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Original Article

A STUDY ON ACUTE TOXICITY OF METHANOLIC EXTRACT OF *MESUA FERREA* L. IN SWISS ALBINO MICE

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ABSTRACT

Objective: To evaluate the Acute toxicological studies of the methanolic extract of dried flowers of *Mesua ferrea* in experimental female Swiss albino mice.

Methods: Acute toxicological studies of the methanolic extract of dried flowers of *M. ferrea* in experimental female Swiss albino mice of four groups were conducted as per OECD guidelines. The parameters were screened such as Physical observation time, Body weight, food and water consumption, Haematological and biochemical parameters. The doses used were 50 mg/kg, 500 mg/kg and 2000 mg/kg.

Results: None of the group examined showed significant change in the body weights and mortality. At the dose levels tested, no toxicity signs were observed in the mice. There is no significant difference between control and treated animals in their haematological as well as biochemical test results. One way analysis of variance (ANOVA) was used to determine the significance between groups.

Conclusion: This study was concluded that the acute toxicity study of *M. ferrea* leaf extract administered orally to mice did not caused any death or acute adverse effect on the clinical observation and mortality to the treatment animals.

Keywords: *M. ferrea,* methanolic extract, experimental female Swiss albino mice, acute toxicity.

INTRODUCTION

Many modern medicinal agents have been taken from nature. Herbal medicines are assumed to be of great importance in the primary health care of individual and communities [1]. Epidemiological studies consistently show that increased consumption of plant-based, antioxidant-rich foods, i.e., fruits, vegetables, whole grains, and nuts, is associated with the reduced risk for several chronic diseases. Present estimates indicate that about eighty percent of the world's population relies on traditional medicine for health care delivery [2]. Studies of medicinal plants using scientific approaches showed that various biological components of medicinal plants exhibit a variety of properties and can be used to treat various ailments. However, a number of studies have reported the toxic effects of herbal medicines [3].

Medicinal plants play a key role in the human health care. About 80% of the world population relies on traditional medicine which is based on plants [1]. Herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness. These drugs are either single plant extracts or fractions or mixtures of extracts from different plants. The plant extracts are standardized for their safety and efficacy [4].

Natural herbs and their constituents have given first knowledge about the treatment of new diseases. Hence, natural medicine like Ayurvedic drugs still remain a popular practice in many countries like India, China etc. Natural medicines have wide important roles in this country [5].

Plant-based traditional knowledge has become a recognized tool in search for new sources of drugs and neutraceuticals. Traditional people are getting the benefits of this practice from ancient times [3]. But, the uses and the safety profile of all of the Ayurvedic medicines are not ensured scientifically. The high degree of efficacy and safety with herbal medicines make them more acceptable compared to other therapeutic invention [1]. Moreover, the conflict between traditional medicines and allopathic medicine are needed to be addressed scientifically in the in vivo and in vitro experimental model.

M. ferrea is an evergreen tree grows up to 20-30 meters in height and it is belongs to the family "Clusiaceae". Paste of its leaves can use against severe head ache and cold. Decotion of bark and roots of this plant are useful in gastritis. Leaves and flowers can be a relief from scorpion stings. The main aim of our study was to evaluate the extract for their toxic effects before it can be used for applications that are of importance to the public.

MATERIALS AND METHODS

Plant material

M. ferrea plant leaves are collected from Konny Forest (Reserve), Pathanamthitta, Kerala. Plant sample was identified from Botanical Survey of India, Southern Regional Centre, Coimbatore- 641 002 (Voucher No. : BSI/SRC/5/23/2013-14/Tech./1712). The leaves were shade dried for 15 days. The dried plant material was ground coarsely and stored for further use. The powdered samples were extracted with methanol in a soxhlet apparatus. Crude methanolic extract was used in this study to evaluate its toxicity effect.

Experimental animals

Study was performed as per Organization for Economic Co-operation and Development (OECD) guidelines 423[6] (OECD, 2011). The institutional ethical committee of KMCH College of Pharmacy, Coimbatore, Tamil Nadu, India approved the protocol for these experiments under number (KMCRET/Ph.D/14/2012-2013). Experiments were performed using healthy young adult female Swiss albino mice, nulliparous, non-pregnant and weighing 25-30 g. Female mice were chosen because of their greater sensitivity to treatment.

Grouping of animals

The animals were randomly divided into four groups each containing six mice. They were identified by the markings in different body part. Animals were marked on head, body, tail, head and body, body and tail and one mouse with no marking to ease the observation.

Caging and Diet

The animals were allowed to stay in polypropylene cages (55 x 32.7 x 19 cm), with sawdust litter in a temperature controlled environment (23 ± 2 ºC). Lighting was controlled to supply 12 h of light and 12 h of dark for each 24-h period. Each cage was identified by a card. This card stated the cage number, number and weight of the animals it contained, test substance code, administration route and dose level. The animals were fed with standard laboratory animal food pellets with water ad libitum.

Mode of administration and test substance volume

The test substance was administered in a single dose by gavage using specially designed mice oral needle. Animals were fasted 3 h prior to dosing (only food was withheld for 3 h but not water).Following the period of fasting, animals were weighed and test substance was administered orally at a dose of 50, 500 and 2000 mg/kg. After the administration of test substance, food for the mice was withheld for 2 h. The administration volume was 1ml/kg body weight of the animal. Based on the body weight of the animal on the day of treatment, the quantity of the test substance was calculated.

Physical observation time

Animals were observed individually after at least once during the first 30 min, periodically during the first 24 h, with special attention given during the first 4 h, and daily thereafter, for a total of 14 days. All the animals were observed at least twice daily with the purpose of recording any symptoms of ill-health or behavioral changes.

Body weight, food and water consumption

The body weights of each animal were recorded once weekly. The differences of the body weights were recorded. The amounts of test substance to be given were calculated again weekly based on the new body weights of the experimental mice to ensure a constant dose volume/kg body weights of the test substance given to the mice. The amounts of food and water intake were recorded.

Haematological and biochemical parameters

The blood samples of each animal from each group were collected through capillary tube and introduced in to a centrifugation tube which contains EDTA. Separation of serum was done by centrifugation of blood samples at 10000 rpm for 10 min. The serum was carefully removed and subjected to haematological parameters such as RBC, WBC and Hb percentage, differential cell count, MCV, MCHC, hematocrit, MCH, platelet count and biochemical parameters such as SGOT, SGPT, alkaline phosphatase and bilirubin were estimated according to standard procedures.

Statistical analysis

All findings such as body weight changes, food and water consumption, haematology and blood chemistry were tabulated and analyzed. Statistical analysis was carried out by using Graph Pad Prism (6.0 Version).One way analysis of variance (ANOVA) was used to determine the significance between groups at $p<0.05$.

RESULTS AND DISCUSSION

The use of herbal preparations as a treatment of diseases is very common. *M. ferrea* plant parts have been used traditionally by some population. The plant is used in inflammation and septic conditions [7].In Thai traditional medicine, it is used to treat fever, cold, asthma and as carminative, expectorant, cardiotonic, diuretic and antipyretic agent [8]. The methanolic extract of dried flowers of *M. ferrea* (100 and 200 mg/kg) was screened for *in vivo* antioxidant and hepatoprotective activity in experimental female Swiss albino mice. The ethanolic extract of flowers showed potent inhibitory activity (96.03%) at 100 µg/ml against nitric oxide (NO) assay [9].The aqueous extract of *M. ferrea* leaves was screened for its activity against fibroblast cell lysis after *Heterometrus laoticus* scorpion venom treatment [10]. Xanthones from *M. ferrea* were screened for antiulcer activity by pyloric ligation method in Swiss albino mice [11]. Ethanol and petroleum ether extract is used for sore throat, cough and asthma [12, 13, 14].

Toxicology of *M. ferrea* was also carried out in mice. The doses used were 50 mg/kg, 500 mg/kg and 2000 mg/kg. None of the group examined showed significant change in the body weights and mortality.

Physical observation time

At the dose levels tested, no toxicity signs were observed in the mice [15]. Physical observation of the extract treated animals throughout the study indicated that none of the them showed signs of toxic effect such as changes on skin and fur, eyes and mucus membrane, behaviour pattern, tremors, salivation, diarrhoea, sleep and coma. No mortality was observed in any of the mice.

Body weight, food and water consumption

The body weight of the treatment and control animals were as shown in Table 1. There were gradual increases in body weight of treatment and control mice [16]. The body weight of the treatment mice were not significant different as compared to the control (Table 1). The food and water consumption of the treatment animals were also not significantly different as compared to the control measured throughout the study (Table 2).

Table 1: Body weight of control and drug treated animals.

	Control	50mg/kg	500mg/kg	2000mg/kg
Initial	19.4±0.7483	26.4±0.6782***	26.8±1.2806***	23.6±0.9273**
Day 3	22.72±0.7670	27.94±1.2496***	28.08±1.8950***	25.5±1.3034**
Day 7	21.84±0.9682	27.82±1.5170***	27.22±1.7953***	25.3±1.6543**
Day 10	22.6±1.1979	28.16±1.514***	27.8±1.8804***	25.8±1.6946**
Day 14	23.88±1.1555	25.9±1.5391***	29.36±2.322***	25.16±1.9983**

Values are expressed as the mean ± S.E.M.; Statistical significance (p) calculated by one way ANOVA. ***P< 0.001, **P< 0.01, *P< 0.05, NS – non-significant calculated by comparing treated group with control group.

Table 2: Food and water intake of control and drug treated animals.

	Control	50mg/kg	500mg/kg	2000mg/kg
Food intake	12.36±0.468	4.156±0.321***	5.132±0.758***	5.538±0.775***
Water intake	17.24±1.852	2.96±0.278	3.587±0.4349	4.007±0.4286

Values are expressed as the mean ± S.E.M.; Statistical significance (p) calculated by one way ANOVA followed by Dennett's ***P< 0.001, **P< 0.01, *P< 0.05, NS – non significant.

Haematological and biochemical parameters

Haematological and biochemical parameters are presented in Table 3 and 4 respectively. Results revealed there is not much variations in drug treated animals from control group. There is no significant difference between control and treated animals in their haematological as well as biochemical test results. Results are comparable to control animals.

Table 3: Haematological parameters of control and drug treated animals.

	Control	50mg/kg	500mg/kg	2000mg/kg
RBC (106l)	8.952±0.0932	8.958±0.2461	9.132±0.1841	8.75±0.4115
WBC(103l)	7.9±1.1730	7.3±1.3992	13.38±2.357	12.66±1.7918
HB (g/dl)	15.8±0.2097	16.18±0.5112	15.86±0.1860	15.56±0.9266
PCV (%)	47±1.0954	49.22±1.486	48.2±0.5830	48.2±2.7092
RDW (%)	28.2±0.6964	27.4±2.2438	29.06±0.5436	27.64±0.4130
Lymphocyte (%)	22.8±6.9455	22.6±5.6	16.6±4.9558	14.8±2.6907
Monocyte (%)	10.6±1.0770	11±0.8366	9.4±0.6	11±2.213
Polymorphs (%)	50±8.0808	49.6±6.2337	57.6±6.7572	58.4±6.0133
***Eosinophil* (%)**	16.6±1.860	16.8±1.9339	16.4±1.6	15.8±2.5573
MCH (Pg)	17.52±0.2989	18.04±0.5653	17.34±0.1777	15.72±2.1866
MCHC (g/dl)	33.62±0.4127	31.64±0.6801	32.86±0.0678	31.48±1.678
MPV (fl)	7.32±0.2222	7.28±0.2817	7.32±0.1392	8.02±0.365
MCV(fl)	52.52±1.3066	56.72±1.701	52.84±0.4781	56.5±1.4289

Values are expressed as the mean ± S.E.M.; Statistical significance (p) calculated by one way ANOVA followed by Dunnett's ***P< 0.001, **P< 0.01, *P< 0.05, NS – non significant calculated by comparing treated group with control group.

Table 4: Biochemical data of control and treated animals.

	Control	50mg/kg	500mg/kg	2000mg/kg
SGOT	50±0.03	57.600±1.498*	75.600±0.4**	87.91±0.792***
SGPT	42.8±0.5830	61.31±0.2991*	67.000±0.774**	72.55±0.4971***
ALP	127±1.2649	149.200±1.781*	150.53±0.4***	229.14±9.341***
Bilirubin	0.74±0.04	0.840±0.04	0.953±0.097	1.2500.0517

Values are expressed as mean ± standard deviation; p value less than 0.05, ($p < 0.05$): significant value, WBC- white blood cell, RBC- red blood cells, HB- haemoglobin, PCV-platelet count volume, MCH- mean cell haemoglobin, MCHC- mean corpuscular haemoglobin concentration, MPV- Mean platelet volume, MCV- mean corpuscular volume.

CONCLUSION

It was concluded that the acute toxicity study of *M. ferrea* leaf extract administered orally to mice did not caused any death or acute adverse effect on the clinical observation and mortality to the treatment animals. Body weight, haematological and biochemical investigations on the tested animals did not showed any significant changes from control animals. This data will be monitored in the sub-acute toxicity study.

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