Acute Oral Toxicity Assessment of Borassus flabellifer Polysaccharides in Wistar Rats

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Abstract
Borassus flabellifer (Asian palmyra palm) is a widely consumed tropical plant rich in bioactive polysaccharides with potential applications in pharmaceutical and nutraceutical formulations. However, scientific data regarding the safety of isolated polysaccharide fractions remain limited. The present study aimed to evaluate the acute oral toxicity of Borassus flabellifer polysaccharides (BFF-PS) in Wistar rats following the OECD 425 limit test. A single oral dose of 2000 mg/kg body weight was administered to female rats, and animals were observed for 14 days for clinical signs of toxicity, behavioural changes, mortality, body-weight variation, and organ abnormalities. Hematological, biochemical, and histopathological evaluations were also performed. No mortality or treatment-related toxic manifestations were observed throughout the study period. Body-weight gain, hematological indices, renal and hepatic biochemical markers, lipid profile parameters, and histological architecture of vital organs remained within normal physiological limits. The findings indicate that BFF-PS is non-toxic at the tested dose, with an estimated oral LD_{50} greater than 2000 mg/kg, supporting its safety for further pharmacological and formulation studies.

Keywords: Borassus flabellifer polysaccharide; Acute oral toxicity; LD_{50} estimation; safety evaluation.

Introduction

Natural polysaccharides have gained considerable attention in recent years due to their broad applicability in pharmaceutical, cosmetic, and nutraceutical fields. Their favorable characteristics, such as biodegradability, biocompatibility, low toxicity, and functional adaptability, make them suitable for use as excipients and bioactive agents [1,2]. Several plant-derived polysaccharides have been reported to exhibit beneficial biological properties, including antioxidant, immunomodulatory, and metabolic regulatory effects [3]. Borassus flabellifer Linn., commonly referred to as the Asian palmyra palm or toddy palm, is a tropical plant extensively cultivated and consumed in South and Southeast Asia. Different parts of the plant, particularly the fruit pulp, sap, and seeds, are traditionally used as food and in indigenous medicinal practices [4]. Nutritional and phytochemical investigations have demonstrated that the plant is a rich source of carbohydrates, polysaccharides, minerals, and other bioactive constituents [5]. Previous studies have highlighted the antioxidant, anti-inflammatory, immunomodulatory, and prebiotic potential of extracts derived from Borassus flabellifer [6-8].

Although the plant has a long history of dietary and traditional use, purified polysaccharide fractions may display distinct biological responses depending on factors such as extraction technique, molecular weight distribution, degree of branching, and purity [9]. Consequently, it is essential to establish the toxicological safety of such polysaccharides before their incorporation into pharmaceutical formulations or therapeutic applications.

The Organization for Economic Co-operation and Development (OECD) Guideline 425, also known as the Up-and-Down Procedure, is an internationally accepted method for assessing acute oral toxicity while minimizing animal usage [10]. This guideline enables the estimation of the median lethal dose and the identification of potential toxic effects following single-dose exposure.

At present, scientific data regarding the acute oral toxicity of Borassus flabellifer polysaccharides (BFF-PS) are limited**.** Hence, the present study was designed to evaluate the acute oral toxicity of BFF-PS in Wistar rats at a limit dose of 2000 mg/kg body weight, in accordance with OECD Guideline 425. The study focused on clinical signs, mortality, behavioural changes, body weight variations, biochemical and hematological parameters, and histopathological examination of vital organs.

Materials and Methods

Plant Collection and Preparation

Fresh, ripe fruits of Borassus flabellifer Linn. were collected from local agricultural farms in Hyderabad, India, during the peak fruiting season. The fruits were authenticated based on their morphological characteristics. The edible pulp was manually separated from the fruits, cut into small pieces, and dried in a hot-air oven at 45–50 °C until a constant weight was obtained. The dried material was then pulverized using a laboratory grinder to obtain a fine powder. The

powder was passed through a 60-mesh sieve to ensure uniform particle size and stored in airtight containers at room temperature until further use.

Extraction of Polysaccharides

Polysaccharides were extracted from the dried fruit powder using a hot-water extraction method**.** Briefly, 100 g of the powdered material was extracted with distilled water under controlled heating, followed by filtration. The aqueous extract was subjected to ethanolic precipitation by adding ethanol in a 1:3 (extract: ethanol) ratio and allowed to stand until complete precipitation occurred. The precipitated polysaccharides were collected by filtration, washed repeatedly with ethanol to remove impurities, and dried. The dried polysaccharide fraction was stored in airtight containers until further use for toxicity studies [11].

Preparation of Dose

For acute oral toxicity studies, the dried Borassus flabellifer polysaccharide (BFF-PS) was freshly prepared by suspending the required quantity of polysaccharide in distilled water**,** which served as the vehicle. The suspension was prepared immediately before administration to ensure uniformity and stability of the dose [12].

Preliminary phytochemical investigation

Preliminary phytochemical screening of the polysaccharide-rich extract was carried out using standard qualitative chemical tests as described in the literature [13-16].

Animals and Ethical Approval

Healthy, nulliparous adult female Wistar rats weighing 230–250 g were used for the acute oral toxicity study. The animals were procured from Wockhardt Research Centre**,** a CPCSEA-registered breeder**,** and housed in the Animal House Facility of Y. B. Chavan College of Pharmacy under standard laboratory conditions. Prior to the commencement of the experiment, the rats were acclimatized for seven days**.**

Animals were maintained at a controlled temperature of 22 ± 2 °C**,** relative humidity of 50–60%**,** and a 12 h light/12 h dark cycle**.** Rats were housed in polypropylene cages with clean rice husk bedding and were provided free access to standard pellet diet and purified drinking water throughout the study.

All experimental procedures, animal handling, and disposal were carried out in strict accordance with the guidelines prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India**.** The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Y. B. Chavan College of Pharmacy**,** under approval number CPCSEA/IAEC/Pharmaceutics/65/2025–26/213**.**

Acute Oral Toxicity Assay (OECD Guideline 425)

The acute oral toxicity of Borassus flabellifer polysaccharides (BFF-PS) was evaluated in accordance with OECD Guideline 425 (Up-and-Down Procedure)**.** Healthy, nulliparous adult female Wistar rats were used for the study, as females are generally considered more sensitive to toxic substances, as recommended by the guideline. Following overnight fasting (with free access to water), animals were administered a single oral dose of 2000 mg/kg body weight of the polysaccharide suspension by oral gavage**.** The control group **(**n = 5**)** received an equivalent volume of distilled water as the vehicle. After dosing, animals **(**n = 5) were observed individually and continuously during the first 30 minutes, periodically during the first 4 hours, and thereafter once daily for 14 consecutive days for signs of toxicity. Observations included changes in skin, fur, eyes, mucous membranes, behavioural patterns, tremors, salivation, convulsions**,** urine color and other clinical signs of toxicity. Animals were also monitored daily for food and water intake, body weight changes, locomotor activity, and mortality**.** All observations were recorded systematically. At the end of the 14-day observation period, all animals were humanely euthanized using ether anesthesia**,** followed by sacrifice. Vital organs, including the liver, kidneys, heart, lungs, and spleen**,** were excised, weighed, and preserved in 10% neutral buffered formalin for histopathological examination.

Hematological analysis:

Blood samples were collected from both vehicle control and BFF-PS–treated rats into EDTA-containing tubes for hematological analysis. The following hematological parameters were evaluated: hemoglobin (Hb) concentration, red blood cell (RBC) count, white blood cell (WBC) count, platelet count, differential leukocyte count (DLC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

Biochemical analysis:

Biochemical evaluations are essential components of toxicological investigations, as they provide sensitive and reliable indicators of organ function and metabolic status**.** Liver function tests (LFTs), including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin, and serum protein level**s**, are commonly employed to assess hepatic integrity and the liver's capacity for metabolism and detoxification. Renal function tests (KFTs), such as serum urea and creatinine, are used to evaluate glomerular filtration efficiency and overall kidney health. In addition, assessment of the lipid profile, including total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL), provides insight into lipid metabolism and potential cardiovascular risk. Collectively, these biochemical parameters offer a comprehensive evaluation of systemic toxicity and organ-specific effects following exposure to test substances [17-19].

Histopathological study

Histopathological examination was carried out to evaluate microscopic alterations in major organs following treatment. Vital organs, including the liver and kidneys**,** along with other relevant tissues, were carefully excised and fixed in 10% neutral buffered formalin**.** The fixed tissues were processed using standard paraffin-embedding techniques, sectioned at a thickness of 4–5 μm, and stained with hematoxylin and eosin (H&E)**.** The stained sections were examined under a light microscope for the presence of histological changes such as inflammation, necrosis, cellular degeneration, and alterations in normal tissue architecture**.** These observations provided supportive evidence for the assessment of organ-specific toxicity and facilitated correlation between biochemical findings and tissue-level changes [20-21].

Statistical analysis

All experimental data are expressed as mean ± standard deviation (SD)**.** Statistical comparisons between the vehicle control and BFF-PS–treated groups were performed using an unpaired Student's **t**-test. For body-weight data recorded at multiple time points, two-way repeated-measures ANOVA was applied, followed by appropriate post hoc analysis. A p-value < 0.05 was considered statistically significant. All statistical analyses were carried out using GraphPad Prism software [22]**.**

3. Results

3.1 Phytochemical investigation

Preliminary phytochemical screening of the ethanol-precipitated extract of Borassus flabellifer fruit pulp showed positive reactions for carbohydrates and reducing sugars, while tests for alkaloids, flavonoids, phenolics, saponins, steroids, proteins, and amino acids were negative. These findings confirm that the extract is a polysaccharide-rich preparation with minimal interference from other phytochemicals.

3.2 Acute oral toxicity and clinical observation

Administration of Borassus flabellifer polysaccharide (BFF-PS) at a single oral limit dose of 2000 mg/kg body weight did not result in any mortality or treatment-related clinical signs throughout the 14-day observation period**.** All treated animals remained active and healthy, with normal posture, grooming, locomotor activity, and sensory responses. No abnormalities were observed in skin, fur, eyes, mucous membranes, salivation, respiration, or behaviour patterns when compared with the vehicle control group.

3.2.1 Clinical Signs and Behavioural Observations

All animals in the BFF-PS–treated group exhibited normal behavioural and physiological responses throughout the 14-day observation period. No abnormalities were observed in parameters such as skin and fur condition, eyes, mucous membranes, respiration, salivation, locomotor activity, posture, grooming behaviour, sleep pattern, urine colour, or faecal consistency when compared with the vehicle control group. There were no signs of tremors, convulsions, diarrhea, lethargy, or mortality during the study period. The detailed clinical and behavioural observations are summarized in Table 1**.**

Table 1. Clinical and behavioural observations of rats following acute oral administration of BFF-PS (2000 mg/kg)

Parameters	**30 min**	**4 h**	**24 h**	**48 h**	**7 days**	**14 days**
Coma	ABS	ABS	ABS	ABS	ABS	ABS
Convulsions / Tremors	ABS	ABS	ABS	ABS	ABS	ABS
Eyes	NA	NA	NA	NA	NA	NA
Faeces consistency	NA	NA	NA	NA	NA	NA
Fur and skin	NA	NA	NA	NA	NA	NA
Itching	ABS	ABS	ABS	ABS	ABS	ABS
Mortality	0	0	0	0	0	0
Mucous membrane	NA	NA	NA	NA	NA	NA
Respiration	NA	NA	NA	NA	NA	NA
Salivation	NA	NA	NA	NA	NA	NA
Sleep	NA	NA	NA	NA	NA	NA
Somatomotor activity & behaviour	NA	NA	NA	NA	NA	NA
Urination (colour)	NA	NA	NA	NA	NA	NA

ABS: Absent; **NA:** No abnormality observed; **0:** No mortality recorded.

3.2.2 Effect of BFF-PS on Body Weight

Body weight measurements recorded on Days 0, 7, and 14 showed a gradual and comparable increase in both control and BFF-PS–treated rats. No statistically significant difference in body-weight gain was observed between the groups during the study period ($p > 0.05$), indicating normal growth and metabolic status following BFF-PS administration **(**Figure 1**).**

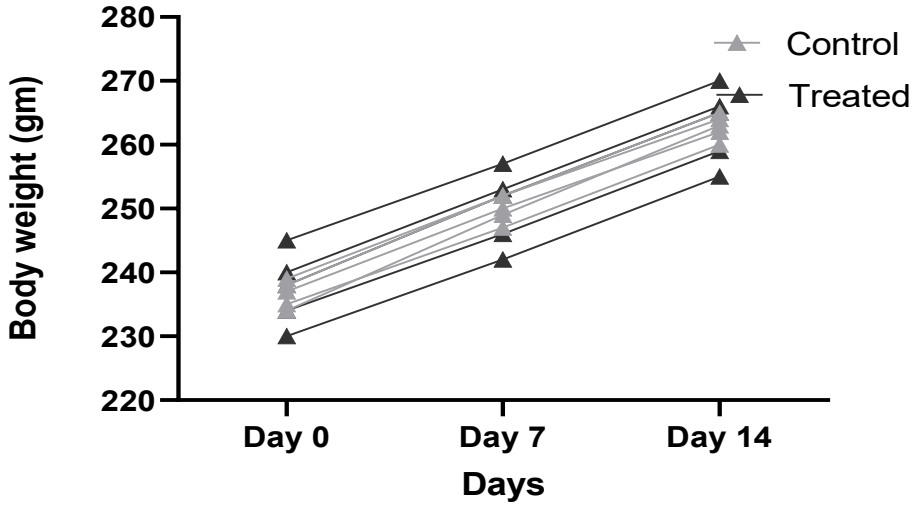
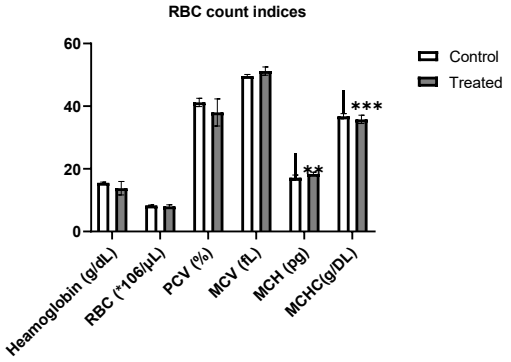


Figure1: Effect of BFF-PS on body weight of rats during the 14-day acute oral toxicity study. **Body weight was recorded on Days 0, 7, and 14 following a single oral administration of BFF-PS (2000 mg/kg). Data are expressed as mean ± SD (n = 5). No significant difference was observed between vehicle control and BFF-PS–treated groups (p > 0.05).**

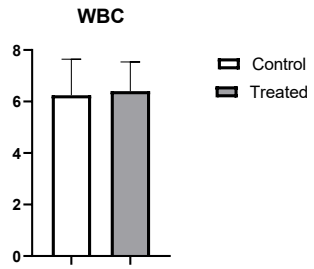
3.2.3 Effect of BFF-PS on Hematological Parameters

Hematological evaluation revealed no adverse or clinically relevant alterations in blood parameters following acute oral administration of BFF-PS. Parameters including hemoglobin concentration, red blood cell count, packed cell volume, white blood cell count, differential leukocyte count, and platelet count remained within normal physiological ranges in treated animals when compared with the vehicle control group **(**Figure 2A–2D**).**

Although minor variations were observed in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH)**,** these changes were statistically significant ($p < 0.05$) but remained within normal physiological limits and were not associated with changes in related hematological indices, indicating no toxicological significance.



A



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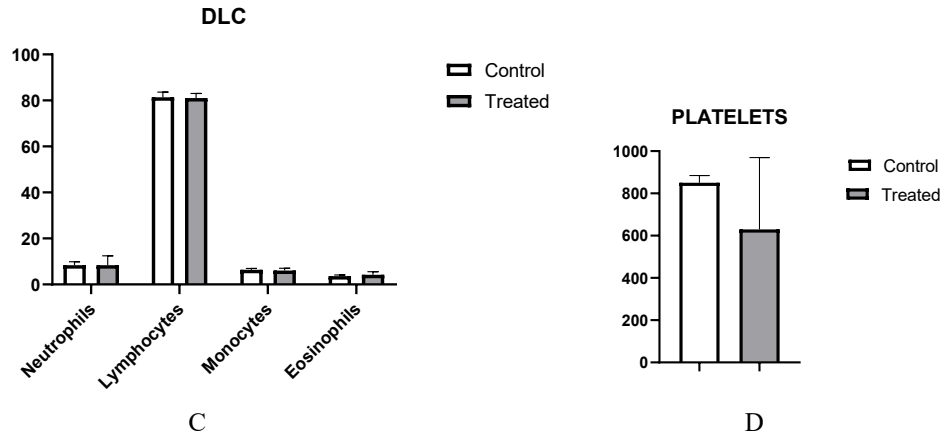
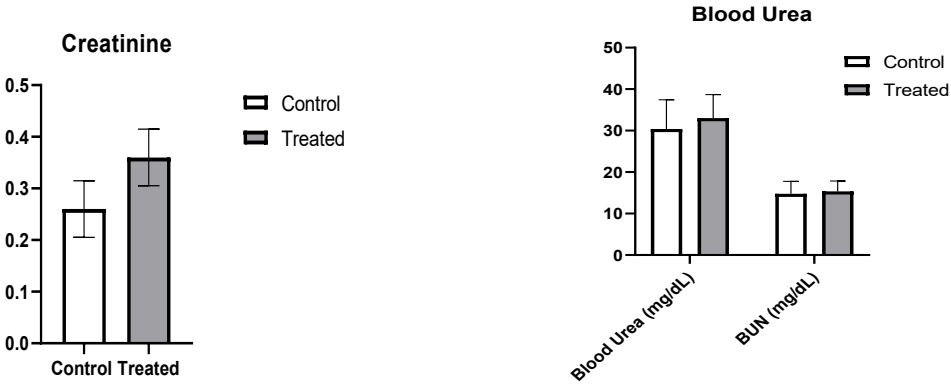


C D

Figure 2. Effect of BFF-PS on hematological parameters of rats following acute oral administration.
(A) RBC indices including hemoglobin, RBC count, PCV, MCV, MCH, and MCHC;
(B) Total white blood cell (WBC) count;
(C) Differential leukocyte count (DLC);
(D) Platelet count.
Data are expressed as mean ± SD (n = 5). Statistical analysis was performed using an unpaired Student's t-test. $p < 0.05$ was considered statistically significant. Minor but statistically significant changes were observed in MCV and MCH; however, values remained within normal physiological limits.

3.2.4 Effect of BFF-PS on Renal Function Parameters

Renal function markers assessed after BFF-PS administration showed no evidence of renal toxicity. Serum blood urea and blood urea nitrogen (BUN) levels did not differ significantly between treated and control groups ($p > 0.05$) **(**Figure 3a**).** A marginal increase in serum creatinine was observed in the treated group ($p < 0.05$); however, the values remained within normal physiological limits and were not accompanied by corresponding alterations in urea or BUN levels, suggesting preserved renal function **(**Figure 3b**).**

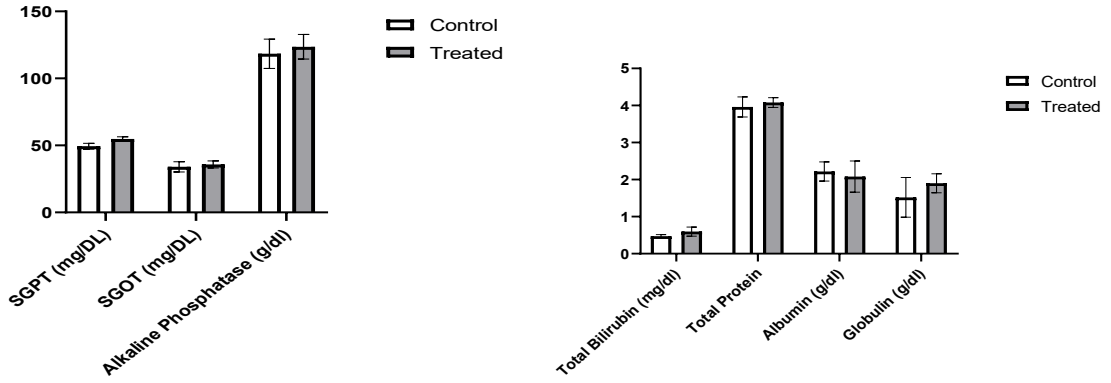


A B

Figure 3: Effect of BFF-PS on renal function parameters in rats.
(A) Blood urea and blood urea nitrogen (BUN);
(B) Serum creatinine levels.
Data are expressed as mean ± SD (n = 5). Statistical analysis was performed using an unpaired Student's t-test. $p < 0.05$ was considered statistically significant. Creatinine levels showed a marginal increase but remained within physiological limits.

3.2.5 Effect of BFF-PS on Liver Function Parameters

Evaluation of liver function parameters demonstrated that acute oral administration of BFF-PS did not induce hepatotoxic effects. Serum levels of SGPT (ALT), SGOT (AST), and alkaline phosphatase (ALP) showed no statistically significant differences between treated and control groups ($p > 0.05$) **(**Figure 4a**).** Similarly, total bilirubin, total protein, albumin, and globulin levels remained within normal physiological limits, indicating intact hepatic synthetic and excretory functions **(**Figure 4b**).**



A B

Figure 4: Effect of BFF-PS on liver function parameters in rats.
(A) Serum SGPT (ALT), SGOT (AST), and alkaline phosphatase (ALP);
(B) Total bilirubin, total protein, albumin, and globulin levels.
Data are expressed as mean ± SD (n = 5). No statistically significant differences were observed between control and treated groups ($p > 0.05$).

3.2.6 Effect of BFF-PS on Lipid Profile

Assessment of serum lipid profile revealed no significant changes in total cholesterol, triglycerides, HDL, LDL, or VLDL levels in BFF-PS–treated rats compared with vehicle controls ($p > 0.05$). All lipid parameters remained within normal physiological ranges, suggesting that acute exposure to BFF-PS does not adversely affect lipid metabolism or cardiovascular risk markers **(**Figure 5**).**

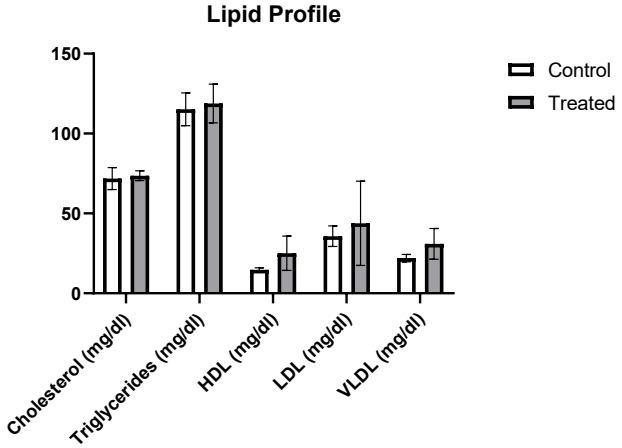
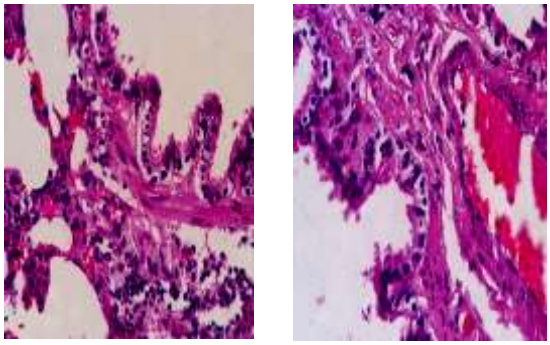


Figure 5. Effect of BFF-PS on lipid profile of rats following acute oral administration. Serum levels of total cholesterol, triglycerides, HDL, LDL, and VLDL were assessed. Data are expressed as mean ± SD (n = 5). Statistical analysis was performed using an unpaired Student's t-test. No statistically significant differences were observed compared with the control group ($p > 0.05$).

3.2.7 Histopathological Findings

Histopathological examination of vital organs, including the brain, liver, kidneys, lungs, and heart**,** revealed normal histoarchitecture in both control and BFF-PS–treated groups. No evidence of inflammation, necrosis, cellular degeneration, or structural abnormalities was observed in the examined tissues. These findings confirm the absence of treatment-related pathological changes and support the biochemical and hematological results **(**Figure 6**).**

Lungs



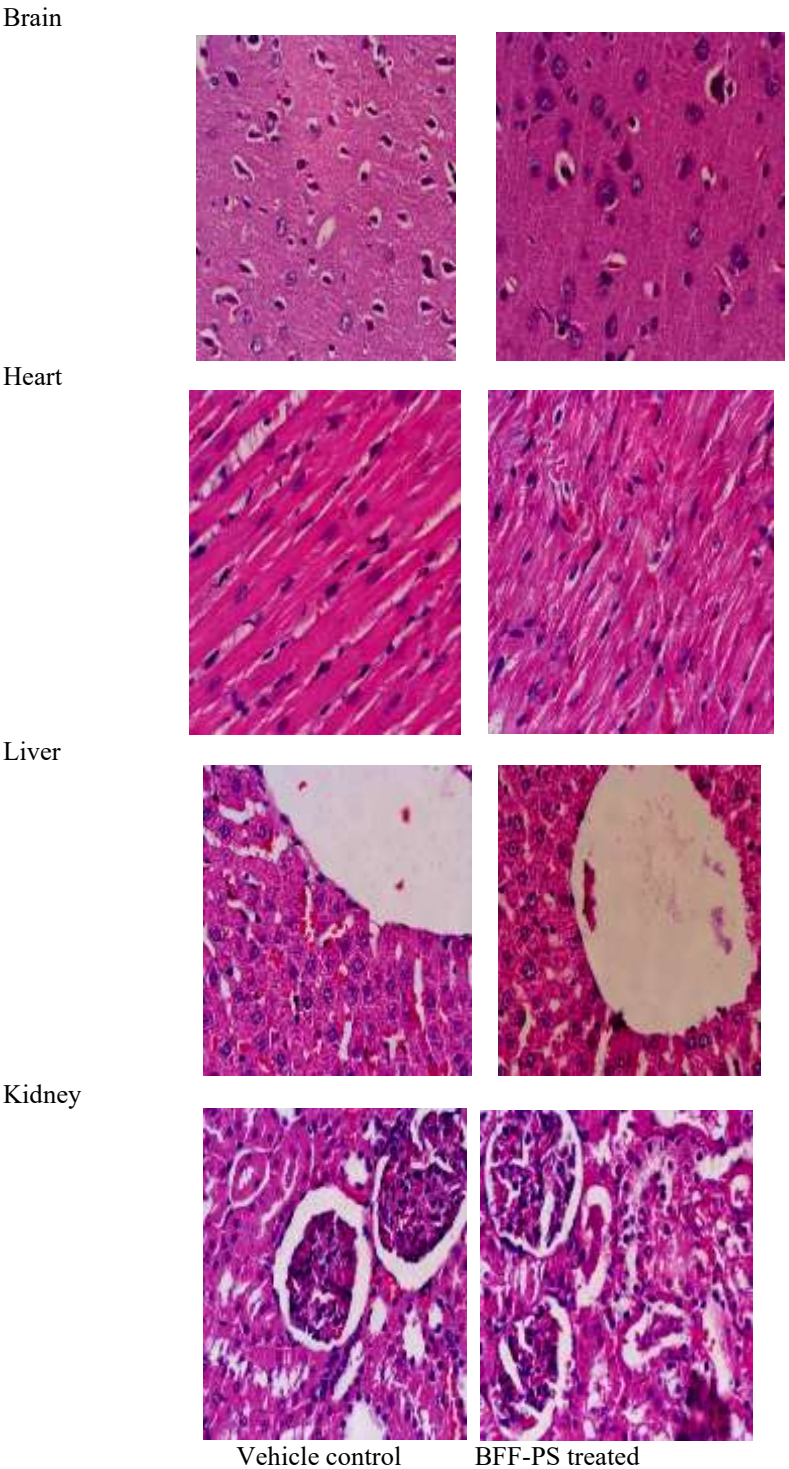


Figure 6: Histopathological evaluation of vital organs following acute oral administration of BFF-PS. **Representative photomicrographs of the brain, liver, kidneys, lungs, and heart from vehicle control (Distilled water) and BFF-PS–treated rats showing normal histoarchitecture with no evidence of inflammation, necrosis, or structural abnormalities (H&E staining).**

Summary of Results

Overall, the results demonstrate that Borassus flabellifer polysaccharide is non-toxic at a single oral dose of 2000 mg/kg, with no mortality, no significant clinical abnormalities, and no biologically meaningful alterations in hematological, biochemical, lipid, or histopathological parameters.

4. Discussion

The present study evaluated the acute oral toxicity profile of Borassus flabellifer polysaccharide (BFF-PS) in rats using the OECD 425 limit test at a dose of 2000 mg/kg body weight. The absence of mortality and treatment-related clinical signs throughout the 14-day observation period indicates that BFF-PS possesses a wide margin of safety, with an estimated LD_{50} value greater than 2000 mg/kg. Behavioral observations, including locomotor activity, sensory responses, and autonomic functions, remained normal in treated animals, suggesting that BFF-PS does not exert acute neurotoxic or systemic adverse effects [23]. Body weight gain in BFF-PS–treated rats was comparable to that of control animals, reflecting normal growth and metabolic status and further supporting the non-toxic nature of the extract. (Figure 1) Hematological evaluation revealed no adverse alterations in major blood parameters, including hemoglobin concentration, RBC count, PCV, WBC count, DLC, and platelet count. Although minor variations were observed in certain erythrocyte indices, such as MCV and MCH, these changes were statistically significant but remained within normal physiological ranges and were not accompanied by corroborative changes in related parameters, indicating no toxicological relevance [24]. (Figure 2) Biochemical analysis demonstrated that renal and hepatic function markers were largely unaltered following BFF-PS administration. A marginal increase in serum creatinine was observed; however, this change was within physiological limits and was not associated with significant alterations in blood urea or BUN levels, suggesting preserved renal function [25]. (Figure 3) Assessment of liver function parameters is a critical component in evaluating the safety of test substances, as the liver plays a central role in xenobiotic metabolism. In the present study, oral administration of Borassus flabellifer polysaccharide (BFF-PS) at a limit dose of 2000 mg/kg did not result in any significant alterations in serum hepatic enzyme levels, including SGPT, SGOT, and alkaline phosphatase, when compared with control animals.(figure 4A) Additionally, serum bilirubin levels and protein profile parameters such as total protein, albumin, and globulin remained within normal physiological limits.(figure 4B) The absence of significant changes in these biochemical markers indicates that BFF-PS does not induce hepatocellular injury or impair liver synthetic function following acute exposure[26]. Evaluation of serum lipid parameters is important in toxicity studies to assess potential disturbances in lipid metabolism and cardiovascular risk. In the present study, administration of Borassus flabellifer polysaccharide (BFF-PS) at a limit dose of 2000 mg/kg did not produce any statistically significant changes in serum total cholesterol, triglycerides, HDL, LDL, or VLDL levels when compared with the control group. All lipid parameters remained within normal physiological ranges, indicating that BFF-PS does not adversely affect lipid metabolism following acute oral exposure [27]. (Figure 5)

Following sacrifice at the termination of the study, the brain, liver, kidneys, lungs, and heart were collected for histopathological evaluation. Microscopic examination showed normal histoarchitecture with no evidence of lesions in the brain, heart, kidneys, or liver of treated animals compared with the vehicle control group [28]. (Figure 6)

5. Conclusion

In accordance with the OECD Guideline 425 (Acute Oral Toxicity—Up-and-Down Procedure), the present study demonstrates that the aqueous extract of Borassus flabellifer polysaccharide (BFF-PS) is safe and non-toxic following a single oral administration at a limit dose of 2000 mg/kg body weight in rats. No mortality or treatment-related clinical signs were observed during the 14-day observation period. Hematological, biochemical, and histopathological evaluations revealed no biologically significant alterations in vital organs, including the brain, heart, liver, kidneys, and lungs.

Based on these findings, the oral LD_{50} of BFF-PS is estimated to be greater than 2000 mg/kg, indicating a wide margin of safety. The results support the potential use of BFF-PS in pharmaceutical and nutraceutical applications and justify further investigation through sub-acute and chronic toxicity studies in compliance with OECD guidelines.

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7. Conflict of interest

I declare that there is no conflict of interest regarding the publication of this manuscript. I have no financial, personal, or professional relationships that could be perceived to influence the work reported in this paper.

8. Funding source

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