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Evaluation of Toxic Effect of Terminalia Chebula Fruit Extract in Albino Rats

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ABSTRACT: *The present study aims to analyse the phytochemicals and test the toxic effect of the fruit extract of Terminalia chebula 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 increase or decrease in the body weight of the extract treated groups compared to control group rats. The food intake and water intake of the control and extract treated rats showed slight variations but not showed the abnormal intake of food and water throughout the experimental period. 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 chebula 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 chebula fruit upto 2000 mg/kg body weight was used for further evaluation studies*.

KEYWORDS: *Terminalia chebula*, fruit extract, phytochemical analysis, *Rattus norvegicus*, toxicity studies.

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I INTRODUCTION

India is richly endowed with a wide variety of plants having medicinal value. These plants are widely used by all sections of the society either directly as folk remedies or indirectly as pharmaceutical preparation of modern medicine (Rajesh et al., 2010). Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years (Mahidol et al., 2003). Medicinal plants are plants that have at least one of their parts (leaves, stem, barks or roots) used for therapeutic purposes (Bruneton, 1993). Recently, medicinal plants have become important for the treatment of different disease conditions, such as diabetes, malaria, anaemia (Fola, 1993). The availability and relatively cheaper cost of medicinal plants, makes them more attractive as therapeutic agents when compared to 'modern' medicines (Agbor and Ngogang, 2005). Phytochemicals with biological activity have had great utility as pharmaceuticals and pharmacological actions (Rajesh et al., 2010). In recent years, because of their easy availability and cost effectiveness, there has been a dramatic rise in use of herbal drugs/preparations in the developed countries besides having desired pharmacological effectiveness with high level of safety and low toxicity profile (Kadam et al., 2012).

Plants have been used in medicines since time immemorial. India has a rich heritage of using medicinal plants in traditional medicines, as in the Ayurveda, Siddha and Unani systems besides folklore practices. The earliest inscription of the medicinal uses of plants is found in the "Rigveda", which is one of the oldest repositories of human knowledge. Fairly comprehensive information on the curative properties of some herbs has been found recorded in "Charak Samhita" and "Sushruta Samhita" (Kamboj, 2000). The plant kingdom is a virtual goldmine of biologically active compounds and it is estimated that only 10-15% of existing species of higher plants have been surveyed. Many plants have been successfully used in the treatment of various diseases. The ancient record is evidencing their use by Indian, Chinese, Egyptian, Greek, Roman and Syrian dates back to about 5000 years. In India, around 20,000 medicinal plant species have been recorded recently, but more than 500 traditional communities use about 800 plant species for curing different diseases (Kamboj, 2000). Phytochemistry have been instrumental in rationalization of the use of various herbal medicines, however unscreened herbal products still find their way to markets owing to their high demand. The quest to unravel the mysteries of bioactive properties of medicinal plants and the comprehension of their nutritional and toxicological constituents have been a subject of intense renewed interest for many scientists all over the World (Ugbogu et al., 2016).

Phytochemicals come in a variety of forms and different vegetables have higher concentrations of a particular phytochemical than others. Some of the main phytochemicals include: carotenoids, isoflavonoids, steroids, lignans, anthraquinones, gums and resins (Rajesh et al., 2010). The most important of bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Doss, 2009). 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. Thus the present study focused on the preliminary phytochemical analysis to know the bio-active compounds of the medicinal plant Terminalia chebula and in order to know bio-safety of the Terminalia chebula, acute toxicity test is to be performed through in vivo studies.

II MATERIALS AND METHODS

The selected plant material for the present study is fruits of Terminalia chebula. The process of extraction and formulation is as described by Sohini et al., (1996). The fruit powder of Terminalia chebula was pulverized and extracted as a whole preparation in a Soxhlet apparatus using polar (ethanol and acetone) and non-polar (benzene) solvents. The different extracts of Terminalia chebula fruit 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, W1is the weight of the container alone and W0 is the weight of the initial dried sample).

Preliminary phytochemical screening

The fruit powder of Terminalia chebula 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 (Kokate, 1994; Harborne, 1973; Rajpal, 2002; Raaman, 2006).

Drug dosage calculation is followed by the method of Erhirhie et al., 2014. Experiments on animals are necessary in drugs discovery and development as well as to advance medical and biological knowledge (Baker et al., 1979). Dosage calculation and stock solution preparation based on dosage rationale formula are prerequisites to drug administration in experimental animals (Erhirhie et al., 2014).

Experimental set up for acute toxicity study

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 University, Thanjavur and brought to the laboratory and maintained under controlled environment. The rats were randomized into control and experimental groups and housed in different plastic cages. 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.

Normal healthy male albino rats fasted for 12 hours were randomly divided in to control and extracted treated groups. There were three different types of extracts (ethanol extract, acetone extract and benzene extract of Terminalia chebula) separately tested for their toxic effect. In each extract, 2000mg/kg were separately administered orally to the rats *for* 14 days. The rats were observed for clinical signs and symptoms of toxicity and mortality from the time of extract administration to 14^{th} day. Behavioural changes, changes in body weight, daily food intake and water intake were observed over a period of 14 days. At the end of the experiment, all experimental animals were fasted overnight, cervically dislocated, and blood samples were collected by cardiac puncture into tube with anticoagulant ethylene di-amine tetra acetic acid (EDTA) for haematology. Blood samples in test tubes containing EDTA were immediately processed for haematological parameters using Automated Haematological Analyzer. Total and differential white blood cell count (WBC), red blood cell count (RBC), the haemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), Red cell distribution width, and platelet count (PLC) were determined. And the vital organs such as lungs, liver, kidney and heart tissues were removed and washed with ice cold saline and, weighed and preserved in 10% formalin solution for histological studies.

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).

III RESULTS AND DISCUSSION

Yield Percentage:

The yield of crude ethanol extract of T. chebula fruit (20.76%) is the highest among the three samples whereas yield of crude acetone extract of T. chebula fruit (10.98%) and the crude benzene extract of the T. chebula fruit is 7.25%.

Preliminary Phytochemical analysis:

Phytochemical analysis concluded on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities (Sofowra, 1993). In the present study acute toxic effect of ethanol, acetone and benzene extract of Terminalia chebula fruit was evaluated. It revealed the presence of many phytochemical compounds which possessed various medicinal activities. The ethanol extract of T. chebula fruit powder revealed the presence of phytochemicals such as protein, phenol, flavonoids, tannin, sterol and carbohydrates. The acetone and benzene extract of T. chebula fruit powder showed the presence of protein, phenol, flavonoids, tannin, saponin, terpenoids and carbohydrates. The phenolic compounds are one of the largest and most ubiquitous groups of plants metabolites (Singh et al., 2008). They possess biological properties such as anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammations, cardiovascular protection and the improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities. Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds (Krings and Berger, 2001). Natural antioxidants mainly come from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc., (Ali et al., 2008). Flavonoids have antioxidant activities as well as health promoting effects viz., anti-allergic, anti-cancer, anti-oxidant, anti-inflammatory, anti-thrombotic, vasoprotective, tumor inhibitory and anti-viral effects (Salah et al., 1995; Okwu, 2004; Hodek et al., 2002). Phytochemicals such as saponins, terpenoids, flavonoids, tannins, steroids and alkaloids have atiinflammatory effects (Akindale and Adeyemi, 2007). Thus the phytochemicals present in the plant extract revealed that the plant drug possess anti-inflammatory, antibacterial, antioxidant, anti-allergic, anticancer, etc., may be used to treat several diseased conditions especially used for skin diseases.

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

Herbal medicine preparations are "natural" and are therefore intrinsically harmless. However, their effects can be very powerful and potentially lethal if used incorrectly and their use as a substitute for conventional medicines may be ineffective (Bateman et al., 1998). In the present study, acute toxic effect of ethanol, acetone and benzene extract of Terminalia chebula fruit powder was evaluated. There were no noticeable changes in the general behaviour, toxicity signs and mortality observed in rats treated with test drug orally at 2000 mg/kg body weight for a period of 14 days.

Toxic effect on 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 ± 8.60 and at the end of the experiment it increased up to 216.3 ± 10.98g. The ethanol, acetone and benzene extract treated rats showed the body weight of 193.4 ± 8.41g, 190. 4 ± 4.92g and 190.1 ± 4.15g, and at the end of experimental period it reached about 195.9 ± 8.67g, 193.8± 4.80g and 193.8 ± 4.02g, respectively.

Toxic effect on food intake

The mean food 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 (16.8 ± 1.35g, 18 ± 2.56g, 17.7 ± 1.67g) but it showed decreased level of food intake in week II (16.2 ± 19.0g, 15.5 ± 18.2g, 16.2 ± 20.5g) respectively when compared to food intake in week I (19.3 ± 2.59g), and week II (18.9 ± 2.09g) of control rats.

Toxic effect on water intake

Mean water intake of control and different extracts treated rats was shown in Figure 1. 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 (22.81± 1.59ml, 19.0 ± 23.0ml and 19.8 ± 23.8ml, respectively) when compared to the water intake of the rats in initial day (23.7 ± 1.72ml, 23.5 ± 1.90ml and 23.9 ± 2.10ml, 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.

Toxic effect on 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 (3.67 ± 0.226, 3.69 ± 0.229and 3.71 ± 0.736 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 (0.7 ± 0.18, 0.7 ± 0.28 and 0.7 ± 0.10g/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 (0.5 ± 0.06 and 0.5 ± 0.07g/100g body weight of right and left kidney respectively).

Toxic effect on haematological parameters

Analysis of blood parameters with respect to animal studies has a high relevance and predictive value for human (Rhiouani et al., 2008; Koshy et al., 2011). 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 (Maglhaes et al., 2008; Oyedemi et al., 2011). 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 (9.02 ± 0.350 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 ± 0.005%) when compared to control (0.2 ± 0.010%) 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).

Effect of different extracts of T. chebula on histoarchitecture of vital organs:

Photomicrography of lungs, heart, liver and kidney of control and different extract of plant powder treated rat groups are shown in Plates 1-4. 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. The cell cords were separated by narrow blood sinusoids. Sinusoidal capillaries (sinusoids) separate the sheets of hepatic cells and empty into the central veins. The hepatic cells were thicker and the sinusoids appear as light areas between the cords of cells. The nuclei of hepatic cells were large and spherical, bi-nucleated cells also found. Histological sections of kidney of all the groups showed that the glomeruli, tubules, blood vessels and interstitium appear normal. No pathological changes were observed in test herbal drugs treated rat kidney. There was no macroscopic change of central organ (such as appearance, colour and size) considered to be related to the treatment. The histopathological examinations of lungs, heart, liver and kidney revealed no remarkable morphological alteration in all treatment and control groups.

IV CONCLUSION

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 damaged cells, blood vessels and tubules in all the extract treated rats. Thus the present study revealed that the Terminalia chebula fruit powder extract at 2000 mg/kg body weight does not produce any toxic effect in the ethanol, acetone and benzene extract treated rats.

Figure 1: Toxic effect of ethanol, acetone and benzene extract of *T.chebula* treatment on body weight, food intake and water intake in albino rats.

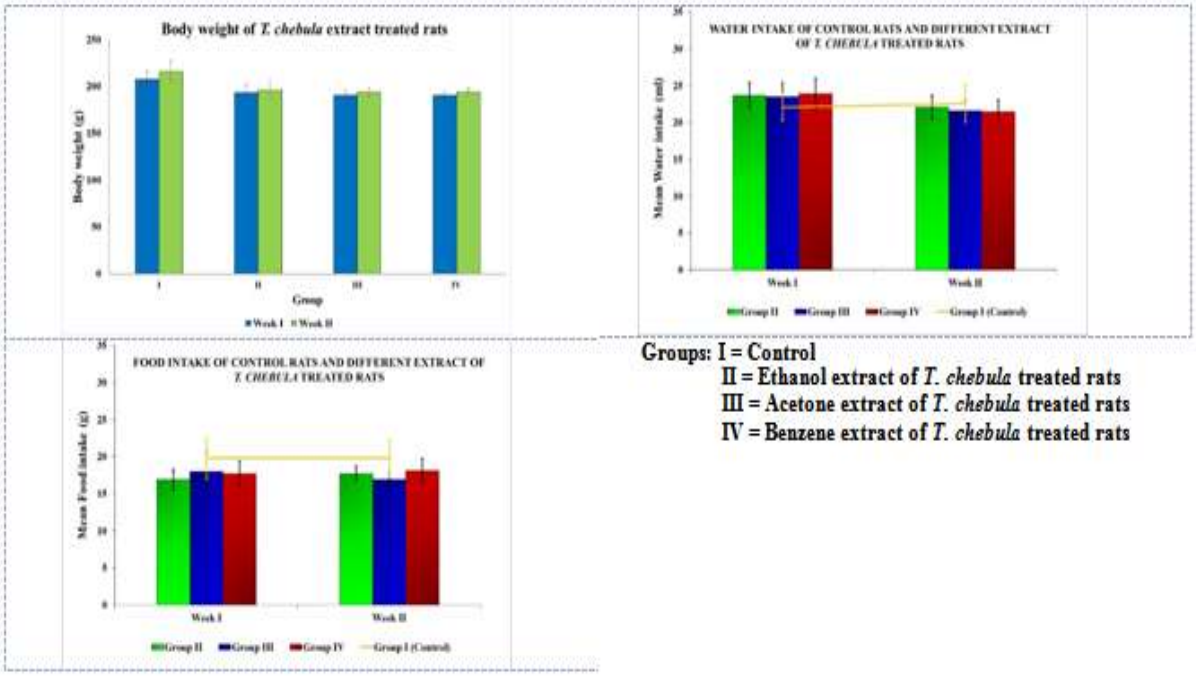


Figure 2: Toxic effect of test drugs on relative weight of liver, heart, lungs and kidney in albino rats.

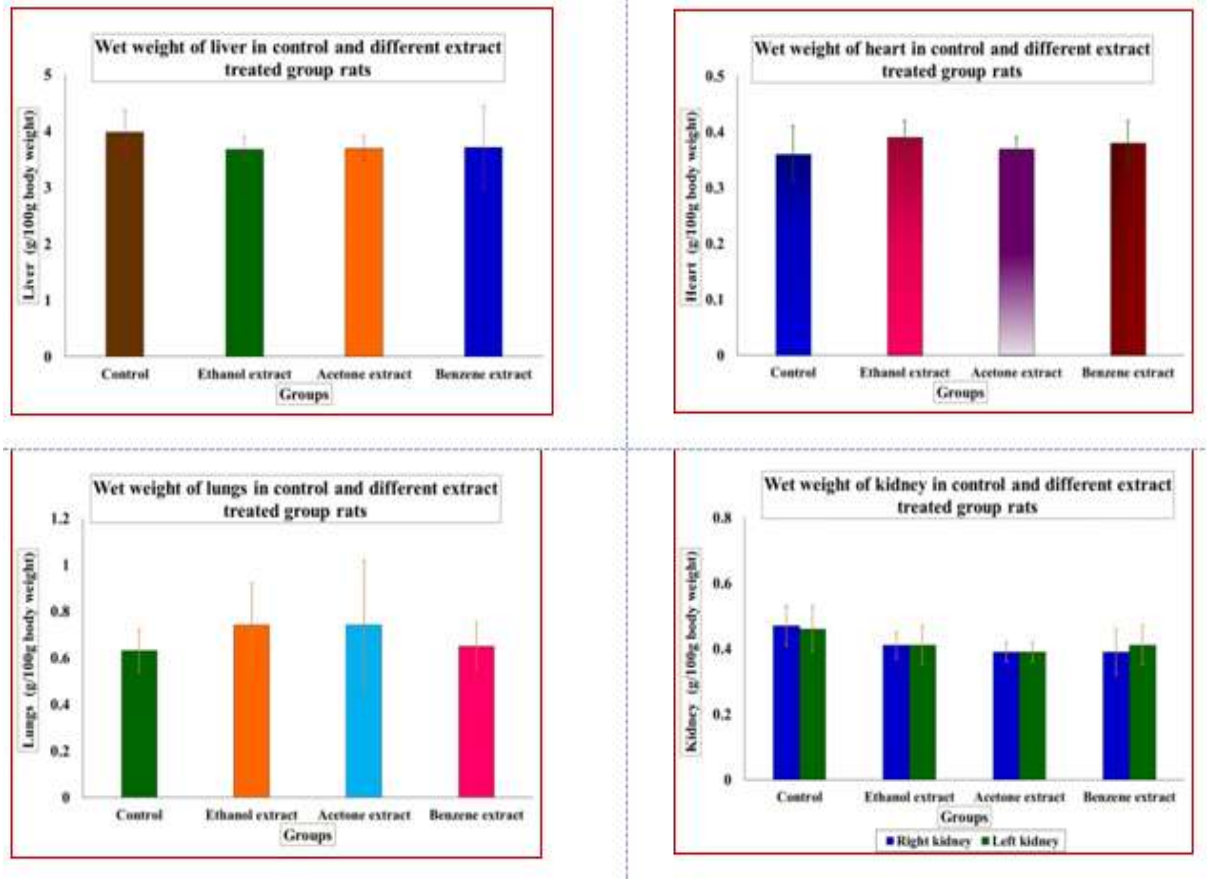


Figure 3a: Toxic effect of test drugs on haematological parameters (WBC- TC and DC, RBC, Haemoglobin and Haematocrit) in albino rats.

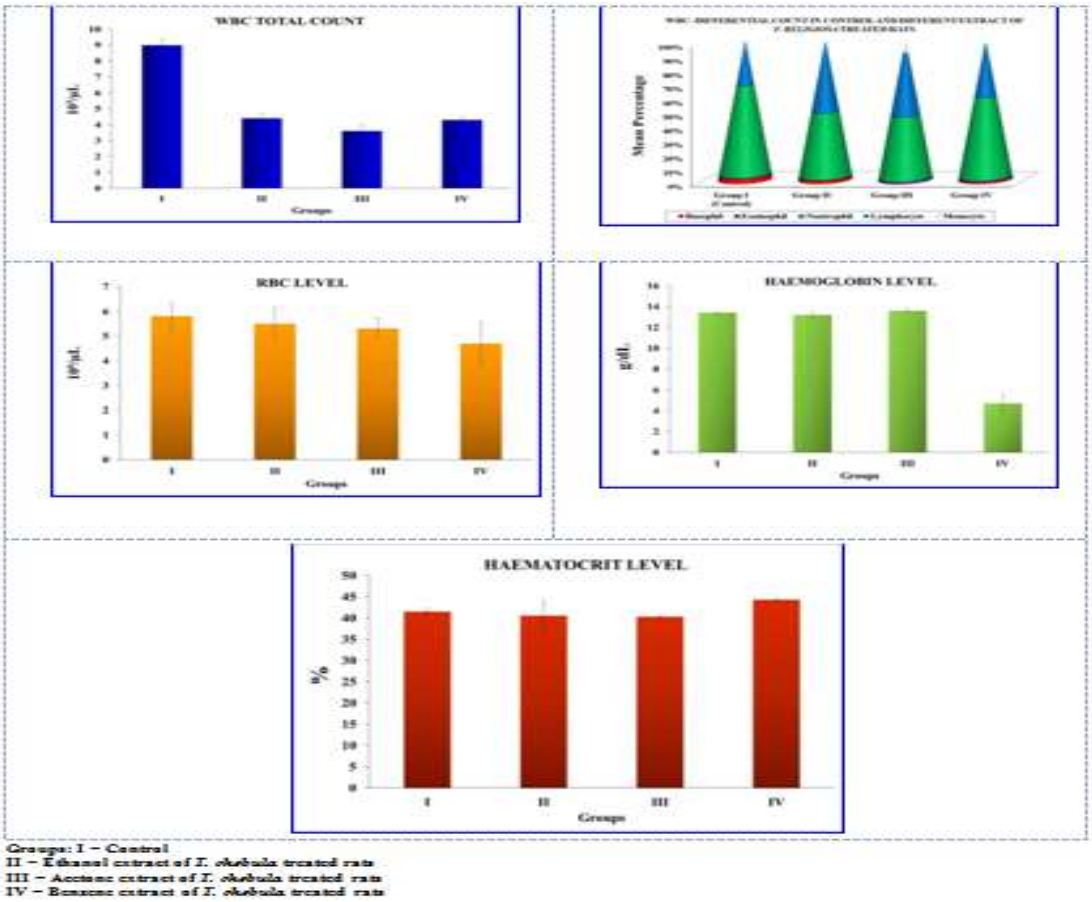


Figure 3b: Toxic effect of test drugs on haematological parameters (MCV, MCH, MCHC level and platelet count) in albino rats.

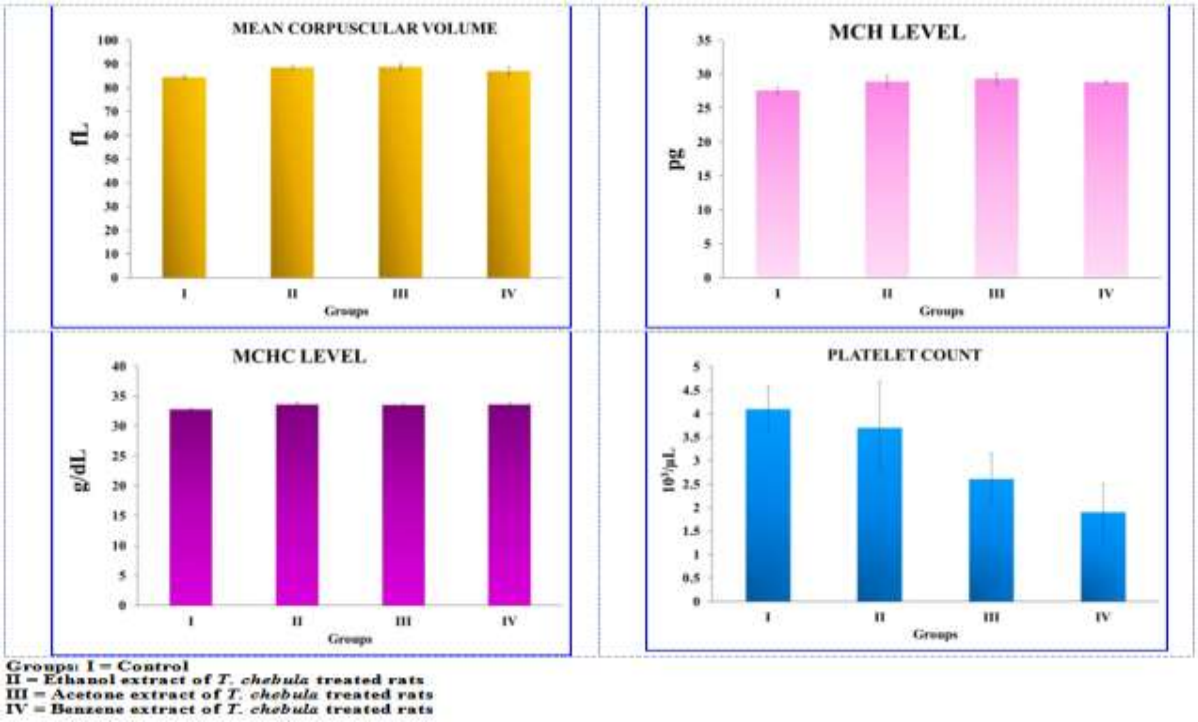
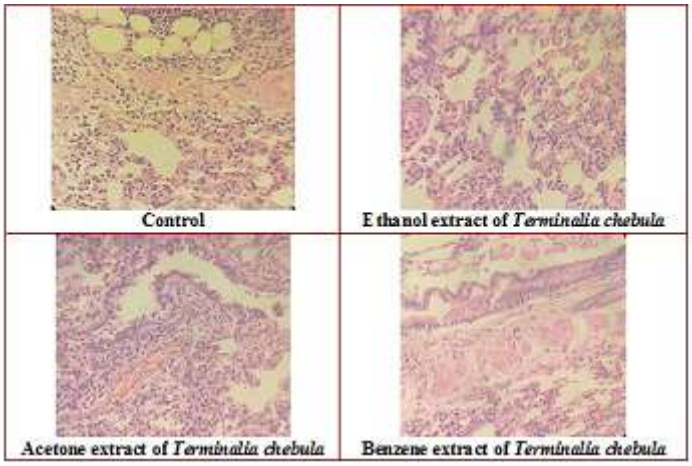
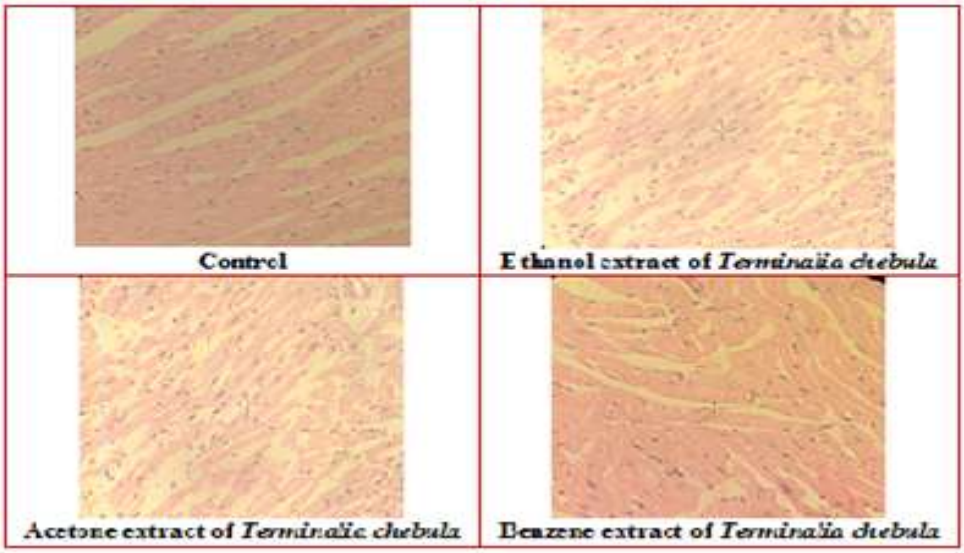


Plate 1: Acute toxic effect of different extracts of *Terminalia chebula* on histoarchitecture of lungs.



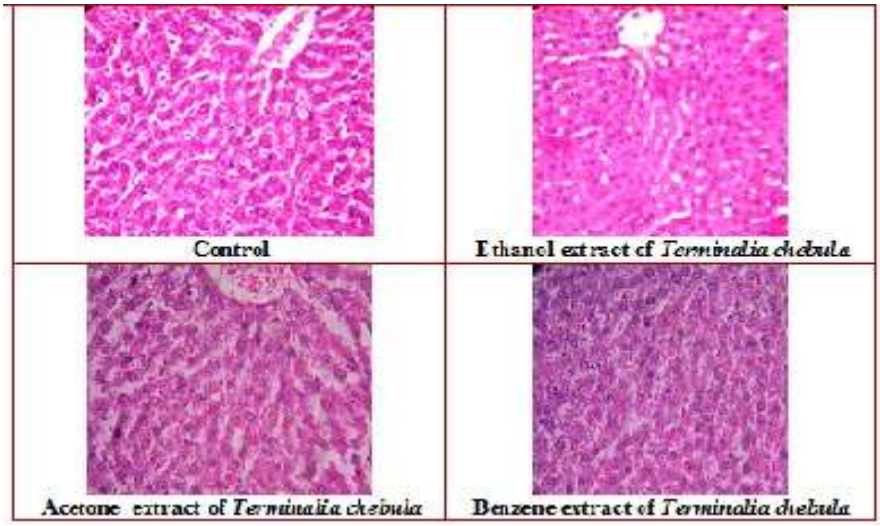
(Images showed the normal alveolar cells in both control and extract treated groups)

Plate 2: Acute toxic effect of different extracts of *Terminalia chebula* in histoarchitecture of heart



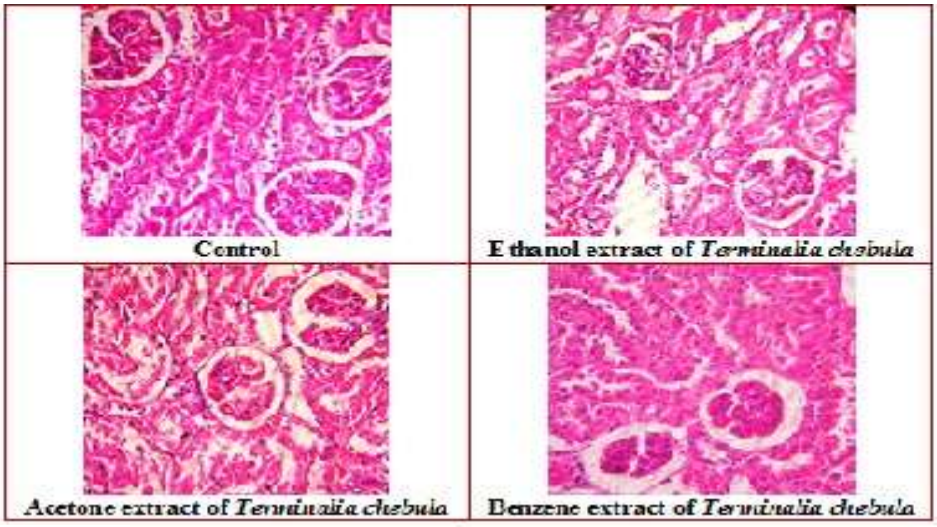
(Images showed the normal cardiac cells in all the groups)

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



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

Plate 4: Acute toxic effect of different extracts of *Terminalia chebula* on histoarchitecture of Kidney



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

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