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ACUTE AND SUBACUTE TOXICITY STUDIES OF METHANOLIC EXTRACT OF *CLEOME GYNANDRA LINN* ON EXPERIMENTAL ANIMALS

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ABSTRACT

Cleome gynandra has been extensively used for many ailments in Ayurveda and Siddha systems. In the present study the acute and sub-acute oral toxicities were investigated on methanolic extract of *Cleome gynandra* in experimental animals. Acute toxicity study was carried out according to OECD guideline no. 423. The different doses like 500, 1000 and 2000mg/Kg b.w were administered orally and observed for 24 hrs and 14 days. In sub-acute oral toxicity study, evaluations were carried out after administering daily oral doses of 200, 400 and 800mg/kg body weight for 28 days. Body weights of the rats observed weekly, biochemical and relative organ weights of the rats were observed on 29th day. By observing the biochemical parameters finally concluded that high doses of methanolic extract of *Cleome gynandra* may exhibit mild organ toxicity effects.

INTRODUCTION:

Herbal medicines are alternative for treatment of various diseases due to their assumed acceptability, effectiveness, affordability, safety and low cost[1]. But, the use of herbal products should be based on scientific origin; or else they would be of no use and precarious. Furthermore, the irrational use of these herbal products may cause serious toxicity for humans. Unfortunately, many people underestimate the toxicity of natural products and do not realize that these agents could be as toxic or more than synthetic drugs[2]. A typical example for a toxic herbal product are the leaves of *Atropa Belladonna*[3] and *Digitalis purpurea*[4], which show severe systemic toxicity if taken orally. Toxicological studies help to make decision whether a new drug should be adopted for clinical use or not. OECD 401, 423 & 425 does not allows the use of drug clinically without its clinical trial as well as toxicity studies. Depending on the duration of drug exposure to animal's toxicological studies may be three types such acute, sub-acute and chronic toxicological studies[5]. *Cleome gynandra*

(Capparidaceae) is used as a medicinal plant and can be found in all over world. It grows as a weed in paddy fields and also in road sides and in open grass lands. In India it is never cultivated but grows spontaneously everywhere[6]. This study aims to evaluate the acute and sub-acute toxicity of methanolic extract of *C. gynandra* on Experimental rats.

Materials and methods

Collection and identification of plant material

Fresh samples of *C. gynandra* were collected from local area of Kadapa, Andhara Pradesh, India. The botanical identity was confirmed at the Department of Botany, SV University, Tirupathi, India by a plant taxonomist. Voucher specimen (no. SV-30475) of the plant is kept in our lab, for reference purposes.

Preparation of the extract

The plant material was washed, air-dried for four weeks and pulverized into coarse powder by using pestle and mortar. The coarse powder was further processed to fine particles with an electric blender. Fifty grams of *C. gynandra* fine powdered sample was extracted with 250 mL methanol for 48 h by using Soxhlet apparatus at 65°C. The extract was evaporated under reduced pressure by using a rotary evaporator and further concentrated in a water bath at 65°C.

Experimental animals

Both sexes of albino rats weighing about 150 to 180 g were used in the study. The study protocol was reviewed and approved by the institutional animal ethical committee of PRRM College of pharmacy (1423/PO/a/11/CPCSEA). Animals were obtained from invivo biosciences, bangalore. Rats were housed in polyacrylic cages (38x23x10 cm). They were housed in an air conditioned room and were kept in standard laboratory conditions under natural light and dark cycle (approximately 12 h light/ 12 h dark) and maintained humidity 60±5% and an ambient temperature of 25±2%. All experiments were performed between 9:00 am to 4:00 pm. The animals were free access to standard diet and water *ad libitium* and allowed to acclimatize for one week before the experiments. The commercial pellet diet contained 22% protein, 4% fat, 4% Fiber, 36% carbohydrates and 10% ash w/w.

Acute oral toxicity Study

The acute oral toxicity study was carried out for methanolic extract of *C.gynadra* using the fixed dose method according to OECD guideline no. 423. Healthy adult albino rats weighing between 150 to 200 g were used for the study. Animals were divided into four groups of three animals each and kept fasted overnight. The different doses like 500, 1000 and 2000mg/Kg body weight were administered to the group II, III and IV respectively. The animals were observed keenly for about 24 hr for any signs of toxicity or mortality and also observed for 14 days without giving drug[7].

Sub acute toxicity study

Experimental design

The animals were divided into 5 groups of 10 rats in each group.

Group I Served as control group provided standard diet and water *ad libitium*

Group II Administered with MECG (200mg/kg, p.o)

Group III Administered with MECG (400mg/kg, p.o)

Group IV Administered with MECG (800mg/kg, p.o)

Group V (Satilite group) Administered with MECG (800mg/kg, p.o)

The rats were observed daily for any signs of toxicity, and their body weights were also recorded weekly throughout the experimental period[9]. The MECG was administered to Group-V for 28 days and next 14 days were observed for delayed toxic effect without drug administration.

Biochemical analysis

Commercial kits from Erba Diagnostics, Mumbai, India, were respectively used for the assay of liver and kidney indices

Statistical analysis

Data were expressed as Mean ± SEM (n=10). The results were analyzed using one way ANOVA in graph pad prism ver.5.0. To determine the level of significant difference between each treatment and the control group using Tukey test.

Results

Acute oral toxicity study

Acute toxicity study performed as per the OECD Guidelines 423, the results reveal that the methanolic extract of *C.gynandra* has been found to be non toxic up to dose level 2000 mg/kg body weight of experimental animals. No mortality was observed during either on first day and up to 14 days of observation.

Sub acute toxicity study

Body weight: During the experimental period all rats showed a significant increase in body weight compared to their initial values. No mortality was observed during the whole experiment period (Table 1).

Relative organ weight

Sub acute oral administration of methanolic extract of *C.gynandra* for 28 days showed in significant and dose dependent decrease in weight of the liver , kidney and partial decrease in weight of heart and brain of animals in group-IV and V compare to group-I (Table 2).

Biochemical parameters

Discussion

Toxicity is an expression of being poisonous, indicating the state of adverse effects led by the interaction between toxicants. Most of the herbal preparations do not have drug regulatory approval to demonstrate their safety and efficacy[8]. It is therefore pertinent to establish the safety of medicinal plant preparations through toxicological assessments. The visceral organs like kidney, liver, heart and brain are very essential organs for human life. Alteration in organ-to-body weight ratio may be as a result of organ damage[9]. In the present study, acute oral administration of MECG to rats at a dose level of up to 2000mg/kg did not cause any mortality or toxic symptoms up to 14 days of observation. Similar kind of results was reported at different LD_{50} values for different plant extracts. The oral LD_{50} of ethanol extract of *Vitex leucoxylon* leaf (>3000mg/kg), cold water infusion extract of the same plant (1050mg/kg), ethanolic extracts of *Ailanthus excelsa* (1000mg/kg), *Toddalia asiatica* (350mg/kg) and *Araucaria bidwilli* (250 mg/kg) have been reported[10]. Sub-acute doses of MECG (200, 400 and 800 mg/kg) caused noteworthy increase in the body weights of the experimental rats.

Table no:01 Effect of EEMCF on body weight

Groups	Body weight (gm) on				
	0 day	7th day	14th day	21st day	28th day
Group-I	257±22.78	276±28.38	257±22.89	235±19.66	267±25.11
Group-II	280±21.89	267±21.56	230±11.48	216±13.71	302±25.03
Group-III	262±26.37	240±23.75	228±8.077	210±11.07	275±24.86
Group-IV	232±20.83	274±24.81	238±6.823	243±8.265	260±20.74
Group-V	222±19.81	290±25.89	271±6.9212	264±6.985	245±19.77

Table no:02 Effect of MECG on relative organ

S.no	Body Organs	Group-I	Group-II	Group-III	Group-IV	Group-V
1	Liver	10.62±0.245	10.03±0.226	9.66±0.186	9.355±0.197***	8.897±0.489***
2	kidney	1.658±0.168	1.463±0.141	1.418±0.123	1.178±0.097***	0.8867±0.0811***
3	Brain	1.732±0.068	1.557±0.064	1.362±0.103	1.098±0.0317	0.9143±0.0263
4	Heart	1.127±0.049	1.062±0.042	0.987±0.987	0.852±0.0296	0.774±0.0217

Values are Mean± S.E.M of 10 animals in each group. One way ANOVA used.
*= P<0.05; **= P<0.01;***=P<0.001 Compared to Group-I

Table no:03 Effect of MECG on biochemical parameters

S.no	Parameters	Group-I	Group-II	Group-III	Group-IV	Group-V
1	TotalCholesterol	111.1±11.04	85.72±5.55	134.4±13.76	264.8±8.23***	322.2±28.40***
2	Triglycerides	36.83±5.375	59.54±4.13	87.92±7.29**	159.3±7.35***	171.0±14.63***
3	HDL	82.53±3.568	78.27±8.13	62.67±4.64	33.74±1.98***	33.18±3.18***
4	LDL	51.48±1.68	72.77±4.66	79.43±9.13	159.3±17.31***	145.6±8.89***
5	VLDL	8.33±0.81	8.283±1.27	10.16±1.55	38.55±3.15**	35.93±2.86**
6	Glucose	108.8±8.84	136.0±14.27	109.9±6.82	39.06±6.042***	38.23±3.74***

Values are Mean± S.E.M of 10 animals in each group. One way ANOVA used.
*= P<0.05; **= P<0.01;***=P<0.001 Compared to Group-I

This may be an advice that the drug does not influence the feed utilization ratio of the animals. MECG causes significant and dose dependent decrease in weight of the liver, kidney and partial decrease in weight of heart and brain of animals in group-IV and V compare to group-I. It can be done by may be the formation of inflammation and granuloma of visceral organs. Assessment of liver and kidney function is a very vital index in evaluating the toxicity of drugs and plant extracts. Kidney function indices evaluated in this study were serum urea and creatinine concentrations. This correlates with the findings of Muhammad *et al*[11], who carried out an investigation on the acute and sub-chronic toxicity of kernel extract of *Sclerocarya birrea* in rats. The methanolic extract of *C.gynandra* showed a significant and dose dependent increase in total cholesterol, triglycerides, LDL, VLDL, direct bilirubin and significant and dose dependent decrease in HDL levels which indicates liver inflammation, degeneration and possible for peripheral and cardio vascular disorders at usage of prolonged

higher doses[12] . In this study glucose levels decreased significantly and dose dependently (P<0.001) at dose of 800 mg/kg body weight it warns to affect with hypoglycemia at usage of prolonged higher doses[13].

Conclusion: *C. gynadra* at high doses caused elevation of some serum biochemical parameters. The plant is though a promising agent in pharmaceutics, but can cause mild organ damage at high doses.

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