Acute Toxicity Evaluation of *Ficus religiosa* Bark Extract on Albino Rats

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Abstract: The present study aims to test the acute toxic effect of the bark extract of *Ficus religiosa* 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 changes in the body weight of the extract treated groups compared to control group rats as well as the food intake and water intake showed slight variations 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 macroscopic and microscopic 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 *Ficus religiosa* bark 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 *Ficus religiosa* bark upto 2000 mg/kg body weight was used for further evaluation studies.

Key words: Phytochemical analysis, Toxicity studies, *Ficus religiosa, Rattus norvegicus.*

I. INTRODUCTION

Plants play a key role in sophisticated ancient traditional medical systems such as traditional Chinese medicine and Ayurveda of India, and have also been central in the Greco-Roman medical tradition, which developed into modern biomedicine. Plants have been used in medicines since time immemorial. Fairly comprehensive information on the curative properties of some herbs has been found recorded in "Charak Samhita" and "Sushruta Samhita" (Kamboj, 2000). Currently, 80% of the world population depends on plant-derived medicine for the first line of primary health care for human alleviation (Rekha and Vidyasagar, 2014). 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 (Zheng and Wang, 2001 and Cai *et al*., 2003).

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 are naturally occurring in the medicinal plants and vegetables that have defense mechanism and protect from various diseases. Easy availability of herbal medicine has led to their increased use (Murugi *et al*., 2012). This has resulted to increased reports of their suspected toxicity and adverse events. Such unwanted reactions can be due to side effects; reactions occurring as a result of overdose, over duration, tolerance, dependence-addiction; hypersensitivity, allergic and idiosyncratic reactions; mid-term and long-term toxic effects. It is such reaction that necessitates toxicity evaluation (Musila *et al.*, 2017).

The main goal of the present study is to investigate the phytochemical compounds of ethanol, acetone and benzene extract of *Ficus religiosa* bark through preliminary phytochemical screening and evaluate the acute toxic effect of the ethanol, acetone and benzene extract of *Ficus religiosa* bark through *in vivo* studies.

II. MATERIALS AND METHODS

The bark powder of *F. religiosa* was extracted by Soxhlet extraction method using ethanol, acetone and benzene. The crude extract is subjected to analyse the preliminary phytochemicals (Kokate, 1994; Harborne, 1973; Rajpal, 2002; Raaman, 2006). Drug dosage calculation is followed by the method of Erhirhie *et al*., 2014. 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.

2.1. Experimental design

Toxicity determination for each extract was conducted separately using modified method of Lorke (1983). Normal healthy female albino rats fasted for 12 hours were randomly divided in to control and extracted treated groups. They were lodged in separate rat cages and 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. Blood was collected through heart punching method for haematological analysis and the vital organs such as liver, kidney and heart tissues were removed and washed with ice cold saline and weighed, and preserved in 10% formalin solution for histological studies.

2.2. Evaluation of toxicity

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

3.1. Yield percentage:

The yield percentage of crude ethanol extract of *F. religiosa* bark (4.94%) is the highest among the three samples whereas yield of crude acetone extract of *F. religiosa* bark (3.43%) and the crude benzene extract of the *F. religiosa* bark is 1.47%.

3.2. Preliminary phytochemical analysis:

The preliminary phytochemical screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Further, these tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds (Varadarajan *et al*., 2008). The secondary metabolites (phytochemicals) and other chemical constituents of medicinal plants account for their medicinal value (Olaleye, 2007). The phytochemical screening revealed the presence of triterpenoids, protein, phenol, carbohydrates and tannins in the ethanol extract; triterpenoids, protein, carbohydrates and phenol in an acetone extract and triterpenoids and tannins in the benzene extract. The presence of different phytoconstituents in the three different extracts may be responsible for the therapeutic properties of *F. religiosa*. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers. Since these compounds were found to be present in the extracts, it might be responsible for the potent antioxidant capacity of *F. religiosa*. Phytochemicals such as saponins, terpenoids, flavonoids, tannins, steroids and alkaloids have anti-inflammatory effects (Manach *et al*., 1996; Latha *et al*., 1998; Liu, 2003; Akindale and

Adeyemi, 2007). Similarly, the ethanol, acetone and benzene extracts of the bark powder of *F. religiosa* contains tannin and terpenoids and in such a way this plant extract may use to treat inflammatory disease. The phenolic compounds are one of the largest and most ubiquitous groups of plants metabolites (Singh *et al*., 2007). 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 (Han *et al*., 2007). Similarly the bark extract of *F. religiosa* contains phenolic compounds which may be used treat the inflammatory conditions.

3.3. Behavioural changes:

In the present study, acute toxic effect of ethanol, acetone and benzene extract of *Ficus religiosa* bark 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.

3.4. 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 216.3 ± 10.98g. The ethanol, acetone and benzene extract treated rats showed the body weight of 191.2 ± 17.30g, 192. 4 ± 5.28g and 190.2 ± 6.09g, and at the end of experimental period it reached about 200.2 ± 18.70g, 197.0± 6.35g and 193.9 ± 5.87g, respectively.

3.5. Food intake:

The mean food intake of control and different extracts treated rats during the experimental period was illustrated in 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 (15.8 ± 1.68g, 17.3 ± 1.65g, 20.4 ± 1.95g) but it showed decreased level of food intake in week II (20.1 ± 1.95g, 20.7 ± 1.02g, 17.5 ± 1.51g) respectively when compared to food intake in week I (19.8 ± 2.83g), and week II (19.7 ± 2.45g) of control rats.

3.6. Water intake:

Mean water intake of control and different extracts treated rats was illustrated 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 (21.8 ± 1.98ml, 19.9 ± 1.75ml and 21.1 ± 2.54ml, respectively) when compared to the water intake of the rats in initial day (23.5 ± 1.61ml, 23.2 ± 1.20ml and 22.7 ± 2.49ml, 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.

3.7. 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 ± 0.228and 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.

3.8. Haematological parameters:

Blood can act as a pathological and physiological indicator of animal health (Jorum, 2016). Total WBC count and the differential count except lymphocytes and basophil of the extract treated rats was decreased when compared to the control rats (9.03 ± 0.350 10^3/µL). Basophil was totally absent in the extract treated rats and the lymphocyte count of benzene extract treated rats was decreased (0.13 ± 0.02%) when compared to control (0.2 ± 0.01%) and the ethanol, and acetone extract treated rats (Figure 3). In this study, a significant normal cell level in the MCV, MCH, WBC, RBC, PCV and MCHC indicates the plant *Ficus religiosa* is non-toxic.

3.9. 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

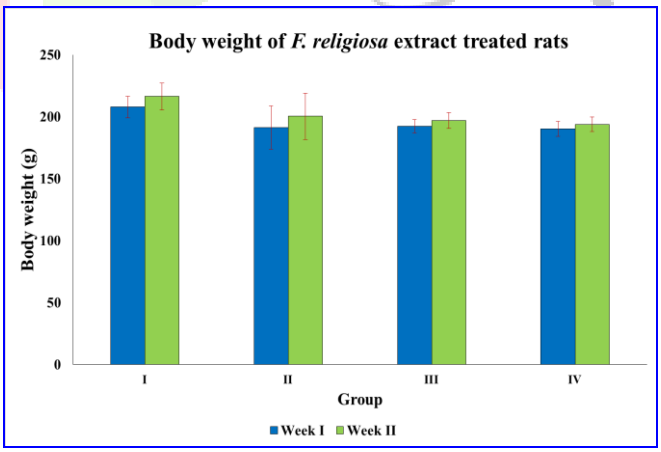
treated groups. The muscle of the heart exhibited alternative light and dark bands and possessed normal central nucleus in all the extract treated rats. The liver of control rat showed normal hepatic lobules, hepatocytes and central vein. The cell cords were separated by narrow blood sinusoids. Sinusoidal 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, binucleated 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.

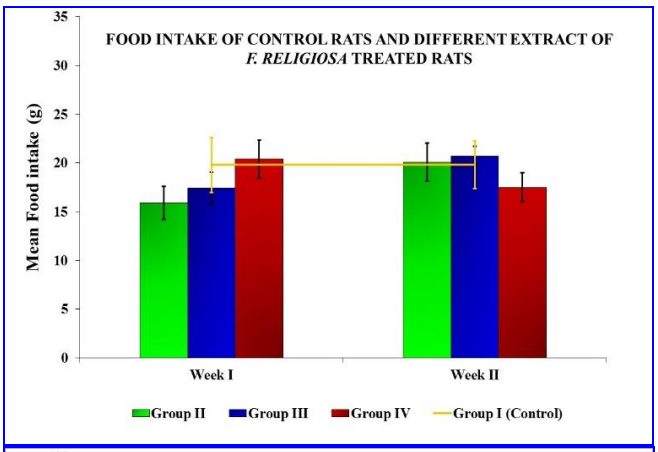
The liver is the organ most commonly involved in the metabolism of endogenous and foreign compounds. Blood is transported to the liver through the portal vein which carries blood containing digested nutrients from the gastro intestinal tract and the hepatic artery which carries oxygenated blood from the lungs (Yang, 2014). The results clearly shown the plant *Ficus religiosa* doesn't cause any toxic effect. The relationship between the function of cells and organs is reflected in the organisation of tissues, visualised under the microscope. Hence histology supports the study of cell biology at all levels. Histology is also very important in diagnosis of disease and hospitals have associated laboratories and systems for examining and reporting on tissue resections and biopsies. In the present study, histopathology evaluation of *Ficus religiosa* on the liver and kidney was done after it was fed to the female albino rats and indicated that the extract did not adversely affect the morphology of the rats' organs. As indicated earlier, kidney, heart, lungs and liver tissues from control group for all toxicity studies showed normal renal, cardiac, lung tissue and hepatic morphology as well as its internal cells appearance. In fact, all animals in group treated with 2000 mg/kg extract for acute toxicity presented no morphology and physiological changes in kidneys and liver tissues as well. Based on the histopatholgy results, this showed that the treatment of *Ficus religiosa* extracts did not show any toxicological significance as no significant histopathological changes were observed in the kidney, heart, lungs and liver tissues for all toxicity studies.

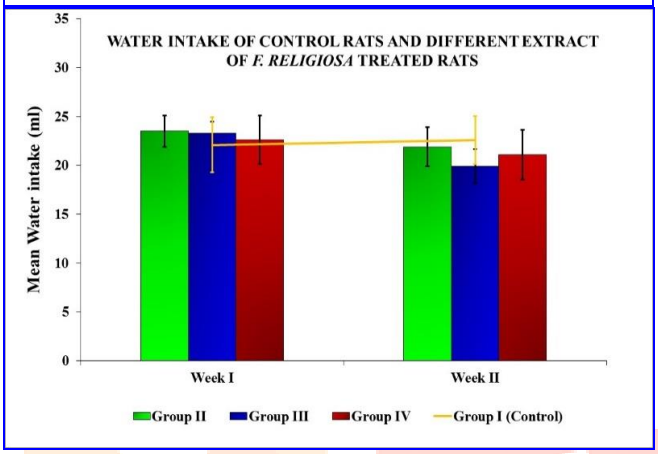
IV. Conclusion:

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 *Ficus religiosa* bark 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 *F. religiosa* treatment on body weight, food intake and water intake in albino rats.

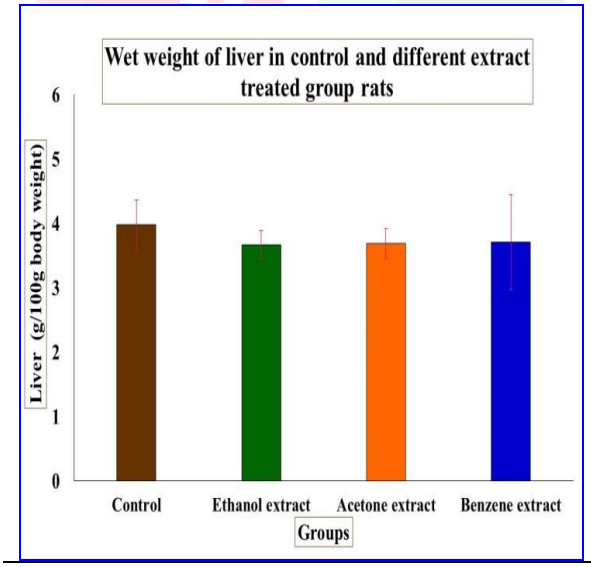


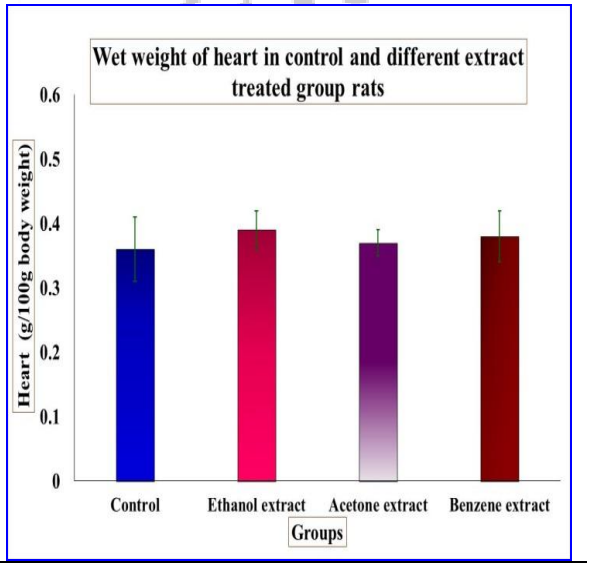


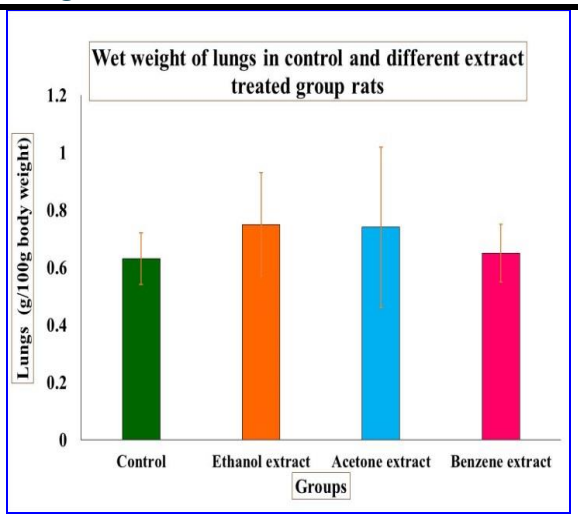


Groups: I = Control
II = Ethanol extract of *F. religiosa* treated rats
III = Acetone extract of *F. religiosa* treated rats
IV = Benzene extract of *F. religiosa* treated rats

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







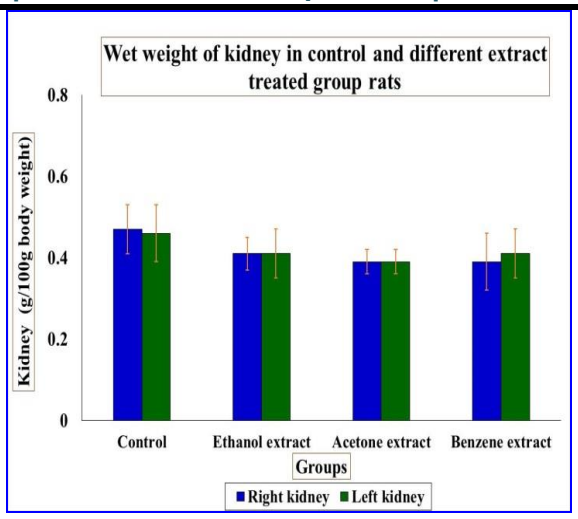
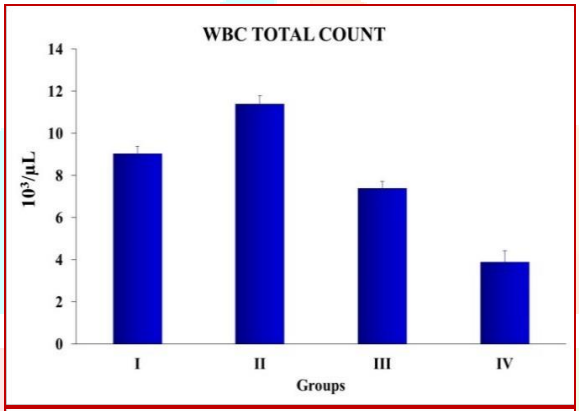
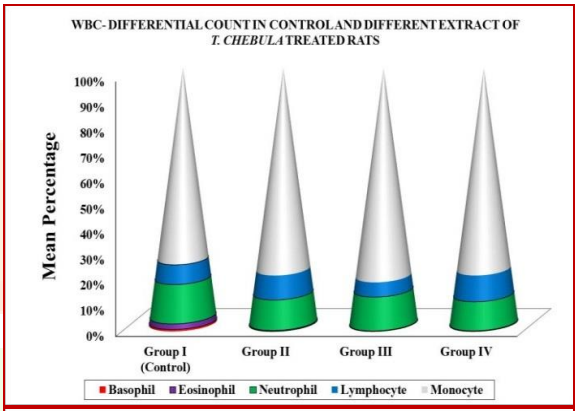
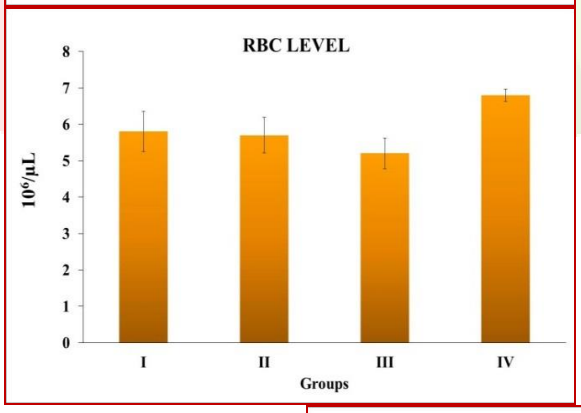
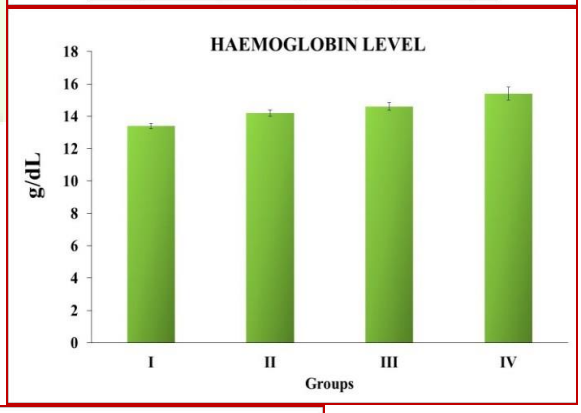


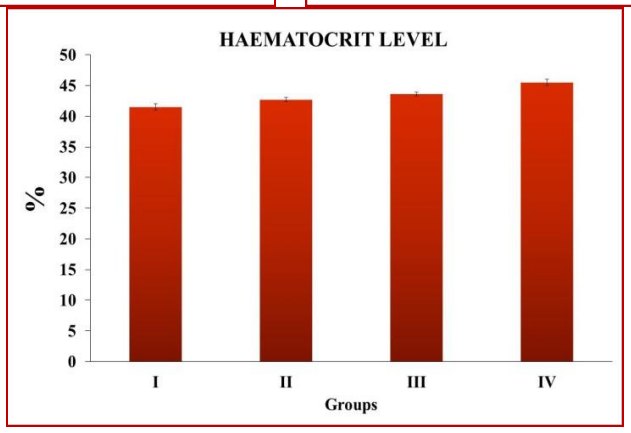
Figure 3a: Toxic effect of test drugs on haematological parameters (WBC- TC and DC, RBC, Haemoglobin and Haematocrit) in albino rats.







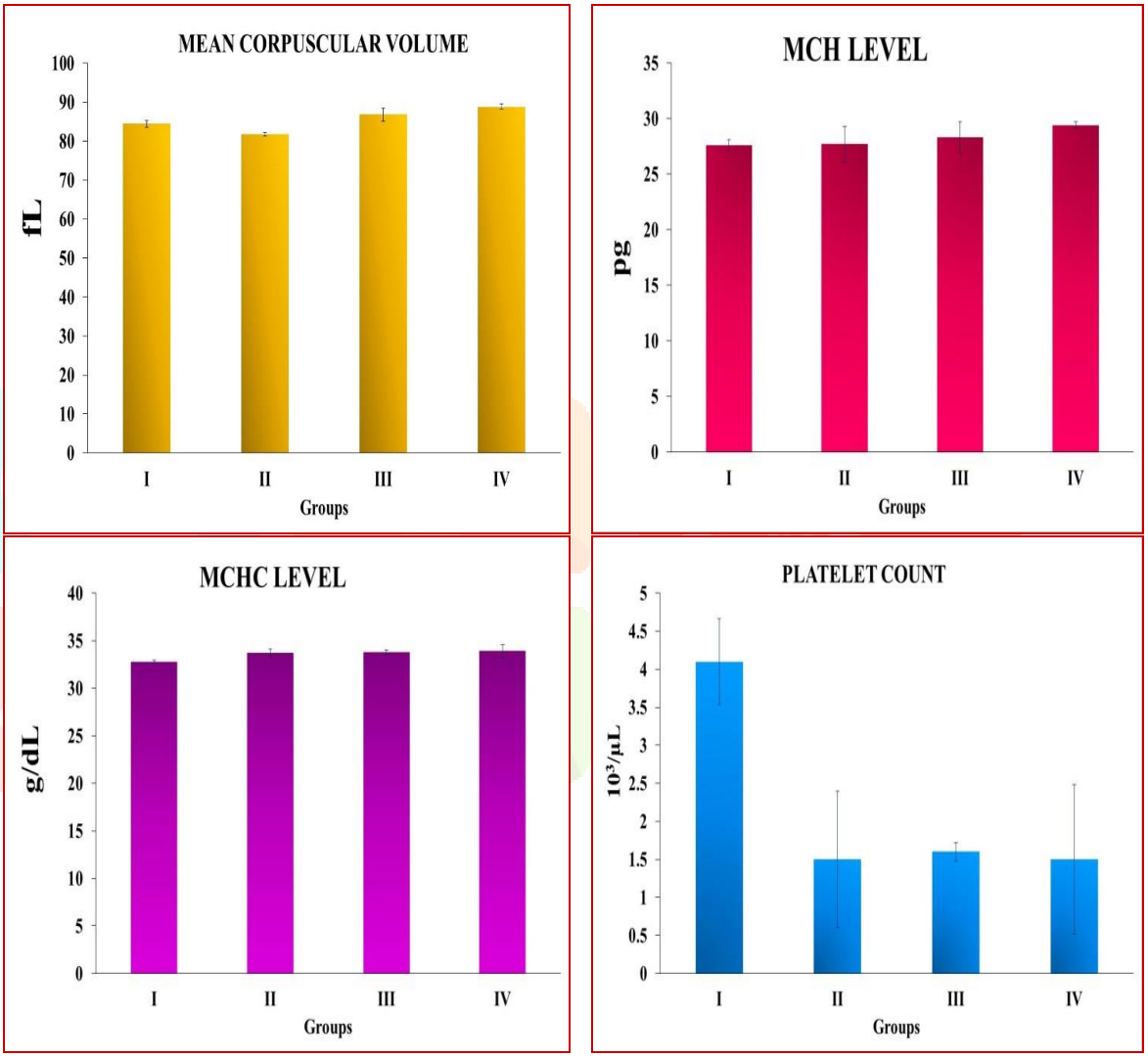




Groups:

I = Control
II = Ethanol extract of *F. religiosa* treated rats
III = Acetone extract of *F. religiosa* treated rats
IV = Benzene extract of *F. religiosa* treated rats

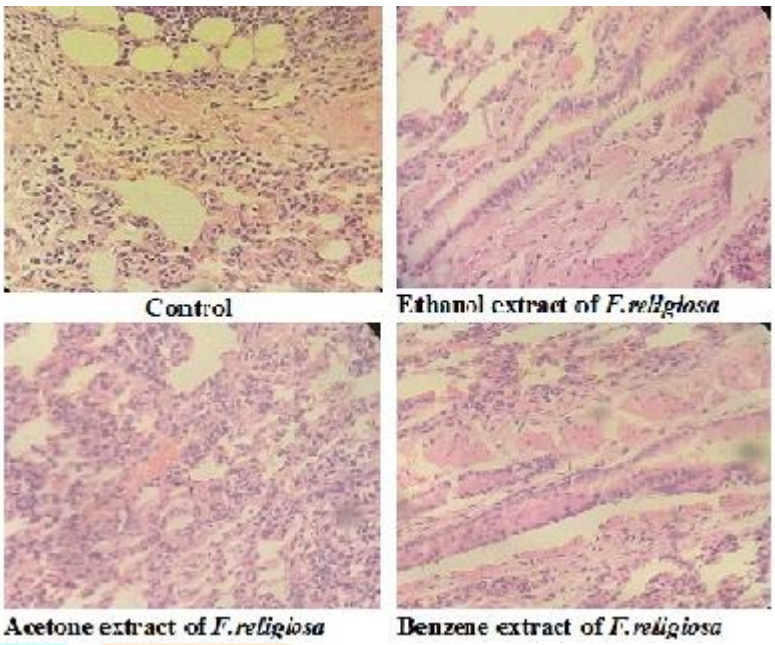
Figure 3b: Toxic effect of test drugs on haematological parameters (MCV, MCH, MCHC level and platelet count) in albino rats.



Groups:

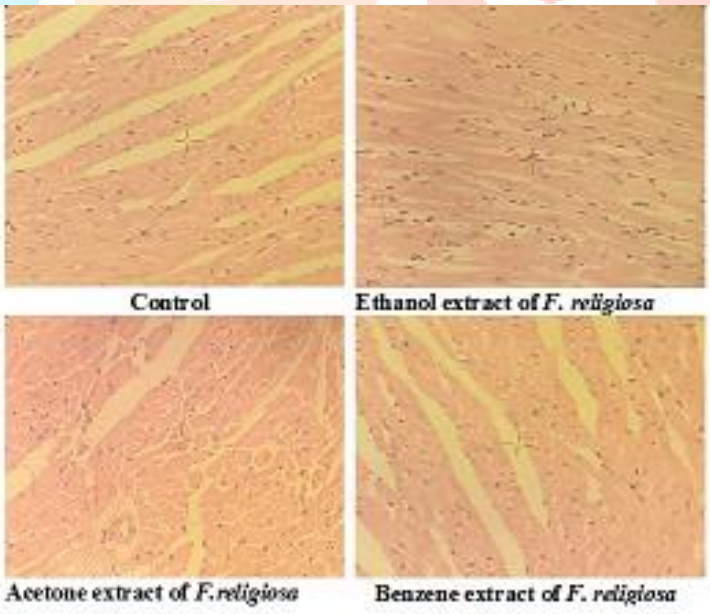
I = Control
II = Ethanol extract of *F. religiosa* treated rats
III = Acetone extract of *F. religiosa* treated rats
IV = Benzene extract of *F. religiosa* treated rats

Plate 1: Acute toxic effect of different extracts of *F. religiosa* on histoarchitecture of lungs.



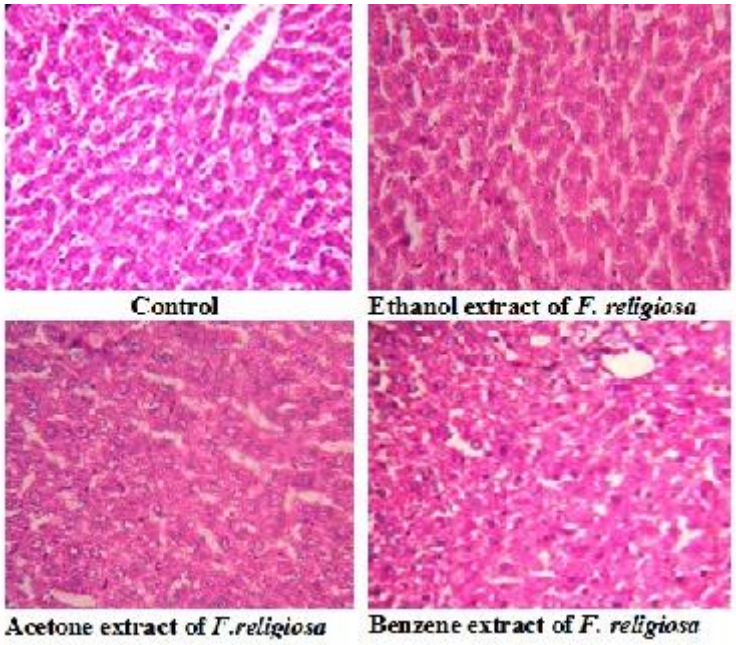
(Images showed the normal alveolar cells in both control and extract treated groups)

Plate 2: Acute toxic effect of different extracts of *F. religiosa* on histoarchitecture of heart.



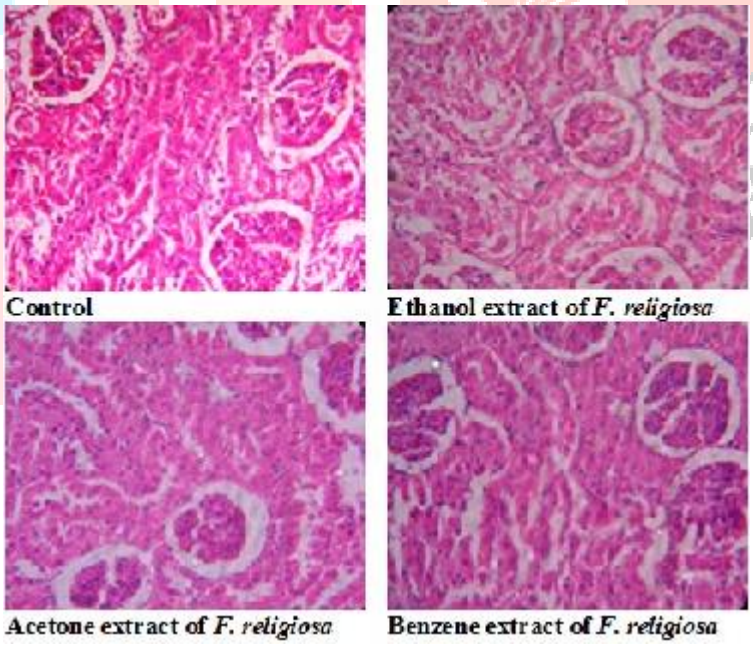
(Images showed the normal cardiac cells in all the groups)

Plate 3: Acute toxic effect of different extracts of *F. religiosa* 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 *Ficus religiosa* on histoarchitecture of kidney.



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

V. ACKNOWLEDGEMENT

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VI. REFERENCES

[1] Akindale, A. J. and Adeyemi, O. O. 2007. Antiinflammatory activity of the aqueous leaf extract of *Byrsocarpus coccineus*. *Fitoterapia*, 78(1): 25-28.

[2] Cai, Y., Mei Sun and Harold Corke. 2003. Antioxidant activity of betalains from plants of the *Amaranthaceae*. *Journal of Agricultural and Food Chemistry*, 51(8): 2288–2294.
[3] Erhirhie, E.O., Ekene, N. E. and Ajaghaku, D. L. 2014. Guidelines on dosage calculation and stock solution preparation in experimental animals' studies. *Journal of Natural Sciences Research*, 4(18): 100-106.
[4] Harbone, J. B. 1973. Phytochemical methods. A guide to modern techniques of plant analysis. *Chapman*, 279.
[5] Jorum, O. H., Ngugi Piero, M. and Alex Machocho, K. 2016. Haematological Effects of Dichloromethane-Methanolic Leaf Extracts of *Carissa edulis* (Forssk.) Vahl in Normal Rat Models. *Journal of Hematology and Thromboembolic Diseases*, 4(1): 5-2.
[6] Kamboj, V. P. 2000. Herbal medicine. *Journal of Current chemical and pharmaceutical Sciences*, 78(1): 35-9.
[7] Kokate, C. K. 1999. Practical Pharmacognosy. *Vallabh Prakashan Publication*, 111-116.
[8] Latha, R., Mary, T., Geetha. and Varalakshmi, P. 1998. Effect of *Vernonia cinerea* less flower extract in adjuvant-induced arthritis. *General Pharmacology: The Vascular System*, 31(4): 601-606.
[9] Liu and Rui Hai. 2003. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *The American Journal of Clinical Nutrition*, 78(3): 517-520.
[10] Lorke, D. 1983. A new approach to practical acute toxicity testing. *Archives of Toxicology*, 54(4): 275-287.
[11] Manach Claudine., Françoise Regerat, Odile Texier., Georgine Agullo., Christian Demigne and Christian Remesy. 1996. Bioavailability, metabolism and physiological impact of 4-oxo-flavonoids. *Nutrition Research*, 16(3): 517-544.
[12] Murugi Njagi Joan., Ngugi Mathew Piero., Kibiti Cromwell Mwiti., Ngeranwa Joseph, N. J., Njagi Eliud Mwaniki, N., Njue Wilson, M., Maina David. and Gathumbi Peter Karuri. 2012. Hypoglycemic effects of *Caesalpinia volkensii* diabetic mice. *Asian Journal of Phytomedicine and Clinical Research*, 5: 69-74.
[13] Musila, F. M., Joseph Nguta, M., Catherine Lukhoba, W. and Saifuddin Dossaji, F. 2017. Antibacterial and antifungal activities of 10 Kenyan *Plectranthus* species in the Coleus clade. *Journal of Pharmacy Research*, 11(8): 1003-1014.
[14] Olaley Mary Tolulope. 2007. Cytotoxicity and antibacterial activity of methanolic extract of *Hibiscus sabdariffa*. *Journal of Medicinal Plants Research*, 1(1): 009-013.
[15] Raaman, N. 2006. Phytochemical Techniques. *New India Publishing Agency, International Standard Book Number*, 81(89422): 30-8.
[16] Rajpal, V. 2002. Testing and extraction methods of Medicinal Herbal, Standardisation of Botanicals. *Estern Publishers, New Delhi*, 2: 124.
[17] Rekha, S and Vidyasagar, G. M. 2014. Anti-Candida activity of medicinal plants. A review. International *Journal of Pharmacy and Pharmaceutical Sciences*, 5(4): 9-16
[18] Singh, G., Sumitra Maurya and Cesar Catalan, A. N. 2007. A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents. *Food and chemical toxicology*, 45(9): 1650-1661.
[19] Ugbogu. E. A., Emmanuel Okezie., Iheanyichukwu Elekwa., Friday Uhegbu., Emmanuel Akubugwo., Chinyere Godwin Chinyere., Faith Ewuzie and Chizoba Jennifer Ugorji. 2016. Toxicological assessment of the aqueous dried leaf extracts of *Senna alata L.* in Wistar rats. *African Journal of Pharmacy and Pharmacology*, 10(34):709-717.
[20] Varadarajan, P., Rathinaswamy, G. and Asirvatahm, D. 2008. Antimicrobial properties and phytochemical constituents of Rheo discolor. *Ethnobotanical Leaf*, 12: 841–845.
[21] Xiang Hua Han., Seong Su Hong., Ji Sang Hwang., Myung Koo Lee., Bang Yeon Hwang and Jai Seup Ro. 2007. Monoamine oxidase inhibitory components from *Cayratia japonica*. *Archives of Pharmacal Research*, 30(1): 13.
[22] Yang, X., Schnackenberg, L.K., Shi, Q. and Salminen, W. F. 2014.HepaticToxicity Biomarkers. *In Biomarkers in Toxicology, Academic Press: Burlington, MA, USA*, 103-112.
[23] Zheng, W and Wang, S.Y. 2001. Antioxidant activity and phenolic compounds in selected herbs. *Journal of Agricultural and Food Chemistry*, 49(11): 5165–5170.