Acute and subacute oral toxicity study of methanolic extract of *Musa balbisiana* Colla fruit pulp in Swiss albino mice

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Abstract: *Musa balbisiana* Colla is extensively found in South-East Asia. Its fruit is widely consumed around the world and it is rich in natural products. The natural products found in the fruit pulp of *Musa balbisiana* Colla can be a better potential candidate for future alternative medicinal drugs to be designed or synthesized. Hence, evaluating and studying the acute, subacute toxicity of *Musa balbisiana* Colla fruit pulp is very important. Swiss albino mice were treated orally with four different doses of methanolic extract of *Musa balbisiana* Colla fruit pulp and after administration they were screened for toxicity for two weeks. Different doses of the extract were given to multiple test groups of mice for 28 days throughout subacute toxicity study. During this study, overall changes of the body and major organ weight, hematological, serum biochemical and histopathological parameters were observed. The study indicated that the LD50 of the methanolic extract of *Musa balbisiana* Colla fruit pulp was exceeding 5000 mg/kg. Subacute toxicity study showed no significant negative impact on food intake, body and organ weights, hematological and serum biochemical parameters, mortality and histopathology of mice of different treatment groups.

Keywords: Acute, Subacute, Histopathology, Hematological, Toxicity.

1. Introduction: Throughout history, numerous countries have relied on traditional medicine as a means to address a diverse array of illnesses. In recent times, the focus has shifted towards the identification and utilization of isolated chemicals and bioactive compounds sourced from plants, serving as the primary foundation for pharmaceutical drugs. These drugs are either derived naturally or created synthetically to mimic naturally occurring compounds. Nevertheless, there exists evidence highlighting the potential adverse effect of certain herbal extracts during the treatment process, raising concerns regarding the safe consumption of herbal medicine. Consequently, it becomes imperative to thoroughly examine the safe use of plant materials prior to their utilization in the management of ailments. Recognizing this necessity, the World Health Organization places significant emphasis on scientific investigations into the toxicity of ethnic herbal medicines as an integral part of the safety assessment process for herbal products (Tran *et al*., 2021).Multiple studies on herbal food and remedies have raised numerous concerns regarding human health and have discussed their antagonistic properties governed by toxic substances such as anti-nutrients, heavy metals etc. on the vital organs (Farsi *et al*.,2013). Lack of quality control and limited knowledge of the complex chemical composition of herbal food or medicines contribute to the evaluation of safety, most importantly toxicity of medicinal herbs. *Musa balbisiana* Colla is a plant that belongs to the family Musaceae, found in wild habitats and also cultivated in South East and Central Asia. Its fruit is widely used as food for consumption and it is reported to have various medicinal and therapeutic properties. In Assam, India, its fruit is popularly known as Bhimkol or Athiyakol. Traditionally, this plant has been used as a dietary supplement for nutrition (Borborah *et al*., 2016). Borborah *et al*. (2016) investigated the phytochemical composition of *Musa balbisiana* Colla fruit. The study reported that the fruit is rich in essential nutrients such as vitamins, minerals and dietary fiber. It also contains bioactive compounds like polyphenols, flavonoids and carotenoids, which contribute to its potential health benefits (Borborah *et al*., 2016). According to another research finding by Kumari *et al*. (2020), the utilization of dried fruit pulp powder of *Musa balbisiana* Colla holds promise as a nutritional approach to prevent heart disease like cardiac hypertrophy by

modulating oxidative stress and inflammation in the hypertrophic heart. Another finding by Irawan *et al*. (2021) highlighted the fact that the ethanolic extract of ripe *Musa balbisiana* Colla fruit pulp is a potential source of antioxidants and an anti-gout agent. Findings by Nhon Hoang *et al*. (2023) have suggested that methanolic, ethanolic and aqueous extracts of *Musa balbisiana* Colla fruit pulp demonstrated excellent performance in exhibiting strong antioxidant, anti-inflammatory, antibacterial, antifungal and anti-diabetic effects.

Although it is taken as medicine and food but the toxicity of the pulp of fruit *Musa balbisiana* Colla has not been completely revealed. The herbal dietary supplements don't fall under the criteria of US-FDA drug-regulatory system, therefore Economic Cooperation and Development (OECD) guidelines are used for evaluation of acute and subacute toxicological evaluations which are of immense importance in order to ensure the safety of drugs or dietary food supplements of herbal origin (Farsi *et al*.,2013). The present study is intended to assess the safety of the standard methanol extract of *Musa balbisiana* Colla unripe dried fruit pulp (MBME). For this study, the Swiss albino mice were selected and examined for acute and the oral 28-day subacute toxicity test in accordance to the OECD guidelines.

2. Materials and methods:

2.1 Plant Material Collection and Identification: The targeted plant species for the present study is *Musa balbisiana* Colla fruit pulp (Musaceae), commonly called 'Bhimkol'. Fresh fruits were collected from Kamrup district of Assam (India) in the month of November 2021. The plant species were identified on the basis of standard literature and authenticated voucher specimen was prepared, poisoned with mercuric chloride and deposit in the Herbarium, Department of Botany, Gauhati University, Assam possessing accession number GUBH20010.

2.2 Preparation of Fruit pulp extract: Fruits of *Musa balbisiana* Colla fruit pulp were collected, thoroughly cleaned, cut into pieces, air dried, coarsely powdered and used in the present study. 10 g fine powder was added to 100 ml of methanol (1:10 ratio) and extracted by cold maceration

extraction. After filtration by filter paper (Whattman No. 1), and the obtained filtrate was evaporated to aridness and the sticky extract (MBME) was recovered and reserved for further use at 4°C (Dharajiya *et al*., 2014).

2.3 Experimental animals: Acute oral toxicity test were performed to determine the LD50 value of MBME. Healthy young adult male Swiss albino mice weighing 20-30g were selected for carrying out the experiment. All experimental procedures were examined and accepted by Institutional Ethical Committee for Animal Welfare having reference no. GUIEC/2021/038 and were conducted according to current guidelines for the concern of laboratory animals of Institutional Animal Ethics Committee (IAEC) having (Ref. No. IAEC/Per/2022/PP-IAEC/ 20222-4/01).

2.3.1. Assignment of animals: The mice were divided into six groups randomly and each group were consisting of six mice (n=6). They were marked with a marker pen for their identification. Group I was selected as the control group and other groups II, III, IV, V, and VI were considered as test groups for the administration of varying concentrations of MBME.

2.3.2. Housing and Nutrition: The experimental animals were kept in cages with food, water, and sawdust litter and the temperature was maintained at optimum level. The lighting condition was carefully regulated to provide a cycle of twelve hours of sunlight followed by twelve hours of darkness, alternating in twenty-four hour intervals.

2.4 Toxicity Studies of MBME: The acute and subacute toxicity study of MBME was performed in accordance with the Organization for Economic Co-operation and Development (OECD) Guidelines No. 425, and 407 correspondingly (O. E. Cooperation, 1981; O. E. Cooperation, 2008).

2.4.1 Acute oral toxicity studies: Swiss albino mice (n = 6/each dose, weight 20g to 30g) were used in this current study and they were randomly grouped into six groups (Group I, Group II, Group III, Group IV, Group V and Group VI), each group containing six male mice. Prior to the experiment, the experimental animals were made to undergo twelve hours of fasting period during

which they were provided unrestricted access to only water. After fasting period, weight of the animals was measured first and then mice of Group II, Group III, Group IV, Group V and Group VI were administrated MBME orally at a dose of 300, 1000, 2000, 3000 and 5000 mg/kg respectively. After administration of MBME, foods of the animals were restricted for 2 hours. Group I was provided vehicle (distilled water) and was considered as normal control group. Changes in fur, skin, mucous membranes and eyes and also mortality were noted for the first 4 hours then consequently for 72 hours and then for 14 days after administration OF MBME. Throughout the entire 14-day duration, the impact of MBME on numerous gross behaviors such as locomotion, body positions, rearing, tremors etc. were carefully observed. On day 1^{st}, 7^{th} and day 14^{th}, the body weights of all mice were recorded.

2.4.2. Sub acute toxicity study in mice: A sub-acute study in Swiss albino mice was conducted for 28 days in accordance with the OECD testing guidelines 407 (O. E. Cooperation, 2008). Four groups were assigned randomly, each containing six male Swiss albino mice. The Group I was orally administered only vehicle (distilled water) every day for 28 days. The rest of the groups received MBME in single doses of 500 mg/kg (group I), 1000 mg/kg (group II), or 2000 mg/ kg (group III) daily for 28 days of duration. The body weight of the mice of each group was documented before the doses were administered on the first day, 7th, 14th and 28th day. Each animal in all the groups was monitored carefully for 28 days for any kind of toxic effect of MBME on gross behaviors, clinical signs, and mortality of each group of animals and recorded.

2.4.2.1 Relative organ weight of mice After sacrificing the animals, some major organs of the body of mice such as the kidney and liver were excised and weighed using an analytical laboratory balance and examined macroscopically for observation of any kind of change. The relative organ weights of animals were calculated by using the formula (Singh *et al.*, 2021):

$$\text{Relative organ weight (row)} = \frac{\text{absolute organ weight (g)} \times 100}{\text{body weight of mice on the day of sacrifice (gm)}}$$

2.4.2.3. Hematological and biochemical analyses: Hematological parameters like white blood cells (WBCs), neutrophils (NP), lymphocytes (LC), eosinophils (EP), red blood cells (RBCs), monocytes (MC), hemoglobin concentration (Hb), and platelet count (PLT) were analyzed using an automatic hematology analyzer (Erba H360):.

2.4.2.4. Statistical analysis: The statistical analysis was performed by using the GraphPad Prism 10.0.0 version. The analysis was done by using ANOVA (one-way analysis of variance). By using Dunnett's test, significant differences between the control and treatment groups were identified.

3. Results:

3.1 Acute toxicity study of MBME

No major significant difference was found in weight gain in the treated groups and control group (Table 1). During the entire observation period, the mice did not reveal any signs of toxicity or mortality up to doses of 5000 mg/kg. Therefore, we observed that LD50 of MBME is more toxic than 5000 mg/kg dose of MBME.

3.2 Sub-acute oral toxicity study of mice

The daily administration of fruit pulp extract at the dose amount of 500, 1000 and 2000 mg/kg of MBME did not cause any mortality in mice. There was also no treatment-related toxicity was identified during expose of mice to fruit pulp extract (MBME).

3.2.1. Effect of MBME on body weight

During sub-acute toxicity study, doses of 500, 1000, and 2000 mg/kg b.wt./day of MBME upon oral administration did not showed any sign of mortality and toxicity in the treated groups of mice for 28 days. No significant differences ($p \leq 0.05$) were noticed in weight gain in MBME-treated groups in comparison with the control group (Table 2).

3.2.2 Effect of MBME on water consumption and food intake in mice

There was no significant difference was observed in food and water consumption of MBME treated groups as compared to normal control groups (Table 3).

3.2.3 Effect of MBME on relative organ weight

The relative weight of the organs such as the kidney, liver, heart, brain, duodenum, and lungs of MBME-treated mice possess no significant difference ($p \leq 0.05$) in comparison to the normal control group even after the treatment duration of 28 days (Table 4).

3.2.4. Effect of MBME on hematological parameters

The data obtained from hematological analysis were presented in Table 5. Many of the parameters of the analysis did not exhibit statistically significant differences in all treatment groups while compared to the normal control.

3.2.5. Effect of MBME on serum biochemical parameters

Some serum biochemical parameter levels such as AST, ALP, ALT, cholesterol, creatinine, blood urea nitrogen (BUN), and uric acid were documented in the Table 6. No significant dissimilarity were noticed in AST, ALP, and ALT, cholesterol, creatinine, blood urea nitrogen (BUN) and uric acid levels at all the given doses of MBME when in contradiction with the normal control group.

3.2.6. Effect of dosage of MBME on antioxidant enzymes in mice liver tissues

Mice of MBME-treated groups for 28 days show negligible significant differences ($P \leq 0.05$) in SOD, catalase, MDA, and GSH (Table 7) levels within the liver tissues of treated groups of animals contrary to the normal control.

3.2.7. Histopathological study of organ tissues

Histopathological observations of the liver and kidney of MBME-treated groups of mice showed normal histological appearances at doses of 500, 1000 and 2000 mg/kg body weight (Fig. 1). No degeneration of hepatic tissue, normal glomeruli and tubular epithelium in kidney tissue were noticed in the tissue sections of the all the given doses of MBME treated group of mice. So, it could be elucidated that these organs did not possess any sign of histological abnormalities related to toxicity after treatment with multiple doses of MBME for 28 days besides the normal control.

Table 1: Effect of MBME on the body weight (g) of mice throughout the acute oral toxicity study

	Body weight (g)		
	First day	**7 days**	**14 days**
Normal Control	24.13±1.83	25.06±1.90	25.96±1.83
300 mg/kg body weight (MBME)	24.78±1.81	25.65±1.73	26.45±1.70
1000 mg/kg body weight (MBME)	25.45±1.85	26.18±1.66	27.01±1.65
2000 mg/kg body weight (MBME)	26.00±1.78	27.01±1.77	27.08±1.45
3000 mg/kg body weight (MBME)	26.56±1.75	27.76±1.67*	27.73±2.03
5000 mg/kg body weight (MBME)	27.4±1.63	26.50±1.37	27.88±1.74

(All values were articulated as Mean ± SD, values that are statistically significant ($p<0.05$) are marked with *.)

Table 2: Effect of MBME on body weight(g) during subacute toxicity study

Body weight (g)					
Day 1		7 Days	14 Days	21 Days	28 Days
Normal control	24.00±1.38	25.21±1.46	25.41±1.34	26.90±1.23	27.48±1.23
500 mg/kg body weight	24.61±1.62	25.31±1.50	26.75±1.60	27.03±1.55	27.66±1.50

(MBME)					
1000 mg/kg body weight (MBME) treated group	25.34±1.52	26.13±1.55	27.02±1.37	27.65±1.19	28.26±1.27
2000 mg/kg body weight (MBME)	25.66±1.23	26.35±1.10	27.20±0.88*	28.00±1.09	28.85±1.00

(All values were articulated as Mean ± SD, values that are statistically significant ($p<0.05$) are marked with *.)

Table 3: Effect of MBME on food and water consumption by test animals during subacute toxicity

Treatment Group	Average Food Intake (g/Day/Mouse)	Average Water Intake (ml/Day/Mouse)
Normal control	4.46±0.21	4.25±0.25
500 mg/kg body weight (MBME)	4.5±0.27	4.28±0.53
1000 mg/kg body weight (MBME)	4.56±0.24	4.62±0.69
2000 mg/kg body weight (MBME)	4.78±0.47	5.05±0.73*

(All values were articulated as Mean ± SD, values that are statistically significant ($p<0.05$) are marked with *.)

Table 4: Effect of MBME on relative organ weight during the subacute toxicity study

Treatment Group	Liver weight (g)	Kidney weight (g)	Heart weight (g)	Lung weight (g)	Brain weight (g)	Duodenum weight (g)
Normal control	3.96±0.16	0.80±0.06	0.49±0.08	0.67±0.03	1.27±0.10	0.53±0.06
500 mg/kg body weight (MBME)	3.95±0.16	0.83±0.05	0.56±0.10	0.68±0.03	1.41±0.13	0.54±0.01
1000 mg/kg body weight (MBME)	4.03±0.23	0.83±0.08	0.47±0.01	0.69±0.04	1.44±0.11*	0.58±0.05

2000 mg/kg body weight (MBME)	4.13±0.24	0.86±0.07	0.56±0.06	0.71±0.06	1.39±0.13	0.56±0.04

(All values were articulated as Mean ± SD, values that are statistically significant ($p<0.05$) are marked with *.)

Table 5: Effect of MBME on hematological parameters of mice during the subacute toxicity study

Hematological parameters	Normal control	500 mg/kg body weight (MBME)	1000 mg/kg body weight (MBME)	2000 mg/kg body weight (MBME)
Hemoglobin (%)	13.05±0.54	13.36±0.52	13.58±0.39	13.81±0.79
RBC (106 /µL)	9.13±0.50	9.28±0.47	9.63±0.43	9.55±0.40
WBC (103 /µL)	12.73±0.90	13.36±0.74	13.65±0.57	13.53±0.87
LC (%)	79.65±2.80	78.25±2.44	76.33±2.33	76.16±5.84
EP (%)	1.68±0.11	1.69±0.09	1.79±0.08	1.83±0.12
NP (%)	21.96±1.36	22.81±1.45	23.88±1.50*	22.63±1.49
MC (%)	1.41±0.12	1.44±0.12	1.52±0.13	1.57±0.13
Platelet (103 /L)	907.16±32.13	870.83±29.84	863.33±43.94	912.50±34.44

(All values were articulated as Mean ± SD, values that are statistically significant ($p<0.05$) are marked with *.)

Table 6: Effect of MBME on biochemical parameters in the serum of mice during the subacute toxicity study

Treatment	AST (IU/dl)	ALT(IU/dl)	ALP (IU/dl)	Cholesterol (mg/dl)	Creatine (mg/dl)	BUN (mg/dl)	Uric acid (mg/dl)
Normal control	41.91± 0.47	36.33± 0.62	76.01± 0.93	146.64± 1 .24	0.34±0.04	10.03±0.31	4.79±0.43
500 mg/kg body weight (MBME)	41.66± 0 .52	35.82± 0.84	75.49± 0.92	145.14± 1 .28	0.32±0.02	9.93±0.34	4.43±0.44
1000 mg/kg body weight	41.35±0.65	35.79± 0.52	75.06± 0.89	145.24±1 .90	0.31±0.02	9.83±0.32	4.27±0.44

(MBME)							
2000 mg/kg body weight (MBME)	41.08±0.72*	34.88± 2.01	74.92± 1.74	145.40±2.68	0.30±v0.02	9.93±0.44	4.40±0.41

(All values were articulated as Mean ± SD, values that are statistically significant ($p<0.05$) are marked with *.)

Table 7: Effect of MBME on antioxidant enzyme SOD, catalase, GSH, MDA content in the liver tissues of mice during subacute toxicity study

Treatment groups	Superoxide dismutase activity (U/mg protein)	Catalase (µmol Hydrogen peroxide oxidized/mg protein)	Glutathione(GSH) (nmol GSH/mg protein)	Lipid peroxidation (nmol MDA/mg protein)
Normal control	72.98±0.64	104.83±2.48	21±2.19	3.80±0.67
500 mg/kg b.w. (MBME)	72.5±1.51	105±1.78	20.5±1.37	3.94±0.54
1000 mg/kg b.w. (MBME)	71.83±1.16	106.16±1.72	21.83±1.16	4.09±0.40
2000 mg/kg b.w. (MBME)	73.16±0.75	106±2.36	21.5±1.87	4.59±0.39*

(All values were expressed as Mean ± SD, values that are statistically significant ($p<0.05$) are marked with *.

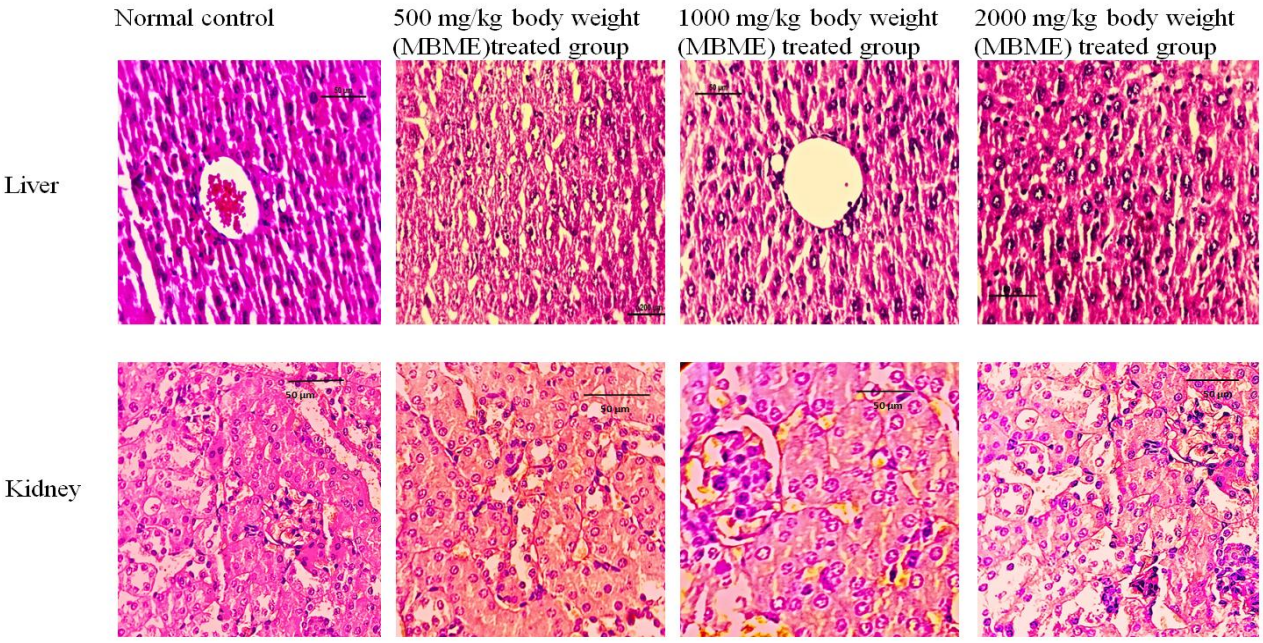

Fig. 1: Histology of the liver and the kidneys following treatment with different doses of MBME during subacute toxicity study.

4. Discussion

So as to establish a standardized safety dosage for natural products, it is crucial to conduct thorough investigations into their diverse toxicities in animals prior to considering them for utilization as alternative medicines in human healthcare. *Musa balbisiana* Colla possesses abundant phytochemical contents and secondary metabolites. The plant is loaded in potassium and chloride, resulting in high alkalinity that holds promising medical applications. Also, *Musa balbisiana* Colla synthesizes polyphenols, flavonoids, tannins, monoterpenoids, sesquiterpenoids, saponins and quinones in various components of the plant (Swargiary *et al*., 2021). Among other parts of *Musa balbisiana* Colla, fruit is widely consumed and it possesses antioxidant, anti-diabetic, anti-urolithic, antibacterial and antiulcer properties (Narzary & Sharma, 2022). In this present study, an acute and subacute toxicity study of the unripe fruit of *Musa balbisiana* Colla were performed. The study of acute toxicity provides valuable data about the drug dosage that induces highest adverse effects and helps determine the minimum lethal dose (Singh *et al*., 2021). The study revealed that when MBME was orally administered to male mice of multiple groups of different doses up to 5000 mg/kg of body weight did not exhibit any signs of toxicity or mortality. Therefore, based on the LD50 value

exceeding 5000 mg/kg, the tested doses of MBME can be considered safe for mice when administered orally.

The evaluation of subacute toxicity is crucial for assessing the long-standing effects of plant yields or products. The current study provides valuable insights into the tested drug's safety level (Manaharan *et al*., 2014). In the subacute toxicity study of MBME and mortality was recorded, and there were no notable differences in body weights of mice observed across the entire doses of MBME compared to normal control. Furthermore, after 28 days of oral administration, the intake of food and water remained unaffected by the administration of MBME. This suggests that there were no disturbances in the metabolism of the animal body of all treatment groups. The absence of negative effects during the subacute toxicity study of MBME, and no mortality in mice confirm the non-toxic nature of MBME.

It is notable that organ weight alterations serve as indicators of toxicity in animals (Olson *et al*., 2000). The outcome of the subacute toxicity study revealed statistically insignificant weight loss of vital organs for instance the liver, kidney, heart, brain, lungs, and duodenum of the mice of all administered doses of MBME observed. The study of hematological parameters provides valuable information regarding the toxic consequence of the extract on the blood of mice. Hematological parameters serve as clear indicators of the physiological and pathological status of any organism. The intensity of toxicity led by the toxic compounds drastically influence the measurement of hemoglobin, total RBC (red blood cells), WBC (white blood cells), LC (lymphocytes count), NP (neutrophils count), MC (monocytes count), EP (eosinophils count) and platelets (Das *et al*., 2015). However, in the case of administering MBME for 28 days, no notable changes were marked in the haematological parameters, indicating that it did not cause any noticeable alteration in the blood composition.

The liver and the kidneys are the vital organs indispensable for the organism's survival. Serum biomarker enzymes for example AST, ALT (primarily found in hepatocytes) and ALP (a

constituent of the endoplasmic reticulum and plasma membrane of various tissues) provide insights into the liver and kidneys health status. Elevated levels of these enzymes indicate liver tissue damage, that may be generated by infection or exposure to toxic substances. During the subacute toxicity study of MBME, administration of the extract did not significantly alter the levels of these enzymes in contrast with the normal control. Estimation of the blood urea nitrogen (BUN), uric acid, and creatinine levels provides indication of renal function. Elevated levels of these parameters suggest impaired renal function. In the subacute toxicity study of MBME, BUN, creatinine, and uric acid levels revealed no statistically significant differences contrary to the normal control. This result indicated that MBME did not exert toxic effects on the kidneys of the tested mice. Also, it is noted that the cholesterol levels exerted no significant variation in all treatment groups plus normal control group which assists the safety of MBME.

Oxidative stress is an alteration in cellular antioxidant levels caused by reactive oxygen species (ROS) that can lead to the inactivation of antioxidant enzymes. Evaluating the antioxidant enzyme levels (SOD, GSH and CAT) and MDA (an indicator of lipid peroxidation) provides valuable insights into the antioxidant status of tissues (Khandker *et al*., 2022). However, in the case of oral dosage of MBME, there were no significant alterations in these antioxidant enzymes as well MDA levels observed in both the treated and normal control groups. This points out that oral dosage of MBME did not impart any toxic effect on the mice liver tissues. Moreover, histological analyses of the liver and the kidneys of mice of all the treatment groups contrast to the normal control revealed normal structures leading to the conclusion that MBME did not induce any toxic effects in Swiss albino mice.

5. Conclusion

On the basis of the above findings, it could be summarized that the methanolic extract derived from the unripe fruit pulp of *Musa balbisiana* Colla is safe and non-toxic when administered acutely and sub chronically. The different extract doses did not demonstrate any adverse or lethal effects on

mice. No notable changes were observed in respect of the whole body, relative organ weights plus in histopathological, hematological, biochemical and morphological parameters. Furthermore, the fruit extract did not stimulate any remarkable fluctuation in the antioxidant enzymes levels in the liver tissues of mice of both treatment and normal control groups. As an outcome, this investigation supports the safe use of the methanolic extract of *Musa balbisiana* Colla (the fruit pulp) at doses up to 2000 mg/kg of body weight. The future prospect of this study includes the study of different medicinal properties this plant extract against a variety of diseases.

Author's Contributions: Formulation of concepts for the study, execution of the experiments, data analysis, and manuscript preparation was carried out by the first Author Nabanita Baruah. Madhubanti Das (second author) contributed to data analysis. Third, fourth and fifth author contributed to improvement of the manuscript. Sixth author Prof. Kandarpa Kumar Saikia Supervised the whole work.

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Data Availability: It is subjected to rational demand of the viewer to the author.

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