IJPSR (2024), Volume 15, Issue 1 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 15 May 2023; received in revised form, 17 August 2023; accepted, 21 November 2023; published 01 January 2024

IN-VIVO TOXICITY PROFILE OF METHANOLIC EXTRACT OF *TERMINALIA ARJUNA* (ROXB. EXDC) BARK IN MALE ALBINO WISTAR RATS

P. Roy Chowdhury, S. Sarkar and D. Choudhuri *

Department of Human Physiology, Tripura University (a Central University), Suryamaninagar, Agartala - 799022, Tripura, India.

Keywords:

Terminalia arjuna, Acute toxicity, Sub-acute toxicity, LD_{50}

Correspondence to Author:
Dipayan Choudhuri

Professor,
Department of Human Physiology, Tripura University (a Central University), Suryamaninagar, Agartala - 799022, Tripura, India.

E-mail: dipayanchoudhuri@tripurauniv.ac.in

ABSTRACT: The bio-active compounds present in *Terminalia arjuna* (Roxb. ExDC) have anti-oxidant, anti-proliferative, anti-obesity, anti-hyperglycemic, and lipid-lowering activity. In addition, it has cardioprotective, hepatoprotective, and renoprotective properties. The study evaluated *in-vivo* toxicity profile of the methanolic extract of *Terminalia arjuna* (META) bark on male albino Wistar rats before undertaking further investigation on the use of this extract as a potential therapeutic agent against such conditions. Animals were administered with 300 and 2000 mg/Kg b.w./day of META bark for 14 days; and 250, 500, and 1000 mg/Kg b.w./day of META bark for 28 days for acute and sub-acute toxicity respectively. The outcome of the acute toxicity study revealed no signs of mortality or general behavioral alteration in treatment groups in comparison to the control group. The sub-acute toxicity study revealed, there is no significant influence ($p<0.05$) of META bark in food consumption and percentage weight gain in the animals. No significant changes ($p<0.05$) in hematological parameters as well as in biochemical parameters were observed. Histopathological examination showed normal cellular architecture of vital organs in treatment groups as compared to the control group. These results indicated the short-term and long-term administration of META bark did not cause any toxicity in male albino Wistar rats.

INTRODUCTION: Nature has bestowed us with a huge wealth of medicinal plants to ensure good health. Herb and herbal elements are one of the precious gifts of nature to mankind [1]. In India, the practice of plant-based herbal medicine dates back at least 2600 BC [2]. In India, the legacy of using herbal medicine is passed from one generation to another which can be seen in the Ayurveda, Siddha, and Unani methods of treatment [3].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.15(1).96-04

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.15(1).96-04

It is well documented that, in India, about 7500 plants are used in the practice of traditional folk medicine; especially in tribal folk medicine [4]. Herbal medicine is comparatively safer and pocket friendly than modern synthetic drugs. Therefore, in recent years, it has been seen that the majority of people are choosing the use of plant-based herbal medicine over modern synthetic drugs [5].

Terminalia arjuna (Roxb ExDC) is widely used as traditional herbal medicine to ameliorate acute and chronic health issues. This deciduous and evergreen plant belongs to the family Combretaceae and is easily available throughout India [6]. The plant parts contain several bioactive chemical components. From the medicinal point of view, the most essential part of the plant is the stem

bark since; it is a rich source of flavonoids, glycosides, polyphenols, tannins, triterpenoids, Saponins, and sterols. The bark of the plant also contains potent bioactive components arjunoic acid, arjunic acid, arjungenin, which have several pharmacological activities [1, 6]. Previous study reports suggest bioactive compound present in the plant has anti-oxidant, anti-proliferative, anti-obesity, anti-hyperglycemic, and lipid-lowering activity as well as cardioprotective, hepatoprotective, renoprotective property [7]. Despite the rising number of reports on the medicinal benefits of the *Terminalia arjuna* bark, there is very limited information available about the in vivo toxicological effect of the plant extract. Therefore, the present study is aimed to investigate the acute and sub-acute toxicity of META bark in male albino rats of the Wistar strain. The outcome of the present study would serve as a very important baseline to select a safe and suitable dosage of this plant extract in animals.

MATERIALS AND METHODS:

Test Reagents: Acetic anhydride, sulfuric acid, ferric chloride, lead acetate, Dragendroff's reagent, Molich reagent, ninhydrin, sodium nitroprusside, pyridine, NaOH, glacial acetic acid were procured from Himedia Laboratory Pvt. Ltd., India.

Collection and Identification of Plant Materials: The bark of *Terminalia arjuna* (Roxb. ex DC) was collected from Ramnagar, Agartala, west Tripura and it was identified by the taxonomist (Prof. Badal Kumar Datta) of the Botany Department at the Tripura University, Suryamaninagar. The voucher specimen (4439) was deposited in the Department of Botany, Tripura University.

Preparation of META Bark: The plant sample was thoroughly washed with distilled water, and dried in a hot air oven at the temperature of 40^{O}C. Then the bark was ground to a fine powder and stored in an airtight container. 1000 gm of *Terminalia arjuna* bark was dissolved in 1500 ml of methanol in a clean glass container and kept undisturbed for 72 hours. Then the solvent was filtered with the help of Whatmans' No. 1 filter paper. Then the mother liquor was concentrated under the reduced pressure of the rotary evaporator at 40^{O}C temperature and then the crud extract was stored at 4^{O}C for phytochemical analysis and toxicological study. The extraction yield in percentage was calculated by the formula [(W1/W2) X 100] given by [8]. Where W1 is the net weight of extracted bark powder in gm and W2 is the total weight of bark powder in gm taken for extraction.

Preliminary Bio-active Component Screening of META Bark: For the establishment of the phytochemical profile of the META bark by the detection of the presence of several bio-active compounds different qualitative tests were performed according to standard protocol [9].

Experimental Animals: The study was conducted on male albino rats of the Wistar strain weighing about 150–180 gm. Then the animals were acclimatized under control conditions in the Tripura University animal house at least one week before the onset of the study. The animals were maintained at a constant temperature (22±2°C) and a 12-hour light/dark cycle. The experiment was carried out according to the guidelines of CPCSEA and approved by Institutional Animal Ethics Committee (Ref. No. TU/IAEC/2022/I/2-5). Before the start of the experiment, the individual body weight of all the animals was recorded for aerate dosage of META bark. The animals were fed with a standard rat diet according to AIN-93G standard rat diet composition and water *ad libitum*. Throughout the experiment, individual food and water consumption was recorded daily and individual body weight was recorded weekly. The percentage change in body weight of the animals was calculated as per the body weight before the administration of the test drug and weekly throughout the period experiment by the formula-

Change of body weight (%) = Final body weight – Initial body weight / Initial body weight × 100

Acute Toxicity Test (Determination of LD_{50}): Animals were divided into three groups, one served as the control group and the rest of the two served as treatment (Treatment-I and Treatment-II) group. After overnight fasting, Animals in the control group were administered with vehicle (distilled water) for relative analysis as per the OECD guideline 425 [10]. The Treatment-I group orally received the 1st test dose at 300 mg/Kg b.w. of META bark for 14 days. 48 hours after the 1st dose, the Treatment-II group orally received the 2nd test

dose at 2000 mg/Kg b.w. of META bark for 14 days. All the animals were carefully monitored throughout the experiment to check the development of any clinical or toxicological signs and symptoms mentioned in the OECD guidelines.

Sub-acute Toxicity Test: Animals were divided into four groups, one served as the control group and the rest of the three served as the treatment (Treatment-I, Treatment-II, and Treatment-III) group. After overnight fasting, animals in the control group were administered with vehicle (distilled water) for relative analysis as per the OECD guideline 407 [11]. The treatment-I, II, and III received 250, 500, and 1000 mg/Kg b.w. of META bark for 28 days.

Animal Sacrifice, Blood, and Tissue Collection: All precautions were taken during the entire period of study, and as per the ICMR guidelines, all the animals of the sub-acute toxicity study were sacrificed at the end of 28 days for cervical dislocation following ether anesthesia [12]. The animals were sacrificed by decapitation and fresh blood was collected immediately by cardiac puncture. After the collection of blood samples in EDTA-coated and plain vacutainers.

Blood in the plain vacutainer was left to coagulate at 4˚C and then it was centrifuged at 3000 rpm for 15 minutes to collect serum. The liver, Kidney, heart, spleen, and testis were extracted from each animal and weighed to avoid weight loss due to evaporation and will be frozen at -80°C for further analysis. The relative weight of the organ (ROW) of each animal was estimated by using the formula-

Relative weight of organ = Absolute organ weight (gm) / Final body weight of the animal (gm) × 100

Preparation of Liver Tissue Homogenate for Analysis of Tissue Biochemical Parameters: Liver tissue homogenate was prepared by following the standard protocol [13].

Analysis of Hematological Parameters: Hematological parameters like total white blood cell (WBC) [1], total red blood cell (RBC) 1, and total platelet count [1] were measured following the standard method by using a Neubauer hemocytometer. The hemoglobin concentration (Hb) [1] was estimated by using Sahli's hemoglobinometer. The pack cell volume (PCV) [1], mean cell volume (MCV) [1], mean cell hemoglobin (MCH) [1] and mean corpuscular hemoglobin concentration (MCHC) [1] were also measured. Blood clotting time was determined with the help of a capillary tube by following the Wright method [1].

Analysis of Biochemical Parameters: Serum protein [14], serum albumin [14], serum glutamic oxaloacetic transaminase (SGOT) [15], serum glutamic pyruvic transaminase (SGPT) [15], serum alkaline phosphatase (ALP) activity [15], acid phosphatase (ACP) activity [15], urea [16] and creatinine [16] were determined.

In addition, tissue biochemical parameters such as tissue alkaline phosphatase (ALP) activity [15] and Tissue acid phosphatase (ACP) activity [15] were determined.

Analysis of Histopathological Parameters: To perform histopathological analysis for sub-acute toxicity all the vital organs were fixed in 10% buffer formalin solution followed by tissue processing with graded alcohol and xylene.

The processed tissues were embedded in paraffin wax and the tissue sections were sliced to a thickness of 5µm by using a rotating microtome (Leica). The tissue sections were stained with hematoxylin and eosin (H &E) stain and then analyzed under the lance of the microscope (Leica DM400 BLED) at 40X magnification.

Statistical Analysis: All the experimental test results were presented as mean ± standard error of the mean (SEM). The difference between each group was analyzed by using one-way ANOVA followed by Tukey HSD Posthoc test with the help of Statistical Program for the Social Sciences (SPSS) 16.0 for Windows. The level of statistical significance was considered at $p<0.05$.

RESULTS:

Extraction Yield in Percentage: Approximately 3.7% META was obtained from bark powder.

Preliminary Bio-active Component Screening of META Bark: Preliminary bio-active component screening of the plant extract revealed the presence of several bio-active components **Table 1**.

TABLE 1: PRELIMINARY BIO-ACTIVE COMPONENT SCREENING OF META BARK

Bio-active Components	Test	Result
Triterpenoids	Liebermann Burchard test	Present**
Phenolics and tannins	Ferric chloride test	Present**
Flavonoids	Lead acetate test	Present**
phytosterols	Salkowaski reactive test	Present**
Saponins	Foam test	Present**
Alkaloids	Dragendroff,s test	Present**
Carbohydrates	Molish,s test	Present*
Proteins	Ninhydrin test	Present*
Lactones	Legal's test	Present**
Glycosides	Keller-Killiani	Present**

Effect of META Bark on General Sign and Behavior: Oral gavages of META bark at a dose of 300 and 2000 mg/Kg b.w. did not show any signs of mortality or morbidity **Table 2**.

TABLE 2: EFFECT OF META BARK ON GENERAL SIGN AND BEHAVIOR

Observation	Control		Treatment I (300mg/Kg b.w.)		Treatment II (2000mg/Kg b.w.)	
	4hrs	24hrs	4hrs	24hrs	4hrs	24hrs
Fur	No alteration	No alteration	No alteration	No alteration	No alteration	No alteration
Skin	No alteration	No alteration	No alteration	No alteration	No alteration	No alteration
Eyes	No alteration	No alteration	No alteration	No alteration	No alteration	No alteration
Behavioral Pattern	Normal	Normal	Normal	Normal	Normal	Normal
Sleep	Normal	Normal	Normal	Normal	Normal	Normal
Salivation	Normal	Normal	Normal	Normal	Normal	Normal
Lethargy	Not Observed	Not Observed	Not Observed	Not Observed	Not Observed	Not Observed
Diarrhea	Not Observed	Not Observed	Not Observed	Not Observed	Not Observed	Not Observed

Effect of META Bark on Food Consumption and Percentage Weight Gain: During the acute (300, and 2000 mg/Kg b.w.) and sub-acute (250, 500, and 1000 mg/Kg b.w.) toxicity experiments, the pattern of percentage body weight gain showed a uniform increase in all the animals. No significant ($p<0.05$) changes were found in daily food consumption and as well as percentage body weight gain in animals of each treatment group compared to animals in the control group **Fig. 1**.

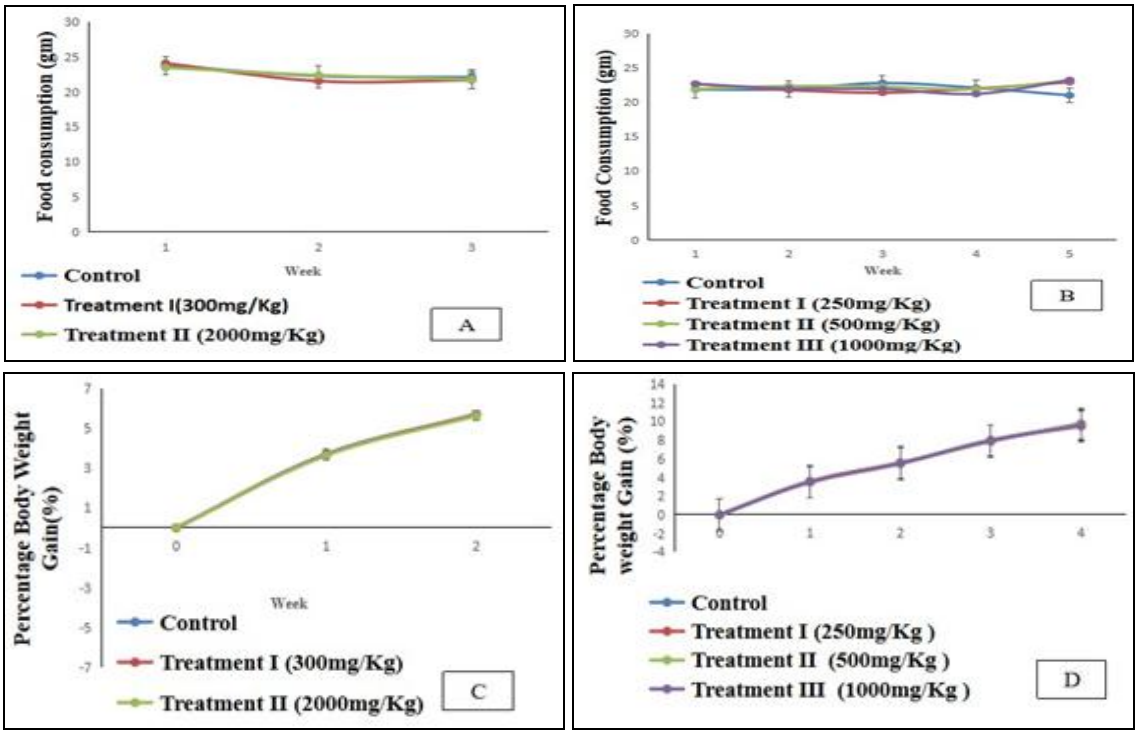

FIG. 1: EFFECT OF META BARK ON DAILY FOOD CONSUMPTION AND PERCENTAGE OF BODY WEIGHT GAIN

Effect of META bark on (A) daily food consumption (acute toxicity) of Control, Treatment I (300 mg/Kg b.w.), Treatment II (2000 mg/Kg b.w.), (B) daily food consumption (Sub-acute toxicity) of Control, Treatment I (250 mg/Kg b.w.), Treatment II (500 mg/Kg b.w.), and Treatment III (1000 mg/Kg b.w.). (C) percentage body weight gain (acute toxicity) of Control, Treatment I (300 mg/Kg b.w.), Treatment II (2000 mg/Kg b.w.), (D) percentage body weight gain (Sub-acute toxicity) of Control, Treatment I (250 mg/Kg b.w.), Treatment II (500 mg/Kg b.w.), and Treatment III (1000 mg/Kg b.w.). Values represented as Mean±SEM of 6 animals in each group at the significance level $p<0.05$.

Effect of META Bark on Relative Organ Weight of Vital Organ of each Animal: Administration of META bark (250, 500, and 1000 mg/Kg b.w.) neither exhibits significant ($p<0.05$) changes in the serum biochemical parameters (**Table 5**); nor tissue biochemical parameters (**Fig. 2**) of the animals in different treatment groups as compared to the animals in the control group.

TABLE 3: EFFECT OF META BARK ON RELATIVE ORGAN WEIGHT OF VITAL ORGANS OF EACH ANIMAL (GM/100GM B.W.)

Animals (6)	Weight of Liver	Weight of Kidney	Weight of Heart	Weight of Spleen	Weight of Testis
Control	3.12±0.02	0.61±0.01	0.74±0.01	0.51±0.02	1.26±0.02
Treatment- I	3.10±0.02	0.59±0.02	0.73±0.01	0.52±0.01	1.24±0.02
Treatment- II	3.10±0.02	0.58±0.02	0.74±0.01	0.50±0.01	1.25±0.02
Treatment- III	3.10±0.02	0.56±0.02	0.72±0.01	0.51±0.02	1.24±0.04

Values represented as Mean±SEM of 6 animals in each group at the significance level $p<0.05$.

Effect of META Bark on Hematological Parameters: Administration of META bark (250, 500, and 1000 mg/Kg b.w.) exhibits no significant ($p<0.05$) changes in the hematological parameters of animals in each dosage group as compared to animals in the control group **Table 4**.

TABLE 4: EFFECT OF META BARK ON HEMATOLOGICAL PARAMETERS

Parameters	Control	Treatment I	Treatment II	Treatment III
WBC count ($10^3\mu$)	4.07±0.31	3.95±0.22	3.91±0.32	3.96±0.29
RBC count ($10^3\mu$)	7.45±0.03	7.60±0.05	7.41±0.04	7.71±0.07
Platelet count ($10^3\mu l$)	1001.80±0.36	1001.97±0.26	1002.26±0.32	1002.39±0.26
Hb count(gm/dl)	13.69±0.36	13.80±0.44	13.71±0.27	13.88±0.44
PCV (%)	41.95±0.55	42.24±1.30	41.98±1.07	42.23±1.57
MCV(fl)	55.42±0.61	56.44±1.50	55.25±1.78	56.67±1.75
MCH(pg)	18.44±0.20	18.15±0.98	18.50±0.59	18.67±0.60
MCHC(gm/dl)	32.63±0.38	32.67±0.87	33.65±0.27	33.86±0.24
Clotting Time (min)	3.64±0.01	3.64±0.01	3.64±0.01	3.64±0.01

Values represented as Mean±SEM of 6 animals in each group at the significance level $p<0.05$.

Effect of META Bark on Biochemical Parameters: Administration of META bark (250, 500, and 1000 mg/Kg b.w.) neither exhibits significant ($p<0.05$) changes in the serum biochemical parameters **Table 5**; nor tissue biochemical parameters **Fig. 2** of the animals in different treatment groups as compared to the animals in the control group.

TABLE 5: EFFECT OF META BARK ON SERUM BIOCHEMICAL PARAMETERS

Parameters	Control	Treatment I	Treatment II	Treatment III
Protein (g/dl)	7.10±0.25	7.27±0.39	7.55±0.40	7.41±0.40
Albumin (mg/dl)	4.43±0.27	4.49±0.42	4.95±0.33	4.53±0.49
SGOT (IU/L)	9.84±0.33	9.56±0.65	9.39±0.65	9.33±0.1
SGPT (IU/L)	11.40±0.45	10.98±0.51	10.52±0.51	10.47±0.37
ALP (IU/L)	46.31±0.72	45.27±0.72	44.80±0.93	44.77±0.91
ACP (IU/L)	6.08±0.11	5.96±0.09	5.78±0.17	5.79±020
Urea (mg/dl)	6.14±0.17	5.66±0.30	5.49±0.27	5.51±0.25
Creatinine (mg/dl)	0.46±0.01	0.44±0.01	0.43±0.01	0.45±0.01

Values represented as Mean±SEM of 6 animals in each group at the significance level $p<0.05$.

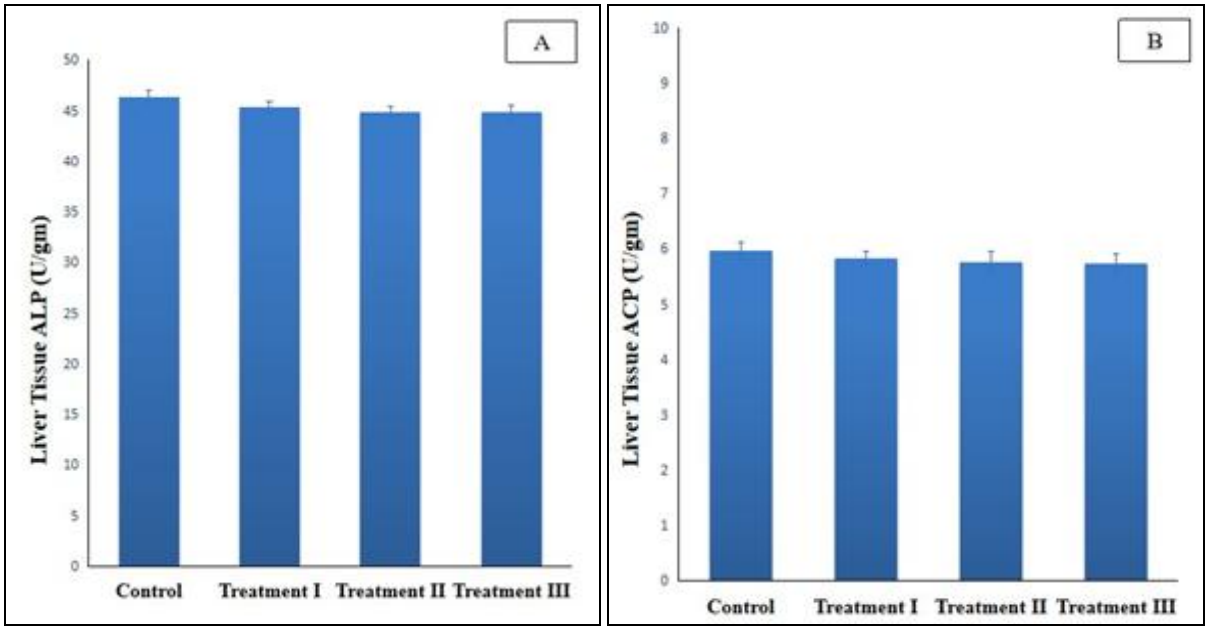

FIG. 2: EFFECT OF META BARK ON LIVER TISSUE ALP AND ACP

(A) Effect of META bark on liver tissue ALP of Control, Treatment I (250 mg/Kg b.w.), Treatment II (500 mg/Kg b.w.), and Treatment III (1000 mg/Kg b.w.). (B) Effect of TA bark extract on liver tissue ACP of Control, Treatment I (250 mg/Kg b.w.), Treatment II (500 mg/Kg b.w.), and Treatment III (1000 mg/Kg b.w.). Values represented as Mean±SEM of 6 animals in each group at the significance level $p<0.05$.

Effect of META Bark on Histopathological Parameters: The microscopic analysis of vital organs showed normal histological architecture of the liver, kidney, heart, spleen, and testis in Treatment I (250mg/Kg b.w.), Treatment II (500 mg/Kg b.w.) and Treatment III (1000 mg/Kg b.w) as compared to the control group **Fig. 3**. Microscopic images were viewed under 40X magnification.

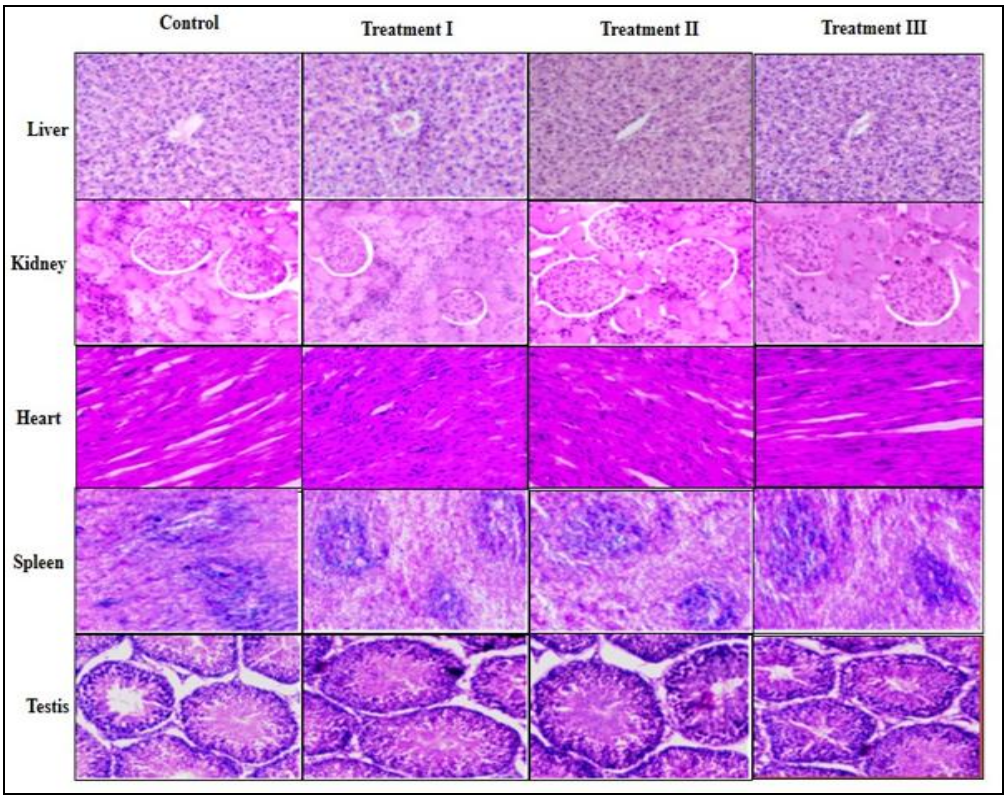

FIG. 3: EFFECT OF META BARK ON THE HISTOPATHOLOGICAL ARCHITECTURE OF VITAL ORGANS

DISCUSSION: *Terminalia arjuna* is an herbal medicinal plant that is well known to cure several acute and chronic diseases. In this present study, the preliminary bioactive component screening revealed that the plant extract contains triterpenoids, Saponins, flavonoids, phenolics, alkaloids tannins, phytosterols, lactones, and glycosides in high concentrations while relatively low concentrations of carbohydrate and protein. Similar kinds of results have also been observed in some previous studies. They have reported the presence of phytosterol, triterpenoids, Saponins, flavonoids, lactones, phenolics, and glycosides in a higher concentration as compared to the presence of alkaloids, protein, and carbohydrates [17].

In the first phase of the experiment i.e., acute toxicity study, animals were treated with 300, and 2000mg/Kg b.w. of META bark for 14 days [10].Both doses showed no mortality. Observation of physical and clinical parameters plays a vital role in the identification of toxicity-related symptoms. Animals in all the groups showed no major changes in the physical as well as clinical parameters. Therefore, the present study suggests that the LD_{50} value of META bark should be greater than 2000 mg/Kg b.w. throughout the experimental period, no significant ($p<0.05$) variation in food consumption, as well as no significant ($p<0.05$) alteration in percentage body weight, were found in each treatment group as compared to the control group.

In the second phase of the experiment i.e., sub-acute toxicity study, animals were treated with 250, 500, and 1000 mg/Kg b.w. of META bark respectively for 28 days [11]. Evaluation of food consumption after administration of an herbal drug is an important parameter to assess the toxicity effect of a drug. Reduction in normal food consumption and water intake indicates a disturbance in the normal metabolism of carbohydrates, protein, and fat [18]. Interestingly, throughout the experimental period, no significant ($p<0.05$) variation in food and water consumption was found when compared to the animals in the control group in the present study. The unaltered food and water consumption anticipate administration of META bark did not possess any adverse effect on metabolic processes in experimental animals. Administration of potentially toxic substances can possess a slight decrease in percentage weight gain [18]. But, in the present sub-acute toxicity study, without any significant alteration, all the animals in each dosage group continued to gain a normal body weight for the entire period of the experiment, compared to the animals in the control group. The relative weight of vital organs exhibits great importance in vivo toxicity studies. Any alteration in the vital organ-to-body weight ratio is considered a treatment-related toxic effect [19]. Interestingly, the present study displayed no significant variation in ROW in the animals in each dosage group as compared to the animals in the control group.

The continuous rise in body weight and normal organ weight anticipate administration of META bark did not possess any adverse effect on the animal's normal growth and development. Blood is the major transportation medium for nutrients and drugs in the living system. Since blood cells and different protein present in the blood primarily encounter any toxicants, blood cells in living beings are critically sensitive to exposure to toxic substances [20]. Interestingly, the present study showed no significant ($p<0.05$) variation in the production and development of hematopoietic cells in animals of each dosage group treated with META bark as compared to animals in the control group. Therefore, the present study revealed that META bark has a normal role in the process of hematopoiesis. Some recent study with META bark also supports our experimental findings [1].

Plant extract contains multiple phytochemical compounds, some of which may start to react with proteins and enzymes present in the liver and kidney. For that reason, it becomes very important to investigate liver and kidney functions [15, 16]. Decreased levels of total protein and albumin are an indication of toxicity [14]. In our study, no significant ($p<0.05$) changes were observed in the level of total protein and albumin of animals in each dosage group treated with META bark as compared to animals in the control group. Furthermore, our observation is supported by some previous scientific research data [1]. SGOT, SGPT, and ALP are the major enzymes produced in the liver. Elevated serum SGOT, SGPT, ALP and ACP; tissue ALP and ACP indicate the development of hepatic toxicity.

In the present study, no significant ($p<0.05$) changes were observed in the level of serum SGOT, SGPT, ALP, and ACP as well as tissue ALP and ACP in animals in each dosage group treated with META bark as compared to animals in the control group. In toxicity studies, the estimation of serum urea and creatinine levels are great parameters to predict the physiological condition of the kidney. The elevated serum urea and creatinine indicate the development of renal toxicity [21]. Interestingly, the present study showed no significant ($p<0.05$) changes in the level of urea and creatinine in animals in each dosage group treated with META bark as compared to animals in the control group. Few previously available scientific data also support our results [16]. The outcomes of serum and tissue biochemical analysis suggest META bark has no adverse effect on the synthesis of liver and kidney biochemical components.

Histopathological analysis of vital organs with Hematoxylin and Eosin (H & E) gives a basic idea to judge the safety of any herbal drug [1]. Under microscopic analysis, the liver of META bark animals displayed a typical architecture without any signs of cellular damage when compared to the kidneys of animals in the control group. Moreover, no signs of cellular injury, accidental cell death, congestion, fatty acid deposition, or hemorrhage around the central vein and liver sinusoids were observed. Under microscopic analysis, the kidney of META bark animals showed a normal glomerular architecture without any signs of injury when compared to the liver of animals in the control group. An adequate number of healthy nephrons with clearly visible nucleoli were observed.

An adequate number of glomerulus, distal convoluted tubules (DCT) and proximal convoluted tubules (PCT) were observed. Furthermore, no signs of interstitial and intraglomerular congestion and or tubular shrinkage were observed. Under microscopic analysis, the heart of META bark animals showed a normal architecture of cardiac fibers without any myocardial distortion when compared to the spleen of animals in the control group. Under microscopic analysis, the spleen of META bark animals showed a normal architecture with an adequate amount of red pulp and white pulp surrounding a central arteriole when compared to the testis of animals in the control group. Similarly, the testis showed normal architecture of seminiferous tubules having numerous germ cells when compared to the heart of animals in the control group **Fig. 3**. These findings are in agreement with some previously available scientific study reports [1].

CONCLUSION: In this study, the administration of META bark at a dose of 300 and 2000 mg/Kg b.w. for 14 days (acute toxicity) and 250, 500, and 1000 mg/Kg b.w. for 28 days (sub-acute toxicity) neither exhibit any physical and general behavioral changes nor displayed sings of mortality in any of the animals during the entire period of study. No significant alteration in percentage body weight gain and relative organ weight was observed. Administration of META bark neither showed any significant fluctuation in biochemical and hematological parameters. The histopathological study disclosed that it did not exhibit major alterations in the architecture of the liver and kidney, heart, spleen, and testis. This present toxicological examination suggests that the META bark do not produce any toxic effect in animals even at high dose. Hence, the META bark holds great significance in drug formulation to treat many critical human diseases.

ACKNOWLEDGMENT: Department of Human Physiology, Tripura University for providing instrumental facility.

CONFLICTS OF INTEREST: The authors declare that there are no conflicts of interest.

REFERENCES:

1. Aamir K, Khan HU, Hossain CF and Afrin MR: Oral toxicity of arjunolic acid on hematological, biochemical and histopathological investigations in female Sprague Dawley rats. The Journal of Life and Environmental Sciences 2019; 8: 8045-8052.
2. Murugan P, Salim A, Chenthamara D, Robert B and Subramaniam S: Therapeutic and pharmacological efficacy of selective Indian medicinal plants - a review. Phytomedicine Plus 2021; 1: 100029-100031.
3. Laldingliani TBC, Thangjam NM, Zomuanawma, Bawitlung L, Pal A and Kumar A: Ethanomedicinal study of medicinal plants used by mizo tribes in champai district of mizoram, India. Journal of Ethanobiology and Ethanomedicine 2022; 18:22-24.
4. Shi Y, Zhang C and Li V: Traditional medicine in India. Journal of Traditional Chinese Medical Sciences 2021; 8: 51-55.

5. Versheny N, Jain D, Janmeda P and Mitra D: Role of medicinal plants in pharmaceutical sector: an overview. Global Journal of Biochemistry and Biotechnology 2021; 10: 18-24.
6. Kumar V, Sharma N, Saini R, Mall S, Zengin G, Sourirajan A, Khosla PK, Dev K and Shazly ME: Therapeutic potential and industrial applications of *Terminalia arjuna* bark. Journal of Ethnopharmacology 2023; 10: 116352-116356.
7. Rajaram V, Ambalavan N, Rajeshkumar S and Mahendra J: Antimicrobial and anti-inflammatory activity of *Terminalia arjuna*. Bioinformation 2023; 19: 184-189.
8. Dwivedi MK, Sonter S and Mishra S: Antioxidant, antibacterial activity, and phytochemical characterization of Carica papaya flowers. Beni-Suef University Journal of Basic and Applied Sciences 2022; 9: 23-25.
9. Lukubye B, Ajayi CO, Wangalwa R and Rugunda GK: Phytochemical profile and antimicrobial activity of the leaves and stem bark of *Symphonia globulifera* L.f. and *Allophylus abyssinicus* (Hochst.) Radlk. BMC Complementary Medicine and Therapies 2022; 22: 223-227.
10. OECD, test no. 425: Acute oral toxicity: up-and-down procedure, OECD guidelines for the testing of chemicals. 2022; 4:23.
11. OECD, test no. 412: Repeated Dose 28-day Oral Toxicity Study in Rodents, OECD guidelines for the testing of chemicals. 2022; 4:24.
12. Indian Council of Medical Research (ICMR). Use of animals in scientific research. 2022.
13. Das S and Choudhuri D: Effect of dietary calcium on adipogenesis program and its role in adipocyte dysfunction in male Wistar rats. Proceedings of the National Academy of Sciences India Section B- Biological Sciences 2022; 90: 631-639.
14. Mishra V and Heath RJ: Structural and biochemical features of human serum albumin essential for eukaryotic cell culture. IJMS 2021; 22: 8411-8412.
15. Das S and Choudhuri D: Calcium supplementation shows a hepatoprotective effect against high-fat ddiet by regulating oxidative-induced inflammatory response and lipogenesis activity in male rats. Journal of Traditional Complementary Medicine 2021; 10: 511-519.
16. Das S and Choudhuri D: Dietary calcium regulates the risk renal injury in high fat diet induced obese rats by regulating renal lipid metabolism, oxidative stress and inflammation. Archives of Physiology and Biochemistry 2022; 128: 1039-1049.
17. Kumar V, Chandel SR and Guleria S: Comparative analysis of phytochemicals, antimicrobial and antioxidant activity of different species of Terminalia from Himachal Pradesh, India. Vegetos 2021; 34: 528-539.
18. Reber E, Gomes F, Vasiloglou MF, Schuetz P and Stanga Z: Nutritional risk screening and assessment. Journal of Clinical Medicine 2019; 8: 1065-1071.
19. Lazic SE, Semenova E and Williams DP: Determining organ weight toxicity with Bayesian causal models: improving on the analysis of relative organ weights. Scientific Reports 2020; 10: 6625-6626.
20. Cattaneo D, Marriott DJE and Gervasoni C: Hematological toxicities associated with linezolid therapy in adults: key findings and clinical considerations. Expert Review of Clinical Pharmacology 2023; 16: 219-230.
21. Griffin BR, Faubel S and Edelstein CL: Biomarkers of drug-induced kidney toxicity. Therapeutic Drug Monitoring 2019; 41: 213-226.

How to cite this article:
Chowdhury PR, Sarkar S and Choudhuri D: "*In-vivo* toxicity profile of methanolic extract of *Terminalia arjuna* (roxb. exdc) bark in male albino Wistar rats". Int J Pharm Sci & Res 2024; 15(1): 96-04. doi: 10.13040/IJPSR.0975-8232.15(1).96-04.