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PRELIMINARY PHYTOCHEMICAL SCREENING AND TOXICITY EVALUATION OF DIFFERENT EXTRACTS OF *TERMINALIA BELLERICA*

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ABSTRACT
The present study aims to test the acute toxic effect of the fruit extract of *Terminalia bellerica* using three different solvents *viz* ethanol, acetone and benzene by examine the changes in behaviour, body weight, food intake, water intake, haematological parameters (WBC total count, WBC differential count, RBC, Hb, HCT, MCV, MCH, MCHC and platelet count) and histological changes in the vital organs such as lungs, heart, liver and kidney. No behavioural changes or any toxic symptoms and mortality was observed throughout the experimental period. There is a slight variations in the body weight, food intake and water intake of the extract treated groups compared to control group rats. The haematological parameters showed significant difference among the different extract treated rats and control rats, but the levels are not exceeded from the normal range. The microscopic and macroscopic examination of the vital organs such as lungs, heart, liver and kidney showed normal cell structures, blood vessels and nuclei. Thus the present study revealed that the ethanol, acetone and benzene extracts of *Terminalia bellerica* fruit did not produce any toxic effects at the high dose of 2000mg/kg body weight and is found to be safe. Thus it is concluded that the plant extract of *Terminalia bellerica* fruit upto 2000 mg/kg body weight was used for further evaluation studies.

KEYWORDS: *Terminalia bellerica*, Toxic effect, Albino rats, Body weight, Food and water intake, Haematology, Histology.

INTRODUCTION
Plants are significant and perennial sources of food and medicines that are used for the treatment of various human diseases.[1] The medicinal plant is considered as a rich resources of ingredients which can be used in drug development and synthesis. Moreover, some plants are considered as important source of nutrition and as a result these plants are recommended for their therapeutic values.[2] The plant parts used for medicinal purposes are root, seed, fruit, bark and leaf.[3] Medicinal plants have many characteristics when used in treatment namely, synergic medicine, support for complex cases; preventive medicines have identified population as half a million plants around world.[2] It is considered that because of the structural and biological diversity of their constituents, terrestrial plants offer a unique and renewable resource for the discovery of potential new drugs and biological entities.[4]

Phytochemistry is the study of phytochemicals, which are chemicals derived from plants. It is used to describe the structures, the functions of compounds in human and plant biology and the biosynthesis of these compounds. Phytochemicals are classified into primary and secondary constituents.[5] Primary constituents include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlorophyll's etc.[5] Secondary constituents are the remaining plant chemicals such as alkaloids, terpenes, flavanoids, ligands, plant steroids, saponins.[6] When screening for biologically active plants constituents, the selection of the plant species to be studied is obviously a crucial factor for the ultimate success of the investigation. Formation of color or change in intensity of color as well as precipitate formation was used as inference.[7] Treatment of various ailments using medicinal plants has been from prehistoric times.[8] However, there is limited report of the proper evaluations of the toxicity of these medicinal plants. Major bioactive compounds responsible for these toxic effects include alkaloids, cardiac glycosides, phorbol esters, lectins and cynogenic glycosides. Previous studies had reported the cases of acute poisoning of patients admitted to hospitals and resulted into death mainly due to ingestion of toxic medicinal plants.[9] Toxicological studies help to decide whether a new drug should be adopted for clinical use or not depending on the duration of exposure of animals to drug, toxicological studies may be of three type viz. acute, sub-acute, and chronic. Toxicity depends not only on the toxic properties of the substance. The relationship

between these two factors is important in the assessment of therapeutic dosage in pharmacology and herbalism [10]. Thus the present study focused on the preliminary phytochemical analysis to know the bioactive compounds of the medicinal plant *Terminalia bellerica*, and in order to know biosafety of the plant extract, acute toxicity test is to be performed through *in vivo* studies.

MATERIALS AND METHODS

The present study was carried out from November 2017 to January 2018. The selected plant material for the present study is the fruit of *Terminalia bellerica.* The process of extraction and formulation of the traditional remedy is as described by Sohini.[11] The powder of *T. bellerica* was pulverized and extracted as a whole preparation in a Soxhlet apparatus using polar (ethanol and acetone) and nonpolar (benzene) solvents. The different extracts of *T. bellerica* were concentrated to a dry mass by vacuum evaporator and stored in desiccator.

The percentage yield was obtained using this formula W2-W1/W0× 100. Where, W2 is the weight of the extract and the container, W1 the weight of the container alone and W0 the weight of the initial dried sample. The powder of *T. bellerica* was subjected to analyse the preliminary phytochemicals such as alkaloids, carbohydrates, Fixed oils and fats, flavonoids, glycosides, phenolic compounds, protein, steroids, saponins, tannins and terpenoids according to the standard methods.[12] Drug dosage calculation is followed by the method of Erhirhie.[13]

Healthy adult male Wistar Albino rats, *Rattus norvegicus* (150-200 mg/kg b.wt.) were used for the present study. The rats were obtained from SASTRA Deemed University, Thanjavur and brought to the laboratory and maintained under controlled environment. All animals were fed with standard pellet feed and water *ad libitum*. The principles of animal care (Ethical Committee's Approval No.001/HCC/IAEC/DST-NPDF/2017) were followed throughout the experimental period.

Experimental design

Toxicity determination for each extract was conducted separately using modified method of Lorke.[14] Normal healthy male albino rats fasted for 12 hours were randomly divided in to control and extracted treated groups. In each extract, 2000mg/kg were separately administered orally to the rats for 14 days.

Rats were divided into control and extract treated group. They were lodged in separate rat cages (Tarsons make 43 x 27 x 15cm size cage). The rats were treated orally with 2000 mg extract/kg body weight by oral gavage needle. The rats in both the test and control group were allowed to access food and water easily. At the end of the experiment, rats were sacrificed for blood collection. The vital organs such as liver, kidney and heart tissues were removed and washed with ice cold saline. The organs were weighed and preserved in 10% formalin solution for histological studies.

Evaluation of toxicity

The rats were observed for clinical signs, symptoms of toxicity and mortality from the time of extract administration to 14th day. Behavioural changes, changes in body weight, daily food intake and water intake were observed over a period of 14 days. Further at end of the experiment all animals were sacrificed. Toxicity of the test drug was confirmed by changes in body weight, food intake, water intake, relative end organ weight, haematological parameters and histoarchitecture of vital organs.

Statistical analysis

Values were represented as Mean ± Standard deviation. All statistical analyses were performed by using windows based SPSS package (Statistical Package for Social Sciences / Statistical Product and Service Solutions).

RESULTS AND DISCUSSION

Yield Percentage of extraction

The yield of crude ethanol extract of *T. bellerica* fruit is 11.35% whereas yield of crude acetone extract of *T. bellerica* fruit is 5.49%, and the percentage yield of the benzene extract of *T. bellerica* fruit is 3.42%.

Preliminary Phytochemical analysis

Plants are endowed with free radical scavenging molecules, such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity.[15] Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. For example, phytochemicals such as saponins, terpenoids, flavonoids, tannins, steroids and alkaloids have antiinflammatory effects.[16] The present study also showed that the presence of phenols, falvonoids, saponins, sterols, tannins, protein, carbohydrates and oil. Flavonoids possess anti-allergic, anti-inflammatory, antiviral and antioxidant activities.[17] The preliminary phytochemical analysis of the different extracts of the study plant (*T. bellerica*) revealed the presence of many phytochemical compounds which possessed various medicinal activities. The ethanol extract of *T. bellerica* fruit powder revealed the presence of phytochemicals such as protein, phenol, flavonoids, saponin and terpenoids. The acetone extract of *T. bellerica* fruit powder showed the presence of protein, phenol, flavonoids, tannin and carbohydrates while the benzene extract of the fruit powder of *T. bellerica* showed the presence of phytochemicals such as tannin, terpenoids and oil.

Acute toxic effect of the plant extract on behavioural changes in rats

Acute toxicity studies give an early indication of the possible target organs.[18] There were no noticeable changes in the general behaviour, toxicity signs and symptoms. No mortality observed in rats treated with test drug orally at 2000 mg/kg body weight for a period of 14 days.

Body weight

Weekly body weight changes among the different extract treated rats and control rats are shown in Figure 1. The control rats and the extract treated rats showed normal increase in their body weight throughout the experimental period. The body weight of control rats are 207.8 ± 8.59 and at the end of the experiment it increased up to 216.2 ± 10.98g. The ethanol, acetone and benzene extract treated rats showed the body weight of 196.9 ± 14.90g, 195.9 ± 11.25g and 187.2 ± 3.47g, and at the end of experimental period it reached about 199.5 ± 14.87g, 198.5± 12.24g and 191.4 ± 3.94g, respectively.

Food and water intake

The mean food intake and water intake of control and different extracts treated rats during the experimental period was shown in Figure 1. The control rats showed normal food intake throughout the experimental period. The food intake of ethanol, acetone and benzene extract treated rats showed slight increase in week I (16.1 ± 2.01g, 17.6 ± 1.71g, 17.8 ± 1.80g) but it showed decreased level of food intake in week II (19.7 ± 2.20g, 17.1 ± 1.37g, 17.5 ± 1.94g) respectively when compared to food intake in week I (19.8 ± 2.83g), and week II (19.7 ± 2.45g) of control rats.

The control rats showed a normal increase in the water intake throughout the experimental period (22.1 ± 1.85ml and 22.6 ± 2.57ml in week I and II respectively). Ethanol, acetone and benzene extracts treated rats showed decreased level of water intake in week II (22.1 ± 1.23ml, 21.2 ± 1.73ml and 22.3 ± 1.47ml, respectively) when compared to the water intake of the rats in initial day (24.3 ± 2.02ml, 23.6 ± 1.64ml and 22.3 ± 1.84ml, respectively). However, the extract treated rats showed the decreased trend of water intake at the end of the experimental period, it showed more or less similar to the water intake of the control rats.

Organ weight

Effect of treatment of the plant extract on relative organ weights are shown in Figure 2. The relative weight of liver of ethanol, acetone and benzene extract treated rats (4.10 ± 0.919, 4.0 ± 1.62 and 3.6 ± 1.14 g/100g body weight, respectively) was observed to be more or less similar to that of control rats (3.98 ± 0.399 g/100g body weight). The mean relative weight of heart of ethanol, acetone and benzene extract treated rats was observed to be similar to that of control rats (0.4 ± 0.05g/100g body weight). The mean relative weight of lungs of the ethanol, acetone and benzene extract treated rats showed slight increase (0.9 ± 0.30, 0.7 ± 0.41 and 0.7 ± 0.15g/100g body weight, respectively) when compared to the control rats (0.6 ± 0.09g/100g body weight). The relative weight of both right and left kidney showed slight increase when compared to the control rats.

Haematological parameters

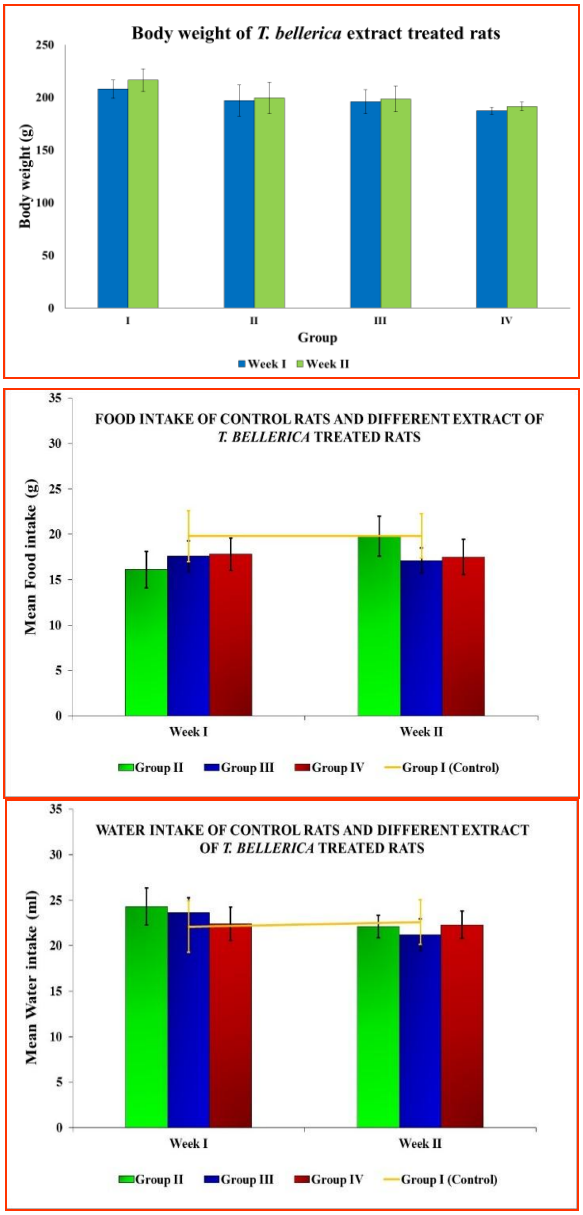
Haematological parameters of control and extract treated rats are shown in Figure 3a and 3b. The total WBC count and the differential count except lymphocytes and basophil of the extract treated rats was decreased when compared to the control rats (9.0 ± 0.35 10^3/µL). Basophil was totally absent in the extract treated rats and the lymphocyte count of benzene extract treated rats was similar (0.2 ± 0.010%) when compared to control (0.2 ± 0.001%) and the ethanol, and acetone extract treated rats. The WBC differential count values in the ANOVA table is expressed in arcsine values. The total RBC count, haemoglobin, haematocrit, MCV, MCH, MCHC showed increased levels when compared to control rats but the levels are not exceeded from the normal range. The platelet count of the control and the extract treated rats differed from each other but the values are within the normal range. All the haematological parameters of control, ethanol, acetone and benzene extract treated groups showed significant difference except MCH (one way ANOVA; $p<0.05$, SNK test).

Histoarchitecture of vital organs

The assessment of haematological parameters could be used to reveal the deleterious effect of foreign compounds toxins, chemicals and plant extracts on the blood constituents of animals. They are also used to determine possible alterations in the levels of biomolecules such as enzymes, metabolic products, haematology, normal functioning and histomorphology of the organs.[19] Photomicrography of lungs, heart, liver and kidney of control and different extract of plant powder treated rat groups are shown in Plate 1-4. No pathological changes were observed in the vital organs of test herbal drug treated rats. There was no macroscopic change of central organ (such as appearance, colour and size) considered to be related to the treatment. The control and extract treated rats showed normal alveoli, alveolar duct and blood vessels. The normal bronchi lined by ciliated epithelium are observed in the extract treated groups. The muscle of the heart exhibited alternative light and dark bands and possessed normal central nucleus in all the extract treated rats. The liver of control rat showed normal hepatic lobules, hepatocytes and central vein. Histological sections of kidney of all the groups showed that the glomeruli, tubules and blood vessels appear normal.

The results of this study showed no changes in the behaviour, no toxic symptoms, and changes in the body weight, food intake, water intake, and relative organ weight. However, the haematological parameters differed from each other but it does not exceed from the normal range. The histoarchitecture of the vital organs did not

show any pathological changes. Thus the present study revealed that the *T. bellerica* fruit extract at 2000 mg/kg body weight does not produce any toxic effect and further it will be used for antipsoriatic evaluation.



Groups: I = Control
II = Ethanol extract of *T. bellerica* treated rats
III = Acetone extract of *T. bellerica* treated rats
IV = Benzene extract of *T. bellerica* treated rats
Figure 1: Toxic effect of ethanol, acetone and benzene extract of *T. bellerica* treatment on body weight, food intake and water intake in albino rats.

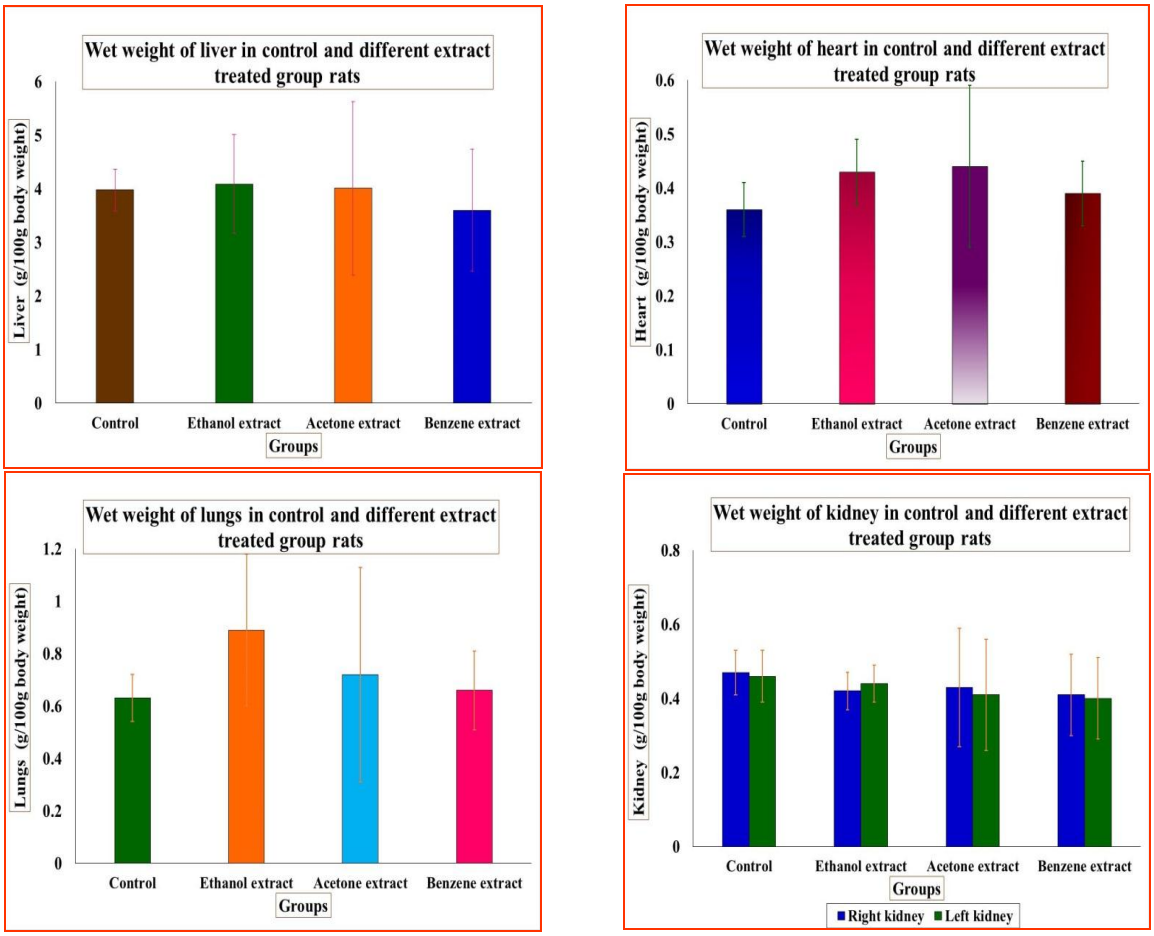
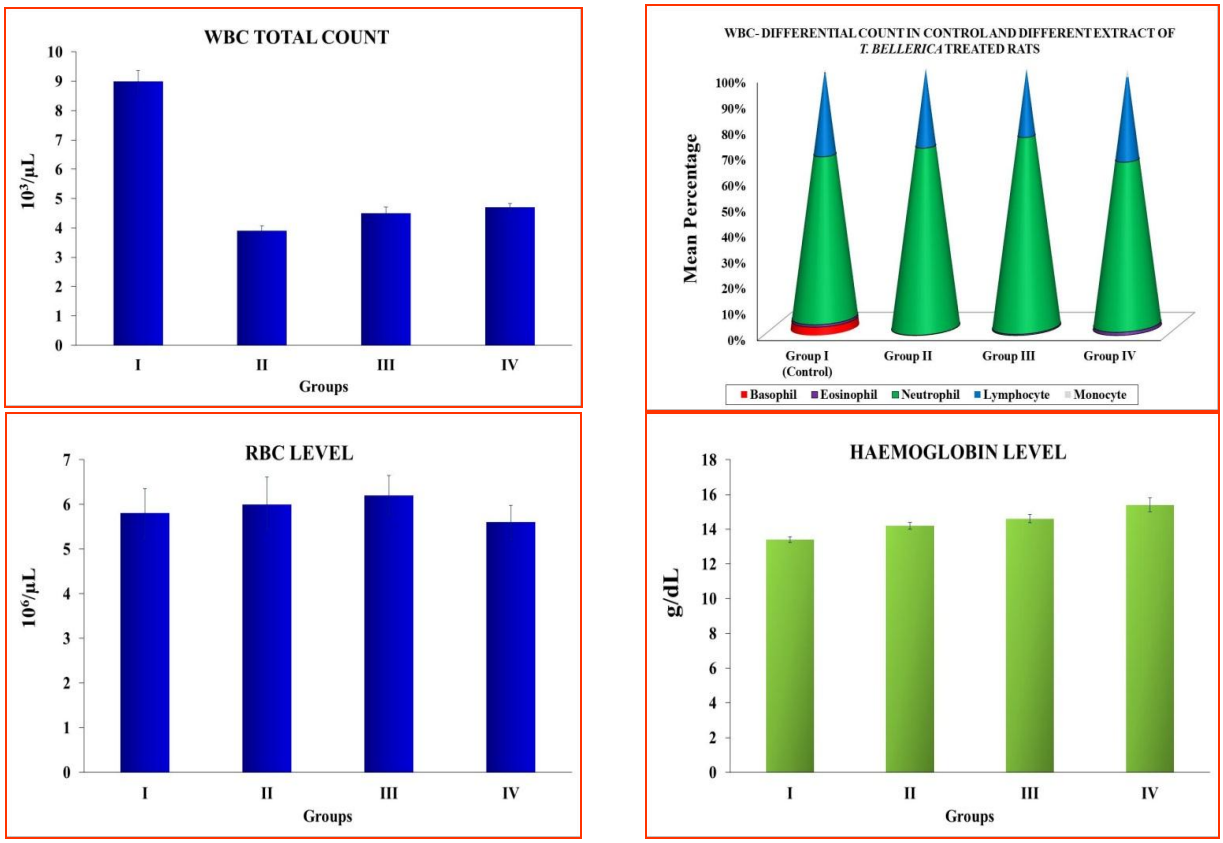
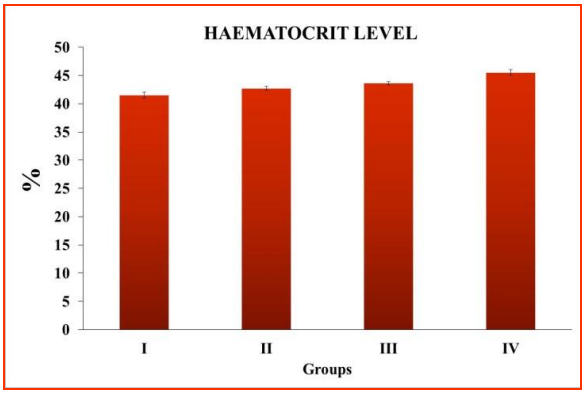
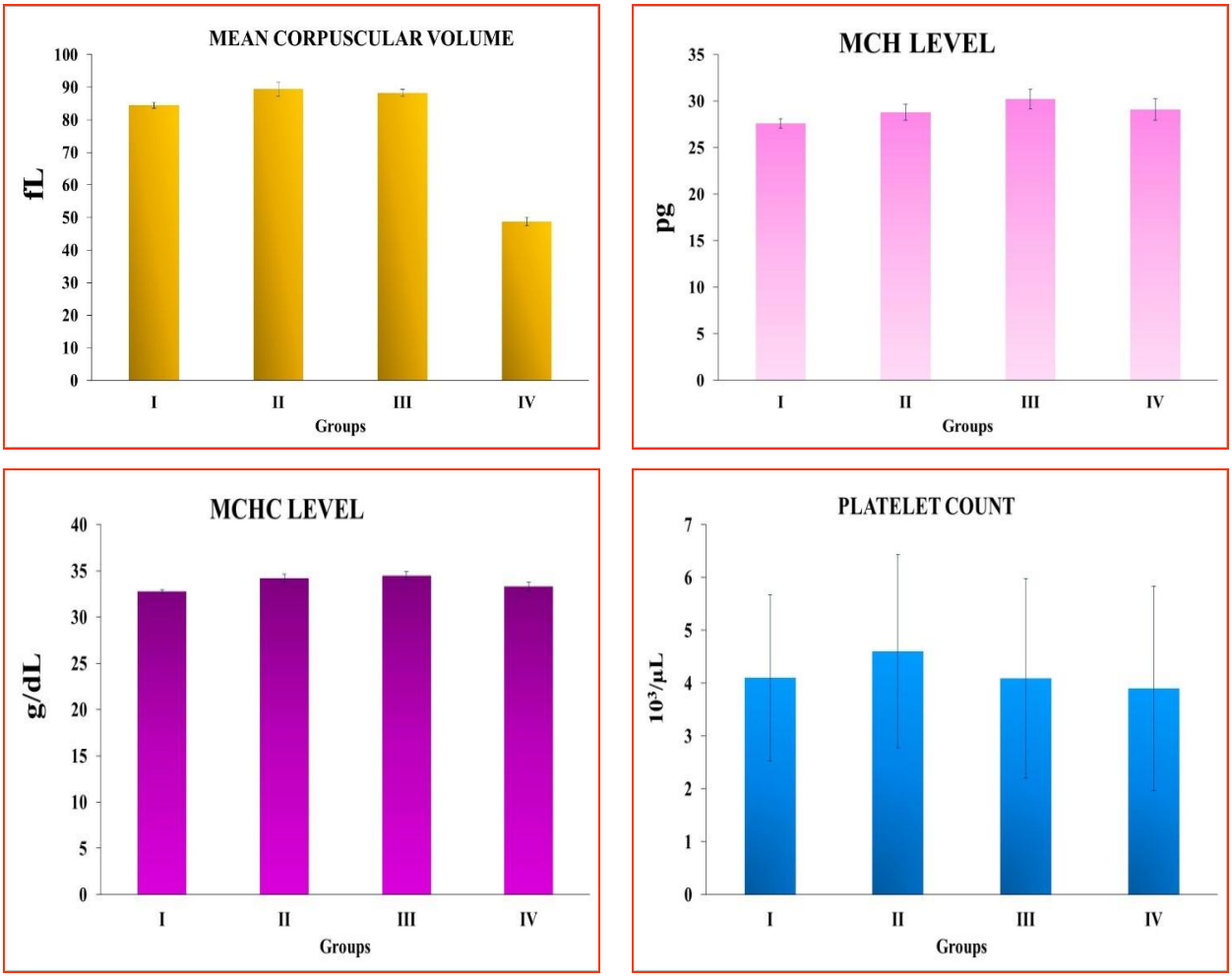


Figure 2: Toxic effect of test drugs on relative weight of liver, heart, lungs and kidney in albino rats.

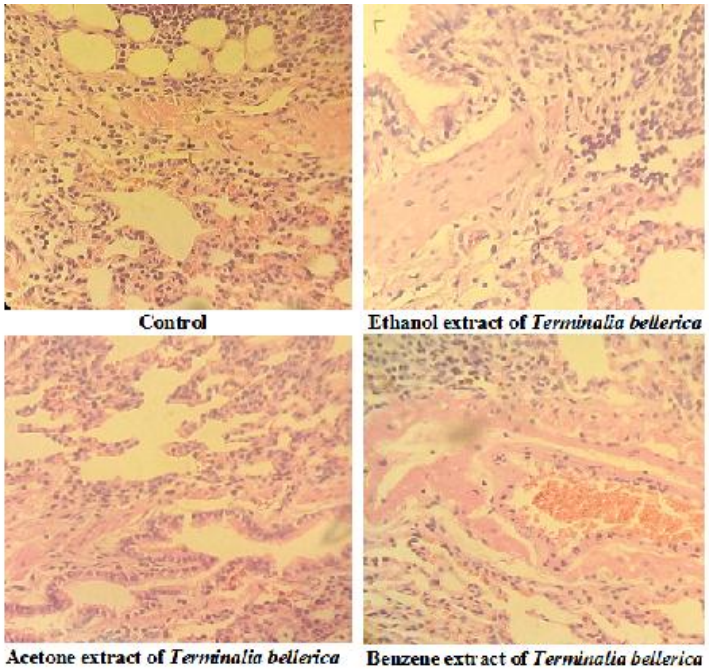




Groups: I = Control
II = Ethanol extract of *T. bellerica* treated rats
III = Acetone extract of *T. bellerica* treated rats
IV = Benzene extract of *T. bellerica* treated rats
Figure 3a: Toxic effect of test drugs on haematological parameters (WBC- TC and DC, RBC, Haemoglobin and Haematocrit) in albino rats.

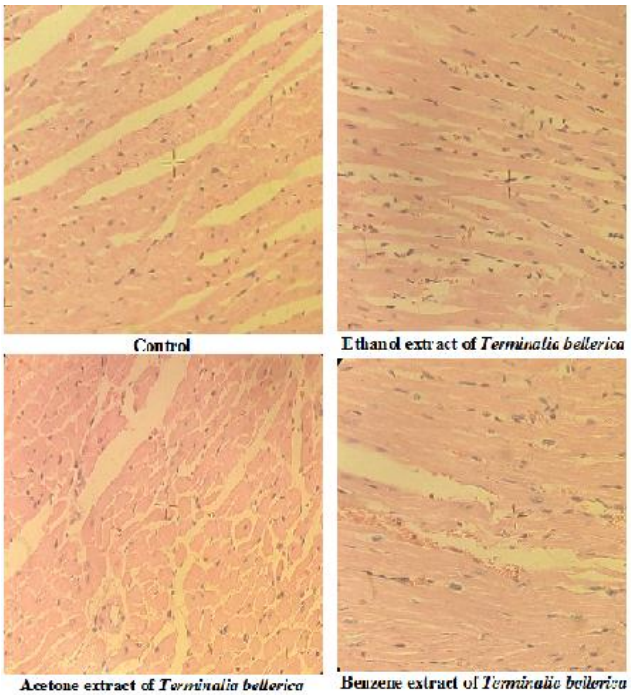


Groups: I = Control
II = Ethanol extract of *T. bellerica* treated rats
III = Acetone extract of *T. bellerica* treated rats
IV = Benzene extract of *T. bellerica* treated rats
Figure 3b: Toxic effect of test drugs on haematological parameters (MCV, MCH, MCHC level and platelet count) in albino rats.



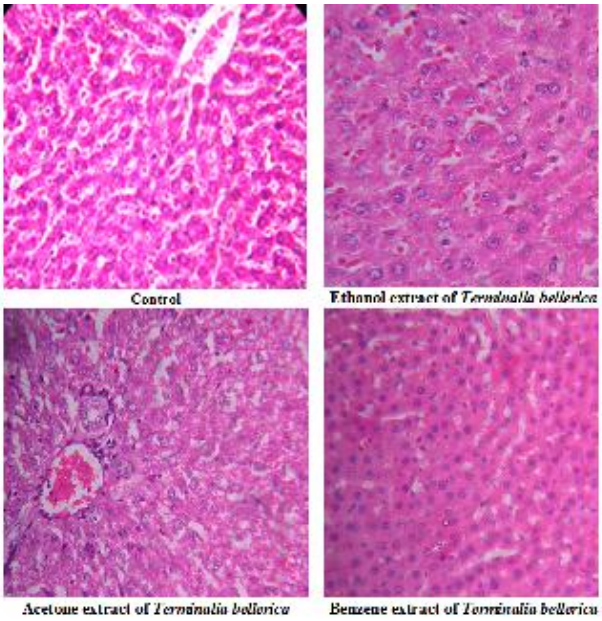
(Images showed the normal alveolar cells in both control and extract treated groups)

Plate 1: Acute toxic effect of different extracts of *Terminalia bellerica* on histoarchitecture of lungs.



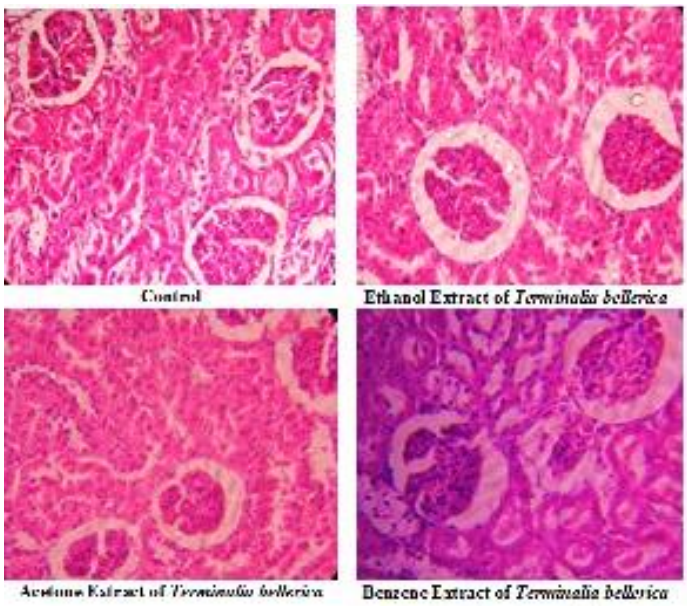
(Images showed the normal cardiac cells in all the groups)

Plate 2: Acute toxic effect of different extracts of *Terminalia bellerica* on histoarchitecture of heart.



(Image showed normal hepatic lobules, hepatocytes, central vein and sinusoids in all the groups)

Plate 3: Acute toxic effect of different extracts of *Terminalia bellerica* on histoarchitecture of liver.



(Images showed normal glomeruli, tubules and blood vessels in all the groups)

Plate 4: Acute toxic effect of different extracts of *Terminalia bellerica* on histoarchitecture of kidney.

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