

Safety Assessment of Methanolic Leaf Extract of *Butea monosperma*: Acute Toxicity in Swiss Albino Mice

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Abstract

Butea monosperma, a traditional medicinal plant, possesses various biological activities such as antioxidant, anti-viral, antimicrobial and specifically antidiabetic. Current work aimed to investigate the acute toxicity study of the methanolic leaf extract of *B. monosperma* in Swiss albino mice. For this purpose, the extract was orally given to the mice as a single dose of 2000 mg/kg of body weight. At the end of the eight days acute trial, dissection was performed and blood sample was taken directly from heart to examine different biochemical (ALT, AST, ALP, Creatinine, Urea, Uric acid) and hematological (RBCs, WBCs, PLT, Hb, Hematocrit, MCV) parameters. Vital organs (liver, kidney, spleen, heart, and lungs) were also removed surgically and examined for histopathology. The level of all biochemical and hematological parameters showed no significant differences as compared to control mice. Histopathological analysis of the liver, kidney, spleen, heart, and lungs also revealed the nontoxic effect of the extract. In conclusion, our findings offer insightful information about the safety of methanolic leaf extract of *B. monosperma* and is highly effective for therapeutic use in future.

1. Introduction

Natural medicines are more widely used than synthetic medications around the globe (Jamshidi *et al.*, 2018). "Herbal medicine" is a therapeutic approach that involves applying or ingesting plants or plant extracts. Several safe and effective drugs have been developed from traditional plants (Sam *et al.*, 2019). *Butea monosperma* is a valuable, dry deciduous forest tree also known as flame of the forest (Akram *et al.*, 2011). The blooms contain many flavonoids, including lutein, which has antioxidant, anti-cancer, hepatoprotective, and anti-diabetic activities (Garapelli *et al.*, 2015).

An extensive investigation describes the toxicological profile of the aqueous extract of *B. monosperma* flowers. It involved acute and sub-acute oral toxicity studies in mice; it identified the extract to be safe with an intraperitoneal LD50 of

3500 mg/kg and no significant toxicity was noted in hematological, biochemical or histological parameters (Sharm *et al.*, 2012). Solvents of successively varying polarity were used to make extract of the root of *B. monosperma*. The extracts was orally administered at the dose of 2000mg/kg/body weight in the adult female mice which had established fertility to determine the anti-implantation activity (Das *et al.*, 2015).

Further evidence is obtained from studies performed on diverse animal models and distinct segments of the *B. monosperma* plant. Research on mice shows that the methanolic extract from the stem bark has strong hepatoprotective properties (Samad *et al.*, 2014). In a model of toxicity caused by carbon tetrachloride, the extract lowered levels of liver function enzymes like alkaline phosphatase, serum glutamate pyruvate

transaminase, and glutamate oxaloacetate transaminase in a dose-dependent way. Another study discovered that the flavonoid fraction of the leaves, extracted with methanol, exhibits significant antioxidant activity *in vitro* and may retain similar properties when evaluated *in vivo* in rats (Divya and Mini, 2014).

The antidiabetic and antioxidant properties of methanolic leaf extract of *B. monosperma* were investigated in Swiss albino mice. Some hematological blood parameters like RBCs and Hb were analyzed in order to investigate the toxicity in mice (Yerragunta *et al.*, 2011). This study aims to elucidate the effects of the methanolic leaves extract of *B. monosperma* on the biochemistry, hematology, and histopathology of Swiss albino mice, consequently enhancing the understanding of its safety and therapeutic potential.

2. Materials and methods

2.1. Collection of plant material

The fresh leaves of *Butea monosperma* were collected from different areas of the University of Okara (Punjab, Pakistan). The plant material was identified by a taxonomist at the Department of Botany, and a voucher specimen has been deposited in the departmental herbarium for future reference.

2.2. Preparation of methanolic extract of *Butea monosperma* leaves

Fresh leaves of the *B. monosperma* were cleaned with tap water and dried under shade at room temperature. These leaves were ground into a coarse powder using an electric grinder, sieved to remove any large particles, and stored in a closed plastic bag for later use. A 50 g leaf powder was dissolved in 75% methanol at 1 g: 10 mL, then let run in a conical flask on an orbital shaker at 24 °C with the rotational speed of 250 rpm for 72 hours. The mixture was filtered using Whatman filter paper No. 1. The dry extract was refrigerated at 4 °C for later use (Nauroze *et al.*, 2023).

2.3. Toxicity profiling in mice model

2.3.1. Maintenance of animals

Male and female Swiss albino mice were acquired from University of Veterinary and Animal Sciences, Lahore, Pakistan. The average mice weighed was 20–30 grams and were 5–6 weeks old. All the mice were handled by following animal care regulations given by the University of Okara's Ethical Research Committee. The mice had access to water, a healthy pelleted feed, and standard habitat conditions (23–25 degrees Celsius, 12 hours of light and 12 hours of darkness). Before starting the experiment, they had a week to acclimate to the lab.

2.3.2. Ethical Approval

Ethical approval was obtained before using animals for experiments, under letter No. UO/ERC/2023/Z12, dated: 22-12-2023, by the Ethical Research Committee, University of Okara, Punjab, Pakistan.

2.3.3. Measurement of body weight

Before starting the experiment, all mice were weighed on a digital weighing balance. Daily weights were taken of the control and treatment groups throughout the acute period. Mouse dose was prepared on the basis of average body weight.

2.4. Acute toxicity study

For this experiment, Swiss albino mice were divided into two groups of five. Experimental mice administered with single oral dose of 2000 mg/kg of their average body weight. The control and treated group mice's diet and water supplies were monitored. For the first 24 hours after the dosing, the mice were analysed for toxicity or unusual behaviour. Starting the next day, the monitoring was done every day for eight days. All mice had to fast for a single night before to being dissected for the experiment. Blood was collected from heart after the mice was anaesthetized with chloroform. Separate blood tubes were used for hematological and biochemical tests (Kanwal *et al.*, 2022).

2.4.1. Biochemical analysis

Blood samples were centrifuged at 3,000 RPM for 10 minutes in standard evacuated biochemical

tubes to collect plasma and serum and the blood samples cooled at room temperature for 5-10 minutes. Commercially available diagnostic kits were used to measure ALT, AST, ALP, uric acid, urea, creatinine, HDL and LDL in mouse serum (Nauroze *et al.*, 2023).

2.4.2. Hematological analysis

The mice' blood samples for hematology were taken in EDTA tubes. The complete blood count (CBC) includes RBCs, PLTs, WBCs, hemoglobin (Hb), hematocrit, and MCV. Blood samples were analysed using a Coulter counter S-plus VI hematological analyser (Nauroze *et al.*, 2023).

2.4.3. Histopathological examination

After dissection, the liver, spleen, kidneys, lungs, and heart were removed surgically and cleaned with phosphate buffer saline. Afterward, the analytical balance weighed each organ in grams. These organs were then immersed in a pH 7.4 and 10% formalin solution for 24 hours. Next, all the vital organs were dehydrated by increasing alcohol exposure (70, 90, and 100%). After xylene extraction, the alcohol was fixed in paraffin wax at 58-60 degrees Celsius. The blocks were sliced into 5-millimeter slices using a microtome. All tissue sections were stained with H&E. After staining, photomicroscopes were used at various magnifications to evaluate the sections (Kanwal *et al.*, 2022).

2.5. Statistical analysis

The data was tabulated as means and SEM. Statistical analysis was done with GraphPad Prism

5 with the unpaired t-test. Statistical significance was assessed by p-values below 0.05.

3. Results

All mice lived for an acute period of eight days after receiving a single oral dose (2000mg/kg of body weight) of the methanolic leaves extract. Both control and treated groups were monitored attentively. There was no unusual change in different behavioral patterns (Table 1). Body weight of both control and treated group was monitored daily to analyze toxicity effects. Table (2) shows that the control and treatment groups had no significant variations in their body weight. Mice organs (kidneys, spleen, liver, lungs, and heart) were surgically removed after dissection. These organs had no alterations in size, shape, color, or composition. No statistically significant difference existed between the control and extract treated groups (Table 3). For oral acute toxicity, mice's blood biochemical profiles showed no significant variations in ALP, AST, ALT, LDL, HDL, Urea, Creatinine, and Uric acid (Figure 1). There was no significant impact of methanolic leaves extract of *B. monosperma* on the level of RBCs, PLT, WBCs, Hb, MCV, and hematocrit in mice's blood (Figure 2). The histopathological examination of the heart, liver, kidneys, spleen, and lungs of dissected mice was performed to ascertain the toxicity of plant extract. The histopathology revealed no damage, suggesting that these organs were unaffected by the methanolic *B. monosperma* leaf extract (Figure 3).

Table 1. The behavioral pattern of extract-treated mice observed during acute toxicity study.

Sr. No.	Parameters	Swiss albino mice	
		Control Group	Treated Group
1.	Drowsiness	Not Found	Not Found
2.	Nasal bleeding	Not Found	Not Found
3.	Lethargy	Not Found	Not Found
4.	Lacrimation	Not Found	Not Found
5.	Convulsions	Not Found	Not Found
6.	Salivation	Not Found	Not Found
7.	Mortality	Not Found	Not Found
8.	Food consumption	Normal	Normal

9.	Water consumption	Normal	Normal
10.	Body weight	Normal	Normal

Table 2. Body weight of control and treated group mice.

Days	Control Group Body Weight (g)	Treated Group Body Weight (g)
Before Dose	24.4 ± 1.6	23.9 ± 1.4
After Dose		
Day-1	23.4 ± 1.2	23.8 ± 1.5
Day-2	23.5 ± 1.2	24.3 ± 1.2
Day-3	23.2 ± 1.6	23.5 ± 1.2
Day-4	23.7 ± 1.6	23.6 ± 1.3
Day-5	22.6 ± 1.5	23.9 ± 1.3
Day-6	23.2 ± 1.5	24.1 ± 1.2
Day-7	22.8 ± 1.2	24.3 ± 1.1

Values are articulated as Mean ± SEM. Values are the mean of five replicates.

Table 3. Organ weight of control and treated group of mice.

Sr. No.	Organs	Control Group	Treated Group
1.	Kidney	0.4 ± 0.0	0.4 ± 0.0
2.	Liver	1.5 ± 0.1	1.6 ± 0.1
3.	Spleen	0.1 ± 0.0	0.2 ± 0.0
4.	Heart	0.2 ± 0.0	0.2 ± 0.0
5.	Lungs	0.2 ± 0.0	0.3 ± 0.0

Values are articulated as Mean ± SEM. Values are the mean of five replicates.

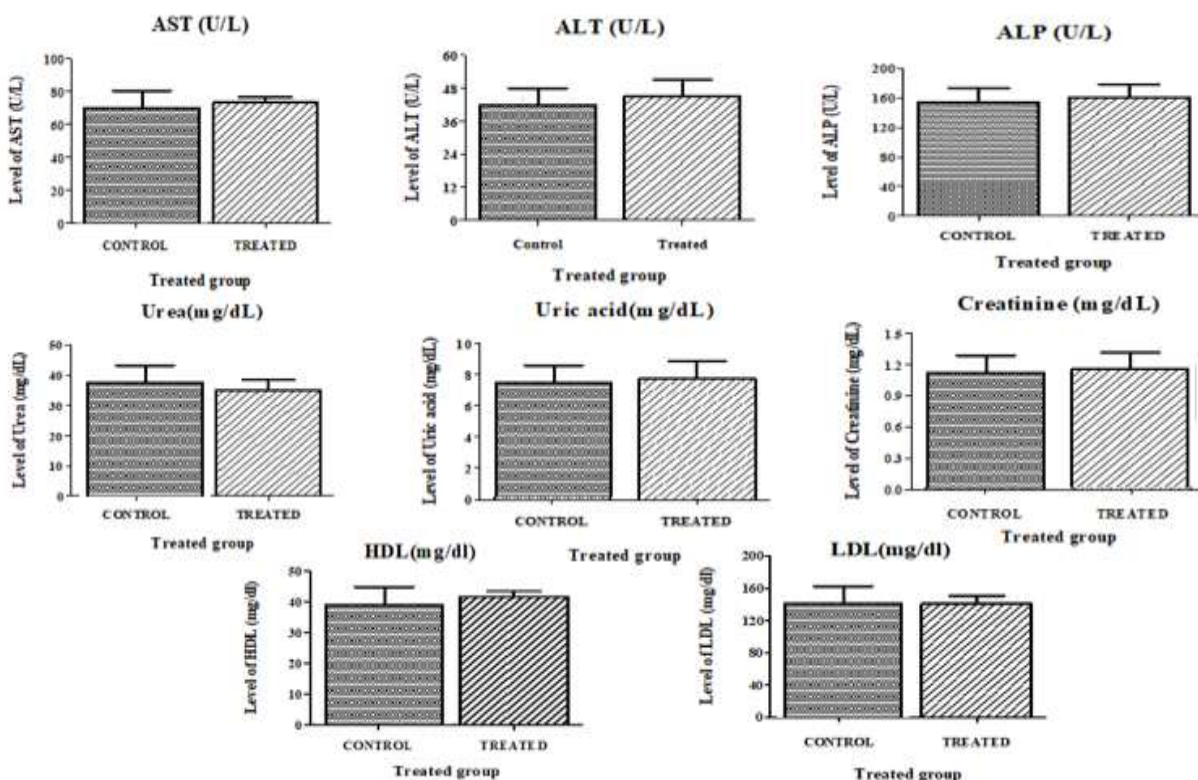


Figure 1. Effect of methanolic leaf extract of *Butea monosperma* on the level of AST, ALT, ALP, Urea, Uric acid, Creatinine, LDL, and HDL of Swiss albino mice in acute toxicity study.

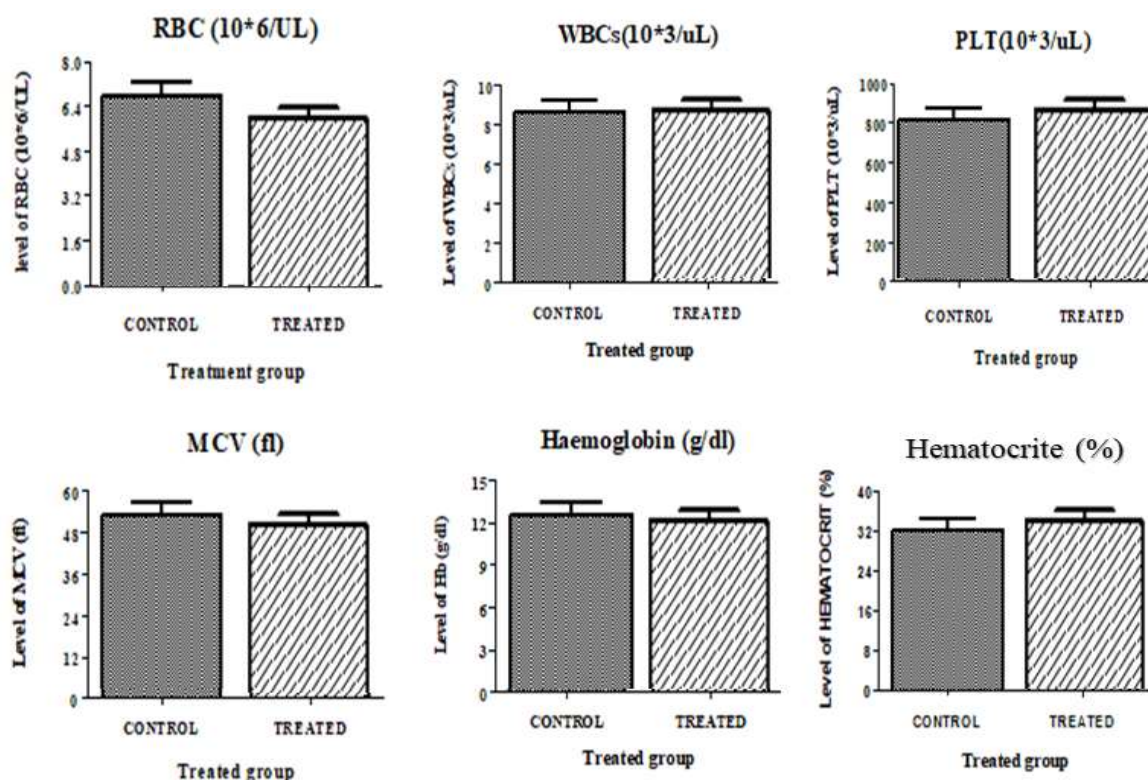


Figure 2. Effect of methanolic leaf extract of *Butea monosperma* on the level of RBCs, WBCs, PLT, MCV, Hb, and Hematocrit of Swiss albino mice in acute toxicity study.

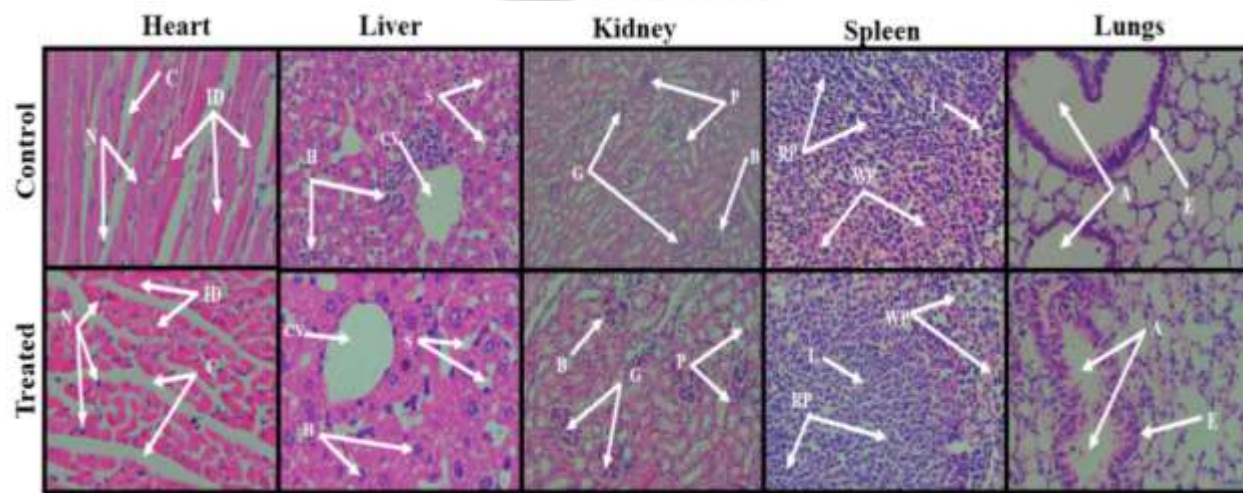


Figure 3: Histopathological comparison of vital organs in control versus *Butea monosperma* leaf-extract treated mice. Hematoxylin-eosin stained sections were examined at 10 x and 40 x. Abbreviations: 'C' for Capillary, 'N' for Nuclei, 'ID' for Intercalated disc, 'H' for Hepatocytes, 'S' for Sinusoidal cells, 'CV' for Central vein, 'G' for Glomerulus, 'B' for Bowman's space, 'P' for Podocytes, 'WP' for White pulp, 'RP' for Red pulp, 'L' for Lymphocytes, 'E' for Epithelium, 'A' for Alveoli.

4. Discussion

The previous study conducted by Zaynab *et al.*, (2018) suggested that herbal medicine has long been used to treat many diseases. Their work examines herbal and traditional medicine, which uses plant extracts to treat disease and promote physiological processes. The main goal of our research was also to explain the use of herbal plants in curing diseases.

According to Sindhia *et al.*, (2010) the moderately sized deciduous tree *B. monosperma*, has anti-inflammatory, hypoglycemic, antibacterial, and antifungal properties. *B. monosperma* has diuretic, aphrodisiac, tonic, and astringent effects. *Butea monosperma* is a plant that is used in medicine in several ways. Flavonoids, glucosinolates, and anthocyanins are the bioactive compounds found in this plant. These substances have properties that fight inflammation, toxicity, and free radicals (Balamurugan *et al.*, 2019).

Methanolic and aqueous extracts were evaluated on mice for acute and short-term toxicity. Three groups of three male mice weighing 25-25 grams were used to study acute toxicity. Oral extract dose was provided to the mice to investigate toxicity effects (Deore *et al.*, 2008). We also use standard methodology to make methanolic leaf extract of *B. monosperma* and provide a single oral administration to the Swiss albino (2000 mg/kg of body weight).

In a previous study Singh *et al.*, (2013) examined the liver biochemistry and histopathology of female Swiss albino mice exposed to a *B. monosperma* aqueous extract. The experiment had five mice per control and treatment group. They found that doses above 2000 mg/kg cause no histopathological changes in the kidneys and liver of female Swiss albino mice. Biochemical parameters were also unaffected. In our research work, same methanolic dose provided to the Swiss albino mice to investigate acute toxicity study. We observed that there were no significant variations in any of the hematological (RBCs, WBCs, PLT, Hb, Hematocrit, MCV) and biochemical parameters (AST, ALT, ALP, HDL, LDL UREA, uric Acid, and Creatinine). Histopathological examination of vital body organs indicating that

the *Butea monosperma* leaf extract is nontoxic and safe for therapeutic use.

5. Conclusion

Our study concluded that the methanolic leaf extract of *Butea monosperma* has a non-toxic and positive safety profile. There were no significant variations were observed in any of serum biochemical and hematological indices measured in acute oral administration of the extract in Swiss albino mice as compared with control group revealing no nephrotoxicity, hepatotoxicity or hematopoietic disruption. Furthermore, the histopathological examination of vital organs showed no signs of necrosis, inflammation and any cellular disruption that strengthen the biocompatibility of methanolic extract. Its non-harmful effects on organs support its extensive historical medical application and provide researchers with a rationale to continue investigating its bioactive compounds for the development of safe, cost-effective pharmaceuticals.

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