Studies on acute, chronic toxicity and *in-vitro* antioxidant activities of *Eranthemum capense* Linn

R Jahnavi, R Manohar*, S Nelson Kumar, C Rajaram and S. Kalpana

Department of Pharmacology, P. Rami Reddy Memorial College of Pharmacy, Kadapa - 516 003, Andhra Pradesh, India.
Professor & HOD Department of Pharmacology P. Rami Reddy Memorial College of Pharmacy Kadapa – 516003 Andhra Pradesh (State), India

***Corresponding author: : Dr. R. Manohar**
reddy.manohar1981@gmail.com

ABSTRACT

The main purpose of this study is to examine the acute and sub-chronic toxicity studies of chloroform and methanol extract of Eranthemum capense Linn (Acanthaceae) on animal models as per the OECD guidelines 407 & 425 respectively and In-vitro antioxidant activities with standard methods. In acute oral toxicity study a single oral doses of 2000 mg/kg body weight of the individual chloroform and methanol extracts was given to rats and observed them for 14 days for the detection of acute changes and for its mortality any. During acute oral toxicity study period no mortality were observed with no signs of acute changes. Based on these acute oral toxicity studies we performed the sub-chronic toxicity study, in this the chloroform and methanol extract of Eranthemum capense Linn separately given daily at doses of 200 and 400 mg/kg body weight for 90 days to detect the changes any at sub-chronic toxicity level. All parameters of treated group were unaltered throughout the study period when compared with that of normal group. This study revealed the safety of oral administration of chloroform and methanol extract of Eranthemum capense Linn. Also chloroform and methanol extract of Eranthemum capense Linn were studied for its in-vitro antioxidant activities like DPPH radical scavenging activity, nitric oxide (NO) radical inhibition assay, lipid per oxidation test, superoxide anion radical scavenging activity and hydroxyl radical scavenging activity. The results uncovered that the extracts of Eranthemum capense Linn shows the better radical scavenging activity with respect to its antioxidant activity on contrasted and that of

standard antioxidants. From the results it may concluded that chloroform and methanol extracts of Eranthemum capense Linn have great antioxidant activities as appeared by in vitro assay.

Key words: Eranthemum capense Linn, acute and sub-chronic toxicity study, in-vitro antioxidant activity.

INTRODUCTION

Traditional medicine has served as a unique health provider for human beings for thousands of years. In the contemporary world herbal remedies still play a significant role in the health care delivery to people living particularly in developing countries, where the availability of health facilities and basic medicines are limited. Anciently plants were utilized for traditional medicine without scientific evidence for its toxicity and medicinal values. In general, history on the traditional utilization of the plant material is expected to enlighten for planning the experimental toxicity studies [1].

In the late 1980s, The Organisation for Economic Co-operation and Development (OECD) and The International Council for Harmonisation (ICH) during the late period of 1980s guided the rules and regulations for carrying the toxicity studies to evaluate the level of toxicity for plant materials, chemical substances [2] and pharmaceutical substances [3]. Toxicity studies is the necessary scientific process for discovery and development of potential toxicants with evidence and evaluation of drug. There are the traditional and scientific methods of determining toxic effects of chemicals and drugs include acute toxicity, subacute and chronic toxicity studies. In acute toxicity study is carried out to determine the short time toxicity effect of a toxicant (1 s to 2 weeks), whereas subacute toxicity study is carried out to know the relative long term effect of a toxicant (4 weeks – 6 months). Chronic toxicity studies are carried out to know the long term effect of a toxicant (1–1½ years). Subacute is sometimes called sub-chronic, sub means under i.e. considered under acute or below chronic [4].

Eranthemum capense Linn is one of the important species used traditionally for various disorders belonging to the family Acanthaceae. The aerial part of the plant provided for antimicrobial studies and anti-inflammatory activity [5]. Still there was lack in scientific study toxicity studies and antioxidant effect of *Eranthemum capense* Linn to substantiate the traditional claim. Hence, the current work was embraced to assess the study toxicity studies and antioxidant activities of chloroform and methanol extracts of *Eranthemum capense* Linn.

MATERIALS AND METHODS

Chemicals and reagent

All the chemicals and reagents used in these acute and chronic and *in-vitro* antioxidant studies were of analytical grade.

Source and authentication of plant material

Fresh aerial parts of *Eranthemum capense* Linn (Acanthaceae) were pull together from surrounded arears of kadapa region and authentified by Dr. A. Madhusudhana Reddy, Professor, Department of Botany, Sri Yogi Vemana University, Kadapa. Andhra Pradesh, India. Voucher specimen (No: EC- 1634) of this plant has been kept in the P. Rami Reddy Memorial College of Pharmacy, Kadapa, Andhra Pradesh, India.

Preparation of plant material

The gathered aerial parts of *Eranthemum capense* Linn was washed with running water, cut into little pieces and shade dried at room temperature to avoid from loss of phytoconstituents of plant. The total shade dried materials pounded for powder and sieved up to 80 meshes. At that point it was homogenized to fine powder and put away in air-tight compartment for further activities.

Preparation of plant extracts

Aerial parts plant powder of the *Eranthemum capense* Linn was extracted successively with two different solvents like chloroform and methanol in a Soxhlet apparatus in batches of 500

gm each. The excess solvent was expelled from extract utilizing a rotary vacuum evaporator and later on concentrated on a water bath. At last dried extracts were put away in desiccators for assess for its toxicity and *in-vitro* antioxidant activities [6].

Animals and maintenance

Wistar Albino rats of either sex, with the body weight of 150-250 gms were acquired from Sri Venkateswara Enterprises, Bangalore, India. Animals were kept up according to rules of NIN animal client manual. Animals are adjusted for 10 days to our creature house, kept up at temperature of 22ºC to ± 2 ºC. The animal was directed by a 12 hours light, 12 hours dark calendar. Five animals are housed per cage estimated 41 cm length, 28 cm width and height of 14 cm. Paddy husk was utilized for bedding and on elective day bedding was changed and washed altogether with water using domex, a disinfectant and detergenic. The rats were feed with a standard pellet diet bought from Suresh organizations, Hyderabad and water not obligatory. The examination convention was investigated and endorsed by the Institutional Animal Ethical Committee (IAEC) and trials were done according to the rules of CPCSEA. Reg. Number: 1423/PO/Re/S/11/CPCSEA, date 25th November 2022.

Acute oral toxicity study

An oral acute toxicity studies of chloroform and methanol extracts of *Eranthemum capense* Linn were performed individually by the Organization for Economic Cooperation and Development (OECD) rule 425 on rodents [7], where the breaking point test portion of 2000 mg/kg was utilized. All the animals were kept 3 hours fasting before to explore different avenues regarding free abundance to water. A solitary oral dosage of 2000 mg/kg body weight of the individual chloroform and methanol extracts were given utilizing oral gavage for present moment (i.e., 48 hr) and long term (i.e., 14 days) to the rats. Before dose administration, the body weight of every rat was resolved and the dose was determined by the body weight. Animals were seen to observe intense changes in morphological and conduct reactions,

unconstrained action, touchiness, corneal reflex, tremors, spasms, salivation, loose bowels, torpidity assuming any and furthermore observed for any mortality throughout toxicity study [8].

Sub chronic toxicity study

The oral sub chronic toxicity study was done by OECD rule 407. The animals were partitioned into 5 groups of 6 rats each were kept in six distinct cages. Group I was kept as should be expected normal control and Group II to V were kept as tested groups which received the dosages as follows.

Group I - animals were kept as control and received normal clean drinking water ad libitum,

Group II - animals received chloroform extract of *Eranthemum capense* Linn (CEEC) 200 mg/kg per orally,

Group III – animals received chloroform extract of *Eranthemum capense* Linn (CEEC) 400 mg/kg per orally,

Group IV – animals received methanol extract of *Eranthemum capense* Linn (MEEC) 200 mg/kg per orally,

Group V – animals received methanol extract of *Eranthemum capense* Linn (MEEC) 400 mg/kg per orally,

All the animals in the above groups get their portions as needs be for the examination time of 90 days [9]. Body weight of every animal in group was recorded at first and for like clockwork span till the most recent day of analysis. Following 90 days' blood tests of exploratory rats in each group was acquired by retro orbital with a capillary into Eppendorf tubes without anticoagulant, centrifuged at 400 x g for 10 min and the serum put away at 4^0C for estimation of different serum biochemical boundaries, for example, glucose, creatinine, total protein, albumin, globulin, bilirubin, SGOP, SGPT and ALP and for electrolytes (phosphorus, chloride and calcium). Other blood tests were gathered into isolated tubes previously covered with

trisodium citrate for haematological investigation, for example, RBC, WBC, haemoglobin, platelets and mean cell volume. Every trial animal was sacrificed in the wake of gathering of blood tests by infusing the phenobarbital infusion for gathering the internal organs to decide the relative organ loads and for histopathological investigations of brain, liver, kidney, heart and lungs.

Serum biochemical parameters

Serum glucose, creatinine, total protein, albumin, globulin, bilirubin, SGOT, SGPT, ALP and electrolytes were determined using a semi-automated analyzer.

Serum glucose determination

Serum glucose levels were determined by using Trinder method (Glucose, GOD-POD) by the addition of reagents present in reagent kit (AGD Biomedicals Pvt. Ltd.). The absorbance of standard and test against reagent blank were measured at 505 nm [10 & 11].

Serum creatinine determination

Serum creatinine levels were ascertained by reagents present in reagent kit (AGD Biomedicals Pvt. Ltd.). The absorbance of standard and test against reagent blank were measured at 520 nm.

Serum total protein concentration

Serum total protein levels were ascertained by using end point assay method acting by the addition of reagents present in reagent kit (Span Diagnostic Ltd.). The absorbance of standard and test against reagent blank were measured at 578 nm. The values of total proteins present in serum were communicated in g/dL.

Serum albumin concentration

Serum albumin levels are ascertained by using bromocresol green, end point assay method by the incorporation of reagents present in reagent kit (span diagnostic ltd.). the absorbance of standard and test against reagent blank were measured at 630 nm. The values of Albumin present in serum were communicated in g/dL.

Serum globulins concentration

Serum globulins levels are ascertained by using the equation:

Globulins = Total proteins – Albumin

And the values are communicated in g/dL.

Serum transaminases (GOT & GPT)

Serum transaminases (GOT & GPT) were decided by the method of Reitman and Frankel [12] by the incorporation of reagents present in reagent kit (Span Diagnostic Ltd). The absorbance of standard and test against reagent blank were measured at 505 nm. Data were communicated as IUL^{-1}.

Serum alkaline phosphatase (ALP)

Serum alkaline phosphatase (ALP) was ascertained by the method of Kind & King [12] by the addition of reagents present in reagent kit (Span Diagnostic Ltd). The absorbance of standard and test against reagent blank were measured at 640 nm. Data were communicated as UL^{-1}.

Serum bilirubin

Serum bilirubin was evaluated by method of Malloy and Evelyn [13]. The two test tubes were hold of and each into was included 0.2 ml of serum test and 1.8 ml of distilled water. To the obscure, 0.5 ml of diazo reagent and to the blank, 0.5 ml of 1.5 % HCl was incorporated. At last, to every cylinder, 2.5 ml of methanol was included and a short time later allowed to address 30 min in ice and absorbance was examined at 540nm. For a standard curve, the above standard was weakened 1 out of 5 ml methanol. The measure of direct responding bilirubin was resolved also by subbing 2.5 ml of water for 2.5 ml of methanol. Qualities were communicated as mg/dl.

Haematological analysis

Red blood cells (RBC), white blood cells (WBC), platelets, haemoglobin (Hg), mean cell volume (MCV) and electrolytes were ascertained with a semi-automated analyzer.

Statistical analysis

All investigations information was communicated as mean ± standard error mean (SEM). This statistical analysis was done utilizing one-way ANOVA strategy accompany by Dunnet-t test with SPSS statistical programming for correlation with the control group. $P \leq 0.05$ was appraise as statistically significant.

***In vitro* antioxidant activity**

DPPH radical scavenging assay

The DPPH radical scavenging movement of chloroform and methanol concentrates of *Eranthemum capense* Linn were assessed by 1, 1 diphenyl 2 picryl hydrazyl (DPPH) strategy [14]. A stock arrangement was set up by dissolving chloroform and methanol extracts in refined water. From these stock arrangements various convergences of 5, 10, 20, 40 and 80 µg/ml working solutions were arranged separately. 0.1 milli molar DPPH solution was set up by dissolving in ethanol. To 1 ml of working solution the 3 ml of individual plant extracts of various fixations were added and afterward the blend was shaken enthusiastically and permitted to remain at room temperature for 20 – 30 minutes. Absorbance was assessed at 517 nm by spectrophotometer. Serial dilutions of standard compound were additionally set up with quercetin as reference standard compound.

Nitric oxide (NO) radical inhibition assay

Various concentrations (5 to 160 µg/ml) of chloroform and methanol extracts of *Eranthemum capense* Linn were arranged independently. 2 ml of sodium nitroprusside (10 mM) in 0.5 ml of saline phosphate buffer was blended in with various concentrations of chloroform and methanol extracts of aerial parts and incubated at 30^0C for two hours. On finish of incubation period add 1 ml of Griess reagent (1% sulphanilamide, 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 2% orthophosphoric corrosive), phosphate buffer (pH - 7.4) was added. The blend was of course incubated at room temperature for 30 – 50 minutes and its absorbance were estimated at 550 nm. Rutin was filled in as standard [15].

Lipid peroxidation assay

Rat liver microsomal part and chloroform and methanol concentrates of *Eranthemum capense* Linn in different concentrations (10 – 160 µg/ml) were set up by the technique for Brouchet *et al* (1998) to decide the thiobarbituric acid receptive substances in this examine. 500 µl of liver microsomal portion, 300 µl of working arrangement of plant extracts and 100 µl of $FeCl_3$ (1mM) were blended. 100 µl vitamin C (1mM) was added at last. Samples were incubated at 37°C for 1 hour and lipid per oxidation was estimated utilizing the response with thiobarbituric acid. The absorbance was estimated at 532 nm. All responses were concluded in three-fold. Vitamin E was utilized as a standard.

Superoxide anion radical scavenging activity

Superoxide anion radical scavenging action of chloroform and methanol concentrates of *Eranthemum capense* Linn were performed by strategy for Nishimiki *et al*. [16]. Sequential dilutions of 5, 10, 20, 40, 80 and 160 µg/ml were arranged independently from chloroform and methanol extracts of *Barleria buxifolia* and *Barleria cuspidata* independently. Every dilution was added by 1ml of nitroblue tetrazolium (NBT) solution and 1ml of nicotinamide adenine dinucleotide (NADH). The response was started by adding 100µl of phenazine methosulphate (PMS) solution and afterward incubated at 25°C for 5 min. The absorbance was estimated at 560 nm against blank. Curcumin was taken as reference compound.

Hydroxyl radical scavenging activity

Working solutions of different concentrations (10, 20, 40, 80 and 160 µg/ml) were set up with chloroform and methanol extracts of *Eranthemum capense* Linn separately [17]. 500 µl of chloroform and methanol extracts at different concentrations were added with 100 µl of 2-deoxy 2-ribose and 200 µl of 1.04 mM ethylene diamine tetra acetic acid (EDTA). Further 200 µM ferric chloride (1:1, v/v) and 100 µl of 1.0 mM hydrogen peroxide were included. At last, 100 µl of 1.0 mM nutrient C was included. All samples were hatched at 37°C. Following one

hour 1 ml of 1% thiobarbituric corrosive (TBA) and 1.0 ml 2.8% trichloroacetic corrosive (TCA) were added to the response combination and incubated at 100°C for 20 minutes. The absorbance was estimated at 532 nm against a blank. Vitamin E at different concentrations was utilized as standard.

RESULTS AND DISCUSSION

Acute oral toxicity study

The acute oral toxicity study was done on Wistar Albino rats of either sex at single portion of chloroform and methanol extracts of *Eranthemum capense* Linn at 2000 mg/kg body weight and persistently checked for 14 days according to OECD rules 425. During this investigation period all the animals were watched for its conduct and its mortality. All the experimental rats show ordinary conduct in their consciousness, touch response, movement, sleeping duration, holding quality, righting reflex, food admission, water utilization, corneal reflex, salivation, pinna reflex, skin shading and sound reaction, and there was missing in reactions, for example, grooming, tremors, diarrhoea, hyper movement, lethargy, convulsions and morbidity as appeared in Table 1. Likewise, all the tested rats made due till the fulfilment of the investigation time frame (14 days) at all degrees of treatment. Along these lines, this outcome says that there was no aggravation in carbohydrate, protein, fat and some other metabolisms. The chloroform and methanol extracts of *Eranthemum capense* Linn at 2000 mg/kg body weight given orally is by all accounts safe and the LD_{50} was considered be 2000 mg/kg body weight. All of these outcomes will propose that the chloroform and methanol concentrates of *Eranthemum capense* Linn at 2000 mg/kg body weight for all intents and purposes non-toxic at single oral dose.

Table 1: Acute oral toxicity study of chloroform and methanol extracts of *Eranthemum capense* Linn at 2000 mg/kg body weight in rats.

S. No.	Observations	Response		
		Control	CEBB 2000 mg/kg b.w	MEBB 2000 mg/kg b.w
1	Consciousness	+	+	+

2	Grooming	-	-	-
3	Touch response	+	+	+
4	Sleeping duration	+	+	+
5	Movement	+	+	+
6	Gripping strength	+	+	+
7	Righting reflex	+	+	+
8	Food intake	+	+	+
9	Water consumption	+	+	+
10	Tremors	-	-	-
11	Diarrhoea	-	-	-
12	Hyper activity	-	-	-
13	Pinna reflex	+	+	+
14	Corneal reflex	+	+	+
15	Salivation	+	+	+
16	Skin colour	+	+	+
17	Lethargy	-	-	-
18	Convulsions	-	-	-
19	Morbidity	-	-	-
20	Sound response	+	+	+
21	Mortality	Alive	Alive	Alive

Note: +=Normal and -=Absent

Sub chronic toxicity study

Sub chronic toxicity studies was therefore executed with chloroform and methanol extracts of *Eranthemum capense* Linn at the doses of 200 mg/kg and 400 mg/kg body weight as per the OECD guidelines 407. The chloroform and methanol extracts of *Eranthemum capense* Linn at 2000 mg/kg body weight given orally is by all accounts safe and the LD_{50} is considered be 2000 mg/kg body weight.

Analysis of body weights of each experimental rats

The outcomes in of any adjustments in body weight of each experimental rat in group at first and for like clockwork span (30 days) till the most recent day of examination are appeared in Figure 1. There were no critical contrasts in mean body weight among the diverse treated groups and the control despite the fact that there will be increment in body weights steadily. These outcomes demonstrated that the chloroform and methanol extracts of *Eranthemum capense* Linn has immaterial degrees of harmfulness on the development of the animals.

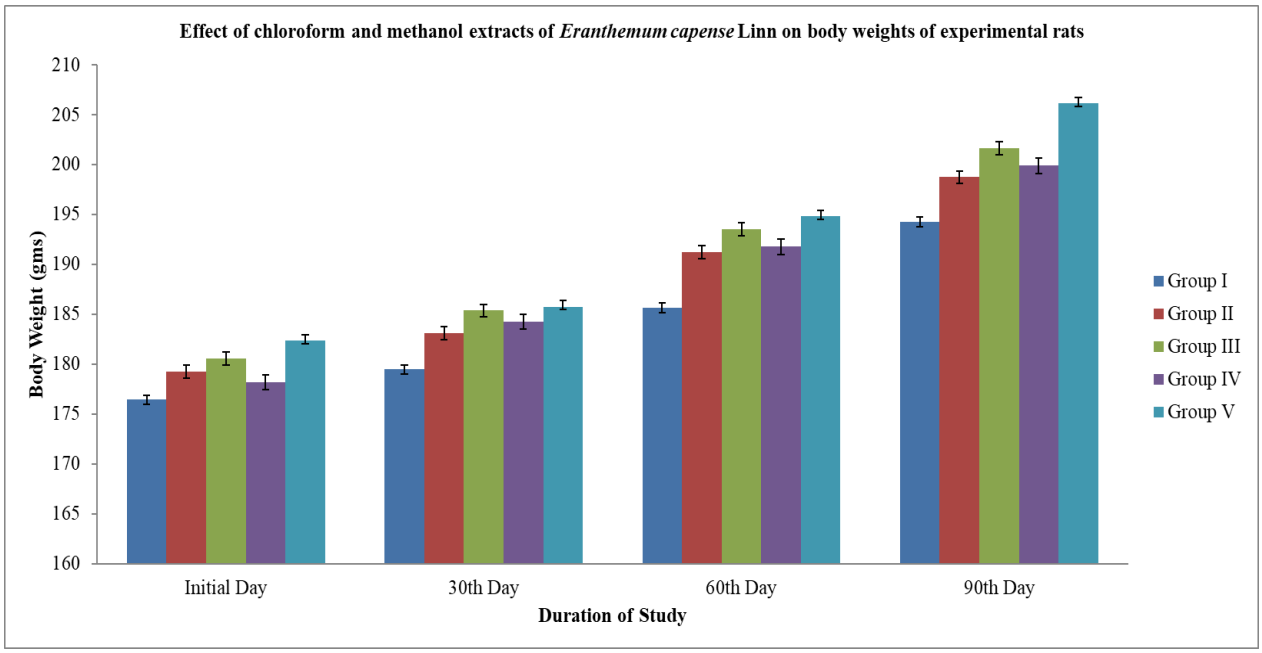

Figure 1: Effect of chloroform and methanol extracts of *Eranthemum capense* Linn on body weights of experimental rats

Measurement of organ weights

The results in of any changes in relative organ weights of each experimental rat in group at end of the experimentation are manifest in Figure 2. The weights of significant organs such as liver, lungs, kidneys and heart were found normal and there were no significant changes in their weight which indicates that nontoxic effect in both control and treated groups. The no significance changes in organ weights and it provides support for the safety of *Eranthemum capense* Linn.

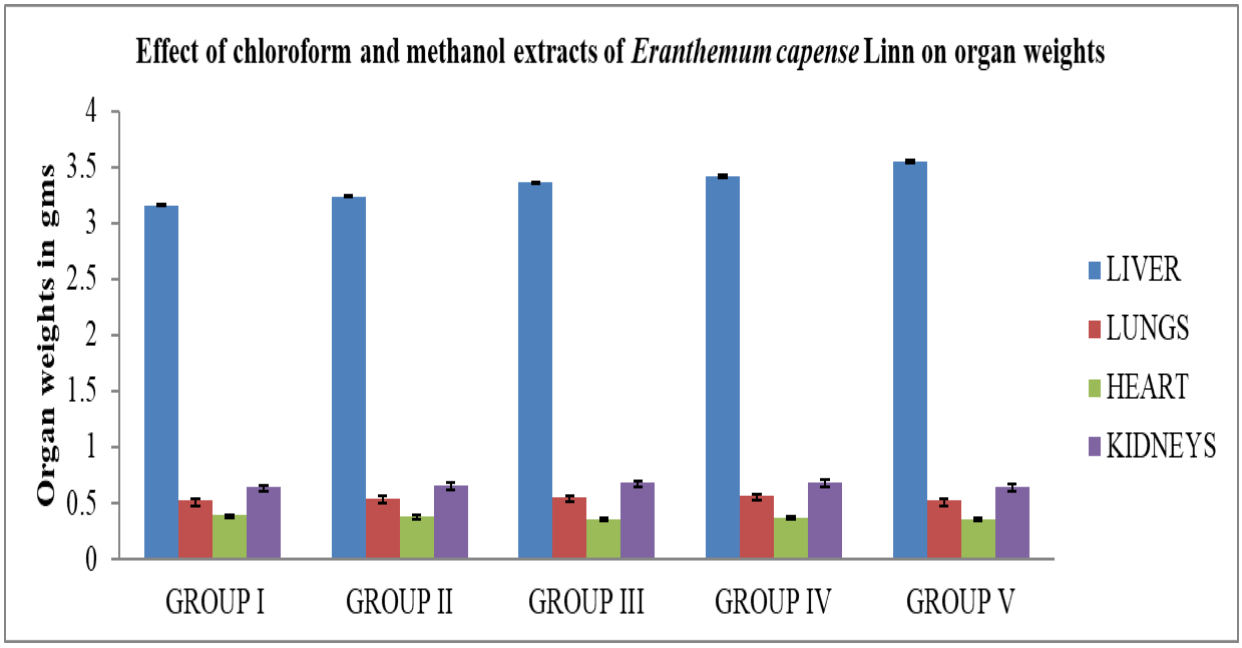

Figure 2: Effect of chloroform and methanol extracts of *Eranthemum capense* Linn on organ weights

Serum biochemical parameters

The results of serum biochemical parameters, for example, glucose, creatinine, total protein, albumin, globulin, bilirubin, SGOT, SGPT, ALP was recorded in Figure 3 and 4 and electrolytes are recorded in Figure 5. Following 90 days of study period, the serum biochemical parameters, for example, SGOT, SGPT, ALP and Bilirubin of divided group don't show significance when contrasted and that of control group. The estimations of these biochemical parameters typically quantify as markers of the best possible liver capacity [18]. In addition, levels of serum total protein, globulin and albumin demonstrated no significant when contrasted the rewarded group animals and that of control group animals. Hypo-proteinaemia, a typical finding in liver damage [19], was additionally not seen in present examination. Additionally, the degrees of creatinine were not essentially extraordinary in the middle of the control and test group of rats. The estimations of serum creatinine will gauge the best possible capacity of urinary system [20], thus with no dangerous in serum creatinine levels of experimental animals shows the correct working of urinary system in animals treated with *Eranthemum capense* Linn. Further, there was no significant in glucose levels in the treated

groups when contrasted with the control group which likewise demonstrates for typical working of liver.

Likewise, the serum levels of electrolytes, for example, phosphorus, chloride and calcium indicated no importance contrast when contrasted the treated group animals and that of control group animals. Which shows that chloroform and methanol extracts of *Eranthemum capense* Linn was protected concerning that of the electrolyte levels.

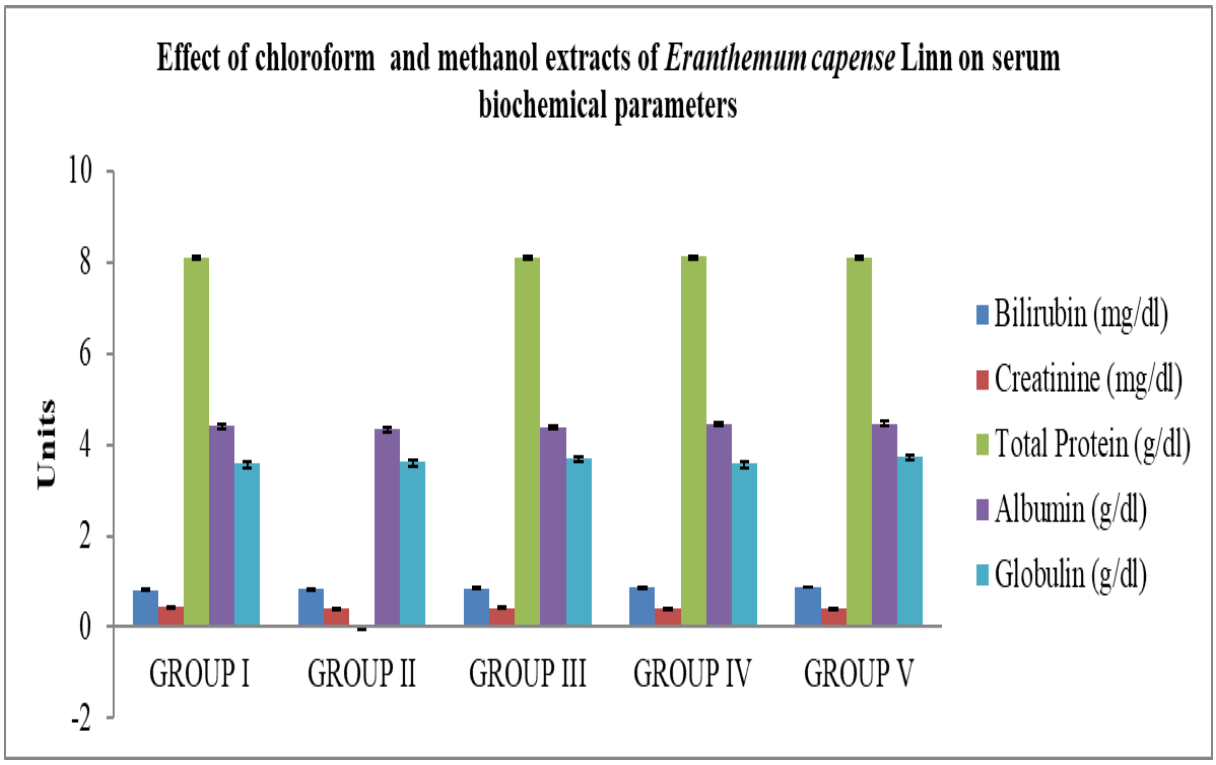

Figure 3: Effect of chloroform and methanol extracts of *Eranthemum capense* Linn on serum biochemical parameters

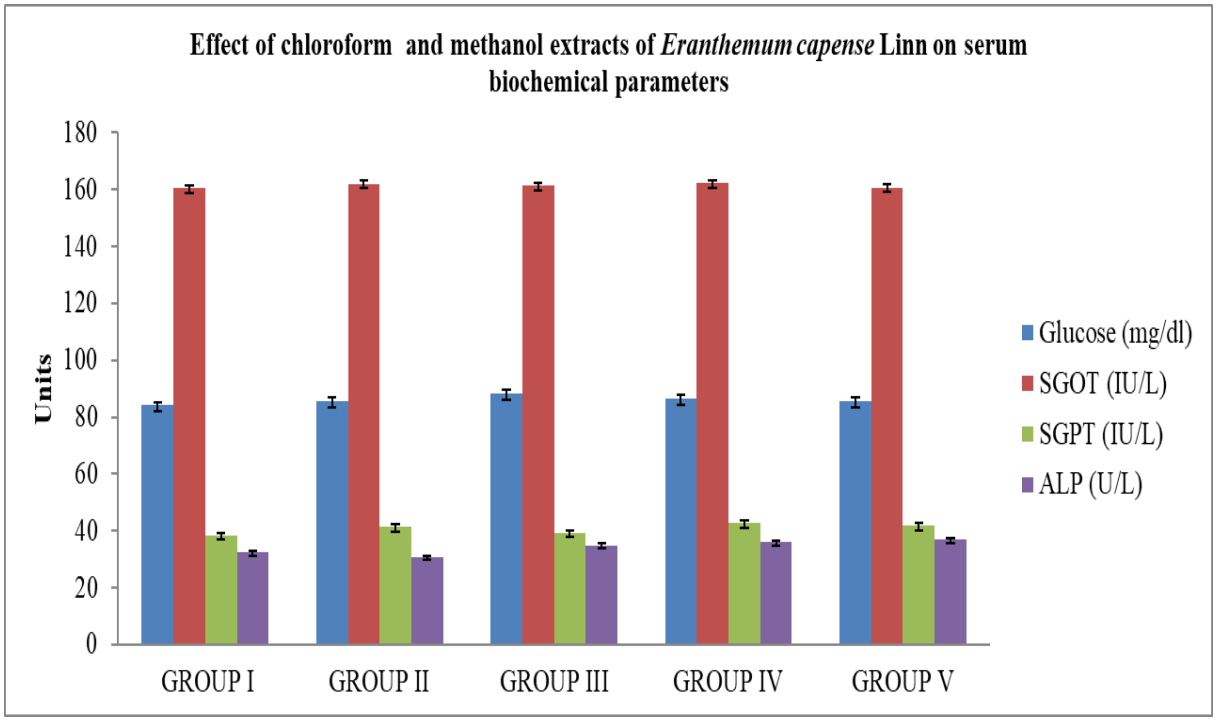

Figure 4: Effect of chloroform and methanol extracts of *Eranthemum capense* Linn on serum biochemical parameters

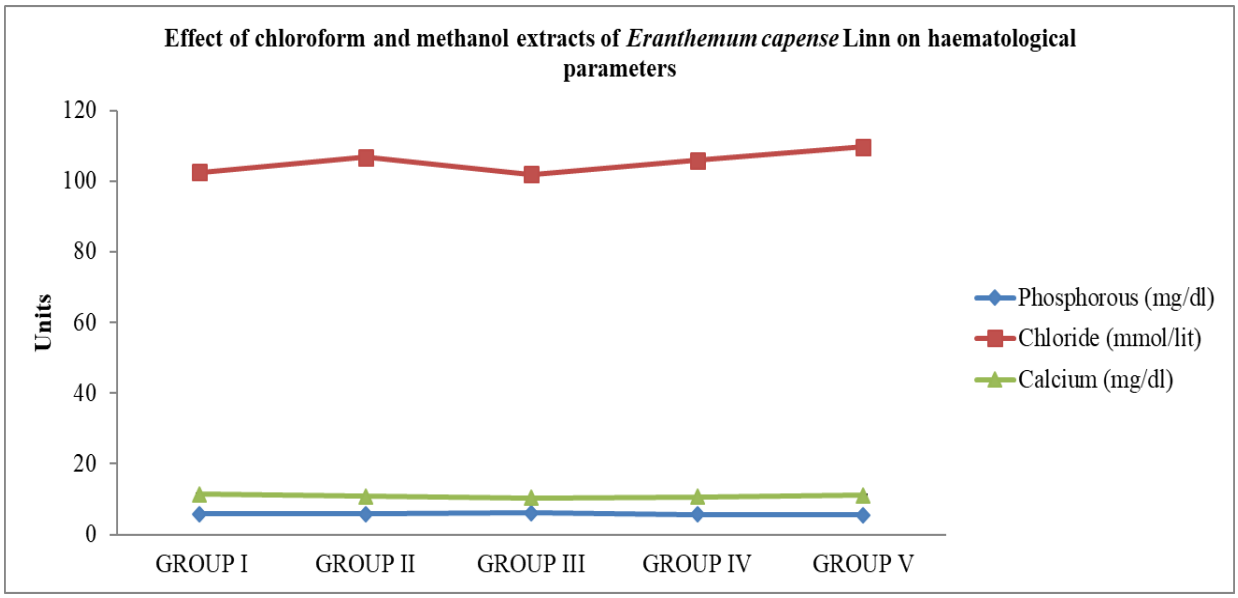

Figure 5: Effect of chloroform and methanol extracts of *Eranthemum capense* Linn on serum electrolyte levels.

Haematological parameters

The results of haematological parameters, for example, RBC, WBC, platelets, haemoglobin and MCV are recorded in Figure 6 and 7. The haematological parameters, for example, RBC, WBC, platelets, haemoglobin and mean cell volume indicated no centrality when rewarded

group animals are contrasted and that of control group animals. Administration of any compound or medication or plant extracts can bring about loss of blood cells and/or hindrance of blood cell synthesis and diminishing in such haematological boundaries in experimental animals has been related with anaemia [21]. The bone marrow is liable for the creation of the blood cell and a few phytochemicals disconnected from plant extracts will influence blood cell levels [22]. As the tested plant extracts didn't show any of unsafe impacts on bone marrow work, with that we may legitimize for all the portions of chloroform and methanol extracts of *Eranthemum capense* Linn didn't initiate anaemia and making it ok for use in this regard.

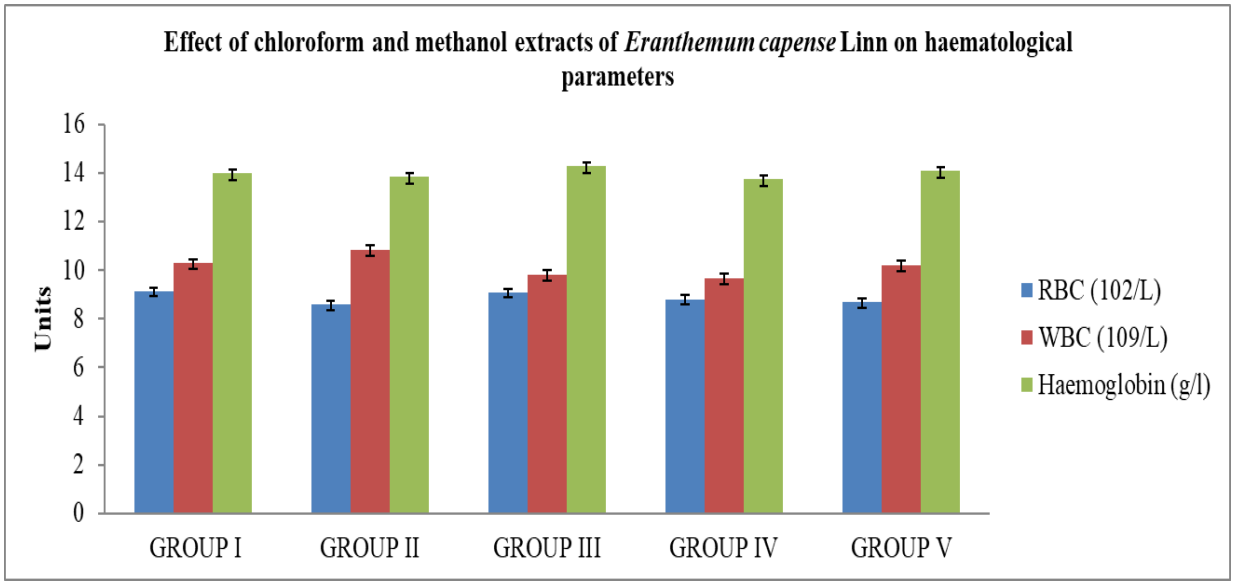

Figure 6: Effect of chloroform and methanol extracts of *Eranthemum capense* Linn on haematological parameters

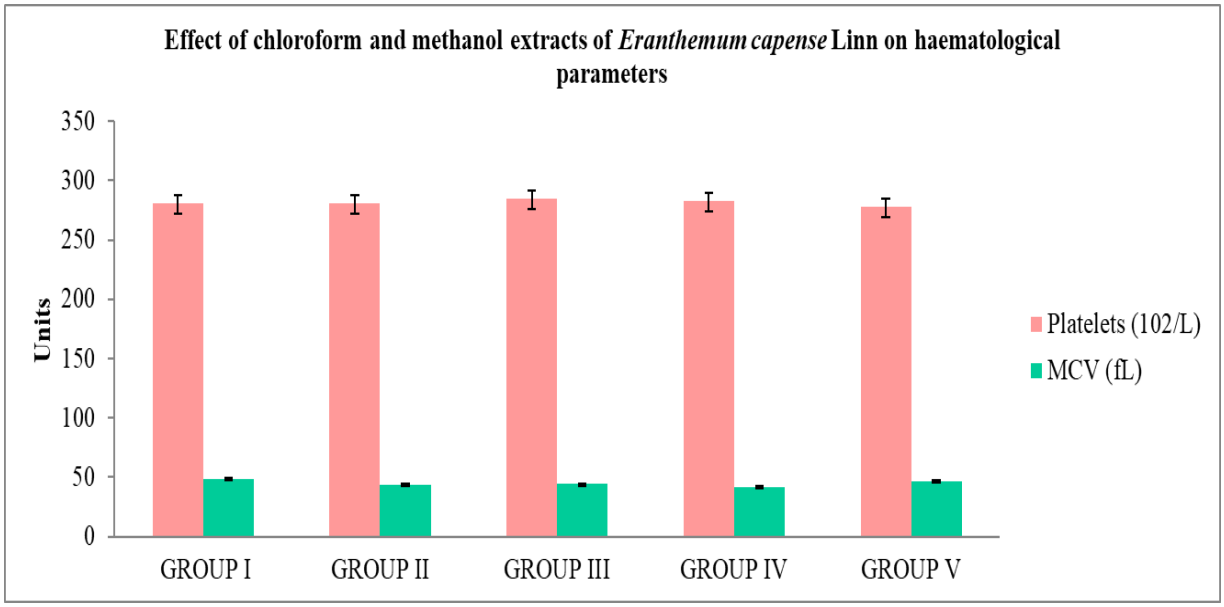

Figure 7: Effect of chloroform and methanol extracts of *Eranthemum capense* Linn on haematological parameters

Histology study

Histological investigations of liver, kidney, lungs, heart and brain tissues in chloroform and methanol extracts of *Eranthemum capense* Linn treated rats were appeared from Figure 8 to 12, which shows no variations from the norm when contrasted the treated and that of control. In this way, Histopathological assessment of chloroform and methanol extracts of *Eranthemum capense* Linn didn't show any unfavourable impact on morphology of tissues which bolsters the biochemical outcomes referenced previously. Hence, it is inferred that *Eranthemum capense* Linn didn't create any harmful impact in Wistar Albino rats of either sex.

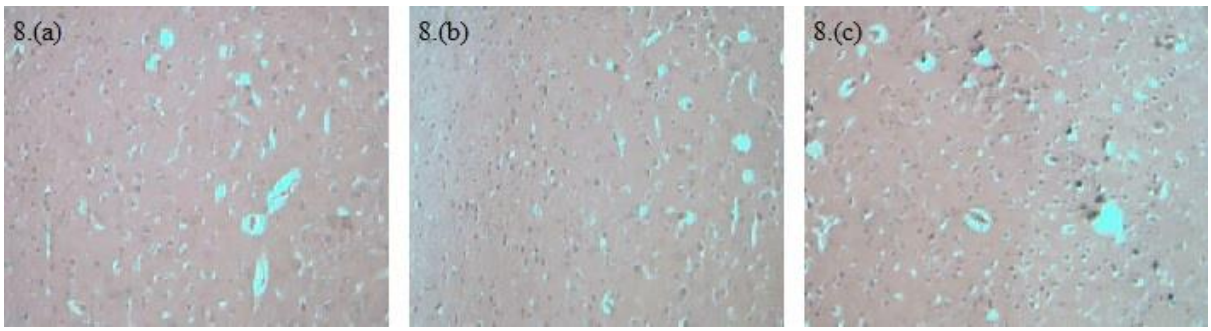

Figure 8: Histology of rat brain treated with *Eranthemum capense* Linn (a) Control group (b) Chloroform extract and (c) Methanol extract at 400 mg/kg b.w. per day.

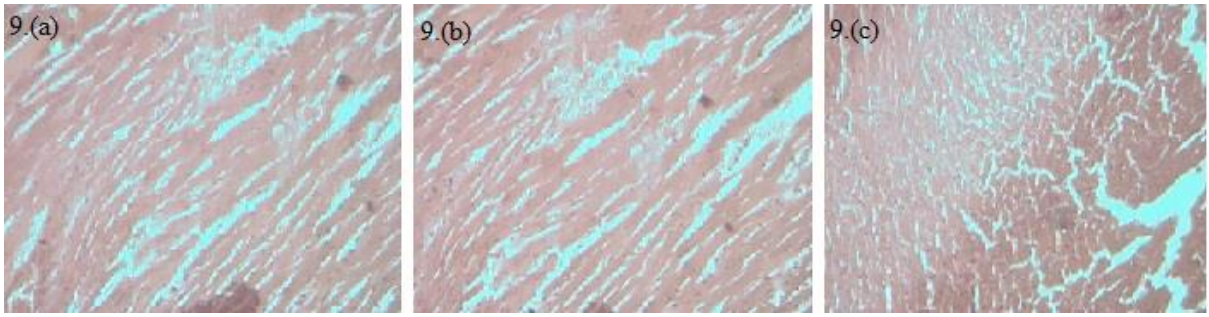

Figure 9: Histology of rat heart treated with *Eranthemum capense* Linn (a) Control group (b) Chloroform extract and (c) Methanol extract at 400 mg/kg b.w. per day.

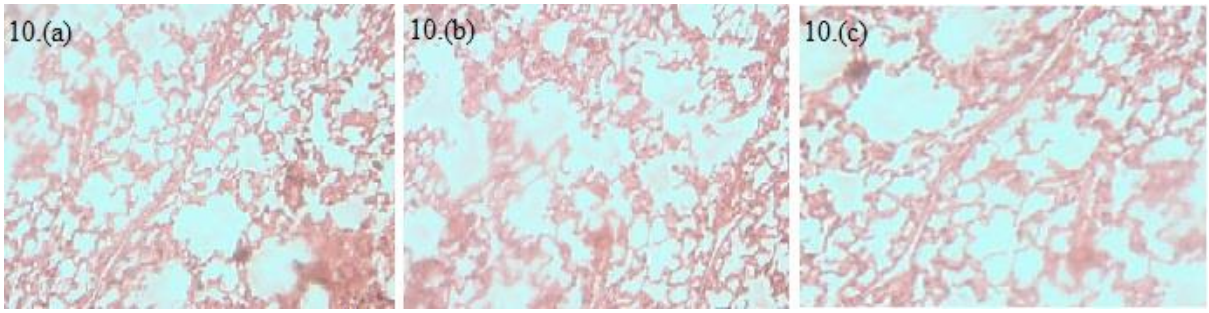

Figure 10: Histology of rat lungs treated with *Eranthemum capense* Linn (a) Control group (b) Chloroform extract and (c) Methanol extract at 400 mg/kg b.w. per day.

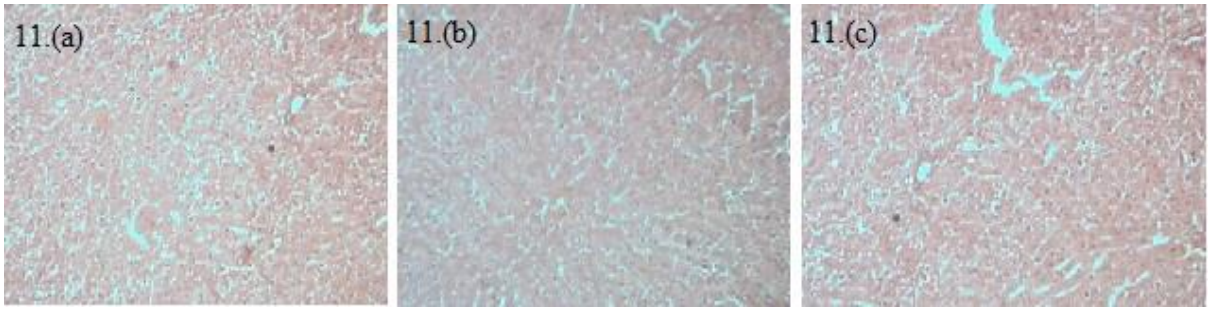

Figure 11: Histology of rat liver treated with *Eranthemum capense* Linn (a) Control group (b) Chloroform extract and (c) Methanol extract at 400 mg/kg b.w. per day.

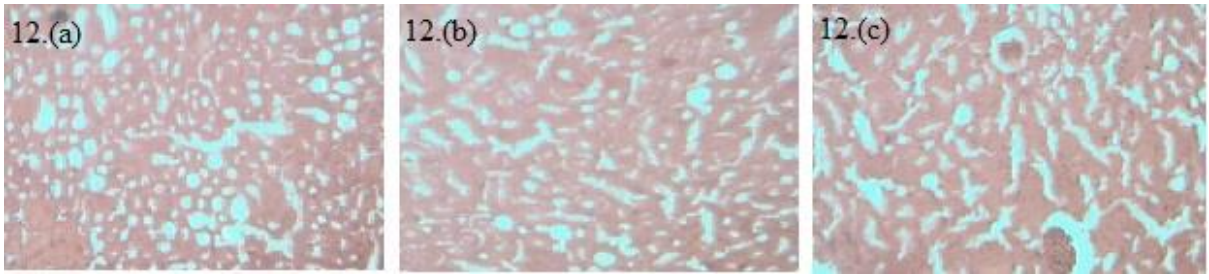

Figure 12: Histology of rat kidney treated with *Eranthemum capense* Linn (a) Control group (b) Chloroform extract and (c) Methanol extract at 400 mg/kg b.w. per day.

CONCLUSION

The results of acute oral toxicity study and subchronic toxicity study of chloroform and methanol extracts of *Eranthemum capense* Linn shows no significant differences in haemotological and biochemical tests in the treated groups on compared with that of control and it will suggest the level of safety of *Eranthemum capense* Linn on animals. Also *in*-vitro antioxidant results of chloroform and methanol extracts of *Eranthemum capense* Linn concluded that it may have antioxidant activity. Accordingly, the outcome shows that the chloroform and methanol extracts of *Eranthemum capense* Linn might be protected and furthermore gives the data to the utilization of *Eranthemum capense* Linn for additional examinations and as an elective arrangement of medication. Present endeavours are composed to separate the dynamic constituents from various extract of plant and explanation of component of activity.

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CONFLICT OF INTERESTS

The authors proclaim that there was no conflict of interest in this research.

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