

## INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES

[ISSN: 0975-4725; CODEN(USA):IJPS00] Journal Homepage: https://www.ijpsjournal.com



#### **Research Article**

# **Toxicological Studies of Methanolic Extracts of** *Ctenolepis garcinii* (Burm. f) Using Rats

### Natarajan P.\*<sup>1</sup>, Melba Y.<sup>1</sup>, Jegan N.<sup>2</sup>, Thiruppathi M.<sup>1</sup>, Edwin Jose<sup>1</sup>

<sup>1</sup>Sankaralingam Bhuvaneswari College of Pharmacy, Sivakasi, Tamil Nadu, India <sup>2</sup>K. M. College of Pharmacy, Madurai, Tamil nadu, India

#### ARTICLE INFO

Received: 22 March 2024 Accepted: 26 March 2024 Published: 02 April 2024 Keywords: Ctenolepis garcinii, Methanolic, Acute and Subacute DOI: 10.5281/zenodo.10908282

#### ABSTRACT

Objective: The present study focused to the acute and subacute toxicity of methanolic extracts of Ctenolepis garcinii burm.f in albino rats. Materials and Methods: In the acute study, a single dose of 2000 mg/kg was administered to albino rat which were observed for 72 h. In sub-acute toxicity study repeated doses of the MECG was administered to albino rats of both genders, separately. The animals received two doses of MECG (200 and 400 mg/kg p.o. respectively) for a period of 28 days. On the 28th day of the experiment, blood sample was collected by cervical decapitation after anaesthetizing them with anaesthetic ether and it was used to identify the hematological and biochemical analysis. Throughout the study, behavior changes, mortality, body weight, food and water consumption were observed and then sacrificed to remove the organ like liver and kidney for histopathological examination. Results and Discussion: There were no morbidity and mortality were reported. In sub-acute toxicity study, there is no any significant changes were observed in haematological and biochemical parameters at 200 and 400 mg/kg/p.o. respectively. Conclusion: It was concluded that no toxicity was observed in methanolic extracts of Ctenolepis garcinii (MECG).

#### **INTRODUCTION**

The pharmacopoeias of many countries of the world include even today a large number of drugs of plant origin [1]. Medicinal plants still play an important role in emerging and developing countries of Asia, both in preventive and curative treatments, despite advances in modern western medicine [2]. Over the years, the use of herbal products globally either as a food supplement or curative medicine has witnessed a phenomenal growth [3]. In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects. A number of medicinal plants, traditionally used for over

\*Corresponding Author: Natarajan P.

Email 🔤 : natarajanmpharm@gmail.com

Address: Sankaralingam Bhuvaneswari College of Pharmacy, Sivakasi, Tamil Nadu, India

**Relevant conflicts of interest/financial disclosures**: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

1000 years named rasayana are present in herbal preparations of Indian traditional health care systems [4]. The WHO has listed 21,000 plants, which are used for medicinal purposes around the world. Among these 2500 species are in India, out of which 150 species are used commercially on a fairly large scale [5]. People in India and China are known to have used plants in organized health care regime for over 5000 years. European herbal medicine blossomed in the Graeco-Roman era and remained the mainstream until six decades ago.

Toxicology can be defined as that branch of science that deals with poisons, and a poison can be defined as any substance that causes a harmful effect when administered either by accident or design to a living organism. Toxicity itself can rarely, if ever, be defined as a single molecular event, but is, rather, a cascade of events starting with exposure, proceeding through distribution and metabolism, and ending with interaction with cellular macromolecules and the expression of a toxic end point [6].

The herbal drugs from the traditional system of medicine have contributed very useful form of drugs over the years and it was aimed to bring the treasure of these traditional systems of medicine. The aim of present study to identify the toxicological risk of selected plant (Ctenolepis garcinii burm.f).

#### MATERIALS AND METHODS

#### **Collection of specimens**

The species for the proposed study is Ctenolepis garcinii (burm.f) leaves and were collected in the month of January to February from the areas near the town of Sivakasi, Virudhunagar district, Tamilnadu, India.

#### **Taxonomic identification**

The species for the proposed study Ctenolepis garcinii (burm.f) was positively identified and authenticated by Dr.V.Ganesan, Dept. of Botany, Ayyanadar Janakiammal College of Arts and Science Sivakasi, Virudhunagar District, Tamilnadu, India. The plant specimen was certified as Ctenolepis garcinii (burm.f) of family Cucurbitaceae.

The leaves of the plant Ctenolepis garcinii (Burm.f) were collected and it was size reduced into small pieces and shadow dried. The dried materials were coarsely powdered before extraction, which was used for further detailed studies [7].

#### **Preparation of the extracts**

About 66 g of Ctenolepis garcinii air dried, powdered material was taken in 1000 ml Soxhlet apparatus and extracted with methanol for 72 hours. At the end of the 3rd day the powder was taken out and it was dried The methanolic extracts were concentrated by distillation and solvent was recovered. After completion of extraction it was filtered and the solvent was removed by distillation under reduced pressure. The extract was dried and stored in desiccators. A reddish brown residue was obtained. The percentage yield was calculated [8].

#### Identification of Phytoconstituents

The MECG were subjected to routine Phytochemicall analysis for the presence of various phytoconstituents [9][10].

#### Selection of suitable animal model

Healthy young Wistar albino rats weighing 100-150 gm (8 to 12 weeks old) were selected for toxicity studies. The rats were kept in properly numbered large polypropylene cages with stainless steel top grill having the necessary facilities and given a standard diet and water ad libitum throughout the experimental period [11]. The animals were maintained with 12 hr light and dark cycle at 220 C ( $\pm$  30C) in a well-ventilated animal house under natural conditions [12]. The experimental protocol has been approved by institutional animal ethics committee, Ref no. SBCP/2012-13/CPCSEA/IAEC-III/06.

#### Acute oral toxicity study



The acute oral toxicity was conducted as per the OECD guidelines 423. Twelve Wistar albino rat 100-150g were used for the acute toxicity study. They were randomly distributed into five groups, containing three animals per group and were on a normal diet provided with water ad libitum. The treated group received orally varying doses (5, 50, 300, 2000 mg/kg/body weight) for 14 days. Animals treated with normal saline 10ml/ kg served as control. They were continually observed for 4h, detect any changes in skin and fur, eyes and mucous membranes, and also autonomic and central nervous system and somatomotor activity and Behavioural patterns, signs of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma also to be noted. The animals were kept 14 days under observation up to after administration methanolic extract of Ctenolepis garcinii (MECG) to find out the delayed mortality [13],[14].

#### Sub-acute toxicity study

The sub-acute toxicity procedure was followed by using OECD guidelines 407. The albino rats of both sexes were randomly selected and put into three groups with six rats in each group. Food was withheld overnight, but water made available. The animals were weighed prior to administration of the extract. First group served as solvent control and was given normal saline 10ml/kg p.o. and other groups were administered methanolic extract of Ctenolepis garcinii (MECG) at 200 and 400 mg/kg p.o. respectively. The test compounds were given once daily orally for 28 days. All the rats were observed for any physiological and behavioural changes and mortality if any. Food and water consumption were checked at an interval of 7 days. All the animals were taken weight once a week upto the end of the day [15].

At the end of the period, the animals in all the groups were sacrificed by cervical decapitation after anaesthetizing them with anaesthetic ether. Blood was collected in heparinized tubes for the analysis of hematological parameters. The collected blood sample was centrifuged for 10 minutes at 4000 rpm at 4°C to obtain the serum for biochemical estimations [16].

#### **Relative organ weights and Histopathology**

After sacrifice, organ weights (liver, kidney, brain, stomach and heart) were recorded and relative organ weights (ROW) were calculated as follows.

#### ROW= Absolute organ weight (g)/Body weight of rat on sacrificed day (g) x 100

Then the liver and kidney were preserved in 10% formalin solution for histological examination [17].

#### STATISTICAL ANALYSIS

Values are expressed as mean SEM. The mean differences in body weight and plasma biochemical analysis were analyzed using one-way ANOVA followed by Dunnest 't' test. The difference between each group were considered statistically significant at P<0.05. All statistical analysis was performed using Graph Pad prism statistical software (version 5.03).

### **RESULTS AND DISCUSSION**

#### Percentage yield

The dried powder of leaves of Ctenolepis garcinii was extracted with methanol by using a Soxhlet apparatus. The extract was dried and finally percentage yield was calculated. The percentage yield of methanolic extract of Ctenolepis garcinii (MECG) was found to be 9.84 (% w/w).

#### **Identification of Phytoconstituents**

In this present investigation, the phytochemical analysis revealed the Alkaloids, carbohydrates, glycosides, tannins, amino acids, flavonoids were present in the methanolic extract of Ctenolepis garcinii (MECG).

#### Acute toxicity study

The results of acute toxicity studies in albino rats were administered with various extracts of Ctenolepis garcinii at the doses of 5, 50, 300 and 2000 mg/kg by oral route. The test extracts were



dissolved in distilled water and administered orally. No acute mortality was observed even at 2g/kg for methanolic extracts of Ctenolepis garcinii. All the animals were found to be normal and there were no gross behavioural changes at the end of the period (14 days). The results were shown in Table-1. From these results, LD50 or the maximum tolerated dose was found to be 2000mg/kg. From this 1/5th and 1/10th of the maximum tolerated dose was selected for the screening toxicological studies. Hence the biological dose was identified as 1/5th and 1/10th of MTD and it was fixed as 200 and 400 mg/kg body weight. The results are shown in Table 2.

Table: 1 Effect of Methanolic extract Ctenolepis garcinii on its behavioural signs of acute toxicity in albino rats.

1 ats.		1																				
Group No	Extracts and dose mg/kg (p.o.)	Skin	Fur	Eyes	Grooming	Apathy	Ataxia	Circling	Compulsive	Locomotor activity	Alertness	Depression	Paralysis	Diarrhoea	Salivation	Piloerection	Gripping	Touching response	Tremors	Convulsions	Sleep	Coma
1	MEC G 5mg/ kg	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
2	MEC G 50 mg/k g	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-
3	MEC G 300 Mg/k g	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-
4	MEC G 2000 Mg/k g	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-
	Tabl	e: 2	Acu	te to	xicit	y stu	dy of	f Me	than	olic e	extra	ict of	Cte	nolej	pis ga	arcin	ii fo	r dos	se sel	ectio	n	
SI	r.	Extr	act		LD	50 or	·MT	D (n	ng/kg	g)	ED:	50 or	·/1/5 <sup>t</sup>	<sup>h</sup> of I	LD5(	) (mg	g/kg)	F	D50 LD5	or/1 50 (m		

Sr. No	Extract	LD50 or MTD (mg/kg)	ED50 or/1/5 <sup>th</sup> of LD50 (mg/kg)	ED50 or/1/10 <sup>th</sup> of LD50 (mg/kg)
1	Methanolic extract	2000	400	200

#### MTD - Maximum Tolerated dose

#### Subacute toxicity study

In subacute toxicity study of methanolic extracts of Ctenolepis garcinia (MECG) at the doses of 200

mg/kg p.o, and 400 mg/kg p.o respectively, there were no deaths during the treatment period either in the control or in the treated groups. Food and



water consumption also did not significantly differ which is shown in the Table- 4 & 5. There was no change in general behaviour or other physiological activities of the animals. Normally, the control and the drug treated groups showed an increase in body weight. The results are shown in Table 3.

#### Haemoglobin (Hb)

There were no significant changes beerved the levels of Hb, RBC, WBC and Platelet on the methanolic extract treated groups at 200 and 400 mg/kg p.o. respectively, when compared to Group-I.The results are shown in Table 6.

#### **Erythrocyte Sedimentation Rate (ESR)**

The MECG (200 & 400 mg/kg p.o respectively) treated groups were compared with the control animals. The slight elevation and significant changes were appeared when compared to Group-I. The results are shown in Table 6.

**Biochemical evaluation of sub-acute toxicity** 

The MECG (200 and 400 mg/kg p.o. respectively) treated groups were compared to Group-I. There was a slight elevation in serum glucose levels, urea, total protein and albumin but no significant changes were appeared. The results were shown in Table-7 The MECG treated groups at 200 and 400 mg/kg p.o. did not show any difference in creatinine, globulin, total bilirubin, Direct and Indirect bilirubin when compared to Group-I. The results are shown in Table 7 & 8. The MECG (200 & 400 mg/kg p.o respectively) treated groups were compared with Group-I. No significant changes were reported in SGPT, SGOT, ALP Cholesterol and Triglycerides. The results are shown in Table 9 & 10.

 Table: 3 Effects of methanolic extract of Ctenolepis garcinii burm.f. on body weight of sub-acute toxicity studies in albino rats

		Dose	Body weight (g)							
Gr. No	Drug treatment	(mg/kg) p.o.	1 <sup>st</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day	21 <sup>st</sup> day	28 <sup>th</sup> day	in body weight		
	Control									
1	(Normal saline)	10	113.3±3.57	119.7±3.43	124.2±2.50	128.3±2.21	133.8±2.24	18.09		
2	MECG	200	128.3±3.57	133.3±3.57	137.7±3.24	142.2±2.94	146.3±2.60	14.02		
3	MECG	400	126.7±4.41	130.5±3.93	134.2±3.51	138.8±3.32	143.3±3.07	13.10		

 Table: 4 Effects of methanolic extract of Ctenolepis garcinii burm.f. on Food intake of sub-acute toxicity studies in albino rats

Gr.	Drug	Dose	Food Intake(g)							
No	Treatment	(mg/kg) p.o.	1 <sup>st</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day	21 <sup>st</sup> day	28 <sup>th</sup> day			
1	Control (Normal saline)	10	43.04±0.54	43.63±0.62	45.18±0.49	47.99±0.63	48.43±0.52			
2	MECG	200	42.18±0.50	43.38±0.34	44.70±0.53	46.29±0.48	48.05±0.43			
3	MECG	400	43.13±0.57	45.82±0.70	46.63±0.59	46.48±0.77	47.24±0.66			



4.50±0.07

4.74±0.05\*

5.32±0.07\*\*

Gr.	Drug	Dose(mg/kg)		l l	Vater Intake (1	ml)	
No	Treatment	p.o.	1 <sup>st</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day	21 <sup>st</sup> day	28 <sup>th</sup> day
	Control						
1	(Normal saline)	10	41.76±0.30	42.27±0.37	42.27±0.37	43.00±0.44	42.43±0.22
2	MECG	200	42.28±0.33	43.03±0.18	43.03±0.18	42.92±0.55	42.77±0.29
3	MECG	400	42.93±0.27	43.48±0.40	43.48±0.40	43.10±0.31	42.85±0.31
Table 6:	Effects of M	ECG on haema	tological para	meters of sub	-acute toxicity	studies in alb	ino rats.
Group No.	Drug Treatmen	t Dose (mg/kg p.o.)	Hb g%	WBC 103/cu.mm	RBC 106/cu.mm	ESR mm/1st hr	Platelet (lakhs/cmm)
	Control						

8.41±0.03

8.45±0.03

8.96±0.03\*

14.19±0.09

14.32±0.10

14.45±0.29\*

Table: 5 Effects of methanolic extract of Ctenolepis garcinii burm.f. on water intake of sub-acute toxicity studies in albino rats

Values were expressed as Mean ± Standard error mean (SEM) of six animals in each group. Statistical analysis was performed using one-way

10

200

400

ANOVA followed by Dunnet's t-test using GraphPad prism 5.0. \*\*p<0.01, \*p<0.05 vs control.

3.13±0.05

3.27±0.07\*

4.17±0.03\*\*

5.68±0.07

5.71±0.08

5.77±0.08

Table:7 Effects of MECG on biochemical parameters of sub-acute toxicity studies in albino rats

Group No.	Drug Treatment	Dose (mg/kg p.o.)	Glucose (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)	Total Protein (g/dL)
1	Control (Normal saline)	10	102.7±0.32	43.59±0.46	0.86±0.02	8.43±0.16
2	MECG	200	106.2±0.44**	43.96±0.52	0.93±0.02	8.64±0.03*
3	MECG	400	105.2±0.19	45.68±0.26*	0.97±0.01*	9.04±0.09**

 $Values were expressed as Mean \pm Standard error GraphPad prism 5.0. **p<0.01, *p<0.05vs mean (SEM) of six animals in each group. control.$ 

Statistical analysis was performed using one-way

ANOVA followed by Dunnet's t-test using

Table:8 Effects of MECG on biochemical parameters of sub-acute toxicity studies in albino rats.

Group No.	Drug Treatment	Dose (mg/kg p.o.)	Albumin (g/dL)	Globulin (g/dL)	Total Bilirubin (mg/dL)	Direct Bilirubin (mg/dL)
1	Control (Normal saline)	10	4.66±0.03	4.75±0.05	0.20±0.02	0.10±0.001
2	MECG	200	4.57±0.01	4.82±0.03	0.19±.009	0.12±0.014
3	MECG	400	5.08±0.14*	4.99±0.11*	0.26±0.006*	0.11±0.02

Values were expressed as Mean  $\pm$  Standard error mean (SEM) of six animals in each group. Statistical analysis was performed using one-way ANOVA followed by Dunnet's t-test using GraphPad prism 5.0. \*\*p<0.01, \*p<0.05vs control.



1

2

3

(Normal

saline)

MECG

MECG

Group No.	Drug Treatment	Dose (mg/kg p.o.)	Indirect Bilirubin (mg/dL)	SGPT (Units/L)	SGOT (Units/L)	ALP (Units/L)	
1	Control (Normal saline)	10	0.12±0.005	45.97±0.22	147.6±0.89	165.0±0.51	
2	MECG	200	$0.14 \pm 0.008$	47.09±0.33	156.5±0.55*	174.6±2.10**	
3	MECG	400	0.13±0.14	47.88±0.36	149.9±0.86*	168.3±0.31*	

Table:9 Effects of MECG on biochemical parameters of sub-acute toxicity studies in albino rats.

Values were expressed as Mean ± Standard error A mean (SEM) of six animals in each group. C Statistical analysis was performed using one-way c

ANOVA followed by Dunnet's t-test using GraphPad prism 5.0. \*\*p<0.01, \*p<0.05vs control.

Table:10 Effects of MECG on biochemical parameters of sub-acute toxicity studies in albino rats.

Group No.	Drug Treatment	Dose (mg/kg)	Cholesterol (mg/dL)	Triglycerides (mg/dL)	GGT (IU/L)
1	Control (saline)	10	42.90±0.67	24.32±0.81	6.25±0.07
2	MECG	200	42.17±0.98	25.73±0.50*	7.20±0.04*
3	MECG	400	43.70±0.74	24.78±0.33	6.26±0.03

Statistical analysis was performed using one-way GraphPad prism 5.0. \*\*p<0.01, \*p<0.05vs ANOVA followed by Dunnet's t-test using control.

Table:11 Effect of MECG on organ	weight of albino rate	s during sub-acute	toxicity studies.
0	8	0	

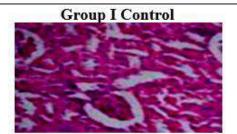
Group No.	Drug Treatment	Dose (mg/kg p.o.)	Brain	Heart	Kidney	Liver	Lungs
1	Control (Normal saline)	10	1.57±0.01	0.40±0.02	1.14±0.01	3.45±0.07	1.26±0.01
2	MECG	200	$1.58\pm0.02$	$0.41 \pm 0.04$	1.13±0.07	3.21±0.07	$1.25 \pm 0.01$
3	MECG	400	$1.64 \pm 0.01 *$	$0.53 \pm 0.02*$	$1.18\pm0.04$	3.63±0.03*	$1.24 \pm 0.07$

Values were expressed as Mean  $\pm$  Standard error mean (SEM) of six animals in each group. Statistical analysis was performed using one-way ANOVA followed by Dunnet's t-test using GraphPad prism 5.0. \*\*p<0.01, \*p<0.05vs control.

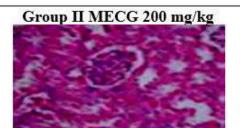
The Histopathology studies of various organ for drug treated groups



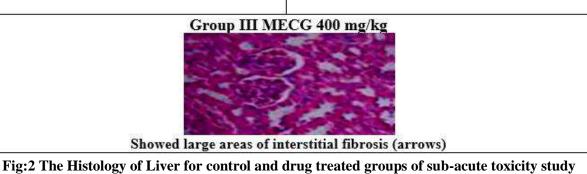
Fig:1 The Histology of Kidney for control and drug treated groups of sub-acute toxicity study

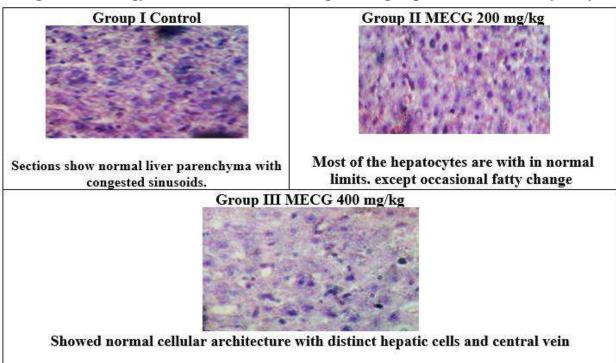


Showed normal kidney histological appearance.



showed minimal histopathological degeneration





#### CONCLUSION

Ctenolepis garcinii belonging to the family Cucurbitaceae is a useful herbal plant to pharmacological and therapeutic point of view. In the current study, an attempt was made to analyze the acute, subacute studies of methanolic extracts of Ctenolepis garcinii in albino rats. The preliminary phytochemical investigation showed the presence of alkaloids, carbohydrates, glycosides, steroids, tannins and flavonoids in the extract of Ctenolepis garcinii. In acute toxicity studies, there was no mortality or morbidity observed in methanolic extracts of Ctenolepis garcinii in the dose level upto 2g/kg. It represents



that the selected plant Ctenolepis garcinii was maximum safe. The results suggest that the haematological. biochemical and histopathological parameters revealed that the plant of Ctenolepis garcinii was not possess any type of toxicity.So, concluded that the above details, the plant of Ctenolepis garcinii is used for in future studies. The plant of Ctenolepis garcinii was used in traditionally for anticancer, analgesic ad anti inflammatory [18] but still not isolate the compound. Finally we conclude that the above plant have not found the toxicity so in future, we isolate the compound to further conform the above activity.

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HOW TO CITE: Natarajan P., Melba Y., Jegan N., Thiruppathi M., Edwin Jose, Toxicological Studies of Methanolic Extracts of Ctenolepis garcinii (Burm. f) Using Rats, Int. J. of Pharm. Sci., 2024, Vol 2, Issue 4, 85-94. https://doi.org/10.5281/zenodo.10908282

