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EFFECT OF *CTENOLEPIS GARCINII* (BURM.F) ON COMPLETE FREUND'S ADJUVANT INDUCED ARTHRITIS IN ALBINO RAT

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ABSTRACT

Objective: *Ctenolepis garcinii* belongs to family Cucurbitaceae has been used by traditionally and also as the indigenous medicine. The present study is to evaluate the Anti-arthritic property of methanolic extract of *Ctenolepis garcinii* (burm. f) in complete Freund's adjuvant-induced arthritis in rats. Methods: The methanolic extract of *Ctenolepis garcinii (burm. f)* (MECG) was administered by the oral dose of 200 and 400mg/kg respectively for a period of 24 days duration. The Biochemical parameters, haematological parameters, and histopathology of the joint tissue were studied. Results: The *Ctenolepis garcinii* extract attenuated the arthritis factors induced by complete Freund's adjuvant by the dose-dependent manner. Conclusion: The current study concludes that MECG extract of both concentrations provides a significant effect of anti-arthritic effects.

INTRODUCTION

Many traditional medicines in use are derived from medicinal plants, minerals and organic matter. A number of medicinal plants, traditionally used for over 1000 years named rasayana are present in herbal preparations of Indian traditional health care systems. In Indian Systems of Medicine (ISM) most practitioners formulate and dispense their own recipes [1].

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. India is the largest producer of medicinal herbs and is called as "Botanical Garden" of the world. The current research focuses on herbal drug preparations and plants used in the treatment of diabetes mellitus, a major crippling disease in the world leading to huge economic losses [2].

The Mohammedan culture enriched the vegetable material medica, which was more enlightened by coming in touch with Greece, Arabia and Persia [3]. People from other countries of the world as China, Cambodia, Indonesia and Baghdad used to come to the ancient universities of India like Takshilla (700 BC) and Nalanda (500BC) to learn health sciences of India particularly 'Ayurveda" From history we learn that since ancient times plants remained major natural resource in the world[4]. Arthritis is a very common disease found in almost all age groups and sexes. It is generally understood as different types of joint pains or joint disease. Arthritis is the most common cause of disability in the present world. More than 20 million individuals in this world suffer from Arthritis and make it very difficult for individuals to be physically active. There are different types

of arthritis, around 200 conditions affect joints, the tissues surrounding the joint, and other connective tissue. It is a rheumatic condition and another name for arthritis is wear and tear [5]. Few cases, arthritis can also affect different types of joints and other organs in the body, leading to a variety of symptoms, including fever, fatigue, weight loss, swelling of glands, loss of flexibility, decreased aerobic fitness, and weakness of the muscles [6]. The aim of present study is to evaluate the Anti-arthritic property of Methanolic extracts of *Ctenolepis garcinii* **(**burm. f) in complete Freund's adjuvant-induced arthritis in rats.

MATERIALS AND METHODS

Taxonomical 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.

Preparation of the extracts

The plant *Ctenolepis garcinii* (burm. f) was collected and it was size reduced into small pieces and shadow dried. The dried materials were coarsely powdered before maceration. After maceration the extract was distillated and crude extract was collected [7].

Selection of animal model

Healthy young wistar albino rats weighing 100-150 gm were selected for study. The rats were kept in polypropylene cages with stainless steel top grill having facilities, and given a standard diet and water *ad libitum* throughout the experimental period. The animals were maintained in 12 hr. light and dark cycle at 22^0C ($\pm$ 3^0 C) in a well-ventilated animal house under natural conditions, they were acclimatized to laboratory conditions for 10 days prior to the commencements of the experiment. Paddy husk was used as bedding material and changed twice a week [8]. The experimental protocol has been approved by institutional animal ethics committee.

ANTI-ARTHRITIC

Arthritis was induced by the injection of 100µl of CFA, (Containing 1mg/ml of heat-killed *Mycobacterium tuberculosis*, 0.85 ml paraffin oil and 0.15 ml of mannide monooleate.) into the sub planter region of right hind paw of rats[9]. The day of CFA injection was considered as day 1. The oral administration of the sample, Indomethacin (10mg/kg, i.p) of all the groups starts from day 14, once daily until day 24[10]. Anti arthritic activity of Ethanolic extract was evaluated the arthritic score on day 0, 4, 7, 10, 12, 14, 16, 19, 21 and day 24. The last day, all the animals were sacrificed under ether anaesthesia the blood was collected by retro-orbital route for haematological parameters. The joints were also cut for histology [11][12].

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 statistical analysis was performed using Graph Pad prism statistical software (version 5.03).

RESULT AND DISCUSSION

Acute toxicity study

The Methanolic extract of *Ctenolepis garcinii* was tested for acute toxicity study in order to fix the dose for the study. Up to 2000mg/kg the extracts were devoid of toxicity. Hence efficacy dose was identified as $1/5^{th}$ and $1/10^{th}$ of maximum tolerated dose and it was fixed as 200 and 400 mg/kg b.w were selected as the dose for testing the activity. The study was performed as per OECD-423 guidelines [13].

Paw volume

The mean difference in paw volume was found to be zero in normal control group. Paw volume difference was very much higher for the negative control group i.e. 0.32 on day 24. The group treated with standard drug was found to be with the 0.02 as the mean change in paw volume. The groups treated with MECG 200 and 400 were 0.10 and 0.05 respectively when compared with the negative control group. The results were shown Table:2

Effect on serum biochemical parameters

The serum level of SGOT was found to be 56.12U/L in Normal control group. The SGOT level of negative control group was increased potentially to 121.01U/L. The Standard treated group (Indomethacin 10mg/kg, i.p) was decreased the elevated level of SGOT to 61.05 U/L when compared to negative control group. The MECG 200mg/kg p.o and 400mg/kg p.o treated groups were decreased the SGOT level to 85.12 U/L and 59.23U/L respectively when compared to negative control group. The standard and MECG 200mg/kg and 400mg/kg p.o groups were decreased and significantly changed. The results were shown in Table:3. The SGPT level of normal control group was obtained 31.21 U/L and the negative control group was increased to 64.21 U/L. Indomethacin 10mg/kg, i.p (Standard treated group) was decreased the level of 32.01 U/L when compared to negative control group. The MECG 200 and 400mg/kg p.o treated groups were decreased the level to 44.02 and 33.27 U/L respectively when compared to negative control group. The results were shown in Table:3. In normal control group of alkaline phosphate was obtained to be 51.12 U/L and the negative control group level was increased to 139.21 U/L when compared to control group. Standard treated group (Indomethacin,10mg/kg, i.p) was decreased the elevated level of alkaline phosphate to 56.23 U/L when compared to negative control group. The MECG 200 mg/kg p.o and 400 mg/kg p.o treated groups were decreased level of alkaline Phosphate to 78.25 U/L and 59.11 U/L respectively when compared to negative control group. The MECG 400mg/kg p.o group obtained the level was almost equal to standard. The results were shown in Table-3

The total protein was found to 6.8 g/dl in normal control group. The level of total protein in negative control group (CFA) was decreased to 3.2 g/dl. The Standard treated group was increasing the level of total protein to 6.5 g/dl when compared to negative control group. The total protein level of MECT 200 and 400mg/kg p.o treated groups were increased to 5.4 and 6.1 g/dl respectively when compared to negative control group. The results were shown in Table-3.

The level of normal control group of Creatinine was found to be 0.37 mg/dl. The Creatinine level of negative control group was increased to 0.65 mg/dl. Standard treated group was decreased the elevated level of creatinine to 0.39 mg/dl when compared to negative control group. The MECG 200mg/kg p.o and 400mg/kg p.o treated groups were decreased the level to 0.49 mg/dl and 0.41 mg/dl respectively, when compared to negative control group. All the group have significantly changed. The results were shown in Table-3.

Effect on Haematological parameters

RBC level was found to be 6.31× 10^6/mm^3 in normal control group and the negative control group was decreased to 4.28× 10^6/mm^3. The standard treated group Indomethacin (10mg/kg i.p.) was increased the level of RBC to 6.12 × 10^6/mm^3 when compared to negative control group. The MECG 200mg/kg p.o and 400mg/kg p.o treated groups were increased the level of RBC to 5.01 and 5.92 × 10^6/mm^3 respectively and significantly changed when compared to negative control group. The results were shown in Table-4.

In WBC level of normal control group was obtained to 8.31× 10^3/mm^3. The

CFA 1mg/ml (negative control group) was increased potentially to 13.1× 10^3/mm^3.The Indomethacin 10mg/kg, i.p (Standard treated group) was decreased the elevated level of WBC to 8.22 × 10^3/mm^3 when compared to negative control group. The MECG 200mg/kg p.o and 400mg/kg p.o treated groups were decreased and significantly changed the level of WBC to 10.12 and 8.13 × 10^3/mm^3 respectively when compared to negative control group. The results were shown in Table-4. In normal control group of haemoglobin value was found to be 11.89 g%. The negative control group was decreased to 6.56 g%. The Standard treated group was increased the level of haemoglobin to 10.27 g% when compared to negative control group. The MECG 200mg/kg p.o and 400mg/kg p.o treated groups were increased the haemoglobin level to 8.01 and 9.94 g% respectively when compared to negative control group. The results of haemoglobin level were found in Table-4.

The level of ESR was found to be 41.14% in normal control group and the negative control group was increased potentially to 65.23%. The Standard treated group indomethacin was decreased the elevated level of ESR to 39.22% when compared to negative control group. The MECG 200mg/kg p.o and 400mg/kg p.o treated groups were decreased the ESR level to 46.21% and 40.47% respectively when compared to negative control group. The results of ESR level were found in Table-4.

Table: 1 Complete Freund's adjuvant (CFA) -induced arthritis in rats

S.No	Group	Drug and dose
1	I	Normal saline (5ml/kg, p.o)
2	II	CFA (1mg/mL) on sub plantar region
3	III	CFA (1mg/mL) + Indomethacin (10mg/kg, i.p)
4	IV	CFA + MECG (200mg/kg, p.o)
5	V	CFA + MECG (400mg/kg, p.o)

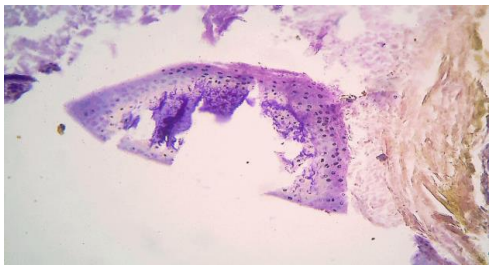
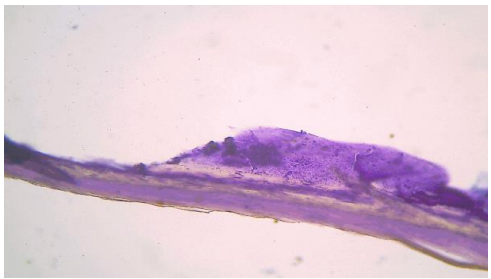


Figure 1 Histopathology of Complete Freund's adjuvant (CFA) induced arthritic paw joint tissue.



Group I Normal saline Sections show bony tissue and surrounding zone of fibro muscular connective tissue. No inflammation is seen.

Sections show bony tissue and surrounding zone of fibro muscular connective tissue. No inflammation is seen.

Drug and dosage	Mean change in paw volume									
	Day 0	Day 4	Day 7	Day 10	Day 12	Day 14	Day 16	Day 19	Day 21	Day 24
Normal saline (5ml/kg, p.o)	0.00± 0.00**	0.00± 0.00**	0.00± 0.00***	0.00± 0.00**	0.00± 0.00**	0.00± 0.00***	0.00± 0.00**	0.00± 0.00**	0.00± 0.00*	0.00± 0.00*
CFA (1mg/mL) on sub plantar region	0.12± 0.04	0.16± 0.06	0.19± 0.01	0.23± 0.03	0.26± 0.05	0.29± 0.01	0.32± 0.04	0.33± 0.01	0.33± 0.03	0.32± 0.02
CFA (1mg/mL) + Indomethacin (10mg/kg, i.p)	0.14± 0.02**	0.13± 0.04**	0.11± 0.03**	0.09± 0.01***	0.08± 0.02***	0.06± 0.03***	0.05± 0.02***	0.04± 0.03***	0.02± 0.06***	0.02± 0.05***
CFA + MECT (200mg/kg, p.o)	0.16± 0.02*	0.15± 0.03	0.14± 0.01**	0.13± 0.06***	0.13± 0.01***	0.12± 0.02***	0.11± 0.06***	0.11± 0.01***	0.10± 0.02***	0.10± 0.01***
CFA + MECT (400mg/kg, p.o)	0.15± 0.02**	0.13± 0.06**	0.10± 0.04***	0.10± 0.01***	0.09± 0.03***	0.07± 0.05***	0.05± 0.01***	0.04± 0.02***	0.04± 0.01***	0.05± 0.03***

Group II Negative control

Table: 2 Effect of MECG on mean paw volume change in Complete Freund's adjuvant (CFA) induced arthritis in albino rats

n=6, Data were expressed as Mean± SEM, one-way ANOVA followed by Dunnett's test, All groups were compared with Negative control, *P<0.05, **P<0.01, ***P<0.001

Table: 3 Effect of MECG on the biochemical parameters in CFA induced arthritis in albino rats

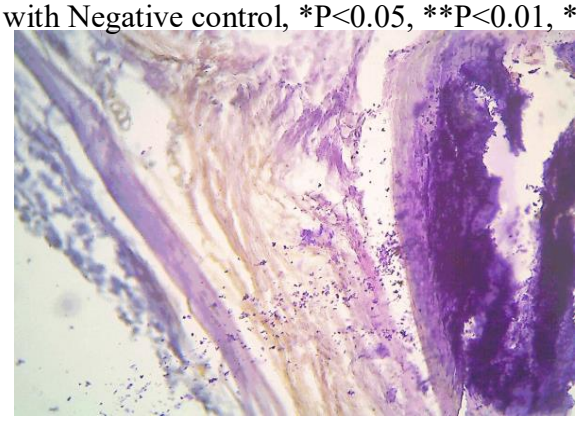
Drug and dosage	SGOT (U/L)	SGPT (U/L)	ALP(U/L)	Total protein(g/dl)	Creatinine (mg/dl)
Normal saline (5ml/kg, p.o)	56.12±1.5***	31.21±0.4**	51.12±0.2***	6.8±0.10**	0.37±0.01***
CFA (1mg/mL) sub plantar region	121.01±1.2	64.21±0.3	139.21±0.3	3.2±0.12	0.65±0.02
CFA(1mg/mL) + Indomethacin (10mg/kg, i.p)	61.05±0.5**	32.01±0.2**	56.23±0.5*	6.5±0.11*	0.39±0.02*
CFA + MECT (200mg/kg, p.o)	85.12±0.3***	44.02±0.1***	78.25±0.4***	5.4±0.17**	0.49±0.04***
CFA + MECT (400mg/kg, p.o)	59.23 ±0.1*	33.27±0.4*	59.11±0.1**	6.1±0.12**	0.41±0.01**

n=6, Data were expressed as Mean± SEM, one-way ANOVA followed by Dunnett's test, All groups were compared with Negative control, *P<0.05, **P<0.01, ***P<0.001

Table: 4 Effect of MECG on hematological parameters in CFA induced arthritis in albino rats

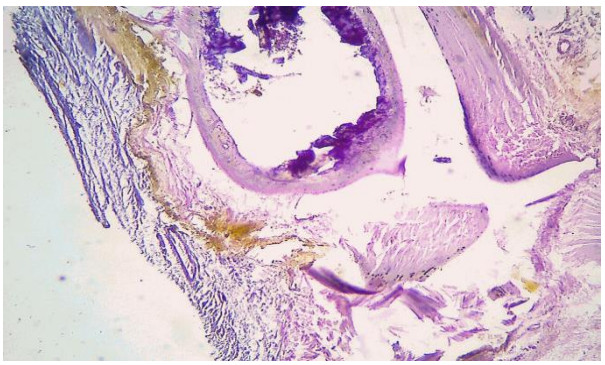
Group	Drug and dosage	RBC (x 10^6/mm^3)	WBC (x 10^3/mm^3)	Haemoglobin (g%)	ESR (mm/hr)
I	Normal saline (5ml/kg, p.o)	6.31±0.12***	8.31±0.24***	11.89±0.02***	41.14±.12***
II	CFA (1mg/mL) on sub plantar region	4.28±0.14	13.1±0.45	6.56±0.11	65.23±1.04
III	CFA (1mg/mL) + Indomethacin (10mg/kg, i.p)	6.12±0.20***	8.22±0.37*	10.27±0.14*	39.22±0.1*
IV	CFA + MECT (200mg/kg, p.o)	5.01±0.13**	10.12±0.24**	8.01±0.10***	46.21±0.12**
V	CFA + MECT (400mg/kg, p.o)	5.92±0.11***	8.13±0.39*	9.94±0.11**	40.47±0.01*

n=6, Data were expressed as Mean± SEM, one-way ANOVA followed by Dunnett's test, All groups were compared with Negative control, *P<0.05, **P<0.01, ***P<0.001



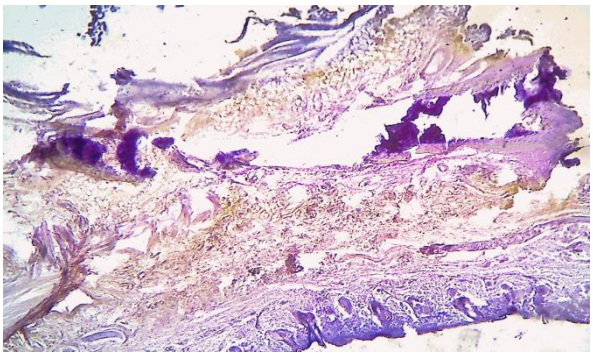
GROUP-III

Sections show bony tissue and surrounding zone of fibro muscular connective tissue. No inflammation is seen.



Group IV MECG 200mg/kg

Sections show bony tissue and fibro muscular connective tissue; No inflammatory changes



Group V MECG 400 mg/kg
Sections show skin, bony tissue and fibro muscular connective tissue. No inflammatory component seen

SUMMARY AND CONCLUSION

In this study, the potential of anti arthritic activity of *Ctenolepis garcinii* was proved by Complete Freund's adjuvant-induced arthritis using rat's model. Findings from this study provide evidence that methanolic extractsof *Ctenolepis garcinii* (MECG) 200mg/kg and 400 mg/kg have anti-arthritic activity but, in a dose, dependent manner. In the dose of aqueous extracts of *Ctenolepis garcinii* 400mg/kg was observed the effectively used to treat the diseases when compared to aqueous extracts of *Ctenolepis garcinii* 200mg/kg. The plant contains many secondary metabolites e.g. flavonoids, steroids, alkaloids and tri-terpenoids. Hence proper isolation of the active principles might help in the findings of new lead compounds in the fields of anti-arthritic and anti-inflammatory drug research. In future studies by isolating the compound are needed to provide the plant as a dosage form for arthritic patients. For this purpose, further works on the *Ctenolepis garcinii* was on progress at The Department of Pharmacology, Sankaralingam Bhuvaneswari College of Pharmacy, Sivakasi.

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