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ACUTE TOXICITY STUDIES AND EVALUATION OF ANTI- INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF LEAVES OF *CELASTRUS PANICULATUS*

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ABSTRACT

The study was performed to evaluate the acute toxicity and anti-inflammatory effect of ethanolic extract of leaves of *Celastrus paniculatus.* The effect of *Celastrus paniculatus*, Ethanolic extract was studied for oral acute toxicity using type of Examinations like Clinical symptoms, Mortality, Body weight, Pathological examination and carrageenan induced paw oedema, method for studying anti-inflammatory activity respectively. The results of the acute toxicity studies indicate that the LD50 of Ethanolic Extract of leaves of *Celastrus paniculatus* (EECF) is more than 2000mg/kg. mortality was not observed in any group. Effect of ethanolic extract of leaves of *Celastrus paniculatus* on anti- inflammatory activity was evaluated by using carrageenan induced paw oedema method and cotton pellet induce glaucoma method. Percentage of anti-inflammatory increased in EEGF groups when compared to carrageenan induced animals. The different concentration of *Celastrus paniculatus* showed prompt anti-inflammatory activity.

KEYWORDS: *Celastrus paniculatus*, Ethanolic extract, acute toxicity, anti-inflammatory activity.

INTRODUCTION

Inflammation is the body's response to an infection, irritation, or foreign material. The inflammatory response is a polyphasic tissue reaction that is part of the host defence mechanism. Anti-inflammatory medicines are evaluated clinically based on their effect on pain, stiffness, or swelling of the affected part, with oedema being the most widely observed and hence the most essential.

Celastrus paniculatus, also known as Jyotishmati or black oil plant, is a herbal plant that has been used extensively in the treatment of a variety of health disorders in traditional medicine. It belongs to the Celastraceae family and is generally known as Jyotishmati or black oil plant. It is a small to medium sized woody species which is native to India and also widely distributed across countries like Malaysia, Thailand, China, Philippines, North eastern part of Australia.[1] The seed oil and fruit are widely used for their calming, sedative, and wound-healing properties. *Celastrus paniculatus* is an example of a traditional medication that tribals utilise to treat a variety of ailments.[2] The bark is a brain tonic, abortifacient, and depurative. The leaves are emmenagogues, and the leaf sap is an effective opium antidote. Celapanin, Celapanigin, Celapagin, Celastrine, and paniculatine are some of the main alkaloids present in the seeds.[3,4] The leaves include alkaloids, a glycoside, and a bright colouring matter, whilst the oil produced from seeds contains sterols, alkaloids, and a bright colouring matter. The oil also contains alkaloids and sesquiterpenes such dipalmitoyl glycerol.[5,6] The present study aimed to determine the acute toxicity studies and anti-inflammatory activity in the ethanolic extract of *Celastrus paniculatus.*

MATERIALS AND METHOD

The *Celastrus paniculatus* plant was collected from herbal garden, Himayatsagar Road, Moinabad in the month of December 2017. Before doing the research, sample was collected and dried under the shade for 12 to

14 days. The plant was were authenticated by Botanical survey of India, Deccan regional Centre Hyderabad-500048, Telangana, India. Freshly collected leaves of *Celastrus paniculatus* were dried for 12 to 14 days at room temperature. The thoroughly dried leaves were powdered using pestle and mortar and weighed. This powdered plant material was used for extraction. Rinse all the glass apparatus by petroleum ether and dry it in the oven at 102^0C. Then keep it in desiccator.

Preparation of extract with ethanol

Weigh 100gm of powdered leaves of *Celastrus paniculatus* and place it in the thimble in soxhlet extractor. Take a 250ml round bottom flask and clean it and fill the flask with sufficient quantity of ethanol. Place the whole setting on a heating mantle and allow the ethanol to boil. Continue the extraction process for almost 3 to 4 hrs. After removing the condensing unit from extraction unit, allow the sample to cool down. Then the solvent was evaporated and the extract was dried under desiccators.

Animals

Wistar Rats of either sex weighing 150-200gms were used, and individually housed in polypropylene cages lined with husk renewed every 24 hr in well-ventilated rooms at 22±3ºC and relative humidity between 30 to 80%, under artificial lighting12:12-hour light period per day and dark cycle in hygienic condition. The rats were fed with standard laboratory pellet diet and drinking water ad libitum. The commercial diet and drinking water were provided. The experimental protocols were duly approved by the Institutional Animal Ethics Committee (IAEC-03/SES/2020/002).

Acute Oral Toxicity Studies

Acute toxicity describes the adverse effects of a substance that result either from a single exposure or from multiple exposures in a short period of time (usually less than 24 hours). To be described as *acute* toxicity, the adverse effects should occur within 14 days of the administration of the substance. Acute toxicity is distinguished from chronic toxicity, which describes the adverse health effects from repeated exposures, often at lower levels, to a substance over a longer time period (months or years). It is widely considered unethical to use humans as test subjects for acute (or chronic) toxicity research. However, some information can be gained from investigating accidental human exposures (e.g., factory accidents). Otherwise, most acute toxicity data comes from animal testing or, more recently, *in vitro* testing methods and inference from data on similar substances.

OECD Guideline 423: Pre-specified fixed doses of 5, 50, 300 or 2000 mg/kg are used. There is an option to use an additional dose level of 5000 mg/kg, but only when justified by a specific regulatory need. Groups of animals are dosed in a stepwise procedure, with the initial dose being selected as the dose expected to produce mortality in some animals. Further groups of animals may be dosed at higher or lower fixed doses, depending on the presence of mortality, until the study objective is achieved; that is, the classification of the test substance based on the identification of the dose(s) causing mortality, except when there are no effects at the highest fixed dose.

Name of Test Compound: Ethanolic Extract of ***Celastrus paniculatus***.
Species/Strain: Rat /Wistar.
Gender: Male and female.
Number of Test Animals: 4 groups each with 6 totally 24.
Mode Of Application: Oral.
Duration And Frequency Of Treatment: Single dose.
Dosage Level: 50, 300, 2000 mg/kg.
Applied Volume: 1 ml100 g.
Vehicle: Distilled water.
Post Treatment Examination Period: 14 days.
Type of Examinations: Clinical symptoms, Mortality, Body weight, Pathological examination.

Grouping

Table 1: Grouping of Animals.

Groups	Dose EECP (mg/kg)	No. Of Animals	
		Male	Female
Control	0	3	3
Low Dose	50	3	3
Medium Dose	300	3	3
High Dose	2000	3	3

Environmental values

Air change rate: 15 times /hour.
Temperature: 22 ± 3 C.
Relative humidity: 30-80 %.
Lighting: artificial with 12 hour dark, 12 hour light period / day.
Feeding conditions: Free food.
Water supply: Tap water of drinking quality.
Measuring of body weights: The weights of the rats were measure at receiving and at randomization of animals, as well as before and after (24 hour) treatment for every 2 days.

General status, behavior, clinical symptoms

During the first 6 hours of the treatment each rat observes for clinical symptoms. Special attention was paid - besides of the general status, and behavior - on alterations of skin and fur, mucous membranes, eyes, circulation, breath, functions of vegetative nervous system, salivation, diarrhea, convulsions. The type and intensity of symptoms and local lesions were recorded individually.

Mortality

The mortality was recorded during the first 6 hours and thereafter twice a day for 14 days.

Evaluation of Anti-Inflammatory Activity of EECP on Carrageenan Induced Paw Edema

Rats of either sex weighing 150-200gms were used. The animals were starved overnight to ensure uniform hydration, the control animals received 5ml of normal saline p.o, test group received the extract at a concentration of 100 & 200mg per kg body weight p.o suspended in the same volume. Standard group received aspirin solution (100mg/kg p.o using gastric canula. Paw edema was induced by injecting 0.1 ml of 1% w/v carrageenan suspended in 1% CMC into sub-plantar tissues of the left hind paw of each rat. The paw thickness was measured before injecting the carrageenan and after 60, 120, 180, 240 min. using vernier caliper. The anti-inflammatory activity was calculated as percentage inhibition of oedema in the animals treated with extract under test in comparison to the carrageenan control group.[6,7,8]

The percentage (%) inhibition of edema is calculated using the formula

$$\textbf{Percentage of inhibition} = T_0 - Tt/T_0 \times 100$$

Where T_t is the thickness of paw of rats given test extract at corresponding time and T_0 is the paw thickness of rats of control group at the same time.

Evaluation of anti-inflammatory activity of EECP on Cotton pellet-induced granuloma

Rats of either sex weighing 150-200gms where selected and anaesthesized with either. Cotton pellets weighing 50 ± 1 mg were sterilized in an autoclave for 30 min at 120 °C. The back skin was shaved and disinfected with 70% ethanol. An incision was made in the lumbar region. Subcutaneous tunnels were formed by a blunted forceps and a sterilized, pre weighed cotton pellet was placed on both sides in the scapular region. The animals were treated with aspirin (100 mg/kg, p.o.) and EECP for 7 days. On 8th day the animals were anaesthetized and the pellets together with the granuloma tissues were carefully removed and made free from extraneous tissues.

The wet pellets were weighed for the determination of wet weight, and then dried in an incubator at 60 °C for 18 h until a constant weight obtained (all the exudates dried); after that the dried pellets were weighed again. The exudate amount (mg) was calculated by subtracting the constant dry weight of pellet from the immediate wet weight of pellet. The granulation tissue formation (dry weight of granuloma) was calculated after deducting the weight of cotton pellet 50 mg) from the constant dry weight of pellet and taken as a measure of granuloma tissue formation. The percent inhibitions of exudate and granuloma tissue formation were determined. Results were subjected to statistical analysis using ANOVA test and percentage increases in the weight of the cotton pellets was calculated.[9,10]

$$\text{Percentage inhibition} = [w_c - w_d / w_d \times 100]$$

Where, w_d = difference in pellet weight of the drug treated group

W_c = difference in pellet weight of the control group.

RESULT AND DISCUSSION

Acute oral toxicity studies of EECP

The results of the acute toxicity studies indicates that the LD50 of Ethanolic Extract of *Celastrus Paniculatus* (EECP) is more than 2000mg/kg. mortality was not observed in any group. After single dose of treatment EECP to all groups persistently increases body weight during 14 days observation period. General appearance and behavioural observations. The appearance and behavioural parameters of animals after drug administration is indicator of the toxicity of the test drug the behavioural patterns of animals were observed for the first 6 h and followed by 14 h after the administration. No significant changes were observed in wellness parameters used for evaluation of toxicity. Skin, fur, eyes, mucous membrane, behavioural pattern, salivation and sleep pattern parameters of the treated animals were found to be normal. No toxic symptom or mortality was observed in any animal. All treated animals lived up to 14 days after the administration of EECP.

Behavioural changes

Table 2: Observation of behavioural changes in all groups.

Signs	Group 1	2	3	4
Skin and Fur	Normal	Normal	Normal	Normal
Eyes And mucous membranes	Normal	Normal	Normal	Normal
Behavior	Normal	Normal	Normal	Normal
Lethargic	Absent	Absent	Absent	Absent
Sleep	Absent	Absent	Absent	Absent
Tremors/ convulsions	Absent	Absent	Absent	Absent
Salivation	Absent	Absent	Absent	Absent
Diarrhea	Absent	Absent	Absent	Absent
Excitability	No	No	No	No
Death	No	No	No	No
Other symptoms	Nil	Nil	Nil	Nil

Anti-inflammatory activity

Anti-Inflammatory Activity of EECP on Carrageenan Induced Paw Edema

All values were shown as mean±SEM. Statistical analysis were performed using one-way analysis of variance (ANOVA) followed by Tukey test. P<0.05 was considered statistically significant.

Table 3: Anti-Inflammatory Activity of EECP on Carrageenan Induced Paw Edema.

Treatments	60 min (mean ± sd)	120min (mean ± sd)	180 min (mean ± sd)
Carrageenan Control	1.56 ± 0.15	2.66 ± 0.15	3.646 ± 0.18
EECP 200mg/kg	1.061 ± 0.13**	1.76 ± 0.13***	2.16 ± 0.12***
EECP 400mg/kg	0.99 ± 0.13***	1.56 ± 0.091***	1.76 ± 0.091***
Aspirin 100mg/kg	0.8 ± 0.09***	1.46 ± 0.067***	1.75 ± 0.13***

Table 4: Percentage inhibition of paw oedema.

Treatments	Percentage of inhibition at 1st hour	Percentage of inhibition at 2nd hour	Percentage of inhibition at 3rd hour
EECP 200mg/kg	28.58%	27.66%	45.82%
EECP 400mg/kg	35.48%	38.77%	52.86%
Aspirin 100mg/kg	45.82%	42%	55.48%

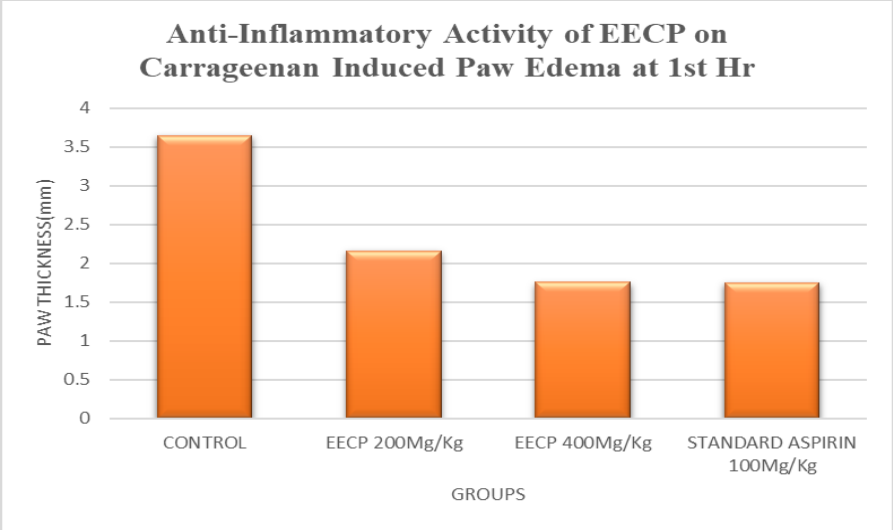

Fig 1: Effect of EECP in Carrageenan Induced Paw Edema At 1st Hr.

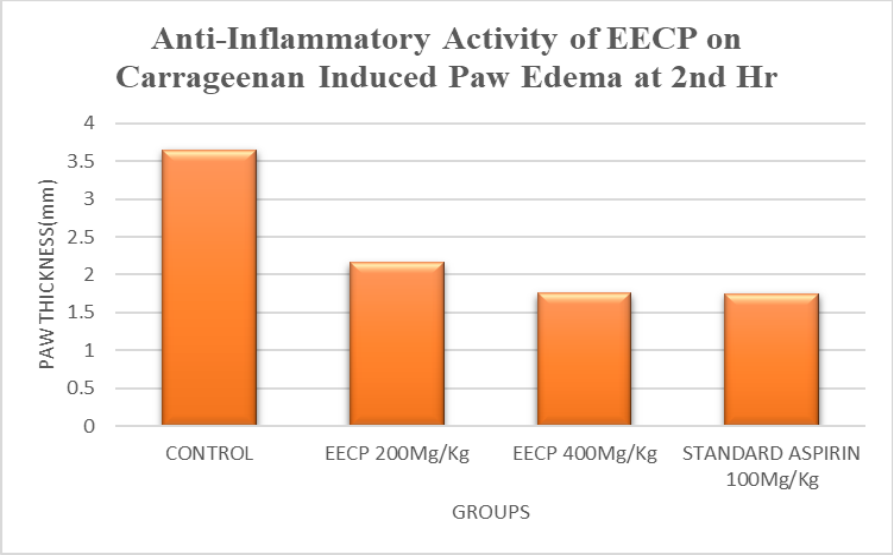

Fig 2: Effect of EECP in Carrageenan Induced Paw Edema At 2nd Hr.

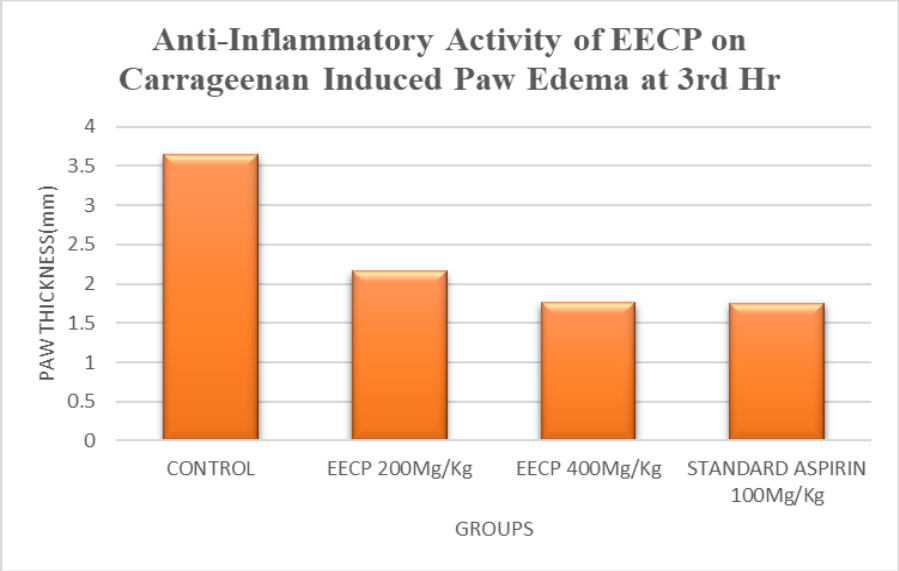

Fig 3: Effect of EECP in Carrageenan Induced Paw Edema at 3rd Hr.

Anti-Inflammatory Activity of EECP on Cotton pellet-induced granuloma.

Table 5: Cotton pellet-induced granuloma.

S. No	Group	Treatment	Weight of cotton pellets at the time of implantation mg	Weight of cotton pellets with granuloma mg (mean ± sem)	Percentage of inhibition of granuloma
1.	I	Control	50 ± 1	112±6.651	_
2.	II	EECP 200mg/kg	50 ± 1	94.75±3.119**	15.30%
3.	III	EECP 400mg/kg	50 ± 1	85.25±1.109***	25.40%
4.	VI	Aspirin 100mg/kg	50 ± 1	72.50±2.398***	39.74%

Significance done by ANOVA Tukey test. *P<0.05, **P<0.01, ***P<0.001 Compared with control, ns = Not significant Compared with control.

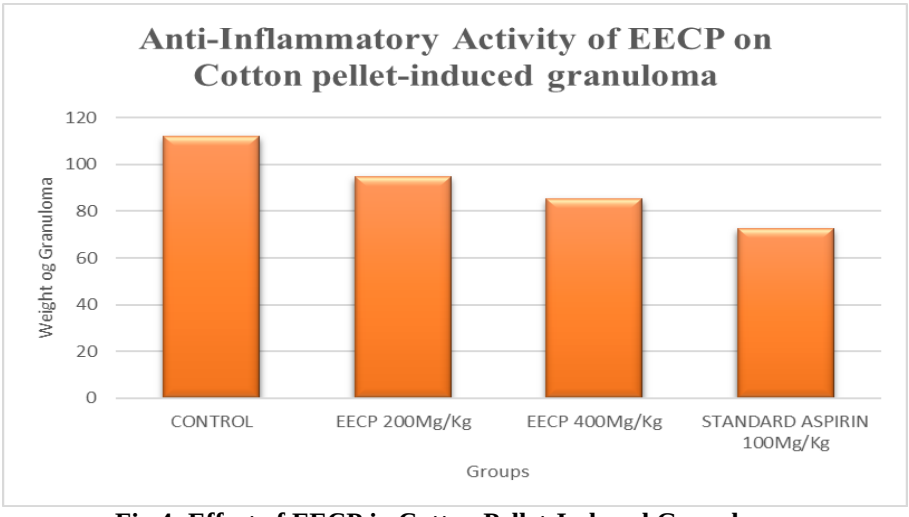

Fig 4: Effect of EECP in Cotton Pellet-Induced Granuloma.

CONCLUSION

All the animals survived by the end of the study; Clinical signs & symptoms did not reveal any major findings of toxicity. The LD50 of the EEGF was greater than 2000mg/kg (Category 5 as per OECD guidelines 420, 423 & 425 for acute Toxicity Studies) and hence it is practically nontoxic. Effect of ethanolic extract of leaves of *Celastrus paniculatus* on anti- inflammatory activity was evaluated by using carrageenan induced paw oedema method and cotton pellet induce glaucoma

method. EECP effectively decreased the Percentage of paw oedema and inhibitions of exudate and granuloma tissue formation in EECP groups when compared to control group animals.

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REFERENCES

1. Misra RC, Kumar S, Pani DR, Bhandari DC. Empirical tribal claims and correlation with bioactive compounds: A study on *Celastrus paniculata* Willd., a vulnerable medicinal plant of Odisha. Indian Journal of Traditional Knowledge, 2012; 11(4): 615-22.
2. Sujana KA, Joseph J. Ethnomedicinal uses of *Celastrus paniculatus* Willd. known to four tribal communities of Wayanad district of Kerala, India. International Journal of Research in Ayurveda & Pharmacy, 2012; 3(4): 573-5.
3. Athira K, Sajeev CR, Saroj KV, Sooraj NP, Sunil KKN, Jaishanker R. Mapping traditional knowledge associated with *Celastrus paniculatus* in India using Geographical Information System (GIS). Journal of Ayurveda Medical Sciences, 2016; 1(2): 55-62.
4. Katekhaye S, Duggal SA, Singh AP. An inside preview of nutritional and pharmacological profile of *Celastrus paniculatus*. Int J Recent Adv Pharm Res., 2011; 1:19-24.
5. Athira K, Sajeev CR, Saroj KV, Sooraj NP, SunilKKN, Jaishanker R. Mapping traditional knowledge associated with *Celastrus paniculatus* in India using Geographical Information System (GIS). Journal of Ayurveda Medical Sciences, 2016; 1(2): 55-62.
6. Deodhar KA, Shinde NW. *Celastrus paniculatus*: Traditional uses and Ethnobotanical study. Indian Journal of Advances in Plant Research, 2015; 2(1): 18-21.
7. Jadhav RB and Patwardhan B., Anti-anxiety activity of Celastrus paniculatusseeds.Indian J Nat. Prod., 19,2003, 16-9. 7.
8. M. N. Ghosh. Fundamentals of experimental pharamacology. 3rd edition. India: Hilton & company, 2005; 175 – 179.
9. Nasrin, M., Alireza, M.N., Alireza, G. 2005. Effects of central and peripheral depletion of serotonergic system on carrageenan induced paw oedema. International Immunopharmacology, 2005; 5(12):1723-1730.
10. Agarwal RB and Rangari VD., Phytochemical investigation and evaluation of anti-inflammatory and anti-arthritic activities of essential oil of Strobilanthus ixiocephana Benth. Indian J ExpBiol., 2003; 41(8): 890-4.