RESEARCH ARTICLE

Acute and Subacute Toxicity Studies of *Diospyros malabarica* Bark Extract by Oral Administration in Mice

[1]Sapna M, [1]Bhargavi G, [2]K.N. Ravindra, [3]Suprada Rao M.S., [4]Rajesh R, [1]Nataraju Angaswamy*.
[1]DoS and R in Biochemistry, Karnataka State Open University, Mysuru, Karnataka, India.
[2]DoS and R in Botany, Karnataka State Open University, Mysuru, Karnataka, India.
[3]DoS in Biochemistry, SBRR Mahajana First Grade College, Mysuru, Karnataka, India.
[4]Chromed Biosciences Pvt. Ltd., Hirehalli, Tumkur, Karnataka, India.
***E-mail**: natabiochem@gmail.com

Abstract

Parts of Diospyros malabarica (DM), particularly the fruits, bark, and leaves, are used to treat burning, diabetes, atherosclerosis, intermittent fever, and cancer because they are rich in phytochemicals. In addition to providing essential nourishment, the wild edible fruit's extract possesses strong antidiabetic, antibacterial, anti-inflammatory, and antipyretic properties. Previous research on animals has shown that DM extracts have antiurolithiatic, anti-diarrheal, analgesic, nephroprotective, hepatoprotective, and antinociceptive properties. Despite this well-known herb's pharmacological benefits, little is known regarding its toxicity. Thus, the purpose of this study is to evaluate the acute and subacute toxicity of DM bark methanol extract in mice. According to OECD guidelines (423, 425, and 407), a 14-day single oral dose for acute toxicity (500, 1000, and 2000 mg/kg b.w.) of extract, and a 28-day, daily oral dose for sub-acute toxicity (500, 750 and 1000 mg/kg b.w.) were investigated. Mice were divided into eight groups (5 female and 5 male, n=10/group). Biochemical markers and body weight were assessed. The morphology and histology of the livers, lungs, spleen, kidneys, and heart were investigated. It was discovered that the oral LD_{50} value of DM was more than 2000 mg/kg. There were no differences in food intake, water consumption, body and organ weight, following administration of DM compared to controls. Similarly, DM did not alter haematological and serological indices, even at higher dosages. Furthermore, histological analyses revealed no discernible variation when compared to control mice. The present investigation demonstrates the non-toxic nature of DM extract and encourages its use in food and pharmacological applications.

Keywords: Acute toxicity; Subacute toxicity, *Diospyros malabarica*; Body weight, Balb/c mice, Haematology, Histology.

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1. Introduction

Medicinal plant products are a natural source of health care for people, and their components possess a range of beneficial health properties [1]. *Diospyros malabarica*, commonly known as the Malabar ebony or Gaub tree, is valued in traditional medicine

for several health benefits. Some of its reported health benefits include antimicrobial, anti-inflammatory, antioxidant, and antidiabetic effects, as well as use as a remedy for coughs and respiratory ailments. Particularly, the tree extract has been extensively used in wound healing and the treatment of digestive issues such as diarrhoea and dysentery. While these benefits are promising, scientific research is still ongoing to fully validate and understand the mechanisms. As a rich source of pharmacologically active bio constituents, *D. malabarica* could be anticipated for its use as a phytomedicine [2]. All organs of this plant, especially bark, fruits, and leaves, are used with reported antioxidant potential in traditional preparations [3]. As a rich source of pharmacologically active bio constituents, *D. malabarica* could be anticipated for its use as a phytomedicine. Traditionally in the Western Ghats of India, *D. malabarica* bark extract has been used to treat inflammation, diarrhoea and dysentery [4].

Toxicology studies are fundamental to protecting human health, ensuring environmental safety, and guiding regulatory decisions. They provide critical insights into how various substances, ranging from pharmaceuticals to industrial chemicals, interact with biological systems, helping to prevent adverse effects and promote well-being. In the realm of drug development, toxicology is indispensable. Before a new medication reaches clinical trials, it undergoes rigorous toxicological assessments to determine its safety profile. These studies identify potential adverse effects, establish safe dosage ranges, and evaluate the impact on vital organs such as the liver, kidneys, and heart. This information is crucial for designing clinical trials that prioritise participant safety and for ensuring that only safe and effective drugs are approved for public use [5]. Toxicological research plays a pivotal role in public health. This knowledge reduces exposure to harmful agents, thereby safeguarding communities [6].

The naturally occurring phytochemicals possess a range of biological activities. However, their potential toxicity is a critical area of research, as they can exhibit beneficial effects at low doses while being toxic at higher concentrations or *vice versa*. Phytochemicals can induce toxicity through various mechanisms. Arsenic, for example, is a known toxicant that can induce oxidative stress through the generation of ROS like hydrogen peroxide and superoxide radicals, impairing cellular antioxidant defences. Similarly, curcumin and other polyphenols can have a dual role; they act as antioxidants at lower concentrations but can cause oxidative stress when consumed in excess. The balance between beneficial and toxic effects often depends on doses.

Conducting acute and sub-acute toxicity studies using OECD (Organisation for Economic Co-operation and Development) guidelines is important for several reasons, such as standardisation, regulatory acceptance, scientific rigour, comparability, compliance and animal welfare. The toxicity studies following OECD guidelines provide reliable, accepted, and ethically conducted safety data crucial for protecting human health and the environment. Leaf, fruit and stem are the most commonly used parts of this tree for evaluation of pharmacological activities both in vitro and in vivo (7). Previous animal studies have demonstrated antiurolithiatic (8), Anti-diarrheal, Analgesic (9), Nephroprotective (10), hepatoprotective (11), and antinociceptive (12) effects of *D. malabarica* extract. Regardless of the pharmacological beneficial effects of *D. malabarica*, detailed knowledge about the toxicity of this famous herb is lacking. Therefore, research including toxicity studies is necessary to assess its potential adverse effects, safe dosage limits, and mechanisms of toxicity.

2. Materials and Methods

Plant material: Fresh stem bark of *Diospyros malabarica* was collected from the Western Ghats, Chikkamangalore region, Karnataka, India, and was authenticated by Prof. Niranjan Raj S, Chairman and a taxonomist at DOS&R in Botany, Mukthagangotri, Karnataka State Open University, Mysore-06. A voucher specimen number (Herbarium no-BOTIDM02) was deposited in the department ledger for further reference and records. The stem bark sample of *DM* was collected and washed under running tap water to remove soil debris and dirt. The stem bark pieces were sliced into small pieces using a knife and hammer. The stem bark pieces were surface-sterilised, air-dried, finely powdered, and stored at 4 ℃ until further use. All the chemicals, reagents and staining dyes used in the present experiment were of analytical grade.

Soxhlet extraction and preparation of extract: The powder prepared from *D. malabarica* stem bark was defatted overnight (n-hexane), followed by methanol extraction using the Soxhlet apparatus (Lab matrix, Karnataka, India). The extract was concentrated under reduced pressure at 60 ± 1°C in a rotary vacuum evaporator (Steroglass, strike 300, Italy) until a solid mass was obtained, and stored in an air-tight container in a refrigerator (4 °C) until further use. The extract was dissolved in saline (1 mg/ml) to prepare the stock solution. The dilutions for oral administration to test mice were prepared from the stock solution.

Experimental animals: Adult Balb/c mice, aged 9-11 weeks (23.8 ± 3.4 grams), were obtained from Chromed Biosciences Private Limited, #38 C, KIAD Industrial Area, Halli, Tumkur, under reference number CBPL-IAEC-111/04/2025. The animals were housed in plastic cages and maintained at 28 ± 2 °C with a relative humidity of 45–55% under a 12 h natural dark/light cycle. The mice had free access to water and food. The sample size of 10 animals per group comprised 5 male and 5 female mice.

Acute toxicity: The assay was conducted in accordance with OECD guidelines (423 and 425). A total of 4 groups of mice (Avg wgt. 21.8±2.5) were used for the study. Groups 1 (control, untreated), 2 (500 mg/kg b.w.), 3 (1000 mg/kg b.w.), and 4 (2000 mg/kg b.w.) were selected. After 12 hours of starvation, a single respective dose of DM extract was administered by oral gavage to each group. Each animal was observed daily for behavioural and general toxicity signs for a total of 14 days. On the 15th day, the mice were sacrificed, and the vital organs (liver, spleen, heart, and kidney) were carefully removed, weighed, and stored in 10% formaldehyde. Blood samples were collected for haematology and serum analysis.

Sub-acute toxicity: The study was conducted in compliance with OECD guidelines (407). The experimental animals were divided into 4 groups. For sub-acute studies, groups 1 (control, untreated), 2 (500 mg/kg b.w.), 3 (750 mg/kg b.w.), and 4 (1000 mg/kg b.w.) were selected. Each group comprised 8–12-week-old mice (Avg. wgt. 21.8±2.5) housed in separate cages and administered the respective daily oral gavage dose of DM extract for 28 days. Each animal was regularly observed for behavioural and general toxicity signs after daily dosing for a total of 28 days. On the 29th day, the mice were sacrificed, and the vital organs (liver, spleen, heart, and kidney) were carefully removed, weighed, and stored in 10% formaldehyde. Blood samples were collected for haematology and serum analysis.

Evaluation of relative body weight: The vital organs were dissected and weighed carefully. The relative organ weight of each animal was calculated by the formula:

Relative organ weight (g/100g) = organ weight(g)/body weight (g)×100

Assessment of haematological parameters: The haematological analysis was performed on blood samples collected in EDTA-coated tubes using an automated

haematology analyser. The haematological parameters analysed included total and differential white blood cell (WBC) count, Red Blood Cells (RBC), Red Cell Distribution Width (RDW), haemoglobin (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count (PLT), and mean platelet volume (MPV).

Histopathological analysis: Representative sections of the liver, spleen and the kidney were made and fixed in tissue cassettes and processed in a tissue processor for twelve hours. The cassettes were embedded in molten paraffin wax and left to form blocks on a cold plate. The paraffin blocks were mounted on a rotary microtome to obtain 4µm-thick sections. These sections were then carefully removed from the microtome knife and put in a water bath to remove folds. The best sections were mounted on labelled slides and placed in an oven at 58 °C overnight to fix. The slides were removed and stained using the Harris haematoxylin & eosin technique. A mounting agent (Dibutyl phthalate in xylene) was added thereafter, and the specimen was finally covered with a cover slip to prevent bubble formation. The slides were examined under a standard light microscope for histological lesions or abnormalities.

Statistical analysis: The computer-guided Statistical Programme GraphPad Prism Software Version 8.0.2 was used for analysis. The analysis involved summarising data using descriptive statistics (mean ± SD). Next, One-way and Two-way ANOVA were performed. Differences in the mean between the treatment groups were considered significant at $p < 0.05$.

3. Results and Discussion

Acute oral toxicity effects of *D. malabarica* stem bark methanol extract

Representative images of the *D. malabarica* plant and its extraction process are given in Figures 1 & 2. After defatting with n-hexane, the methanol extract (22.48% yield) was used for the study. Leaf, fruit and stem are the most commonly used parts of this tree for evaluation of pharmacological activities both *in vitro* and *in vivo* (7). Previous animal studies have demonstrated antiurolithiatic (8), Anti-diarrheal, Analgesic (9), Nephroprotective (10), hepatoprotective (11), and antinociceptive (12) effects of *D. malabarica* extract. Regardless of the pharmacological beneficial effects of *D. malabarica*, detailed knowledge about the poisonous effects of this famous herb is lacking. Hence, the current study was undertaken to evaluate and focus on the acute and subacute toxicity of *D. malabarica* in mice.

Following administration of the extract, the animals showed no signs of lethargy, weakness, abnormal or slow motor and reflex activities. The untreated control group of mice and all three treatment groups showed a resemblance in water and food intake. The extracts caused no mortality and behavioural changes in the mice during the initial treatment period and throughout the study period. No signs of toxicity were observed in the wellness parameters during the 14-day observation period.

The acute toxicity test is the first step in the preclinical assessment and evaluation of the toxic characteristics of a substance. It can provide data on health hazards that are likely to arise from a short-term exposure. Generally, there was an increase in the animals' body weight throughout the study period, as observed in our study. Therefore, the approximate acute lethal dose, LD_{50}, was estimated to be above 2000 mg/kg for *D. malabarica* stem bark methanol extract.

Figure 1: Representative images of *D. malabarica* plant parts.

Figure 2: Pictorial representation of the Soxhlet extraction procedure with *D. malabarica* bark, bark powder and the methanol extract.

Effect of oral administration of *D. malabarica* extract on acute body weight

The body weights and body weight gain of mice treated with *D. malabarica* stem bark methanol extract doses (500, 1000, and 2000 mg/kg body weight) are presented (Figure 3). The mice exhibited a gradual increase in body weight in all animals. The control animals and 500 mg/kg group gained only 2% of their initial b.w. Group 3 and Group 4 animals with 1000 and 2000mg/kg b.w gained about 5% of their initial weight. The observation was similar in both male and female groups. The acute toxicity study, which involved the administration of *D. malabarica* to mice

orally at doses of 500, 1000 and 2000 mg/kg body weight, demonstrated no significant changes in animal behaviour, as well as reductions in body weight in mice even at high doses (2000 mg/kg body weight). The acute toxicity study is utilised to check the harmful effects of an agent to the organism given as a single or short-term exposure [13]. In the present study, the acute toxicity evaluation showed that the oral LD_{50} value of *D. malabarica* extract was 2000 mg/kg. For Hodge and Sterner (2005), with the help of LD_{50} determination, our extract showed practically non-toxic category.

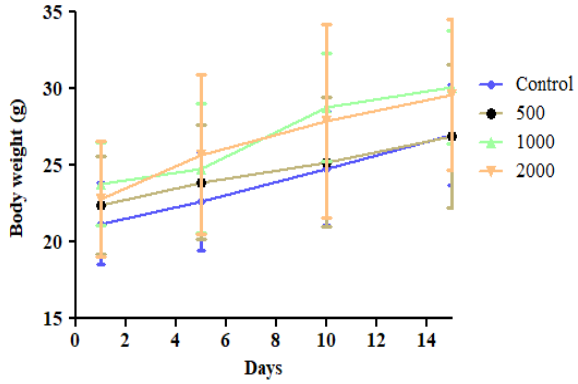


Figure 3: Body weight measurements (g) of mice in the acute toxicity study of the methanol extract of *D. malabarica*. Results are represented as the mean ±SD for n=5.

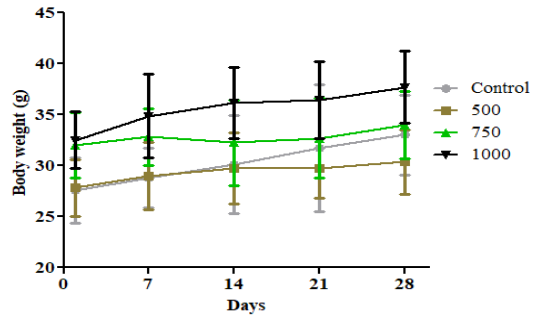


Figure 4: Body weight measurements (g) of mice in the subacute toxicity study of the methanol extract of *D. malabarica*. Results are represented as the mean ± SD for n=5.

Effect of oral administration of *D. malabarica* extract on sub-acute body weight

The repeated dose toxicity tests provide information on toxic effects, identification of target organs, effects on animal physiology, haematology, the biochemical profile, and histopathology. These tests are required by regulatory agencies to characterize the toxicological potential of any substance. *D. malabarica* did not cause any evident sub-acute toxicity at any of the doses used, nor toxicity or death in any of the treated mice. Mice treated sub-acutely with repeated oral treatments of the *D. malabarica* (500, 750 or 1000 mg/kg) showed no significant changes in urinary volume or food and water consumption. Normal and treated mice showed no symptoms of toxicity at the end of the study and throughout the 28 days. According to the results shown, when compared to the control group, the body weights of mice in the treatment with *D. malabarica* with dosages up to 1000 mg/kg did not change substantially ($p > 0.05$) during the investigation period (Figure 4). However, the body weight gains of control and mice treated with 500, were similar (3%), whereas 750, and 1000 mg/kg were all higher than the control. About 5% ($P>0.05$) increase in b.w. was observed during repeated dose administration for 28 days. In this study, during subacute exposure, all animals were active and responded positively to stimuli. No deaths and no clinical signs of local or systemic toxic effects were observed. The behaviour of the animals was recorded daily (general health and clinical signs of toxicity), and no changes were found [14]. The behaviour of all animals in all groups tested was framed as normal for the species.

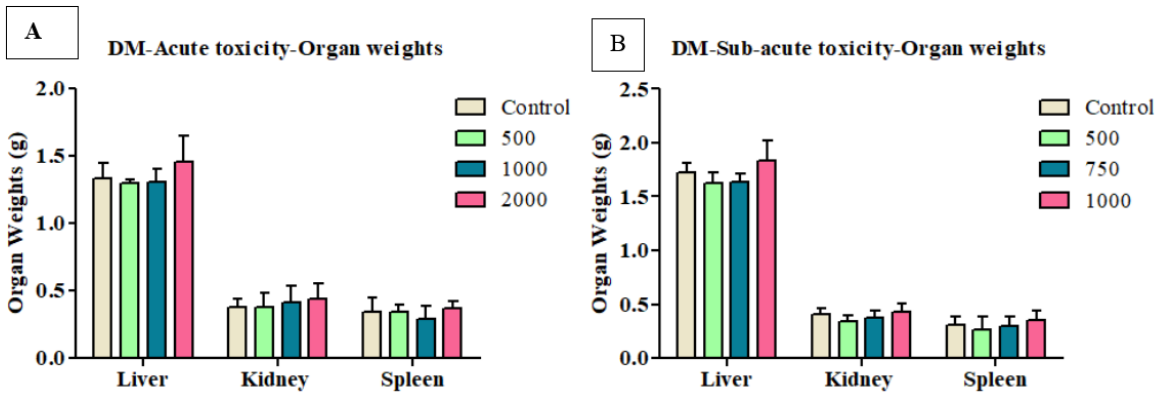


Figure 5: Organ weights (kidney, spleen and liver in grams) of the mice in the acute and sub-acute toxicity study of the methanol extract of *D. malabarica*. Results are represented as the mean ± SD for n=5.

Effect of oral administration of *D. malabarica* extract on acute organ weights

Changes in body and organ weights are a clear indication of damage caused by the ingestion of a toxic substance; the latter being considered the most sensitive indicator of any toxic effect. In our study, there were no significant ($p > 0.05$) variations in the relative weights of the liver, kidney, and spleen of the mice among the different groups compared with the control. Moreover, there was no noticeable difference in mean relative organ weights in mice. No significant difference was found in relative or absolute mouse organ weights (Figure 5A). In general, an increase or decrease in the body weight of an animal has been used as an indicator of an adverse effect of drugs and chemicals [15]. Moreover, the relative organ weight indicates whether the organ has been exposed to injury or otherwise. Impaired organs often have abnormal atrophy [16]. In the present study, the body weight and the relative organ weights of all treated mice did not differ significantly ($P > 0.05$) from those of the control groups. It indicates that the extract did not effect on appetite or adverse effects on the growth of the animals.

Effect of oral administration of *D. malabarica* extract on sub-acute organ weights:

The absolute weights of organs collected from normal mice and mice treated for 28 days with *D. malabarica* extracts. There was no significant difference in the absolute and relative weight of the liver, kidneys, and spleen between the control and treatment groups. Moreover, there was no significant difference in mean relative organ weight of the mouse treatment cohorts. Further, we did not observe perceptible changes in mice cohorts in terms of organ weight and organ morphology following sacrifice (Figure 5B).

The liver and kidneys are vital organs in the body; one is responsible for digestion and waste elimination, whereas the other is responsible for waste elimination alone. It is necessary to know the state of the liver and kidneys in order to assess the toxicity of any new drug, which can be validated through biochemical estimation. The weight, morphological appearance and histology of two organs in correlation with haematological data are routinely utilised as clinical biochemical markers of liver disease (Figure 5-7).

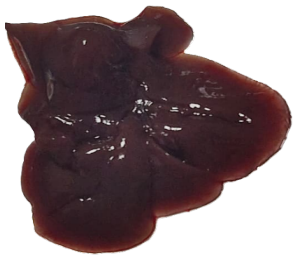
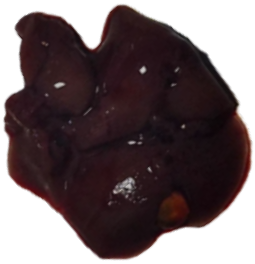
	Spleen	Liver	Kidney
Control			
DM 2000 mg/KG			

Figure 6: Representative morphology of different organs of treated and control mice in acute toxicity studies

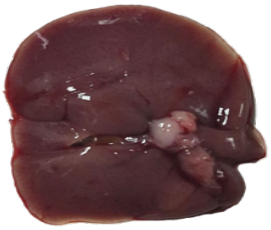
	Spleen	Liver	Kidney
Control			
DM 1000 mg/KG			

Figure 7: Representative morphology of different organs of treated and control mice in sub-acute toxicity studies.

Effect of oral administration of *D. malabarica* extract on haematological indices

Haematological parameters such as WBC, haemoglobin, granulocytes, lymphocytes, monocytes, RBC, platelets, MCV (mean corpuscular volume), and MCH (mean corpuscular haemoglobin) in mice treated with 500, 1000, and 2000 mg/kg body weight of *D. malabarica* were compared with the control. There were no statistically significant ($p > 0.05$) differences in haematological parameters among the different groups. However, granulocyte (4.5 ± 1.56) concentration was significantly decreased in mice treated with 1000 mg/kg body weight of *D. malabarica.* Platelet count was significantly lower in mice treated with 500 mg/kg body weight of *D. malabarica* compared to the controls (Table 1).

Analysis of blood parameters in animal toxicity studies is important for reporting alterations and assessing the relative risk to the hematopoietic system when extrapolating those findings to humans. In the present investigation, haematological values observed in the treated groups were normal compared to the control group. However, some values were significantly different from those of the control group, such as granulocyte and platelet counts. Moreover, the observed reductions in platelet counts may suggest a decline in immune system function. Therefore, these results indicate that the extract has a tendency to cause anaemia and immunological defects in mice. Nevertheless, this trend was not seen in all tested doses and was inconsistent at the highest dose tested, warranting further investigation. Furthermore, no significant differences were observed in mice treated with *D. malabarica* extract at various doses.

Sl No	Parameters	Control	500 mg/kg	1000 mg/kg	2000 mg/kg
1	WBCs (10^3/µl)	9.2 ± 0.85	6.3 ± 0.65	9.9 ± 1.24	9.1 ± 2.5
2	Haemoglobin (g/dl)	7.4 ± 1.24	13.7 ± 2.4	12.5 ± 1.86	9.1 ± 1.64
3	Granulocyte (10^3/µl)	9.6 ± 0.60	8.2 ± 0.76	4.5 ± 1.56	8.8 ± 1.34
4	Lymphocyte (10^3/µl)	71.7 ± 3.65	67.6 ± 0.45	64.8 ± 1.33	82.2 ± 6.1
5	Monocyte (10^3/µl)	8.7 ± 0.60	7.2 ± 1.86	8.5 ± 1.96	6.6 ± 5.5
6	RBC (10^6/µl)	8.42 ± 1.22	8.34 ± 1.54	9.49 ± 0.44	6.12 ± 0.63
7	Platelets (10^3/µl)	574 ± 32	580 ± 35	599 ± 66	456 ± 36
8	MCV (fL)	67.9 ± 0.91	49.2 ± 2.6	49.5 ± 1.6	52.8 ± 2.5
9	MCH (pg)	21.8 ± 1.85	16.5 ± 2.2	16.3 ± 2.09	14.9 ± 2.3

Table 1: Effect of acute oral administration of *D. malabarica* methanol extract on the haematological parameters of mice.

Parameters	Control	500	750	1000
WBCs (10^3/µl)	7.1±0.85	6.3±0.65	8.4±1.24	9.1±2.5
Haemoglobin (g/dl)	16.1±1.24	14.3±2.4	15.5±1.86	16±1.64
Granulocyte (10^3/µl)	13±0.60	12±0.76	15±1.56	18±1.34
Lymphocyte (10^3/µl)	4.0±3.65	6.0±0.45	6.1±1.33	6.9±6.1
Monocyte (10^3/µl)	0.8±0.60	0.7±1.86	0.9±1.96	1.2±5.5
RBC (10^6/µl)	10.4±1.22	9.3±1.54	9.49±0.44	10.2±0.63
Platelets (10^3/µl)	650±32	708±35	723±66	700±36
MCV (fL)	49.2±0.91	45.7±2.6	49.7±1.6	52.8±2.5
MCH (pg)	15.3±1.85	15.6±2.2	16.3±2.09	16.9±2.3

Table 2: Effect of sub-acute oral administration of *D. malabarica* methanol extract on the haematological parameters of mice.

Effect of sub-acute oral administration of *D. malabarica* extract on haematological indices

Haematological parameters such as WBC, haemoglobin, granulocytes, lymphocytes, monocytes, RBC, platelets, MCV, and MCH in 500, 750 and 1000 mg/kg body weight of *D. malabarica* treated mice are compared with the control in Table 2. The results demonstrated that the counts of granulocytes were significantly lower in *D. malabarica* treatment mice compared to controls ($p < 0.05$). On the contrary, the counts of lymphocytes and platelets were significantly higher in all the extract treatment groups compared to the control. Based on the haematology reports, it was feasible to predict that the given doses of extracts could cause toxicity to essential organs in mice. However, since the results are inconsistent and the variations in haematological parameters were not dose dependent, it could be due to disturbances in the kidneys and liver.

Effect of sub-acute oral administration of *D. malabarica* extract on biochemical parameters

The serum biochemical parameters showed no significant differences between the treatment groups and the control animals, except for glucose levels, which were 94 to 125 in the control group and ranged from 124 to 190 in the acute and subacute toxicity groups, respectively, indicating a mild rise compared to the control group. However, the values remained within the range observed in Balb/c mice, as directed by CCSEA rules [17-19]. No significant differences in the serum parameters between the DM treated and control animals were observed in both acute and sub-acute toxicity studies.

Effect of acute and sub-acute oral administration of *D. malabarica* extract on histopathology

Histological qualitative analysis of the mice liver and kidney tissues was carried out to ascertain any aberration as a result of a single or multiple dose exposure to *D. malabarica* stem bark methanol extract after 14 days. Figure 8 and 9 shows photomicrographs of some of the main organs analysed histologically, which are usually associated with the toxicity of food and chemical consumption. The sections of kidney, liver and spleen stained with haematoxylin and eosin revealed normal morphology without inflammatory cell infiltration. The liver appeared normal with preserved hepatic architecture. In mice, signs of degeneration (lesions) and centrilobular necrosis were not observed, even at the highest dose tested, indicating the non-toxic nature of the extract to the liver. This may also indicate that the phytochemical constituent of the *D. malabarica* extract might get detoxified by the liver normally. The kidney morphology for all the mice in the control group remained intact. Further, photomicrographs of the kidneys of mice treated with various doses of *D. malabarica* extract showed no necrosis or inflammation of the renal interstitium. The histological structure of the spleen in all treated mice was comparable to the normal pattern of the organ in controls. No changes within macrophages in the red pulp with abnormal structural architecture were found.

The liver and the kidneys are target organs for toxic chemicals due to their essential functions in bodily detoxification and excretion processes. Thus, they are considered highly useful in toxicity studies because of their sensitivity to harmful compounds and their potential to predict toxicity. Toxicity-related changes in the weights of these vital organs are often accompanied by corresponding histopathological findings.

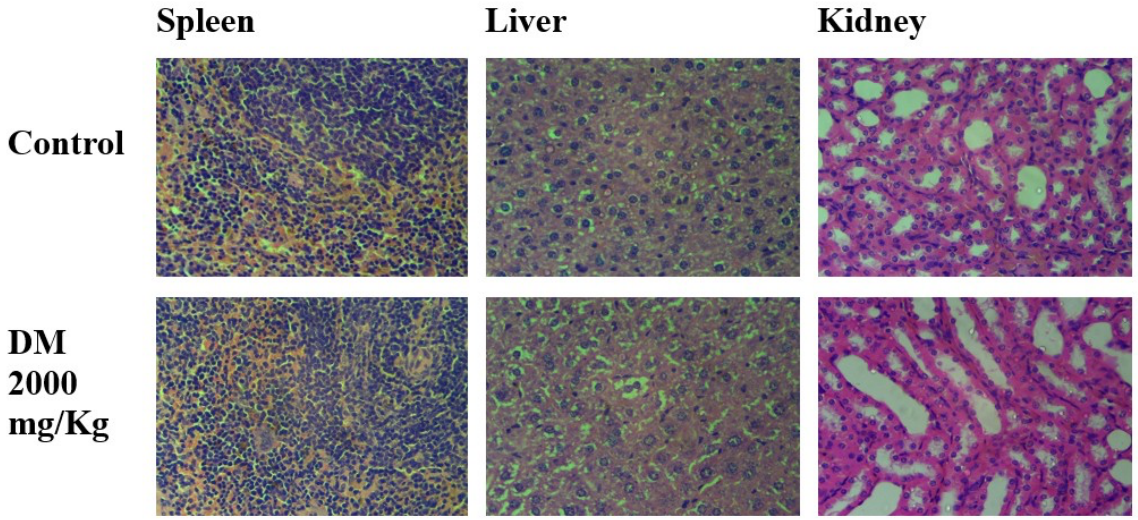


Figure 8: Representative histopathology of different organs of treated and control mice among the acute toxicity studies.

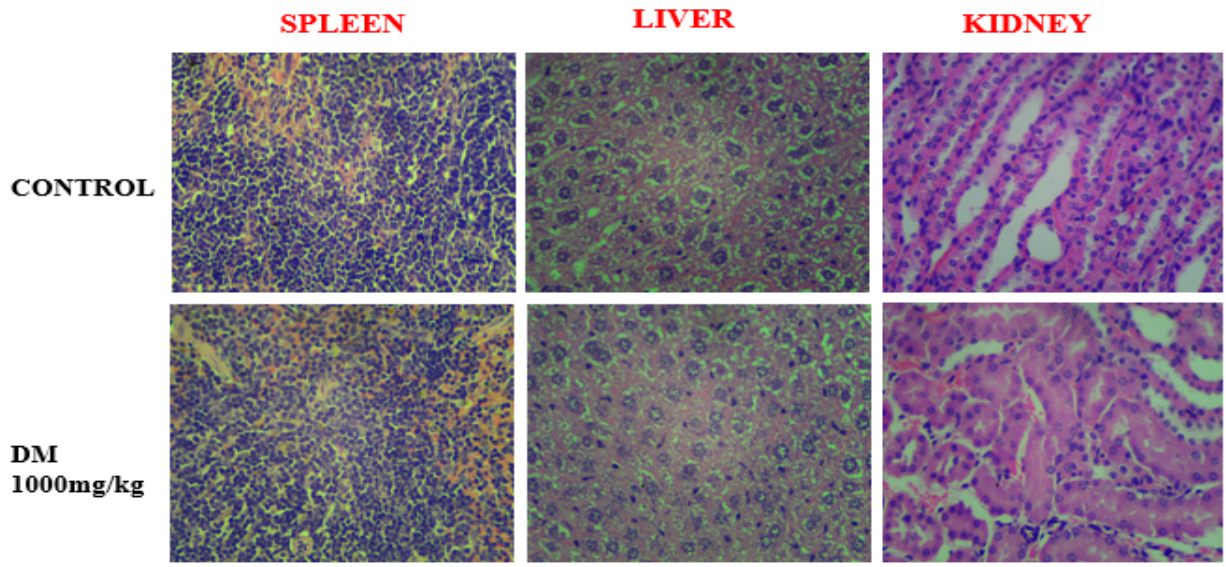


Figure 9: Representative histopathology of different organs of treated and control mice among the sub-acute toxicity studies.

Sl. no.	Parameters	Control	500 mg/kg	DM-1000 mg/kg	DM-2000 mg/kg
1	Alkaline Phosphatase	16.88±0.00	11.69±0.79	15.19±0.79	18.87±3.175
2	Bilirubin	4.94±0.001	4.70±0.001	6.44±0.002	7.54±0.001
3	Cholesterol	124.73±0.00	149.46±0.00	144.09±0.002	134.45±0.001
4	Glucose (503nm)	94.16±0.001	123.04±0.001	132.48±0.001	137.04±0.001
5	LDH	671.20	639.06	770.30	620.23
6	SGOT	64.13±1.51	78.09±5.54	86.68±3.02	93.12±3.02
7	SGPT	24.43±2.01	24.40±1.51	35.24±0.00	27.25±2.52
8	Total Protein	5.2±0.001	7.2±0.001	6.1±0.001	6.9±0.001

Table 3: Effect of acute oral administration of *D. malabarica* methanol extract on the serum biochemical parameters of mice.

Parameter	Control U/L	DM (500 mg/kg) U/L	DM- (750 mg/kg) U/L	DM- (1000 mg/kg) U/L
Alkaline Phosphatase	16.88±0.00	11.69±0.79	17.19±0.79	15.75±3.175
Bilirubin	4.94±0.001	4.70±0.001	4.64±0.002	5.54±0.001
Cholesterol	124.73±0.00	149.46±0.00	139.09±0.002	128.76±0.001
Glucose (505 nm)	124.16±0.001	123.04±0.001	189.68±0.001	153.04±0.001
LDH	571.20	639.06	650.30	600.23
SGOT	64.13±1.51	78.09±5.54	98.73±3.02	88.12±3.02
SGPT	24.43±2.01	24.40±1.51	34.09±0.00	22.25±2.52
Total Protein	5.6±0.001	5.9±0.001	6.1±0.001	5.4±0.001

Table 4: Effect of sub-acute oral administration of *D. malabarica* methanol extract on the serum biochemical parameters of mice.

Conclusion

Neither mortality nor any serious clinical manifestations were seen in the mice after a single oral dose or repeated administrations of the extract. Haematological parameters were also normal, with intact histology of the liver and kidneys. It is therefore safe to conclude that the extracts are relatively safe to use. Therefore, it is recommended that the *D. malabarica* stem bark methanol extract at the given doses does not produce any significant toxic effect and is safe to be considered for use in animal studies and as a constituent of medicinal and food preparations.

This study provides valuable data on the sub-acute effects of *D. malabarica* extract in mice. The doses of 500, 750 and 1000 mg/kg body weight did not cause any mortality or toxic effects in the sub-acute toxicity study on mice. No significant clinical, haematological, or histopathological changes were observed in treated animals versus controls in a 28-day oral sub-acute toxicity study. Finally, it is critical to understand that medicinal plants should be studied and evaluated for toxicity and safety. These findings provide valuable preliminary information on the toxicological profile of *D. malabarica*. As a result, more testing (such as genotoxicity, subchronic toxicity, reproductive toxicity, and component toxicity) is needed before moving forward with clinical trials of this plant.

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