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Research Article

EVALUATION OF ACUTE AND SUB-ACUTE TOXICITY OF METHANOLIC LEAF EXTRACT OF *BAMBUSA VULGARIS* ON MALE WISTAR RATS

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ABSTRACT

Objectives: The study aims to evaluate the potential toxicity of methanolic extract of leaves of *Bambusa vulgaris* in male Wistar rats.

Methods: The methanolic extract was prepared for acute and sub-acute toxicity to determine the lethal dose. During the acute toxicity study, male rats were orally administered with leaf extracts at single doses of 100, 500,800, and 2000 mg/kg according to OECD Guidelines 425 for 14 days. Sub-acute toxicity dose at 200, 400, and 800 mg/kg was studied by daily dosing on male rats for 30 days.

Results: The acute toxicity study revealed no lethal effects and behavioral signs of toxicity at the tested doses indicating that LD50 is <2000 mg/kg. In a sub-acute study, non-significant changes in the body weight ($p<0.05$), body weight gain percentage ($p<0.05$) food intake ($p<0.05$), and relative organ weight percentage ($p<0.05$), and hematological parameters ($p<0.05$) of the rats were observed. A significant ($p<0.05$) increase in ALT level was observed dose at 800 mg/kg. Histopathological analysis showed mild cell distortion in the liver and kidney in male rats treated at 800 mg/kg of extract.

Conclusion: Our study suggests that oral administration of methanolic leaf extract *Bambusa vulgais* is safe. However, a dose of 800 mg/kg may be toxic for the liver and kidney.

Keywords: *Bambusa vulgaris*, Methanolic extract, Acute toxicity, Sub-acute toxicity, LD50 dose.

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INTRODUCTION

Herbal medicine or phytomedicines have been used for mankind since the beginning of the human race and have become an important part of the development of modern civilization [1]. Repeatedly over, 80% of the world's population depend on the plant as their primary source of medication [2]. Chemical drug development is an extremely challenging process that requires huge costs and long-term investment with a high risk of failure in trials. Furthermore, these drugs show many side effects [3]. To deal with these problems, the scientific communities stated to resist and reinvent the alternative medicine which has fewer side effects along with low cost and long-term use. Although the use of various parts of folk plants has shown high potential with promising global demand, there are still concerns about their potential benefits and safety [4]. Herbal medicines are usually considered safe or of low toxicity based on their historical uses by humans [5]. However, it is extremely is important to conduct toxicity studies on any medicinal plants to determine their safety profile before being used for human application [6].

Bambusa vulgaris, known as Golden Bamboo is a fastest-growing evergreen, arborescent plant and is a member of the grass family Poaceae. The leaf of *Bambusa vulgaris* has been used as Indian folk medicine to treat many medical conditions from the ancient period. According to the many studies leaf of *Bambusa vulgaris* are rich in many phytochemical compounds [7]. There are reports of numerous biological activities such as anti-oxidant [8], anti-diabetic [9], anti-inflammatory [10], lipid-lowering effect, and effect in reducing high blood pressure [11]. However, a detail analysis about its toxicity profile is lacking. Therefore, the potential study is designed of both acute and sub-acute oral toxicity of *Bambusa vulgaris* of methanolic extract on male albino Wistar rats.

MATERIALS AND METHODS

Collection of plant material

The leaves of *Bambusa vulgaris* were collected from Tripura University campus, Tripura, India. The leaves were taxonomically identified by taxonomist, of Botany Department, Tripura University, Tripura, Agartala. A voucher specimen (Coll No. TUH.4438) has been preserved in our laboratory.

Procedure of extraction

The collected sample of *Bambusa vulgaris* leaf was thoroughly washed under tap water followed by distilled water. The sample was shade dried for 7 days in normal temperature. Then, it was grounded to fine powder by electrical grinder and was stored in an airtight container. 200 g of leaf powder of the above-mentioned plant material was dissolved in 2000 ml of methanol in an air-tight container for 72 h. Afterward, the dissolved solution was filtered with the help of Whatman N 1 filter paper. The collected filtrate will be dried under reduced pressure at 40°C on a rotary evaporator. The collected dried extract was stored in the refrigerator at 4°C until further use. The extract was resuspended in distilled water on daily basis during administration to experimental animals according to their body weight.

Determination of leave extractive value

The percentage yield of methanolic extracts of the bamboo leaves on dry weight basis was calculated as follows:

$$\text{Yield (\%)} = \frac{\text{Weight of the extract after solvent evaporation}}{\text{Dry weight of the plant materail used for extraction}} \times 100$$

Phytochemical analysis
The presence or absence of the phytochemical constituents of the methanolic leave extracts of *Bambusa vulgaris* performed using the following methodologies was adapted Harborne and Parekh and Chanda [12,13].

Experimental animals
The study was conducted on healthy male albino rats of Wistar strain weighing about 150–170 g. The animals were acclimatized under control conditions in the university animal house at least 1 week before the onset of the study. The animals were maintained at a constant temperature (22±2°C) and a 12-h light/dark cycle for 7 days before experimentation.

Acute and sub-acute toxicity studies

Acute toxicity test

The oral acute toxicity study of the leaf extract of *Bambusa vulgaris* was carried out using the 'Up-and-Down' method of testing in male Wistar rats at single doses at 100, 500, 800, and 2000 (limit test) mg/kg body weight according to the Organization for Economic Development (OECD) guideline no. 425 [14]. Five male rats were used for each dose level in the study. An animal was picked at a time, weighed, and dosed with the equivalent volume of extract dissolved in distilled water. The extract was administered by oral gavage. Treated rats were observed for 14 days to follow the probable appearance of signs of acute poising including mortality and behavioral modifications, respiratory and digestive system changes, morphological changes (eyes color, skin changes, diarrhea, lethargy, coma and sleep, tremors, and convulsion).

Sub-acute toxicity test

Healthy male Wistar rats were assigned randomly to four groups (n=5/group). Group 1 received distilled water (2 mL/kg body wt.) only while groups 2–4 received 200, 400, and 800 mg/kg of the extract by oral gavage for 30 days. Daily food intake was recorded for the rats. The weights of all animals were measured weekly.

Animal sacrifice
At the end of the experimental period, all the animals were fasted overnight and sacrificed the next day according to the animal ethical guidelines of the Indian Council of Medical Research, Govt. of India. The fresh blood was collected immediately by cardiac puncture for biochemical and hematological analyses. The heart, liver, kidneys, spleens, and testes were excised, weighed, trimmed, and immediately kept at −20°C for future experiments.

Measurement of body and organ weights
The animals were weighed on day 1, 15, and 30. The level of weight gain (%) was determined using the relation:

$$\text{Body weight gain}\ (\%) = \frac{\text{Final body weight} - \text{initial body weight}}{\text{Initial body weight}} \times 100$$

Relative organ weight of each organ was calculated using the relation:

$$\text{Relative organ weight}\ (\%) = \frac{\text{Absolute organ weight}}{\text{Body weight at sacrifice}} \times 100$$

Assessment of hematological parameters
The blood samples for hematological analysis were put in bottles containing anticoagulant, ethylene diamine tetra-acetic acid. Parameters analyzed include hemoglobin concentration (Hb count), red blood cell count (RBC count), hematocrit (HCT), mean corpuscular volume, mean corpuscular hemoglobin (MCH), MCH concentration (MCHC), white blood cell count (WBC count), and platelet count were analyzed.

Assessment of serum biochemical parameters
The following serum biochemical parameters including renal function marker urea and createnine were determined using local biochemical kits (Coral Diagnostic kits, India); total protein and albumin were determined by commercial kits (Coral Diagnostic kits, India). Liver enzymes alanine transaminase (ALT) and aspartate transaminase (AST) were assayed by the colorimetric method using commercial kits (Erba, Mannheim) in semi-auto-analyzer.

Histopathological examination
All organs were gently washed in phosphate buffer saline and subjected to fixation in 10% neutral buffered formalin. Further processing was carried out which included dehydration and infiltration followed by embedding in paraffin. 5 (μm) sections were obtained from the paraffin block using a microtome and the sections were stained with Hematoxylin-Eosin (H&E). The histological images of the stained slides were obtained under a bright-field microscope (Leica DM400 B LED, Germany).

Ethical note
The research undertaken was approved by the Institutional Animal Ethics Committee (IAEC) of Tripura University, Agartala, Tripura, India (Ref No. TU/IAEC/2022/I/2-6); and it was performed as per the Organization for Economic Co-operation and Development (OECD) guidelines 425.

Statistical analysis
All the results were expressed as the mean±standard error means (SEM). The differences between the means of groups were analyzed by one-way ANOVA followed by Tukey HSD *post hoc* test using SPSS (Statistical Program for the Social Sciences, Chicago, IL, USA) 16.0 for Windows. Statistical significance was considered at $p<0.05$.

RESULTS

Plant yield
The yield of the *Bambusa vulgaris* leaf methanolic extract was 7.12%.

Phytochemical analysis
The different chemical compounds were presented in leaf extract of *Bambusa vulgaris* (Table 1). Different types of metabolites such as carbohydrate, protein, reducing sugar, alkaloid, terpinoid, phenolic, flavonoids, and glycosides are detected.

Effect of leaf extract on acute toxicity studies in rats
Using 100, 500, 800, and 2000 (limit test) mg/kg body weight doses of the methanolic extract of *Bambusa vulgaris* caused no death in rats and no lethal effects were marked throughout 14 days of study period. Therefore, the extract may be safe at these doses and the oral LD50 considered <2000 mg/kg in rats.

Effect of leaf extract on sub-acute toxicity studies in rats
All the treated male rats at the doses of 200, 400, and 800 mg/kg survived throughout the 30-day study period. Any kind of toxicity signs were not observed in the treated rats compared to the control.

Effect of the extract on food intake in rats
The methanolic leaf extract did not show any kind of significant changes on food intake at all doses consumed by the male rats when compared with the control (Fig. 1).

Table 1: Primery phytochemical analysis of methanolic leaf extract of *Bambusa vulgaris*

Chemical compounds	Tests	Result
Carbohydrate	Molish's test	Present
Protein	Biuret test	Present
Reducing sugar	Fehling's test	Present
Alkaloid	Wagner's test	Present
Terpinoid	Salkowski test	Present
Phenolic	Lead Acetate test	Present
Flavonoids	Shinoda's test	Present
Glycosides	Keller Killiani test	Present

Effect of the extract on body weight and percentage gain in body weight of rats

The initial day, 15th day, and 30th day body weight of control and treatment groups are reported (Fig. 2). There was increase in body weight but did not show any significant changes compared with control group. In addition, there were also non- significant changes in body weight gain percentage compared with control group rats (Fig. 3).

Effect of the extract on relative organ weight

Sub-acute toxicity effect of methanolic leaf extract of *Bambusa vulgaris* on relative weight of vital organs such as heart, liver, kidney, spleen, and testis of treated male rats did not show any significant changes compared to the rats of control group (Fig. 4).

Effect of the extract on hematological Parameters

The hematological parameters studied in control group and the treatment groups did not show any significant changes between the groups (Table 2).

Effect of the extract on biochemical parameters

In treated male rats, there was a significant increase in ($p<0.05$) serum ALT level in Group 4 (800 mg/kg) compared with control. All other parameters were not significantly different when compared to the control (Table 3).

Effect of the extract on histological assessment

Histological evaluation of the heart, spleen, and testis of male rats showed no changes when compared to the control. However, there was mild structural disruption of liver and kidney observed in Group 4 at 800 mg/kg dose (Fig. 5).

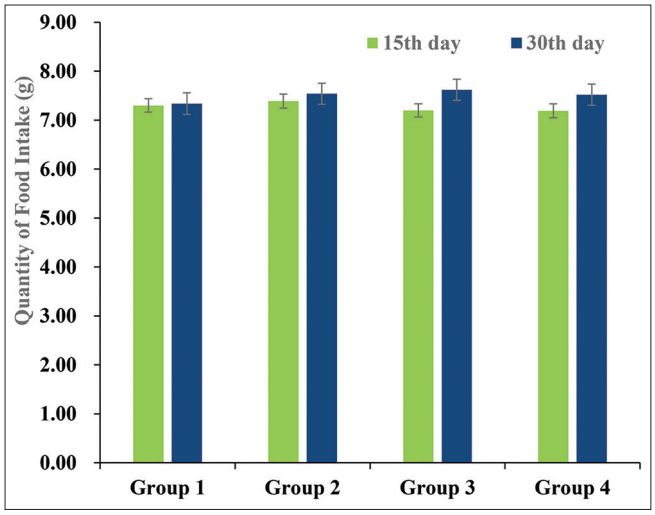

Fig. 1: Representation of sub-acute toxicity effect of methanolic extract of *Bambusa vulgaris* on quantity of food intake (g). Values are represented as mean±SEM (n=5 rats/group)

DISCUSSION

Herbal plants contain a high amount of highly active phytochemical compounds and these have been used for the cure of various diseases from ancient times. The preliminary phytochemical compound analysis of the methanolic leaf extract of *Bambusa vulgaris* contains alkaloids, phenolic, flavonoids, glycosides, and other essential biochemical compounds. Previous phytochemical analysis showed the presence of similar bioactive compounds in the extract that have potential beneficial effects [8]. The main reason for evaluating the safety of any medicinal plant is to identify the nature and significance of adverse effects and to establish the exposure level at which this effect is observed [6]. The results of the acute toxicity study indicate that the methanolic extract of *Bambusa vulgaris* leaf extract was administered oral route to rats at 100, 500, 800, and 2000 mg/kg according to the Organization for Economic Development (OECD) guideline no. 425 did not generate any sign of toxicity and death in the animals. This suggests that the oral LD50 of the plant being higher than 2000 mg/kg may be safe.

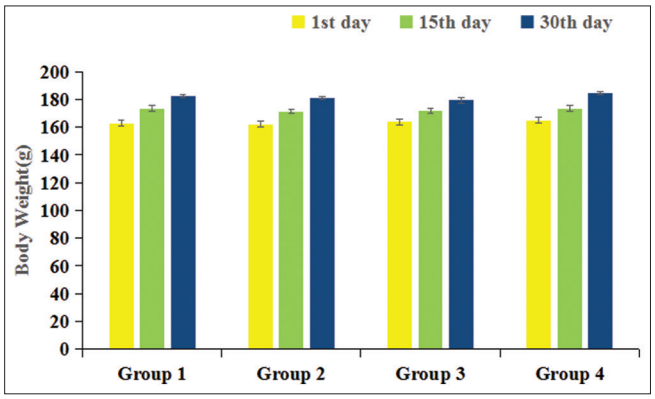

Fig. 2: Representation of sub-acute toxicity effect of methanolic extract of *Bambusa vulgaris* on body weight (g). Values are represented as mean±SEM (n=5 rats/group)

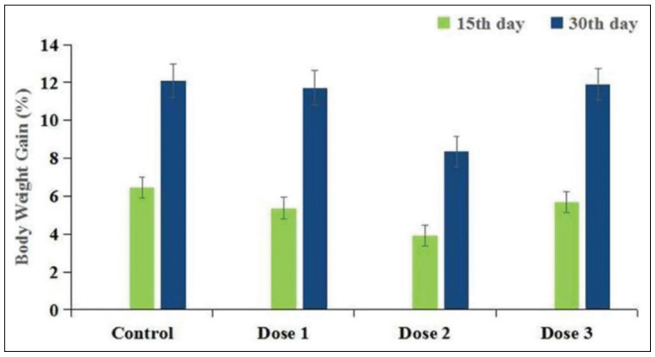

Fig. 3: Representation of sub-acute toxicity effect of methanolic extract of *Bambusa vulgaris* on percentage gain in body weight. Values are represented as mean±SEM (n=5 rats/group)

Table 2: Representation of sub-acute toxicity effect of methanolic extract of *Bambusa vulgaris* on hematological parameters

Hematological parameters	Group 1	Group 2	Group 3	Group 4
Hb concentration (g/dL)	14.58±0.13	14.50±0.12	14.51±0.14	14.54±0.12
RBCs count (10^6/µL)	7.75±0.18	7.76±0.15	7.74±0.18	7.75±0.22
HCT (%)	43.44±2.61	43.44±2.01	43.23±3.04	43.32±2.27
MCV (fl)	56.06±0.48	55.61±0.84	55.86±0.74	55.90±0.64
MCH (pg)	18.81±0.50	18.66±0.72	18.74±0.53	18.76±0.61
MCHC (g/dL)	33.56±0.23	33.37±0.31	33.56±0.20	33.56±0.34
WBCs count (10^3/µL)	6.72±0.84	8.24±2.47	10.76±3.14	10.80±2.29
Platelet count (10^3/µL)	667.20±58.02	662.40±50.04	675.80±40.29	648±31.24

Values are represented as mean±SEM (n=5 rats/group. SEM: Standard error mean, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, WBC: White blood count

Table 3: Representation of sub-acute toxicity effect of methanolic extract of *Bambusa vulgaris* on serum biochemical parameters

Biochemical parameters	Group 1	Group 2	Group 3	Group 4
U (mg/dL)	16.71±1.82	15.98±3.40	16.85±2.02	16.32±1.15
Cr (mg/dL)	0.82±0.04	0.81±0.06	0.85±0.05	0.79±0.01
Total protein (g/dL)	5.81±0.11	5.78±0.16	5.82±0.20	5.80±0.14
Albumin (g/dL)	3.12±0.16	3.14±0.23	3.09±0.15	3.20±0.11
AST (U/L)	136.05±3.21	130.15±4.04	134.95±3.01	137.42±2.94
ALT (U/L)	67.81±1.95	70.41±2.47	68.20±1.20	132.41±2.54*

Values are represented as mean±SEM (n=5 rats/group). AST: Aspartate transaminase, ALT: Alanine transaminase

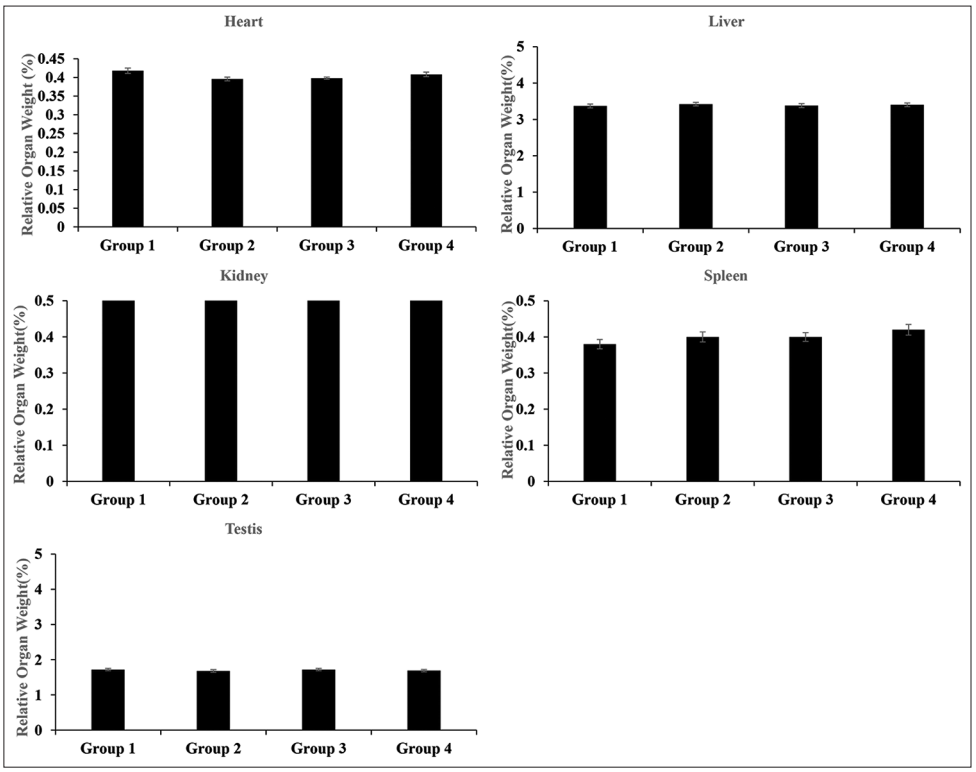

Fig. 4: Representation of sub-acute toxicity effect of methanolic extract of *Bambusa vulgaris* on relative organ weight percentage. Values are represented as mean±SEM (n=5 rats/group)

Acute toxicity data show limited clinical application since cumulative toxic effects do occur even at low doses. Hence, repeated dosing toxicological evolution that is sub-acute toxicity study is almost always invaluable for the safety profile of phytomedicines [15]. Daily administration may cause accumulation in the body with gradual effects on tissues and organs rodents. Sub-acute toxicity testing is essential for assessing the target organs and hematological or biochemical effects of extracts since these effects are usually not observable in acute toxicity testing. It is also useful in establishing human safety, especially in the development of pharmaceuticals [16]. Therefore, in this study, the sub-acute toxicity profile of *Bambusa vulgaris* leaf extract was evaluated in rats using measurement of body and organ weights, hematological, biochemical, and histopathological parameters.

Changes in food intake and changes in body weight have been used as an indicator of the general health status of experimental animals. Oral administration of the methanolic extract of *Bambusa vulgaris* daily throughout 30 days of exposure showed no mortality and no significant changes in food intake and body weight suggesting that there was no effect on the normal growth of rats with administration of these extracts on various doses. These results also found at the acute toxicity study of aqueous extract of *Bambusa vulgaris* leaves on rats observed by Abe [17]. There is a slight decrease in percentage gain in body weight but these changes are also not significant. Our result suggests *Bambusa vulgaris* leaf contains flavonoids and polyphenol compounds which can suppress appetite that may act as anti-obesogenic properties because obese individuals have been shown to have enhanced appetite and reduction of appetite has helped in the prevention of further weight gain [18].

In toxicity studies, organ weight changes or any morphological changes are sensitive indicators of toxicity, effects on enzymes, physiological disturbances, and target organ injury [19]. The gain in relative organ weight causes hypertrophy while the decrease suggests necrosis in the organs. While organ weights provide useful signals that indicate test article-related effects, organ weight data must be interpreted in an integrated fashion with gross pathology, clinical pathology, and histopathology findings [20]. As our results revealed that all the relative weights of organs such as heart, liver, kidney, spleen, and testis of treated animals on multiple doses were not different from the control group at significant levels; therefore, it could be claimed that the extract may be safe and does not show any pathological effects in given doses level.

Hematological parameters analysis is very crucial for the toxicity study of foreign substances including plant extracts. Hematopoiesis is the process of the formation of cellular components of blood. It takes place within the hematopoietic system that includes organs such as the bone marrow, liver, and spleen. In toxicity studies, changes in the hematopoietic system have a crucial effective value when data are translated from animal studies [21]. In this study, administration

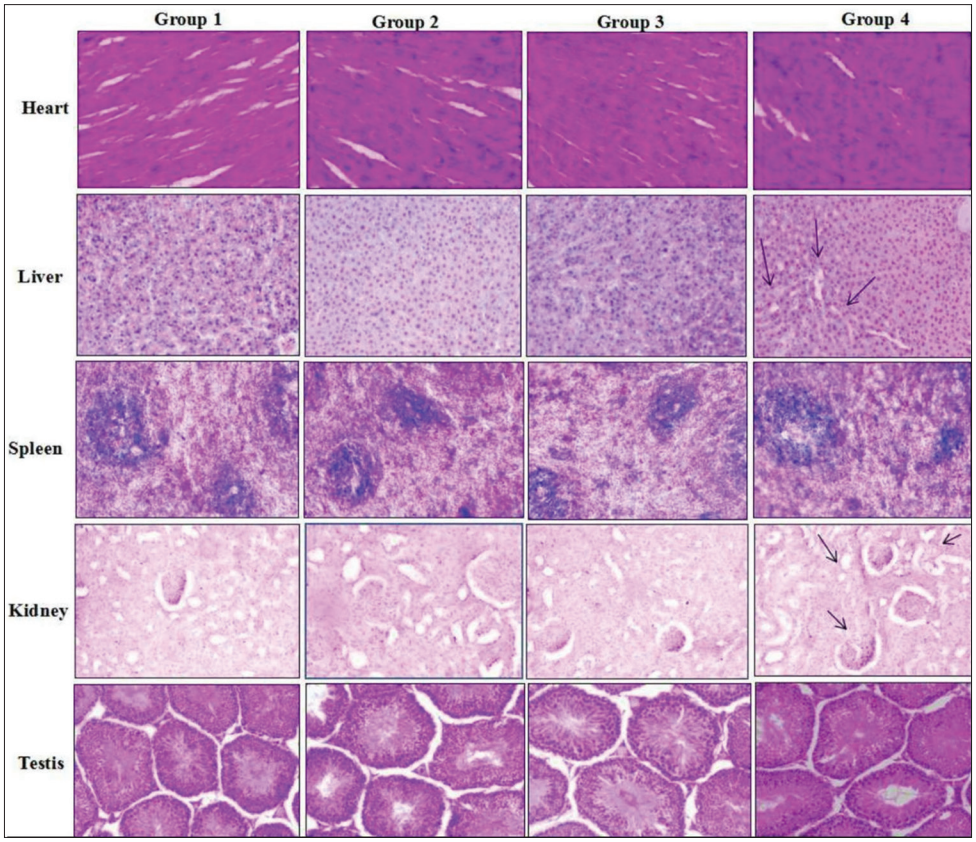

Fig. 5: Histopathological analyses of hearts livers, spleens, kidneys, and testis in control and treated groups were carried by hematoxylin and eosin (H&E) staining. The black arrow indicates the disruption in liver and kidney tissue. Other section did not show any kind of changes

of methanolic extract of *Bambusa vulgaris* in male rats for 30 days produced no significant changes in Hb concentration, RBCs count, HCT, MC, MCH, MCHC, WBCs count, and platelet at 200 mg/kg and 400 mg/kg and 800 mg/kg body weight doses compare with control.

The functions of the liver and kidney play an important role in the survival of animals. The functionality of these organs is measured by serum biochemical analysis, which plays a very conclusive toxicological evaluation of xenobiotics [22]. The status of the liver provides by serum liver function tests. The liver enzymes that are aminotransferases; ALT, and AST demonstrate cellular integrity, while functionality is described by albumin and total protein level [23]. AST and ALT are mainly produced by the liver and any disturbance to the liver may cause an increase in the serum level of these enzymes which causes hepatocellular toxicity whereas, a depiction may cause enzyme inhibition [24]. However, ALT is an important sensitive marker of liver damage since AST is also found in the kidney, testis, and cardiac [25]. In the present study, there is a significant increase in the ALT level at 800 mg/kg. Therefore, it may be mildly toxic since the evaluation of liver enzymes in male rats and histopathological study at similar doses, there is mild damage in the liver. A dose of 800 mg/kg may show mild toxicity in the liver.

The functionality of the kidney is measured by the levels of urea, and creatinine. A high level of serum urea indicates dysfunction of the kidney or dehydration condition of animals whereas; low urea levels are seen during acute liver failure [26]. In animal model toxicity studies, the serum level of creatinine is the most widely used laboratory test to estimate renal function. Creatinine clearance, an indicator of glomerular filtration rate, is used for assessing kidney function [27]. Our extract did not cause any significant change in the creatinine and urea levels when compared with the control suggesting that the extract may not be toxic to the kidney. However, a dose of 800 mg/kg shows mild structural changes in kidney tissue. Hence, this dose may not be safe for a kidney in prolonged use.

CONCLUSION

The oral LD50 of methanolic extract of the leaf of *Bambusa vulgaris* is <2000 mg/kg and is generally considered safe. *Bambusa vulgaris* has also been shown to cause appetite suppression and reduction of body weight and hence can be used as an anti-obesity agent. Prolonged administration revealed that it may safe at dose 200 and 400 mg/kg but at dose 800 mg/kg causes elevation of liver enzymes and mild toxicity to the liver and kidney. The acute toxicity study provides baseline information on doses for further study on methanolic leaf extract of *Bambusa vulgaris* acquired from this present study.

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AUTHOR'S CONTRIBUTION

- SS contributed to data accusation, study design.
- PRC contributed to data analysis and manuscript preparation.
- DC contributed to initial study design and analysis, drafted the initial version of the manuscript, and contributed to its critical revision. All authors approved the final version of this manuscript.

COMPETING INTEREST

The authors declare no conflict of interest.

AUTHORS' FUNDING

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