World Journal of Pharmaceutical Science and Research
www.wjpsronline.com
Research Article
ISSN: 2583-6579
SJIF Impact Factor: 3.454
Year - 2024
Volume: 3; Issue: 3
Page: 01-07

IN-VIVO ANTI-INFLAMATORY AND ANALGESIC ACTIVITY OF ETHANOLIC EXTRACT OF CANTHIUM PERVIFLORUM

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Article Received: 06 March 2024 || ***Article Revised: 27 March 2024*** || ***Article Accepted: 18 April 2024***

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ABSTRACT

This study investigates the potential anti-inflammatory properties of the ethanolic extract of Canthium parviflorum. Ayurvedic medicine has long recognized the benefits of Canthium Parviflorum, a valuable shrub and woody herb. Phytochemical studies have identified various biochemical substances in Canthium parviflorum plant extracts, including flavonoids, glycosides, alkaloids, saponins and terpinoids. The aim of our work is to evaluate the anti-inflammatory effect of Canthium parviflorum in vitro using the protein denaturation method, because terpenoids and flavonoids have an important effect on inflammation. Inflammation and rheumatoid arthritis have been linked to protein denaturation. C. dicoccum whole plant ethanol extract in doses of 250 mg/kg*g p.o. and 500 mg/kg*g p.o. was tested in several models for its anti-inflammatory properties against conventional diclofenac at a dose of 25 mg/kg*g p.o.

KEYWORDS: Cathium perviflorum, flavonoids, glycosides, phytochemical analysis, anti-inflammatory.

INTRODUCTION

Inflammation is a defensive response triggered when the body is threatened by for example pathogens, damaged cells or irritants. Inflammatory diseases include rheumatoid arthritis, atherosclerosis, Alzheimer's, asthma, psoriasis, multiple sclerosis, and inflammatory bowel diseases. The three major groups of drugs used in treatment of inflammatory diseases are corticosteroids, non-steroidal anti-inflammatory drugs [NSAIDs], and disease modifying anti rheumatoid drugs [DMARDs].

Inflammation

Inflammation is an important physiological reaction which occurs in response to a wide variety of injuries agent [e.g. bacterial infection, physical trauma, chemical or any other phenomenon] ultimately aiming to perform the dual function of limiting damage and promoting tissue repair.

Analgesia

Pain is an subjective experience that is the net effect of complex interaction of the ascending and descending nervous system involving biochemical, physiological, psychological and neocortical processes.

Classification of inflammation

1. **Acute inflammation:** Acute inflammation is a short term process which is characterized by the classic science of inflammation which are: swelling, redness, pain, heat and loss of function due to infiltration of the tissue by plasma and leucocytes.
2. **Chronic inflammation:** Chronic inflammation is a pathological condition characterized by concurrent active inflammation, tissue destruction and attempts at repair.

Inflammatory mediators

The inflammatory response is a complex and highly regulated sequence of events that start with an initial production of pro-inflammatory mediators recruit professional inflammatory cells to the site of injury to clear the offending trigger

1. Cyclooxygenase (COX)
2. Prostaglandins (PGs)
3. Arachidonic acid
4. Thromboxane
5. Leukotrienes
6. Poly unsaturated fatty acids (PUFA)
7. Histamine
8. Nitric oxide

AIM AND OBJECTIVE OF THE WORK

1. To study the in-vivo anti-inflammatory and analgesic activity of ethanolic extract of canthium perviflorum.
2. Phytochemical investigative carried out on canthium perviflorum revealed the presence of many active constituents such as phenolic acid, glycosides & flavonoids
3. They differ from each other in chemical constituents viz kaempferol 3-0-A-1 kaempferoal 3-O-P-D-galactoside & kaempferal 3-O-P-D- digalatide.

Plan of work

1. Extraction process by soxhlet apparatus.
2. Acute Toxicity study of ethanotic extract of canthium perviflorum.
3. Phytochemical investigation of ethanolic extract of canthium perviflorum.
4. Evalution of anti-inflammatory and analgegic activity from ethanolic extract of canthium perviflorum.

LITERATURE REVIEWON PLANT

Introduction to plant canthium parviflorum plant-canthium

Botanical name-canthium perviflorum Lamk

Family - Rubiaceae

Toxonomical classification:

Domain	:	Eukaryota
Kingdom	:	Plantae
Sub kingdom	:	Viridaeplantae
Phylum	:	Tracheophyta
Subphylum	:	Euphyllophytina
Infraphylum	:	Radiatopses
Class	:	Magnoliopsida
Subclass	:	Asteridae
Superorder	:	Gentiananae
Order	:	Gentianales
Family	:	Rubiaceae
Subfamily	:	Ixoroideae
Tribe	:	Vangueriea
Genus	:	Canthium
Specific epithet	:	Parviflorum
Botanical name	:	Canthium parviflorum

Profile of canthium parviflorum

Canthium perviflorumis also known as Carray cheddile, has useful traditional medicines for centuries. The root, stem, leaf fruit, seed, bark, flowers are used to treat many aliment, ranging from wound healing to diabetes.

Habitat and Distribution

It is distributed India through the India in forest and dry plins especially western part of peninsula from Gujarat and maharastra, southwards, and in bihar and Orissa.

Synonyms

Canthium parviflorum, Plectronia parviflora, Paederia valli-kara, Webera tetrandra.

Description

The Rubiaceae are trees, shrubs, orinfrequently herbs comprising and interpetiolar about lianous forms. The leaves nearly 450 genera and 6,500 species, including some are simple and usually entire, and are opposite or sometimes whorled; stipules are the flowers are present always bisexual and actinomorphic, often heterostylous, and usually are in cymose inflorescences.

Traditional Uses

The Canthium parviflorum plant is well known for its various medicinal properties in India. The leaves and fruits are edible.

MATERIALS AND METHODOLOGY

Animal Husbandry

Species	:	Rat, Albino
Strain	:	Wistar
Sex	:	Male
Age at initiation of study	:	10 – 12 weeks old
Body weight range	:	200 – 300 g
Source	:	M/s. Mahaveera Enterprices, Hyderabad.
Number of the animals per group	:	6
Number of groups	:	10
Number of animals	:	60
Identification of Animals	:	By rat accession number on body marking by 10% picric acid.

Environmental conditions

Temprature	:	22+3
Relative Humidity	:	40-
Photoperiod	:	12hrs light and 12hrs dark light

Phytochemical screening

Phytochemical screening was carried out for the obtained extracts as per the standard methods.

1. **Detection of Alkaloids:** Extracts were dissolved individually in dilute HCl and filtered.

Mayers test: Using Mayer's reagent (potassium mercuric iodide), the filtrate was subjected to the Mayers test. Alkaloids can be detected by the formation of a yellow-colored precipitate.

2. **Detection of carbohydrates:** In order to identify carbohydrates, each extract was diluted in 5 milliliters of distilledwater and then filtered. To check for the presence of carbs, the filtrates were employed.

Molisch's test: Filtrates were treated with 2 drops of alcoholic alpha napthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates.

3. **Detection of saponins**

Froth test: Froth test: 20 ml of distilled water was added to the extracts, and the mixture was agitated in a graduate cylinder for 15 minutes. The presence of saponins is indicated by the formation of a 1 cm layer of foam.

4. **Detection of tannins**

Gelatin test: 1% sodium chloride-containing gelatin solution was added to the extract. Tannins are present when white precipitate forms.

5. **Detection of phenols**

Ferric chloride test: Extracts were subjected to three or four drops of ferric chloride solution. There are phenols present when a bluish black color forms.

6. Detection of flavonoids

Alkaline reagent test: Extracts were subjected to a few drops of sodium hydroxide solution. There are flavonoids present when a yellow color forms and then turns colorless when diluted acid is added.

Eddy's Hot plate method in mice

The mice of either 6 were weighed and divided into 5 groups. Group -1 serve as control, Group -2 Disease control, Group – 3 and 4 received CP 250 and 500 mg/kg body weight, Group -5 received diclofenac (10mg/kg). The reaction was noted down on the hot plate 30, 60, 90, 120, minutes after treatment. The basal reaction time was taken by observing hind paw licking and jumping response in animal while placed on the hot plate. Which was maintained at constant temperature 55degrees cut off period of 10sec was observed to avoid damage to the paws. The percentage increase or decrease in reaction time was observed at each time interval was calculated.

Percentage increase in reaction time=(Rt/Rc-1)100

Where,

Rt is paw licking/jumping response in treated group Rc is paw licking/jumping response in control group.

RESULTS DISCUSSION

Effect of Ethanolic extract of canthium parviflorum fruit on Serum parameters in formalin induced inflammation:

Groups	Serum Parameters	
	AST (IU/ml)	ALT(IU/ml)
Control	0.09± 0.26	0.05±0.89
Disease control	0.20±0.39	0.12±1.57
EECP (250mg/kg)	0.07±0.28	0.56±1.20
EECP (500mg/kg)	0.065±1.89	0.54±1.42
Diclofenac (10mg/kg)	0.049±1.14	0.46±1.71

The data is shown as mean ± SEM for the six values. *P<0.01. *P<0.001 in comparison to the control group.

Effect of oral dose of EECP on Formalin- induced Paw edema in Rats

Groups	Mean paw thickness on first day (cm)	Mean paw thickness after 7 days (cm)	Increase in pawthickness	% inhibition
Control	0.2±0.03	0.2 ±0.02	-	-
Disease control (2% formalin)	0.5±0.05	1.2±0.04	0.7±0.061	-
EECP (250mg/kg)	0.4±0.04	0.09±0.08	0.5 ±0.093	28.5*
EECP (500mg/kg)	0.4±0.02	0.8±0.03	0.4±0.075	42.5**
Diclofenac (10mg/kg)	0.3±0.06	0.06±0.56	0.3±0.023	57.1**

Mean ± SEM is used to express values. ANOVA was used to determine whether the comparison was statistically significant, and Dunnett's test was then performed. *p<0.05; **p<0.01 for comparison of Group 1, Group 2, 3 & 4.

Effect of Ethanolic Extract of canthium parviflorum on Cotton pellet induced glanuloma

Treatment	Mean dry weight of granuloma (mg)	% Granuloma inhibition
Disease control	45±0.26	-
EECP (250mg/kg)	30±0.20	33*
EECP (500mg/kg)	25±0.16	44*
Diclofenac (10mg/kg)	20±0.12	51*

Mean ± SEM is used to express values. ANOVA was used to determine whether the comparison was statistically significant, and Dunnett's test was then performed. *p<0.05; **p<0.01 for comparison of Groups 1, 2, 3, and 4.

Effect of Ethanolic Extract of canthium parviflorum on 0.6% acetic acid induced writhing in mice

Treatment	No. of Writhings in 30 mins	% inhibition
Disease control (0.6% acetic acid)	33±0.026	-
EECP (250mg/kg)	14±0.032	58*
EECP (500mg/kg)	12±0.034	64**
Diclofenac (10mg/kg)	10±0.042	70**

Mean ± SEM is used to express values. ANOVA was used to determine whether the comparison was statistically significant, and Dunnett's test was then performed. P <0.01, **P <0.001 in relation to the disease control group.

Effect of Ethanolic Extract of canthium perviflorum on Eddy's hot plate method in mice

Treatment	Basal reaction time				
	0min	30min	60min	90min	120min
Disease control	2±0.1	2±0.2	2±0.21	2 ±0.1	2±0.23
EECP (250mg/kg)	2±0.2	2±0.3	4±0.32	4±0.45	5±0.42
EECP (500mg/kg)	2±0.3	4±0.12	7±0.31	8±0.43	10±0.46
Diclofenac (10mg/kg)	3±0.5	7±0.2	9±0.33	10±0.42	12±0.46

Mean ± SEM is used to express values. ANOVA was used to determine whether the comparison was statistically significant, and Dunnett's test was then performed. P <0.01, **P <0.001 in relation to the disease control group.

CONCLUSION

This study's results have shown that CP has strong analgesic and anti-inflammatory properties, which support the use of CP in conventional medicine to treat painful and inflammatory disorders. The findings also provide evidence that the plant's potential benefit may stem from its ability to scavenge free radicals.

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