PHYTOCHEMISTRY AND TOXICITY EVALUATION OF DIFFERENT EXTRACTS OF *EMBLICA OFFICINALIS ON ALBINO RATS*

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Abstract: The present study aims to test the toxic effect of the fruit extract of *Emblica officinalis* using three different solvents *viz* ethanol, acetone and benzene by examine the changes in behaviour, body weight, food intake, water intake, haematological parameters 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. 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 *Emblica officinalis* fruit did not produce any toxic effects at the high dose of 2000mg/kg body weight, and is found to be safe, and used for further evaluation studies.

Keywords: *Emblica officinalis*, Phytochemical screening, Toxicity study, Behavioural changes and Haematological and Histological changes.

I. INTRODUCTION

In all countries of the world, there exists traditional knowledge related to the health of humans and animals (Balick *et al*., 1996). 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). The medicinal value of these plants lies in some chemical substance that produces a definite physiological action on the human body (Edoga *et al*., 2005). The therapeutic potential of plants has surged over the years with volumes of scientifically documented information showing considerable potential for medicinal plants to be used in the treatment of several diseases (Idris Bello *et al*., 2016). Many plants synthesize substances that are useful for the maintenance of health in humans and other animals. These substances, most of which are phenols or their oxygen substituted derivatives such as tannins, secondary metabolites of plants which have been isolated are at least 1200 (Fabricant, 2001).

Plants synthesize these compounds to protect themselves against insect attacks and plant diseases, they have many health benefits in human body (John *et al*., 1995). There is limited report of the proper evaluations of the toxicity of these medicinal plants. Thus, proper phytochemical screening of the plant is necessary because plants can synthesize toxic substances to protect themselves against infections, insects and other organisms which feed on them. Various groups of compounds are responsible for the toxic effects of these plants. Major bio-active 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 (Van Wyk *et al.,* 2002). Recent investigations have also revealed the presence of genotoxic, mutagenic and carcinogenic compounds in many plants used as traditional medicine or food both in vitro and in vivo assays (Demma *et al.,* 2009). However, some toxic plants are used by doctors for the treatment of diseases (Botha and Penrith, 2008). Toxicity data are required to predict the safety associated before the use of medicinal products. 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. 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 (Chanda *et al*., 2015). Thus the present

study focused on the preliminary phytochemical analysis to know the bio-active compounds of the medicinal plant *Emblica officinalis* and in order to know biosafety of the plant extracts, acute toxicity test is to be performed through *in vivo* studies.

II. MATERIALS AND METHODS

The present study was carried out from November 2017 to January 2018. The process of extraction and formulation of the traditional remedy is as described by Sohini *et al* (1996). The powder of *Emblica officinalis* 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 *E. officinalis* 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 *E. officinalis* was subjected to analyse the preliminary phytochemicals such as alkaloids, carbohydrates, Fixed oils and fats, flavonoids, glycosides, phenolic compounds, protein, steroids, saponins, tannins and terpenenoids 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.

Healthy adult female 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 (Tarsons make 43 x 27 x 15cm size cage). There were three different types of extracts (ethanol, acetone and benzene extract of *Emblica officinalis*) separately tested for their toxic effect. The rats were treated orally with 2000 mg extract/kg body weight by oral gavage needle to the rats for 14 days. The rats in both the test and control group were allowed to access food and water easily.

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 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, rats were sacrificed and the blood was collected in EDTA tube for haematological analysis. 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.

2.3. 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

Plants are significant and perennial sources of food and medicines that are used for the treatment of various human diseases (Revathi *et al.,* 2017). The plant parts used for medicinal purposes are root, seed, fruit, bark and leaf (Elavarasi *et al.,* 2017). Herbal drug is a chief constituent in traditional medicine and a common constituent in ayurvedic, homeopathic, naturopathic and other medicine systems (Maiti *et al*., 2011). Phytochemical constituents are secondary metabolites of plants that serve as a defence mechanism against many microorganisms, insects and other herbivores (Rahman *et al*., 1989).

3.1. Percentage of extraction

The yield of crude ethanol extract of *Emblica officinalis* is 12.48% whereas yield of crude acetone extract of *E. officinalis* is 8.32%. The percentage yield of the benzene extract of *E. officinalis* is 2.49%.

3.2. Preliminary Phytochemical analysis

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 anti-inflammatory effects (Akindale and Adeyemi, 2007). The ethanol, acetone and benzene fruit extract of *E. officinalis* also possess the phytochemical such as phenols, protein, saponin, oil and carbohydrates. Tannins are complexes of phenol and water-soluble nature. The action of tannins as free radical scavengers, which is a function of the active oxygen interception forming stable radical, helps prevent many degenerative diseases such as cancer, multiple sclerosis, atherosclerosis, and the aging process itself (Mello and Santas, 2007). The bactericidal and fungicidal activities occur for three general characteristics common to both tannins groups: complexation with metal ions; antioxidant activity and scavenging of free radicals; complexing ability with other molecules, especially proteins and polysaccharides (Mello and Santas, 2007). The ethanol and acetone extract possess tannins. The ethanol and benzene extract of the fruit contains carbohydrates. Similarly, ethanol, acetone and benzene extracts of *S. china* rhizome showed the presence of terpenoids, oil and carbohydrates. Steroids and triterpenoids showed the analgesic properties (Sayyah *et al*., 2004; Malairajan *et al*., 2006). The steroids and saponins are responsible for central nervous system activities (Argal and Pathak, 2006).

3.3. Acute toxic effect of the plant extract:

Acute toxicity is usually an initial study performed: to serve as the basis for classification and labeling, to provide initial information on the mode of toxic action of a substance, to help arrive at a dose of a new compound and to help in dose determination in animal studies (Ukwuani *et al*., 2012). Toxicity study results showed no drastic changes in the body weight, food intake and water intake throughout the experimental period. No toxic signs and mortality was observed in all the different plant extract treated groups. There were no noticeable changes in the general behaviour, no toxicity signs and mortality observed in rats treated with test drug orally at 2000 mg/kg body weight for a period of 14 days.

Weekly body weight changes among the different extract treated rats and control rats are given in the Table 1. The control rats and the extract treated rats showed normal increase in their body weight throughout the experimental period. The mean food intake and water intake of control and different extracts treated rats during the experimental period was illustrated in Table 1. The control rats showed normal food and water intake throughout the experimental period. The food intake of ethanol, acetone and benzene extract of *E. officinalis* treated rats showed slight increase in week I but it showed decreased level of food intake in week II. Ethanol, acetone and benzene extracts of *E. officinalis* treated rats showed decreased level of water intake in week II when compared to the water intake of the rats in initial day. 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.

Dosage calculation and stock solution preparation in preclinical studies, involving the use of experimental animals is important in screening and development of new drugs (Erhirhie *et al*., 2014). Effect of treatment of the plant extract on relative organ weights are given in Table 2. The relative weight of lungs, heart, liver and kidney of ethanol, acetone and benzene extract of *E. officinalis* treated rats was observed to be more or less similar to that of control rats.

Evaluation of hematological parameters represents an important and relevant risk evaluation as the changes in the hematological system have a higher predictive value for human toxicity, when the popular data are translated from animal studies (Olson *et al*., 2000). In addition, hematological analyses provide information about the 360 mg of the extract, translating to an intake of 5.14 mg/kg per day hematopoietic system and immunological responses (Igwebuike and Obidike, 2007). Haematological parameters of control and extract treated rats are depicted in Table 4. 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 (9.03 ± 0.35 10^3/µL). Basophil was totally absent in the extract of *E. officinalis* treated rats and the lymphocyte count of benzene extract treated rats was similar (0.19 ± 0.002%) when compared to control (0.19 ± 0.009%) and the ethanol, and acetone extract treated rats. 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 (SNK test).

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 both extract treated and control 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 and extract treated rats showed normal hepatic lobules, hepatocytes and central vein. The cell cords were separated by narrow blood sinusoids. 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 and blood vessels 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.

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 *E. officinalis* fruit extract at 2000 mg/kg body weight does not produce any toxic effect in the ethanol, acetone and benzene extract treated rats and it can be used for further evaluation.

Table 1: Toxic effect of test drugs on body weight, food and water intake in different groups of the albino rats. Values within the parentheses are range of respective mean.

Parameter	Group	Week 1 (Mean ± SD)	Week 2 (Mean ± SD)
Body weight (g)	I	207.8 ± 8.59 (197.0 – 227.0)	216.2 ± 10.97 (206.0 – 237.0)
	II	186.9 ± 3.75 (183.0 – 195.0)	190.8 ± 5.16 (184.0 – 200.0)
	III	193.4 ± 6.43 (186.0 – 204.0)	196.1 ± 6.14 (188.0 – 205.0)
	IV	188.2 ± 3.55 (183.0 – 195.0)	191.3 ± 3.52 (186.0 – 200.0)
Food intake (g)	I	19.3 ± 2.59 (16.2 - 23.8)	18.9 ± 2.09 (15.8 - 22.2)
	II	19.4 ± 0.56 (18.8 - 20)	18.6 ± 1.79 (15.8 - 20.8)
	III	17.8 ± 2.13 (14.8 - 20.5)	17.1 ± 0.96 (15.5 - 18.5)
	IV	17.4 ± 1.97 (14.5 - 19.5)	18.1 ± 1.74 (15.5 - 20.2)
Water intake (ml)	I	22.1 ± 1.85 (18.8 - 24)	22.6 ± 2.57 (19 - 25.5)
	II	21.9 ± 2.13 (18.8 - 24.5)	21.1 ± 2.51 (17.2 - 24.5)
	III	22.4 ± 2.58 (19 - 25.5)	22.3 ± 1.51 (20.0 - 24.2)
	IV	22.5 ± 2.33 (19.8 - 25.5)	20.7 ± 1.94 (18.2 - 23.5)

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

Table 2: Toxic effect of test drugs on organ weight in different groups of the albino rats. Values within the parentheses are range of respective mean.

Groups	Organ weight (g/100g body weight)				
	Liver (Mean ± SD)	Heart (Mean ± SD)	Lungs (Mean ± SD)	Right Kidney (Mean ± SD)	Left Kidney (Mean ± SD)
I	3.98 ±0.39 (3.40 – 4.26)	0.4 ± 0.05 (0.31 – 0.44)	0.6 ± 0.09 (0.55 – 0.75)	0.5 ± 0.06 (0.38 – 0.53)	0.5 ± 0.07 (0.38 – 0.54)
II	3.96 ± 1.135 (2.70 – 5.46)	0.4 ± 0.09 (0.29 – 0.51)	0.6 ± 0.23 (0.48 – 0.98)	0.5 ± 0.11 (0.33 – 0.59)	0.4 ± 0.12 (0.31 – 0.60)
III	3.88 ± 1.114 (2.81 – 5.19)	0.4 ± 0.16 (0.26 – 0.61)	0.8 ± 0.28 (0.50 – 1.16)	0.4 ± 0.14 (0.30 – 0.53)	0.4 ± 0.11 (0.30 – 0.51)
IV	3.87 ± 1.004 (2.73 – 5.17)	0.4 ± 0.04 (0.30 – 0.40)	0.8 ± 0.21 (0.62 – 1.06)	0.4 ± 0.62 (0.35 – 0.47)	0.4 ± 0.08 (0.34 – 0.50)

Groups: I = Control; II = Ethanol extract of *E. officinalis* treated rats;
III = Acetone extract of *E. officinalis* treated rats; IV = Benzene extract of *E. officinalis* treated rats

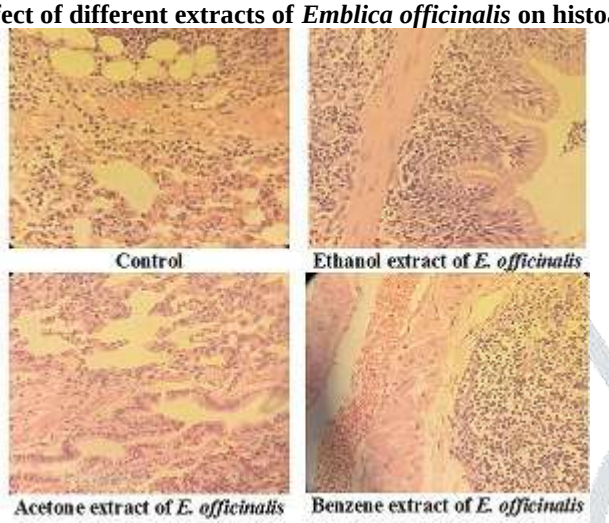
Table 3: Student-Newman-Keuls (SNK) post hoc test results show the toxic effect of ethanol, acetone and benzene extract of *E. officinalis* on haematological parameters of different groups of the albino rats. Mean values are arranged in ascending order. Horizontal lines connect similar means.

Parameters	Groups			
WBC (10^3/µL)	4.075 (III)	4.550 (IV)	7.775 (II)	9.025 (I)
Neutrophil (%)	0.211 (II)	0.222 (IV)	0.225 (III)	0.389 (I)
Eosinophil (%)	0.001 (II)	0.004 (IV)	0.004 (III)	0.006 (I)
Basophil (%)	0.0000 (III)	0.004 (II)	0.004 (IV)	0.019 (I)
Lymphocyte (%)	0.174 (III)	0.19 (IV)	0.19 (I)	0.22 (II)
Monocyte (%)	0.004 (II)	0.004 (III)	0.01 (I)	0.02 (IV)
RBC (10^6/µL)	5.79 (I)	5.98 (IV)	6.75 (II)	6.82 (III)
Haemoglobin (g/dL)	13.23 (II)	13.38 (I)	14.25 (III)	15.25 (IV)
PCV/HCT (%)	40.68 (IV)	40.70 (II)	41.45 (I)	41.98 (III)
MCV (fL)	83.00 (IV)	83.88 (II)	84.35 (I)	85.40 (III)
MCH (pg)	25.45 (IV)	26.8 (III)	27.63 (II)	27.63 (I)
MCHC (g/dL)	32.75 (I)	33.68 (II)	33.68 (III)	35.33 (IV)

Platelet (10^3/µL)	1.53 (IV) ◄►	1.78 (II) ◄►	1.88 (III) ◄►	4.06 (I) ◄►

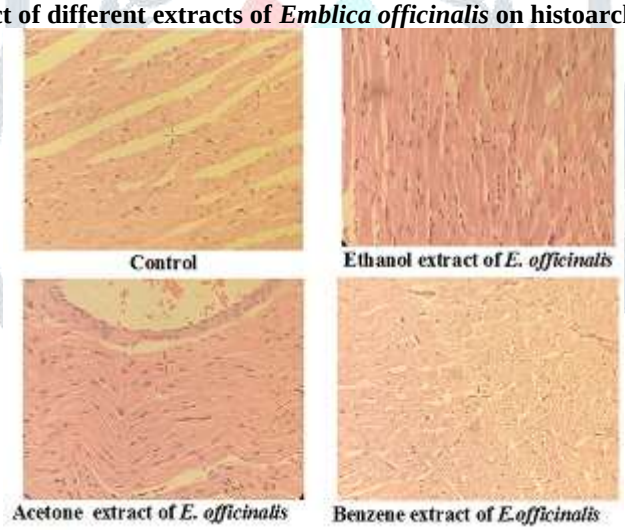
Groups: I = Control rats (Group I); II = Ethanol extract of *E. officinalis* treated rats (Group II)
III = Acetone extract of *E. officinalis* treated rats (Group III)
IV = Benzene extract of *E. officinalis* treated rats (Group IV)

Plate: 1. Acute toxic effect of different extracts of *Emblica officinalis* on histoarchitecture of lungs.



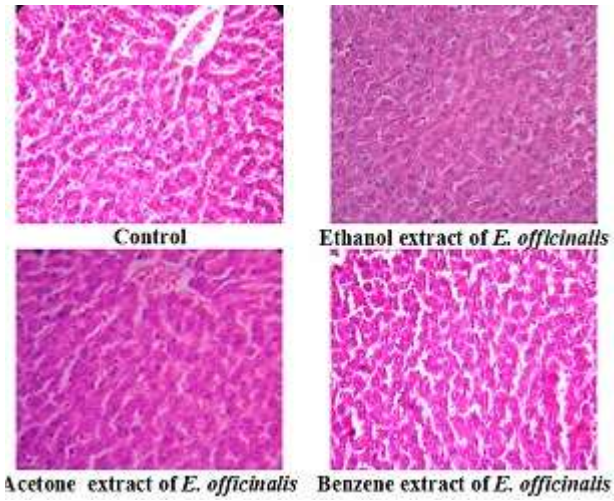
(Images showed the normal alveolar cells in both control and extract treated groups)

Plate 2: Acute toxic effect of different extracts of *Emblica officinalis* on histoarchitecture of heart.



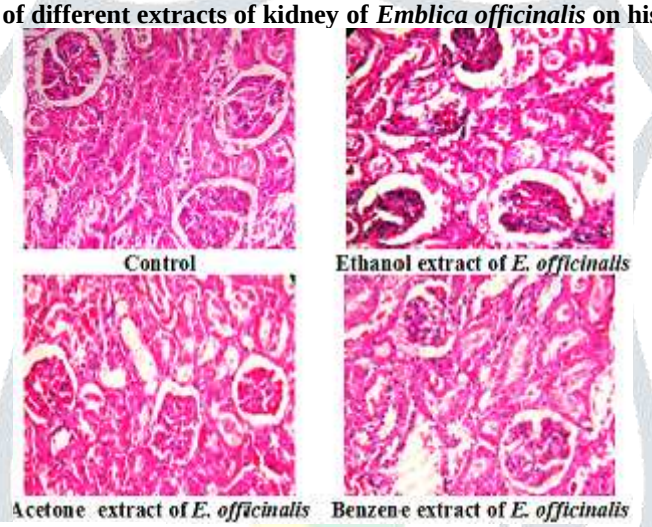
(Images showed the normal cardiac cells in all the groups)

Plate 3: Acute toxic effect of different extracts of *Emblica officinalis* on histoarchitecture of liver.



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

Plate 4: Acute toxic effect of different extracts of kidney of *Emblica officinalis* on histoarchitecture of kidney.



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

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