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A 90-DAY ORAL TOXICITY STUDY OF THE CHLOROFORM EXTRACT OF *GOMPHRENA GLOBOSA* IN RATS

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ABSTRACT

Gomphrena globosa is one of the most widely used folk herb and a constituent in many traditional Indian and Chinese herbal medicines. The present work involves '90-Day Oral Toxicity Study' of the Chloroform Extract of *Gomphrena globosa* (CEGG) in Wistar strain Albino rats. The rats were administered with CEGG at the dose of 1000 mg/kg, p. o., once daily orally for 90 days. The blood samples were collected at 91st day with anticoagulant, as well as without anticoagulant (EDTA). Blood with the anticoagulant was used immediately for the determination of hematological parameters, while blood without the anticoagulant was used for biochemical parameters. There were no significant adverse effects on clinical signs, body weight, food conversion efficiency, and vital hematological indices. There were no significant changes in the hematological parameters such as WBC, lymphocyte, monocyte, neutrophil, eosinophil, basophil, red blood cell count (RBC), Hb, HCT, MCV, and in the levels of total proteins, albumin, ALT, AST, blood urea nitrogen, creatinine, in the CEGG treated group as compared to normal. Significant changes are observed in the MCH, MPV, total cholesterol and triglycerides. The histopathology study did not show any significant changes in the appearance of the cells of various organs investigated

as compared to normal. Thus, the findings reveal that the proper use of CEGG in traditional medicine at an oral dose up to 1000 mg/kg, p. o., may harbor no prolonged toxicity to rats. However, further studies of CEGG are still necessary to assess its oral safety in patients.

KEYWORDS: Gomphrena globosa, 90-day oral toxicity, hematological, biochemical, histopathology.

INTRODUCTION

Cancer is a second leading cause of death in developing countries, with uncontrolled division of cells in the part of the body.[1] The cancer burden in developing countries is raising as a result of increasing population ageing and growth, and adaptation of cancer-associated lifestyle choices including smoking, physical inactivity and 'Westernized diet'.[2] It has been reported in the year 2018 that there were more than eight lakh people died of cancer in India, and in female most deaths were due to breast cancer. The standard methods used currently to cure or control the cancer, exhibit severe toxicity on normal tissues. Therefore, worldwide research is going on to investigate the best effective antitumor agents from different sources. Among the different sources, herbal source remains important in identifying lead molecule in the plants with proven anticancer property that ultimately occupies the platform for clinical use. More than 60% of the commercially available anticancer drugs are related to natural origin. The ethno medicinal plants with anticancer properties reported possess the commonest phytoconstituents like alkaloids, glycosides, terpenoids, stearic, oleic, palmitic acid, flavonoid, β-sitosterol, amino acids, saponins etc.

Different species of the same genus have been reported to possess similar pharmacological activity.[3,4] Literature search on this line, indicates that *Gomphrena martiana* and *Gomphrena macrocephala* have been reported for anti-tumor activity;[5,6] however, the anticancer property of *Gomphrena globosa,* yet another plant belonging to the same genus has not been investigated though the plant has been reported for antihypertensive property and containing saponins, alkaloids, reducing sugars and coumarins.[7] Therefore, it was planned to investigate the anticancer property of *Gomphrena globosa* using chloroform extract of aerial parts of the plant.

Nonetheless, to our knowledge, no data are available so far on the safety assessment of CEGG in animals. Therefore, the present research investigated the potential toxicity of CEGG using a 90-Day Oral Toxicity study in rats.

MATERIALS AND METHODS

Materials

Preparation of CEGG

The aerial parts of *Gomphrena globosa* (Amaranthaceae) were collected freshly in the month of June, in and around Tiruchengode, Namakkal district, Tamil Nadu, India. The plant was authenticated by Dr. G. V. S. Murthy, Joint Director, Botanical Survey of India, Coimbatore, Tamil Nadu (Specimen Number: 1368).

The aerial parts of *Gomphrena globosa* were dried under shade, coarse powdered, and extracted (2500 g) with chloroform by continuous hot extraction method for 20 hours by using Soxhlet apparatus.[8] The extract was concentrated to a dry mass by vacuum distillation and at controlled temperature (40ºC - 50ºC). The crude chloroform extract was 180 g by wt. (Yield-7.2%). The extract was preserved in a refrigerator at 40°C, which was utilized for the present study.

Experimental Animals

Albino rats of Wistar strain was used for this repeated oral toxicity study. The ethical clearance for animal experiments was obtained from the Institution Animal Ethical Committee, Swamy Vivekanandha College of Pharmacy (889/ac/05/CPCSEA dated 29th April 2005). Rats of either sex weighing 125 to 150 g were used for the study. Each animals, at the commencement of their dosing, was between 8 and 12 weeks old and their weight variation was within ± 20% of the mean weight of any previously dosed animals. The temperature in the experimental animal room was 22ºC (± 3ºC) and the relative humidity was between 50-60%. These animals were fed with pellet diet manufactured by Amrut laboratory, Animal Feed Company, Sangli, Maharashtra and drinking water *ad libitum*. They were kept in 12 h/12 h light/dark cycle and maintained for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions.[9-11]

STUDY DESIGN OVERVIEW

90-Day Oral Toxicity study

The study was done on Wistar strain albino rats as per the OECD guidelines No: 408.[12] Albino rats of either sex were divided into 2 groups of 10 animals in each (5 males and 5 females). First group served as solvent control and was given normal saline (1 ml/kg. p.o.) and the other group was administered CEGG at the dose of 1000 mg/kg, p.o. The test extract was given once daily orally for 90 days. All the rats were observed for any physiological and

behavioral changes and mortality if any. Food and water consumption was checked daily. Body weight was recorded on 1st, 30th, 60th and 90th day. Blood samples were obtained (in capillary tubes) by the retro-orbital puncture under light diethyl ether anesthesia, with, as well as without anticoagulant (ethylenediamine tetra acetate).[13] Blood with the anticoagulant was used immediately for the determination of hematological parameters, while blood without the anticoagulant was centrifuged at 4000 rpm for 10 min at 4ºC, and the serum obtained was stored at -20ºC until analyzed for biochemical parameters.

OBSERVATIONS

Clinical Signs

The changes in the physical appearance, behavioral pattern, breath, glandular secretion, excreta, mortality, and signs of illness were continuously monitored throughout the experiment. Additionally, the body weight of each rat was also observed every four days in the study.

Body Weight, Food Consumption, and Food Conversion Efficiency

Body weight was recorded on the first day of administration, then recorded every four days during the 90-day feeding and at the end of the experiment. Food consumption was evaluated over successive periods of 90 days by weighing the rats. In addition, food conversion efficiency was measured as the weight gain per gram of food consumed.

Hematological and Biochemical Analyses

Hematological parameters such as white blood corpuscles (WBC), lymphocyte, monocyte, neutrophil, eosinophil, and basophil, red blood corpuscles (RBC), Hb, HCT, MCV, MCH and MPV were estimated on the next day of the prescribed period. The blood samples were collected from each rat individually into non-heparinized tubes and were allowed to coagulate. Serum was separated by centrifugation and total proteins, albumin, ALT, AST, blood urea nitrogen, creatinine, total cholesterol, and triglycerides were analyzed. Animals were then sacrificed and the vital organs such as heart, lung, liver, spleen, kidney, brain, thymus and adrenal gland were removed, and color of the organs was observed for gross pathological changes, and then they were processed for histopathology examination.

Organs Weight and Histopathology Examinations

At the ending of the experiment, all the rats were euthanized and subjected to necropsy. Pertinent observations on the pathology of each organ were conducted and recorded. Organs

such as heart, lung, liver, spleen, kidney, brain, thymus and adrenal gland were weighed before the histopathology tests. Histopathology procedures were conducted according to standard protocols. After trimming at 2-3 mm thickness, formalin-fixed tissues were embedded in paraffin. Moreover, the tissues were cut at sections in 5 µm thick and stained with haematoxylin and eosin (H&E). Microscopic examination was performed and the findings were compared to parallel sections with the control group.

Statistical Analysis

All values were presented using means ± SEM. The differences between the control and dosage group was statistically evaluated by one way ANOVA followed by Turkey Kramer multiple comparison test. P value < 0.05 was considered to be statistically significant. Statistical analyses were conducted by SPSS 24.0 software.

RESULTS AND DISCUSSION

Clinical Observations

All the experimental animals survived and appeared healthy during the 90-day period of the study. No treatment-related changes were discovered in daily general observations and clinical examinations.

Body Weight, Food Consumption, and Food Conversion Efficiency

After the 90-day oral feeding, no statistically significant changes were found in body weights, food consumed, and food conversion efficiency between the treatment and control groups. The data were presented in Table 1.

Hematology and Biochemical Data

The hematological data were presented in Table 2. No significant changes of hematological parameters were found between the CEGG and control group, except MCH and MPV values. MCH and MPV values were significantly changed ($P < 0.05$) as compared to control.

The serum biochemical parameters were summarized in Table 3. No significant changes of biochemical parameters were found between the CEGG and control group, except total cholesterol and triglycerides values. They were significantly changed ($P < 0.05$) as compared to control.

Organs Weight and Histopathology Examinations

Except lung and spleen, there was no significant weight loss of vital organs of the animals treated with CEGG when compared with control animals. The data were presented in Table 4. The histopathology study did not show any significant changes in the appearance of the cells of various organs investigated, as compared to the normal (Data not shown).

Table 1: Body weight, food consumption, and food conversion efficiency of rats fed with CEGG for 90 days.

Day	Control	CEGG
Day 0	198.78 ± 5.86	201.71 ± 5.21
Day 4	199.84 ± 4.98	202.23 ± 4.98
Day 8	201.13 ± 5.14	203.13 ± 5.24
Day 12	201.96 ± 5.45	204.58 ± 4.75
Day 16	202.48 ± 4.78	205.64 ± 6.13
Day 20	203.74 ± 4.95	206.49 ± 5.84
Day 24	204.57 ± 5.67	207.75 ± 5.94
Day 28	205.83 ± 5.81	208.43 ± 4.68
Day 32	206.73 ± 4.52	209.22 ± 4.27
Day 36	207.86 ± 4.87	210.84 ± 4.57
Day 40	208.47 ± 5.21	211.49 ± 5.61
Day 44	209.65 ± 5.69	212.76 ± 5.91
Day 48	210.12 ± 6.14	213.94 ± 7.12
Day 52	211.14 ± 5.74	215.34 ± 6.42
Day 56	211.89 ± 4.95	216.83 ± 5.17
Day 60	212.08 ± 3.50	217.92 ± 3.37
Day 64	213.45 ± 4.58	218.71 ± 4.85
Day 68	214.86 ± 5.27	219.11 ± 3.64
Day 72	215.27 ± 5.61	219.87 ± 4.69
Day 76	215.94 ± 3.98	220.27 ± 5.82
Day 80	216.43 ± 4.82	221.13 ± 4.67
Day 84	217.62 ± 5.18	221.98 ± 5.93
Day 88	218.84 ± 5.24	222.16 ± 6.24
Day 90	219.53 ± 3.42	222.86 ± 4.26
Total food consumption[a]	1348 ± 48.25	1157 ± 57.63
Average feed conversion efficiency (%) [b]	12.86 ± 0.87	13.29 ± 0.92

Body weight means (g/rat ± SD), food consumed means (g/rat/day ± SD, values in parenthesis), and average food conversion efficiency [(g weight gain) / (g feed consumed) ± SD] of Albino rats (n=5/dose) consuming different concentrations of CEGG.

[a] *Total food consumption: total food consumed since study initiation.*

[b] *Average feed conversion efficiency: total food consumption divided by total body weight gain. The treated group was not significant as compared to control.*

Table 2: Hematology in rats given CEGG for 90 days.

Parameters	Control	CEGG
WBC ($\times 10^9$/L)	9.63 ± 4.97	9.39 ± 2.78
Lymphocyte (%)	78.37 ± 5.67	78.12 ± 4.41
Monocyte (%)	5.21 ± 1.53	5.34 ± 2.81
Neutrophil (%)	14.76 ± 4.12	14.87 ± 5.34
Eosinophil (%)	1.65 ± 0.35	1.66 ± 0.82
Basophil (%)	0.01 ± 0.03	0.01 ± 0.03
RBC ($\times 10^{12}$/L)	8.64 ± 0.54	8.64 ± 0.43
Hb (g/L)	151.80 ± 6.77	152.90 ± 5.46
HCT	43.34 ± 1.76	43.50 ± 2.86
MCV	50.65 ± 1.68	51.63 ± 0.91
MCH	17.58 ± 0.63	18.83 ± 0.52*
MPV	6.58 ± 0.17	6.38 ± 0.31*

Values are means ± standard deviations. The values are significantly different (∗P < 0:05) as compared with the corresponding control group.

Table 3: Serum biochemistry in rats given CEGG for 90 days.

Parameters	Control	CEGG
Total Proteins (g/L)	54.17 ± 2.97	55.36 ± 3.21
Albumin (g/L)	38.42 ± 2.41	37.41 ± 1.96
ALT (U/L)	31.47 ± 5.84	32.24 ± 5.37
AST (U/L)	89.64 ± 10.32	88.15 ± 9.54
Blood Urea Nitrogen (mmol/L)	6.32 ± 1.68	5.95 ± 1.89
Creatinine (µmol/L)	41.69 ± 11.56	42.31 ± 10.68
Total Cholesterol (mmol/L)	1.63 ± 0.24	1.42 ± 0.31*
Triglycerides (mmol/L)	0.85 ± 0.35	0.52 ± 0.29*

Values are mean ± SEM of 5 animals each. The values are significantly different (∗P < 0:05) as compared with the corresponding control group.

Table 4: Organ weights in rats given CEGG for 90 days.

Absolute Organ Weight (g)	Control	CEGG
Heart	1.47 ± 0.07	1.42 ± 0.08
Lung	1.84 ± 0.13	1.68 ± 0.12*
Liver	12.96 ± 1.26	13.24 ± 1.41
Spleen	0.82 ± 0.06	0.73 ± 0.08*
Kidney	2.97 ± 0.21	3.24 ± 0.16
Brain	2.35 ± 0.11	2.26 ± 0.14
Thymus	0.37 ± 0.05	0.38 ± 0.07
Adrenal gland	0.06 ± 0.01	0.05 ± 0.01

Values are means ± standard deviations. The values are significantly different (∗P < 0:05) as compared with the corresponding control group.

DISCUSSION

Cancer is a second leading cause of death in developing countries, with uncontrolled division of cells in the part of the body. It has been reported in the year 2018 that there were more than eight lakh people died of cancer in India, and in female most deaths were due to breast cancer. The standard methods used currently to cure or control the cancer, exhibit severe toxicity on normal tissues. Therefore, worldwide research is going on to investigate the best effective antitumor agents from different sources. Among the different sources, herbal source remains important in identifying lead molecule in the plants with proven anticancer property that ultimately occupies the platform for clinical use. More than 60% of the commercially available anticancer drugs are related to natural origin. Literature search on this line, indicates that *Gomphrena martiana* and *Gomphrena macrocephala* have been reported for anti-tumor activity; however, the anticancer property of *Gomphrena globosa*, yet another plant belonging to the same genus has not been investigated. Therefore, it was planned to investigate the anticancer property of *Gomphrena globosa* using chloroform extract of aerial parts of the plant. Nonetheless, to our knowledge, no data are available so far on the safety assessment of CEGG in animals.

It is well known that toxicological screening and safety evaluation on medical plants is essential before therapeutic applications in disorders and diseases. During the last several decades, acute and long-term oral feeding has been advocated as a fundamental test and applied in many safety assessing studies. Therefore, we firstly conducted the acute oral toxicity study in Albino mice, and the results revealed that the CEGG has no toxicity to Albino mice (data not shown). The present study, followed by the previous acute oral toxicity study, provided evidences of CEGG safety using a 90-Days oral feeding with dosing up to 1000 mg/kg body weight. All toxicity tests in the present study are conducted in compliance with the guidelines of "*Organization for Economic Co-operation and Development*".

During the course of the experiment, rats in all CEGG group and the control group were all survived in good health. No significant drug-related changes associated with clinical symptoms were found using clinical examination.

According to our findings, no significant difference in the body weight and average feed conversion efficiency were found between the CEGG group and control group during the following 90-day period. It is generally accepted that the reduction of body weight gains or losses 10% more than the mean control value is defined as toxicological significance.[14]

Therefore, our study suggests that the CEGG administration had no compound related effects on body weight changes in rats.

As for the hematological examination, the mean platelet volume (MPV) in the CEGG group was significantly lower than the control one. Additionally, compared with the control group, the mean corpuscular hemoglobin (MCH) in the CEGG group was found significantly increased. A previous study suggested that the changes in MPV and MCH were correlated with the changes of blood viscosity and RBC deformability.[15] However, these statistical differences were not considered adverse or related to the consumption of CEGG because these values were comparable between the CEGG group and control group.

In terms of serum biochemical examination, total cholesterol and triglyceride significantly decreased in the CEGG group compared with the control one, which may result from the prolonged fasting in the rat before treatment, inducing the excessive binding of triglycerides to proteins.

Although there were few statistically significant differences between the CEGG group and control group, no significant relationship effects were found in these abnormal indicators. Consequently, the changes of hematology and biochemical indicators in rats should not be considered as the compound-related effects and was not considered biologically significant.

In the analysis of organ weight and histopathology, though significant differences in absolute weights were discovered in lung and spleen, no obvious lesions were found in further histopathology examination and these changes could be considered to be incidental.

Overall, compared with the control group, we found a decrease in the total cholesterol, as well as triglyceride, and a decrease in the MPV and increase in the MCH in the CEGG group. However, no significant correlations were found with the CEGG in these altered values. We attribute this phenomenon to some extraneous factors as well as biological variations arising from inter- and intra-animal components. In addition, other physiological factors including age, sex, restraint, diet, and circadian may also have the effects on the overall variation. The results of our research confirm that there are no adverse effects due to the administration of CEGG.

CONCLUSION

To the best of our knowledge, this is the first study on the safety assessment of CEGG. In the present study, the results suggest that CEGG on prolonged administration may cause a slight change in body weight and hematological examination, but could harbor no significant toxicological effect. Therefore, our study provides some evidence on the safety of the chloroform extract of *Gomphrena globosa* for potential clinical application. In addition, further studies of CEGG are still necessary to assess its oral safety in patients.

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