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Toxicological Evaluations of *Eranthemum roseum* R. Br Linn Root extracts

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ABSTRACT: Present investigation was done to evaluate acute and chronic toxicity of Methanolic extract of ***Eranthemum roseum* (MER),** From the study it was found that the LD_{50} Methanolic extract of ***Eranthemum roseum* (MER**) is greater than 2000mg/kg. In chronic toxicity study three dose levels were selected and administered up to 90 days. Methanolic extract of *Eranthemum roseum* (ER) were administered orally at 180 mg/kg (low dose), 450 mg/kg (medium dose) and 900 mg/kg (high dose) for chronic toxicity of 90 days , and the effects on body weight, food consumption, organ weight, urine analysis, hematology, histopathology and serum biochemical parameters were evaluated. The biochemical parameters like Glucose, Blood Urea, Calcium, SGOT, and SGPT ,Creatinine, Cholesterol, Total Bilirubin, Direct Bilirubin and Total Protein does not show any significant changes as that of the control group of animals. All other parameters like body weight, food consumption, urine analysis, hematology and histopathology do not have any significant change. We can conclude that Methanolic extract of *Eranthemum roseum* (ER) is good enough safe for internal administration even for long term administration.

Key words:*Eranthemum roseum* (ER); acute toxicity study, chronic toxicity study; Dashmuli, Serum Biochemistry .

INTRODUCTION

Eranthemum roseum T.andres, in Journ.Linn .Soc.IX(family: Acanthaceae) is distributed in Konkan,, Chhattisgarh, & local area in the Toranmal Hills of Satpuda in Nandurbar District of Maharashtra. This herb is popular for its valuable tuberous roots. [1] It mainly consist steroid , saponin, aminoacid, protein and carbohydrate. The plant is reported as gastroprotective [2] , Anti-inflammatory & Antioxidant. [3] We found that Triable peoples living in Toranmal Hills of Satpuda in Nadarbur District of Maharashtra(India) tradionally used this plant as Amalapitta (Antiulcer), Anitinflammatory as Folk Medicine. Despite the wide use of *Eranthemum roseum* in folk medicine, no study has been published in the scientific literature about its toxicological profile.

Herbal preparations are commonly employed in developing countries for the treatment of various diseases .Unfortunately, there is limited scientific evidence regarding safety and efficacy to back up the continued therapeutic application of these preparations. The rationale for their utilization has rested largely on long-term clinical experience. [4]

Despite the various uses over long time periods, little toxicological information is available regarding safety following repeated exposure to *Eranthmum roseum*. Currently, we must pay more attention to the safety and potential toxicity of botanicals, including medical plants and edible materials. Therefore, the aim of the present study was to provide scientific data on the safety of *Eranthmum roseum*, focusing on the acute and 90 days (13weeks) sub-chronic toxicity of

methanol extract that was orally administered to Wister rats.

MATERIALS AND METHODS

Plant Material and Extraction

Eranthmum roseum obtained from Toranmaal Hills (Nandurbar Dist.)in Maharashtra. The plant was authenticated by Dr.D.A.Patil, Reader,P.G.Department of Botany S,S.V.P`S Science College Dhule-424 005(MS) .The whole root of *Eranthemum roseum* was dried under shade, powdered. Methanolic extract was prepared by cold maceration procedure. The extract was stored in a refrigerator.

Experimental animals

Swiss albino mice (20 – 30 g) were used for the acute toxicity study and Albino wistar rats (130 -180 g) were used for the chronic toxicity study. Animals were fed *ad libitum* with standard palletized feed and had free access to tap water. They were housed in polypropylene cages and maintained under standard conditions of humidity 35-60%, temperature at 25 ± 3^0C and 12 h light/darkness cycle. The animals were acclimatized for a week before the commencement of the study. The study was approved by Institutional Animal Ethical Committee of R.C. Patel Institute of Pharmaceutical Education & Research Shirpur, India, registered under CPCSEA, India (Registration No.651/02/C/CPCSEA).

Chemicals

Glucose, Blood urea and Bilirubin kits (Ranbaxy diagnostic Pvt. Ltd.), Creatinine, Cholesterol, SGOT, SGPT, Calcium and Total Protein Kits (Beacon diagnostic Pvt. Ltd.) were used in this study.

Toxicity studies

Acute toxicity studies in mice

Groups of 5 Swiss mice were used for each dose. The doses of methanolic Extract of *Eranthmum roseum* (MER) were administered *per os* as described above in 300, 500, 1000, 2000 mg/kg doses. Next, the animals were observed at 15, 30, 60, 120 and 240 min, with no intake of food and water. After that, the animals were observed as for morbidity and mortality, once a day, for 13 days, with food and water intake ad libitum from the collected data the LD_{50} value of *Eranthemum roseum* (MER) was calculated. [5]

Chronic toxicity studies in rats for 90 days.

Wistar rats were used under the same conditions as described for mice. The animals were divided in four groups of 10 animals each (5 males and 5 females). One group was for control and the three others for tests with methanolic extract of *Eranthemum roseum* (ER) in the doses of 180 (low dose), 450 (medium dose) and 900 mg/kg (high dose) for 90 days. During the study, body weight of the animals and food consumption were evaluated weekly and toxicity and mortality events daily.[6] At last day all the animals were placed in the metabolic cages (for 24 hrs.) for the collection of urine (used for urine analysis), the parameters like urine volume, pH and electrolytes were determined.

By the end of 90 days, the animals were not fed for 12 h and sacrificed by cervical dislocation method. Their blood was collected in heparin containing tubes (used for hematological examination) and in tubes without anticoagulants (used for biochemical tests). Hearts, livers, kidneys, spleens, lungs, and thymus were taken out for macroscopic examination, fragments of these organs were fixed in 10% formalin for histopathological examination

Food Consumption and Body Weight

The food consumption of all animals were taken daily and the body weight of each rat was assessed using a sensitive balance during the acclimatization period, once before commencement of dosing, once weekly during the dosing period and once on the day of sacrifice.

Blood analysis

Hematological analysis were performed in total blood was collected in heparinized tubes. The following parameters were evaluated hemoglobin (HB), red blood cells count (RBC), white blood cells count (WBC), platelets count (C PLAQ), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and differential leukocyte count (DLC). [7]

Serum biochemistry

Biochemical analysis was performed in serum obtained after centrifugation of total blood without anticoagulants, at 2500 rpm for 15 min. Standardized diagnostic ready-to-use kits by Ranbaxy® and a Beacon® diagnostics were used in spectrophotometrical determination of the following biochemical parameters Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), cholesterol (COLT), creatinine (CRE), glucose (GLU), total proteins (PROT), urea (URE), bilirubin (BIL) and calcium (CAL). [8]

Urine Analysis

At last day all the animals were placed in the metabolic cages (for 24 hrs.) for the collection of urine , the parameters like urine volume, pH and Sodium, Potassium, Chloride were determined.

Statistical analysis

The results are expressed as means standard error of the mean (SEM). Difference between the groups was statistically determined by analysis of variance (ANOVA) followed by Dunnett's test, with the level of significance set at $P < 0.05$.

RESULTS

Acute toxicity studies in mice

Lethal effects were not observed after administration of methanolic extract of *Eranthmum roseum* (300, 500, 1000, 2000 mg/kg). No behavioral changes were observed during the observation period. No death was observed for all above doses. Therefore, the LD50 value for oral administration of methanolic extract of *Eranthemum roseum* is greater than 2000 mg/kg.

Chronic toxicity studies in rats for 90 days.

The sub-chronic oral administration of methanolic extract of *Eranthemum roseum* resulted in no noticeable changes in the general behavior of the treated rats compared to the control group, and no significant changes in body weight(Table 1) were determined in the treated rats. Rats gained weight with time (as expected),with no significant difference in weight gain at the end of 90-day treatment between controls and rats treated . Both the controls and rats treated with methanolic extract of *Eranthemum roseum* (180, 450, 900mg/(kg day)) appeared uniformly healthy at the end of the experiment as well as throughout the 3-month period.

The effect of MER on hematological parameters of experimental and control rats is presented in Table 2. The results indicate that all hematological parameters measured (hemoglobin (HB), red blood cells count (RBC), white blood cells count (WBC), platelets count (C PLAQ), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and differential leukocyte count (DLC)) remained within physiological range throughout the treatment period (90 days).

Eranthemum roseum (ER) in doses of low, medium and high given daily for 90 days did not produce any significant change in biochemical parameters like in Glucose, cholesterol, Blood Urea, Creatinine, SGOT and SGPT , Total Bilirubin, Direct Bilirubin and Total Protein as compare with that of the control group of animals. (Table 3)

At the last day of the treatment (90^{th} day) all the animals were placed in the metabolic cages for the collection of the urine samples to measured different parameters like pH, Urine volume (24 hrs.) and Urine electrolytes (Sodium, Potassium and Chloride). All the treated groups did not show any significant changes in all above parameters as compare with that of control group of animals (Table 4).

All the treated groups did not produce any significant changes in the weight of important vital organs like liver, lung, kidney, heart, spleen and thymus as compare with that of control group of animals. (Table 5)

There were no significant differences between the control and treated groups in the organ weights of male and female rats. Neither any abnormality was detectable in pathological examinations of the tissues, nor was alterations noticed in the microscopic examination of the internal organs. The cellular appearances were unremarkable in both groups.

DISCUSSION

Herbal formulations are popular among the rural and urban community in India, the reason for the popularity and acceptability being the belief that all natural products are safe . There is evidence that using some ayurvedic medicine, especially those involving herbs, metals, minerals, or other materials involves potentially serious risks, including toxicity. Despite the widespread use, few scientific studies have been undertaken to ascertain the safety and efficacy of traditional remedies . [9]

The present investigation showed that the methanolic extract of the roots of *Eranthemum roseum,* used in the traditional medicine for a number of medical conditions in Triable region of Maharashtra , was relatively non-toxic when administered to mice as a single oral dose, as the calculated LD50 was more than 2000 g/kg, for the oral , and since to be non-toxic in humans, a single oral dose of 2 g/kg . The *Eranthemum roseum* (MER) therefore do not show any toxicity at the acute level. According to Kennedy et al. (1986), substances that present LD50 higher than 2.0 g/kg by oral route can be considered practically non-toxic .

In the chronic toxicity study in rats when Methanolic extract of *Eranthemum roseum* (MER) given orally at various doses for 90 days , there were no significant change in animal behavior, body weight, food consumption, internal vital organs weight in the treated groups as compared to the control. Since, the changes in body weight have been used as an indicator of adverse effects of drugs and chemicals the present results suggest that at the oral doses administered of methanolic extract of the *Eranthemum roseum* (MER) is non-toxic in rat. [10]

In chronic toxicity study for 90 days investigations shows that various parameters like Hematological studies, easily reveal anomalies in body metabolic processes and the blood profile usually furnishes vital information on the response of the body to injury, deprivation and/or stress. No significant difference in blood parameters were observed between treatment groups treated with Methanolic extract of *Eranthemum roseum* (MER) and control group. Generally, all the values remained within the normal limits for this animal species [11] and did not suggest toxic effects in chronic treatment. The determination of such parameters is important in the study of safety of the product with therapeutic purpose. Blood parameters analysis is relevant to risk evaluation as the hematological system has a higher predictive value for toxicity in humans (91%) when assays involve rodents and non-rodents. [12]

Since, there were no adverse effect on the usual markers of liver and kidney toxicity (plasma levels of liver enzymes, ALT and AST, bilirubin, urea and creatinine), it may be concluded that the methanolic extract of Eranthmum roseum did not induce any significant damage to these organs, as compare with that of the control group of animals.

Increase of transaminases (SGOT, SGPT) which are indicator of liver damages, The AST is also found in a large number of tissues, such as heart, lung, skeletal muscle and kidney, whereas ALT is primarily limited to liver, thus the latter is considered a highly sensitive indicator of hepatotoxicity, since the methanolic extract of *Eranthemum roseum* (MER) do not alter in SGOT & SGPT , it can be concluded that ER do not show any hepatoxicity.[12]

Raised urea and non-protein nitrogen level in blood have been observed with impaired renal function or in acute renal failure, since the *Eranthemum roseum* (MER) do not change sigfincantly plasma urea, uric acid and Creatinine , it can be concluded that ER do not show any Renal toxicity.

In the present study, histopathological evaluation of the subchronicoral ingestion of *Eranthemum roseum* methanol extract did not adversely affect the morphology of rat organs. This corroborated the results from biochemical analysis, and oral administration of low, medium & High doses of MER for 13 weeks was well tolerated by the treated rats. This dose is substantially higher than the traditionally used dose of ER. Extrapolation of these results to humans suggests that Eranthmum roseum methanol extract should be relatively safe for usage at doses of 180, 450, and 900mg/kg.

In conclusion, the oral administration of the *Eranthemum roseum methanol* extract at levels of 180, 450, and 900mg/(kg day) to male and female Wister rats for 13 weeks did not result in death and was not associated with adverse effects reflected in the general condition, growth, body and organ weights, haematology, or clinical biochemistry values and did not result in histopathological abnormalities.

According to these results, *Eranthemum roseum* methanol extract could be categorized as a no-observed-adverse-effect level(NOAEL) crude drug that acts harmlessly under the current normal usage which is considered to be of no toxicological concern . Further studies are required to established toxicity of *Eranthemum roseum* (ER) after isolation of active constituents.

Table 1: Effect of Oral Administration methanolic extract of *Eranthemum roseum* (MER) for a period of 90 day on Average Body Weight (g) and Food Consumption (g/rat/day) in rats

Groups	Initial Body weight (g)	Final body weight (g)	Food consumption (g/day/rat)
Control	135 ± 3.74	175 ± 3.80	13.4
Lower Dose (180 mg/Kg)	133 ± 4.16	177 ± 5.01	15.3
Middle Dose(450 mg/Kg)	136 ± 3.31	179 ± 3.84	14.8
Higher Dose(900 mg/Kg)	136 ± 2.53	182 ± 3.60	16.1

$^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ as compared to control, as per one way analysis of variance (ANOVA) and Dunnett's multiple comparison test was used as post hoc analysis. Values are mean ± SEM of n = 10 animals in each group.

Table 2: Effect of Oral Administration of methanolic extract of *Eranthemum roseum* (MER) for a period of 90 day on different Haematological Parameters

Parameter	Control	Lower Dose (180 mg/Kg)	Middle Dose (450 mg/Kg)	Higher Dose (900 mg/Kg)
RBC (10^6 x mm^3)	7.95 ± 0.36	7.10 ± 0.37	7.18 ± 0.25	6.87 ± 0.46
WBC (10^3 x mm^3)	9.65 ± 0.16	9.70 ± 0.22	9.38 ± 0.17	9.72 ± 0.25
Hb (g/dl)	12.1 ± 0.84	11.8 ± 0.66	10.3 ± 0.40	12.1 ± 0.73
Platelet (10^4/µl)	84.2 ± 0.99	84.9 ± 1.17	84.7 ± 1.63	84.8 ± 1.78
PCV (%)	49.7 ± 1.2	48.7 ± 2.8	44.5 ± 3.2	42.9 ± 2.5
MCV (m^3)	67.1 ± 4.8	77.3 ± 5.1	62.2 ± 5.9	59.8 ± 3.5
MCHC (%)	25.1 ± 1.8	23.0 ± 1.3	26.3 ± 1.6	25.1 ± 1.3
Neutrophils (%)	16.3 ± 5.4	13.7 ± 4.3	18.2 ± 3.8	14.2 ± 4.4
Lymphocyte (%)	79.2 ± 4.3	81.4 ± 3.7	78.7 ± 5.3	84.3 ± 3.2
Eosinophils (%)	3.8 ± 0.3	2.7 ± 0.5	2.5 ± 0.6	1.9 ± 0.4
Monocytes (%)	1.1 ± 0.5	2.2 ± 0.6	1.2 ± 0.5	0.9 ± 0.3

$^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ as compared to control, as per one way analysis of variance (ANOVA) and Dunnett's multiple comparison test was used as post hoc analysis. Values are mean ± SEM of n = 10 animals in each group.

Table 3: Effect of Oral Administration of methanolic extract of *Eranthemum roseum* (MER) for a period of 90 day on different Biochemical Parameters

Parameter	Control	Lower Dose (180 mg/Kg)	Middle Dose (450 mg/Kg)	Higher Dose (900 mg/Kg)
Blood Urea (mg/dl)	36.2 ± 0.83	37.4 ± 1.91	38.6 ± 1.19	39.0 ± 2.12
Calcium (mg %)	9.92 ± 0.59	9.34 ± 0.39	9.13 ± 0.34	9.34 ± 0.39
Cholesterol (mg/dl)	109 ± 6.77	108 ± 2.22	109 ± 4.47	109 ± 2.20
Creatinine (mg/dl)	1.57 ± 0.146	1.35 ± 0.103	1.31 ± 0.108	1.37 ± 0.105
Glucose (mg/dl)	76 ± 2.6	74 ± 2.0	73 ± 2.2	74 ± 2.1
SGOT (U/L)	64.5 ± 3.8	60.2 ± 3.7	63.7 ± 6.1	69.2 ± 6.4
SGPT (U/L)	25.2 ± 3.6	22.3 ± 2.9	25.8 ± 2.8	27.3 ± 4.7
Total Bilirubin(mg %)	0.48 ± 0.06	0.53 ± 0.05	0.56 ± 0.09	0.50 ± 0.06
Direct Bilirubin(mg %)	0.16 ± 0.04	0.21 ± 0.06	0.18 ± 0.02	0.23 ± 0.05
Total Protein (g/dl)	7.42 ± 0.38	7.54 ± 0.43	7.87 ± 0.30	7.84 ± 0.24

$^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ as compared to control, as per one way analysis of variance (ANOVA) and Dunnett's multiple comparison test was used as post hoc analysis. Values are mean ± SEM of n = 10 animals in each group.

Table 4: Effect of Oral Administration of methanolic extract of *Eranthemum roseum* (MER) for a period of 90 day on Urine Analysis

Parameter	Control	Lower Dose (180 mg/Kg)	Middle Dose (450 mg/Kg)	Higher Dose (900 mg/Kg)
pH	7.21 ± 0.17	7.83 ± 0.31	7.56 ± 0.35	7.52 ± 0.29
Urine volume	3.8 ± 0.27	3.4 ± 0.34	4.6 ± 0.44	4.3 ± 0.33
Urine electrolytes				
Na+ (mEq/l)	143 ± 7.1	138 ± 4.6	140 ± 4.0	138 ± 5.0
K+ (mEq/l)	2.5 ± 0.37	3.4 ± 0.29	2.8 ± 0.17	3.1 ± 0.15
Cl- (mEq/l)	168 ± 3.6	174 ± 6.9	180 ± 9.2	183 ± 11.4

$^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ as compared to control, as per one way analysis of variance (ANOVA) and Dunnett's multiple comparison test was used as post hoc analysis. Values are mean ± SEM of n = 10 animals in each group.

Table 5: Effect of Oral Administration of methanolic extract of *Eranthmum roseum* (MER) for a period of 90 day on different weight of organs

Organ	Control	Lower Dose (180 mg/Kg)	Middle Dose (450 mg/Kg)	Higher Dose (900 mg/Kg)
Liver	3.632 ± 0.08	3.368 ± 0.09	3.534 ± 0.06	4.121 ± 0.25
Lung	0.436 ± 0.08	0.426 ± 0.03	0.327 ± 0.07	0.438 ± 0.03
Kidney	0.668 ± 0.09	0.747 ± 0.06	0.784 ± 0.06	0.756 ± 0.06
Heart	0.346 ± 0.09	0.361 ± 0.08	0.452 ± 0.04	0.426 ± 0.08
Spleen	0.326 ± 0.06	0.275 ± 0.09	0.243 ± 0.07	0.285 ± 0.05
Thymus	0.149 ± 0.08	0.167 ± 0.03	0.249 ± 0.08	0.197 ± 0.02

$^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ as compared to control, as per one way analysis of variance (ANOVA) and Dunnett's multiple comparison test was used as post hoc analysis. Values are mean ± SEM of n = 10 animals in each group.

REFERENCES

1) Kirtikar, K.R. and Basu B.D., Indian Medicinal Plants.1996, India International book distributors,Deharadun, IInd edition,vol III-1887- 1868
2) Patil.P.H.,Surana.S.J.,``Gastroprotective effect of Eranthmum Roseum.R.BR.LINN root extracr in Albino Rats``; Indian Journal of Pharmacology & Biological Sciences,Vol.3, (1) 2009,81-93.
3) Tatiya.A.U., Desai.D.G., Surana.S.J.,Patil.PH.,``Antiinflammatory & Antioxidant activity of roots of Eranthmum roseum``, Indian Drugs, 44(11)Nov.2007.
4) Calixto, J.B., (2000). Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). *Brazilian Journal of Medical and Biological Research*. Vol. 33, 179-189.
5) Ghosh, M.N., 2005. Fundamentals of Experimental Pharmacology, Calcutta: Scientific Book Agency.
6) Feres, C.A.O., Madalosso, R.C., Rocha, O.A., Leite, J.P, Guimaraes, T.M, 2006. Acute and chronic toxicological studies of *Dimorphandra mollis* in experimental animals. *Journal of Ethnopharmacology*. 112, 56 – 63.
7) Amresha G., Singh P.N., and Rao. C.V. 2008. Toxicological screening of traditional medicine Laghupatha (Cissampelos pareira) in experimental animals. *Journal of Ethnopharmacology,* 116, 454–460.
8) Hilaly, J.E., Israili, Z.H. and Lypussi, B., 2004. Acute and chronic toxicological studies

of *Ajuga iva* in experimental animals. *Journal of Ethnopharmacology*. 91, 43–50.

9) Pattanik, N., Singh, A.V., Panday, R.S., Singh, B.K., Dixit, S.K. AND Tripathi, Y.B., 2003. Toxicological and free radical scavenging activity of tamra bhasma. *Indian Journal of Clin. Bio*. 18(2), 181–189.
10) Teo, S., Stirling, D., Thomas, S., Hoberman, A., Kiorpes, A., Khetani, V., (2002). A 90-day oral gavage toxicity study of d-methylphenidate and d,l-methylphenidate in Sprague–Dawley rats. *Toxicology*. Vol 179, 183–196.
11) Olson, H., Betton, G., Robinson, D., Thomas, K., Monro, A., Kolaja, G., Lilly, P., Sanders, J., Sipers, G., Bracken, W., Dorato, M., Deun, K.V., Smith, P., Berger, B., Heller, A., 2000. Concordance of toxicity of pharmaceuticals in humans and in animals. *Regulatory Toxicology and Pharmacology.* 32, 56–67.
12) Feldman, D.L., Mogelesky, T,C., Sharif, R., Sawyer, W., Jeune, M., 1999. The in vitro and ex vivo antioxidant properties and hypolipidemic activity of CGP 2881. *Atherosclerosis*, 144, 343–355.

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