0.53	12.10±0.66^{ns}
	Hematocrit (Vol%)	41.96 ± 0.89	44.46±0.98^{a}
Biochemical Markers	Creatinine (mg/dL)	0.195 ± 0.02	0.270 ± 0.02^{ns}
	Cholesterol (mg/dL)	191.68±2.38	178.03±4.15^{ns}
	Glucose (mg/dL)	109.16±3.73	102.46±4.33^{ns}
	ALP (IU/L)	152.08 ± 5.37	184.43 ± 8.69^{a}
	AST (IU/L)	79.37 ± 6.04	63.82 ± 6.30^{ns}
	ALT (IU/L)	70.73 ± 2.34	59.98 ± 4.96^{ns}
	TB (IU/L)	0.684 ± 0.01	0.755 ± 0.03^{ns}

AST: Aspartate Transaminase, ALT: Alanine Aminotransferase, ALP: Alkaline Phosphatase, TB: Total Bilirubin, ns: non-significant ($P > 0.05$), a: significant ($P < 0.05$), b: more significant ($P < 0.01$)

values, in mice. Similarly, the levels of biochemical markers such as serum creatinine, cholesterol, glucose, total bilirubin, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) remained unaffected. However, there was a slight increase in alkaline phosphatase (ALP) levels (P<0.05). The numerical data are presented in Table 4.

3.3. Histopathological Study

Histopathological slides of the brain, liver, and kidney from both the control and treated groups were examined to assess the structural damage. No evident damage was observed in the histological slides from the treated animals compared to those from the normal control group. Consequently, the Histology Activity Index (HAI) was calculated as zero, as none of the signs of damage, such as necrosis, fibrosis, degeneration, inflammation, and edema, were present in the histological slides of the treated animals, as shown in Table 5 and Figures 1-3.

4. Discussion

Lavandula stoechas a member of the Lamiaceae family, is a well-known plant from which numerous essential oils have been extracted using methods such as steam distillation and supercritical carbon dioxide extraction (Topal et al. 2008). The essential oils of ***L. stoechas*** contain hundreds of chemical constituents, including fenchone and camphor (Tzakou, Bazos, and Yannitsaros 2009), linalool (Végh et al. 2012), 1, 8 cineole, viridiflorol and myrtenyl acetate (Kırmızıbekmez et al. 2009), as well as camphene, limonene, eucapur, and endobronyl acetate (Sebai et al. 2013). Other identified compounds include pulegone, menthone, menthol, and various steroidal and aromatic moieties (Topcu et al. 2001)

Table 5. Histopathological studies of mice brain, liver, and kidney to assess chronic toxicity.

Tissue	Groups	Scoring of Histopathology Lesion and Individual Histology Activity Index (HAI) for Each Parameter					HAI
		Necrosis	Fibrosis	Degeneration	Inflammation	Edema	
Brain	Control	–	–	–	–	–	0
	Treated	–	–	–	–	–	0
Liver	Control	–	–	–	–	–	0
	Treated	–	–	–	–	+	0
Kidney	Control	–	–	–	–	–	0
	Treated	–	–	–	–	–	0

Control was administered with normal saline 10 ml/Kg P.O for 3 months and Treated Group was administered with Active Fraction of *L. stoechas* AfL.s 18 mg/Kg P.O for 3 months, HAI: Histology Activity Index, –: Not present, +: Very Slight present

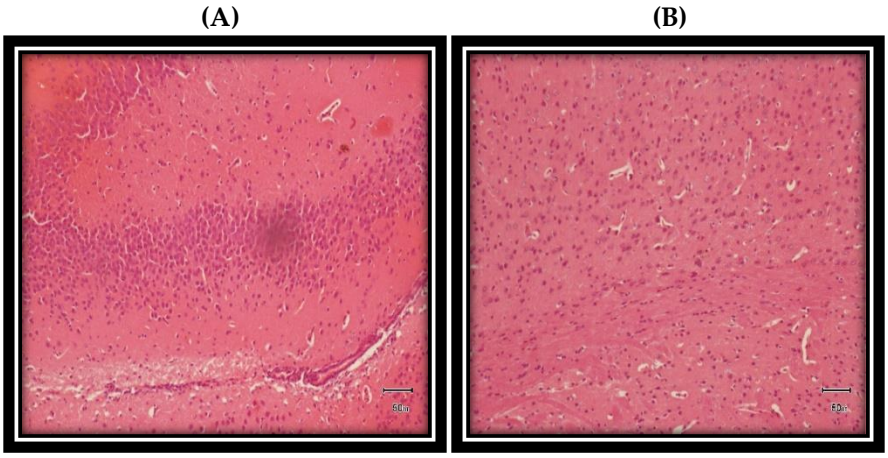

Figure 1: Histopathological studies of mice brain to assess chronic toxicity of active fraction of *L. stoechas* (hematoxylin & eosin staining, magnification power at 100x). (A) Control group (treated with normal saline and normal diet only). (B) Experimental group (treated with AfL.s 18 mg/Kg P.O for 3 months)

and still, research is going on aims to further isolate and identify potential bioactive compounds from different parts of this plant.

L. stoechas, possesses various pharmacological activities. Research has demonstrated its potential in treating brain disorders such as amnesia, epilepsy, nerve pain, palsy, and paralysis (Gilani et al. 2000). In traditional Unani and Ayurvedic medicine, ***L. stoechas*** has been commonly used as a local remedy for central nervous system disorders and is referred to as the "broom of the brain" (Baytop 1999). Our previous studies extensively investigated the plant's role in enhancing memory in albino mice (Mushtaq, Anwar, and Ahmad 2018b, a). The active fraction of ***L. stoechas*** was found to contain phenethylamine and α-tocopherol, which enhance memory by inhibiting acetylcholinesterase and increasing choline acetyltransferase levels in scopolamine-induced hypermnesia mice (Mushtaq et al. 2021).

The current study aimed to investigate the acute and chronic toxicity of ***L. stoechas*** in albino mice. The results indicated that AfL.s is safe at therapeutic doses and only exhibits toxic effects at very high doses exceeding 300 mg/Kg P.O, with an LD_{50} calculated as 325 mg/Kg P.O and a broad therapeutic index (Nelson, Bryant, and Aks 2014, Mushtaq et al. 2021). Administration of an acute toxic dose (300 mg/Kg P.O) resulted in

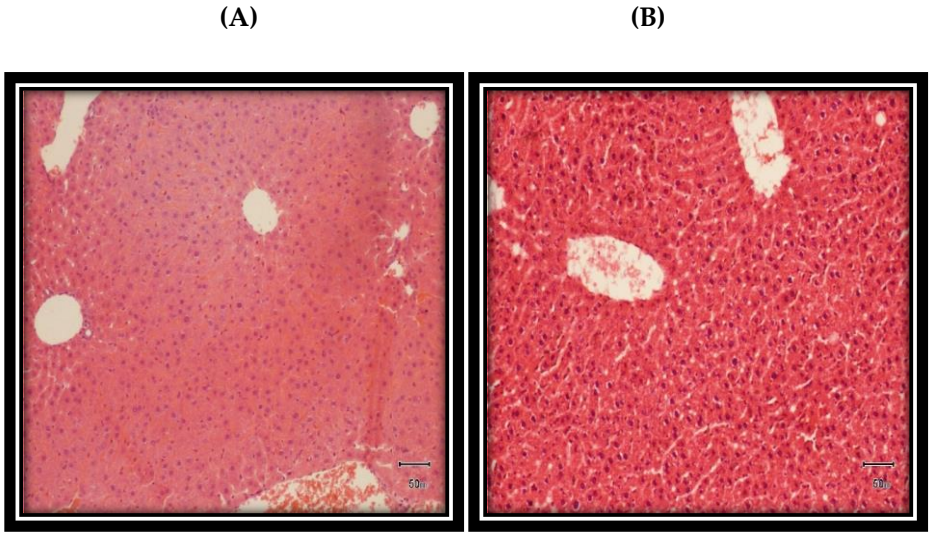

Figure 2: Histopathological studies of mice liver to assess chronic toxicity of active fraction of *L. stoechas* (hematoxylin & eosin staining, magnification power at 100x). (A) Control group (treated with normal saline and normal diet only). (B) Experimental group (treated with AfL.s 18 mg/Kg P.O for 3 months)

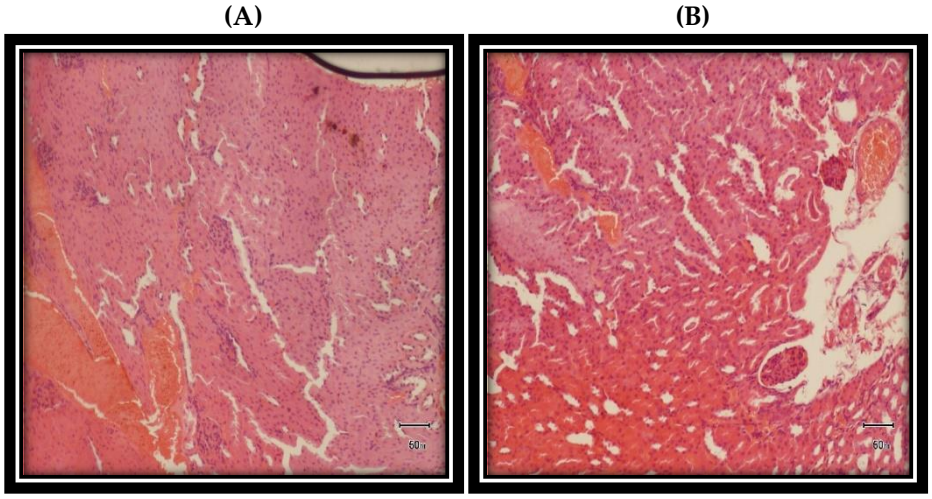

Figure 3: Histopathological studies of mice kidney to assess chronic toxicity of active fraction of *L. stoechas* (hematoxylin & eosin staining, magnification power at 100x). (A) Control group (treated with normal saline and normal diet only). (B) Experimental group (treated with AfL.s 18 mg/Kg P.O for 3 months).

convulsions, hyperactivity, jumping, ataxia, hypersalivation, irritability, and strabismus in mice, which lasted for more than 72 hours. The overstimulation of the brain and behavioral changes observed in mice is attributed to the toxic intake of phenethylamine. Previous studies have shown that high doses of phenethylamine can lead to brain overstimulation, resulting in symptoms such as headaches, confusion, insomnia, hallucinations, and various types of seizures. Intoxication with high doses of phenethylamine is associated with the development of serotonin syndrome and cerebral vasculopathy (Zanda and Fattore 2017, Nelson, Bryant, and Aks 2014). Phenethylamine induces tonic-clonic seizures and hyperactivity at doses of 125-200 mg/Kg i.p. (Dourish and Cooper 1983) and it has been reported to cause death due to brain overstimulation and cardiac arrest (Nelson, Bryant, and Aks 2014).

The results of the acute study indicate that AfL.s is safe even at moderately high doses above the therapeutic dose, producing only hyperexcitation, irritability, and behavioral changes in mice at very high doses (≥300 mg/Kg P.O). The underlying mechanism of CNS overstimulation and behavioral changes in mice is because AfL.s contains phenethylamine (the principal constituent), which prevents the reuptake of serotonin and dopamine at brain synapses (Xie and Miller 2008). The increase in synaptic levels of catecholamines and other biogenic amines contributes to CNS hyperstimulation and other physiological side effects such as jumping, irritability, hypersecretion, and hypersalivation (Ma and Gang 2008).

A chronic toxicity study revealed that AfL.s significantly ($P < 0.05$) reduced the body weight in mice when orally administered at a dose of 18 mg/Kg. This reduction in body weight may be credited to α-tocopherol found in AfL.s, which is a potent antioxidant. Prolonged intake of antioxidants such as vitamin C has been shown to result in weight loss (Johnston et al. 2007). Additionally, chronic administration of phenethylamine has been reported to suppress the appetite center in the brain, leading to reduced food intake and subsequent weight loss (Johnston et al. 2007). It has also been reported that phenethylamine in various forms is found in many weight-reducing supplements (Pawar and Grundel 2017), and as it is the key ingredient of AfL.s, it is responsible for weight reduction in mice.

Chronic administration of AfL.s (18 mg/Kg P.O) resulted in nonsignificant changes in most hematological and biochemical markers. Significant ($P < 0.05$) elevations were observed in the levels of white blood cells (WBCs), hematocrit, and ALP, as shown in Table 4. The levels of total bilirubin and creatinine remained unchanged, indicating no potential damage to hepatic and renal cells with chronic use of AfL.s. α-Tocopherol and phenethylamine are the principal constituents of AfL.s. α-Tocopherol, being antioxidant, protects body cells against damage and is safe even at high doses (5-10 g), causing neither hepatotoxicity nor nephrotoxicity at ordinary doses (Doseděl et al. 2021). Similarly, phenethylamine at normal dose ranges is considered safe and non-toxic for the liver and kidneys (Mossoba et al. 2016). It has also been demonstrated that α-tocopherol, acting as a pro-oxidant, is beneficial for the health of bodily cells and does not have a hemolytic effect (Doseděl et al. 2021). Based on this evidence, our results strongly support that AfL.s do not produce toxic effects upon chronic administration.

Similarly, the results of the histopathological study indicated that oral administration of mice with AfL.s in chronic doses of 18 mg/Kg P.O. did not cause any structural damage to brain cells. No significant pathological signs were observed in the photomicrographs of the kidneys and livers of mice. It has been suggested that antioxidants provide defense against structural damage in the body (Floyd 1999). AfL.s (18 mg/Kg P.O), being a potent antioxidant, prevented cellular damage in the kidneys, liver, and brain of mice.

5. Conclusion

In conclusion, the acute toxicity study revealed that AfL.s is safe when administered at therapeutic doses, with toxic effects only observed at very high doses (>300 mg/Kg P.O). This indicates the wide safety margin supported by a broad therapeutic index, which underscores its safety in clinical application. Chronic administration of AfL.s (18 mg/Kg) orally, led to weight reduction in mice, likely due to α-tocopherol and phenethylamine. The weight reduction, however, did not coincide with any sign of chronic toxicity. The therapeutic doses of the active fraction of *L. stoechas* appear to be safe for prolonged use, as evidenced by the lack of significant hepatic or renal damage, despite some hematological changes. The histopathological analysis further reinforced the safety profile of AfL.s, confirming its protective antioxidant effects on the brain, liver, and kidney tissues. No structural damage was observed in these organs,

suggesting that AfL.s do not induce harmful effects even with chronic administration. Overall, these findings support the safety and potential therapeutic benefits of AfL.s for long-term use.

Conflict of Interest

The authors declared no conflict of interest.

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Study Approval

The study was approved vide letter no; GCU-AEC-682/24. by the Government College University Lahore.

Consent Forms

NA

Data Availability

All the data related to the manuscript is available with the authors.

Author contributions

AM, HSS, and UFG conceptualized the study. AM, TR, QUA, and SHK performed the experimental work. AH and QUA helped in statistical analysis. AM and TR wrote the manuscript.

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