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

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ABSTRACT

Inflammation is a defensive response triggered when the body is threatened by for examplepathogens, damaged cells or irritants. Inflammatory diseases include rheumatoid arthritis, atherosclerosis, Alzheimer's, asthma, psoriasis,multiple sclerosis, and inflammatory bowel diseases, and many of these inflammatory diseases are becoming common throughout the world Pain is an unpleasant subjective experience that is the net effect of a complex interaction of the ascending and descending nervous systems involving biochemical, physiological, psychological, and neocortical processes. Pain can affect all areas of a person's life including sleep, thought, emotion, and daily activities.The present study was carried out to evaluate the anti inflammatory and analgesic activity of a natural plant canthium perviflorum fruits ethonolic extract on male albino rats. A group of animals will be examined for health and healthy Wistar rats will be selected for the study & will be randomly assigned to different grou*ps* based on the body weight.Phytochemical screening and pharmacological evaluation on rats has been done and it has shown the significant anti inflammatory and analgesic activity and it is further requires elaborated studies on the plant.

KEYWORDS: Diclofenac, Paw edema method, Carboxy methyle cellulose, Formalin.

INTRODUCTION

Inflammation is a defensive response triggered when the body is threatened by for examplepathogens, damaged cells or irritants. These responses are essential for humans in

combating infections and for promoting healing and restoration to normal function in the event of injury. Unfortunately, these defensive responses can occasionally go wrong, leading to different inflammatory diseases.

There are two main strategies for research in the area of natural products; older- and modern strategy. In the older strategy, the chemistry of compounds is in focus and selection of natural sources is based mainly on ethnopharmacological information as well as traditional uses. Isolation and identification of compounds are performed before biological activity testing (primarily in vivo). The modern strategy, so-called bioactivity-guided isolation is, as the name indicates, more focused on bioactivity. Biological assays (mainly in vitro) are used to target the isolation of bioactive compounds. Selection of organisms is based on ethnopharmacological information and traditional use, but might also be randomly selected. In modern strategy natural marine sources are particularly utilized.

Canthium Parviflorum, is a valuable medicinal and woody plant which has been valued for centuries in ayurvedic medicine. *Canthium* is a genus of flowering plants is the Rubiaceae family. Canthium Parviflorum, Known by several common names including carray cheddile, coromandel canthium.

Inflammation is an important physiological reaction which occurs in response to awide variety of injurious agents (e.g. bacterial infection, physical trauma, chemicalsor any other phenomenon) ultimately aiming to perform the dual function of limitingdamage and promoting tissue repair. Inflammatory processes are required for immune surveillance, optimal repair, and regeneration after injury. The inflammatory process protects our body from diseases by releasing cells and mediators that combat foreign substances and prevent infection.

MATERIALS AND METHODS

ANIMALS

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.

Husbandry

Environmental conditions

Temperature : 22± 3º

Relative Humidity : 40-70%

Photoperiod : 12 hours light and 12 hours dark light.

Housing

Rats will be housed in solid bottom autoclaved polypropylene cages (sizes: approximatelyL 425 x B 266 x H 185 mm), three rats per cage with stainless steel top grill having facilities for pellet feed and drinking water in polycarbonate bottle during acclimatization and throughout the study duration. Corn cob will be used as bedding material and will be changed thrice a week.

Diet

Rodent pellet feed will be provided to the animals, and the animals will be given mineral water by a water dispensing bottle.

Acclimation of animals

Animals were kept for one week, before enrollment into the study ,Food ,housing and water to the animals are also taken care.

Randomization and Grouping

A group of animals will be examined for health and healthy *Wistar* rats will be selected for the study & will be randomly assigned to different grou*ps* based on the body weight.

Study design

The select Rats were divided into normal control, disease control, standard, test group1and test group2. Disease induction is done. Administration of the extract & standard drugs were done at time.

Chemicals used: Diclofenac sodium, Formalin, Acetic acid

Vehical used : Sodium carboxy methyl cellulose.

Plant extract : *Canthium perviflorum fruits*

Plant extract preparation

Collection of *Canthium perviflorum*fruits

Canthium perviflorum fruits are collected from local area.

Preparation of ethanolic extract of fruit of *Canthium perviflorum*

Ethanolic extract of fruits of *canthium perviflorum* was performed according to the method of National Institute of Health and Family Welfare (NIHFW), New Delhi. Fresh fruits of *Canthium perviflorum* dried in an incubator for 6 days at 400º C, crushed in an electrical grinder to have coarse powdered. Then 100 gm fruit powders of *C. perviflorum* was suspended in 500 m L of ethanol then extracted for 18 hr in a soxhlet apparatus and a deep red ethanolic extract was obtained. The suspension was then filtered by coarse sieve filter paper. The filtrate was evaporated to dryness under reduced pressure in a rotary evaporator. A deep brown material was obtained. It was stored at 0-4ºc until used.

Preparation of dose formulation

Diclofenac

The required quantity of diclofenac will be using analytical balance and transferred into motor and pestle.Desired quantity of normal water added and triturated well to get final concentration of 25mg/ml solution.

Dose preparation of Ethanolic extract of *Canthium Perviflorum* (EECP)

The required quantity of EECP will be weighed using an analytical balance and transferred to a motor and pestel. The desired volume of 0.5%*w/v* carboxy methyl cellulose sodium viscosity (CMC-Na) in water will be added and triturated to get the final concentration of 250 and 500 mg/kg body weight suspensions. The suspension formulation will be transferred to a centrifuge tube to obtain homogeneous suspensions.

EXPERIMENTAL PROTOCOL

Treatment schedule for formalin induced paw edema method

Groups	**Treatment**	**Dose**		**Duration** (Days)	**No.of Rats** (Male)
		2%Formalin (ml)	Treatment mg /kg		

G1	Control	-	Vehicle	7	6
G2	Disease control (0.1 ml)	0.1	-	7	6
G3	Diclofenac	0.1	10	7	6
G4	EECP+2%Formalin	0.1	250	7	6
G5	EECP+2%Formalin	0.1	500	7	6

Treatment schedule for Cotton pellet induced Granuloma method

Groups	**Treatment**	**Dose**		**Duration**(Days)	**No.of Rats** (Male)
		Cotton pellet	**Treatment mg/kg**		
G1	Control	-	Vehicle	7	6
G2	Disease control	10mg	-	7	6
G3	Diclofenac	10mg	10	7	6
G4	EECP+ cotton pellet	10mg	250	7	6
G5	EECP+ cotton pellet	10mg	500	7	6

Analgesic activity:

Treatment schedule for acetic acid induced writhing method

Groups	**Treatment**	**Dose**	
		0.6%Acetic acid	**Treatment(mg/kg)**
G1	Control	-	Vehicle
G2	Disease control	0.1ml	-
G3	EECP+6% acetic acid	0.1ml	250
G4	EECP+6%acetic acid	0.1ml	500
G5	Diclofenac	0.1ml	10

Tabel 4. 9: Treatment Schedule of Eddy's hot plate method

Groups	**Treatment**	**Dose(mg/kg)**
G1	Control	Vehicle
G2	Disease control	-
G3	EECP	250
G4	EECP	500
G5	Diclofenac	10

Screening models for Anti inflammatory activity

Formalin-induced paw edema

The anti-inflammatory effect of CP in chronic inflammationwas evaluated by formalin-induced paw edema. In this model, chronic inflammation was induced byinjecting 0.1 ml of 2% formalin intothe sub-plantar area of the right hind paw of ether anaesthetizedrats.To the divided groups given EECP (250and 500 mg/kg, p.o.), diclofenac(10 mg/kg. p.o.) anddistilled water 2 ml/kg were given 30 min prior to formalininjection and continued for seven consecutive days. All drugsand the vehicle were given orally once daily using oral gavageneedle. In this model, the increase in paw edema was measuredby vernier caliper

method. In vernier calipermethod, the paw thickness was measured before and6 days after induction of inflammation by using vernier caliper. The difference in paw thickness after and before induction ofinflammation was calculated and presented as mean increasein paw thickness (mm). The ability of the anti-inflammatorydrug to suppress paw inflammation was expressed as a percentageof inhibition of paw edema.

Cotton pellet induced granuloma in rats

The effect of Ethanol extract of *Canthium perviflorum* fruit on the chronic phases of inflammation was assessed in the cotton pellet induced granuloma rat model. Autoclaved cotton pellets weighing 10mg each were implanted subcutaneously. One on each side of the abdomen of the animal, through a small ventral incision of rats anesthetized with ether. The different groups of rats were administered with EECP (250and 500 mg/kg, p.o.) and diclofenac (10 mg/kg. p.o.) once daily for 7 consecutive days from the day of cotton pellet insertion. The control group received vehicle (distilled water, 10 ml/kg, p.o.). On the eighth day the animals were sacrificed and the cotton pellets were removed, dried at60°C for 24 h and their mass was determined. The results are expressed as mg granulation tissue formed per 100 g body weight.

Biochemical analysis

On the eighth day, the animals were sacrificed under mild ether anesthesia and blood was collected in clean centrifuge tubes. The serum was obtained by centrifugation and used for the estimation of various biochemical parameters by using different analytical methods.

Estimation of SGOT

Estimation of SGPT

Estimation of Alanine aminotraferase (ALT)

Estimation of Aspartate aminotransferase(AST)

Models for Analgesic Activity

Acetic acid induced writhing method

Male albino mice weighing between 20-25 g were selected for the study. The animalswere divided into 5 groups (n=6 in each group). All animals received 0.1 ml acetic acid 0.6 % v/v. i.p. and first group served as control, Group II serve as disease control, Group V received Diclofenac. The groups III, IV, received 250 and 500 mg/kg body weight of hydro alcoholicextract of *Canthium perviflorum* (cp)respectively 30 minutes prior to the

administration of acetic acid i.p. The writhing effect was indicated by the stretching of abdomen with simultaneous stretching of at least one hind limb.

This was observed for 30 minutes and change in number of writhings in test group compared with standard treated and control treatment.

The percentage inhibition was calculated by using the formula

Percentage inhibition = $1- (N_T /N_C) X 100$

Where N_T is average numberof writhings in treated group

N_C is average numberof writhings in control group.

Eddy's hot plate method in mice

The mice of either sex were weighed and divided into five groups. Group I serve as control, group II Disease control, group III and IV received CP 250 and 500 mg/kg body weight, group v received diclofenacs (10mg/kg).The reaction was noted down on the hot plate 30,60,90,120, mins 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 55º.cut off period of 10 seconds 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)x 100

Where,

R_t is paw licking/ jumping response in treated group

Rc ispaw licking/ jumping response in control group.

RESULTS

The physical nature, color characterstic and % yield of each individual extract are found as given in the table.

Percentage yield of extract

Crude extract	Nature of extract	Colour	Weight
Canthium perviflorum	Semisolid	Deep green	23%

Phytochemical evaluation tests

Phytochemical studies of Ethanolic extract *Canthium perviflorum*

TEST	Ethanolic extract of *C.perviflorum*
Alkaloids	+
Saponins	+
Tannins	+
Steroids	-
Flavonoids	+
Glycosides	+
Terpinoids	+

+ = present - = absence

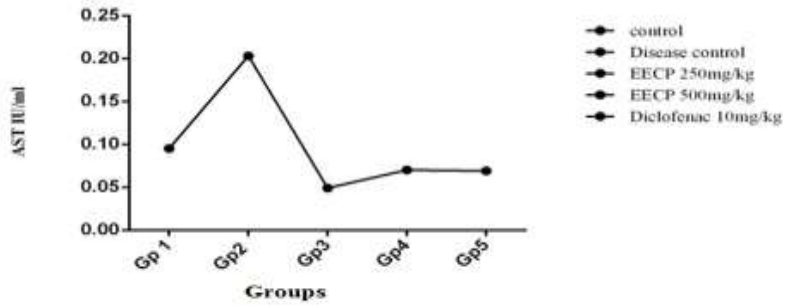
Acute toxicity studies of *canthium perviflorum*

Treatment	Dose mg/kg	No.of mice	No. of mice death	Signsof toxicity	LD50
Control	5ml/kg	10	-	-	-
Ethanolic extract of *C.Perviflorum*	100	10	-	-	>2000mg/kg
	500	10	-	-	
	1000	10	-	-	
	1500	10	-	-	
	2000	10	-	-	

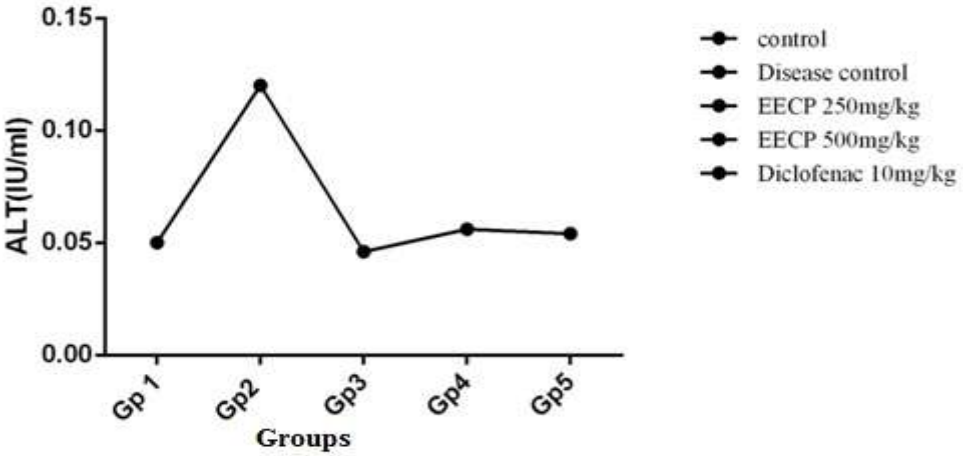
Effect of Ethanolic extract of *canthium perviflorum* fruit on serum parameters in formalin induced inflammation

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**

Values are expressed as mean ±SEM (n=6).*P<0.01. **P<0.001 as compared with control (one- way ANOVA followed by Dunnet's test).



Effect of EECP on AST level in formalin induced inflammation



Effect of EECP on ALT level in formalin induced inflammation

Increased in level of AST, ALT level in control group. Diclofenac treated groups shows decreased in level of AST and ALT level (P<0.001).EECP (250mg/kg) show significant decrease in level of AST and ALT (P<0.01). EECP (500mg/kg) treated group shows decreased in level of AST and ALT level (P<0.001) compared to control group.

ANTI INFLAMMATORY STUDIES

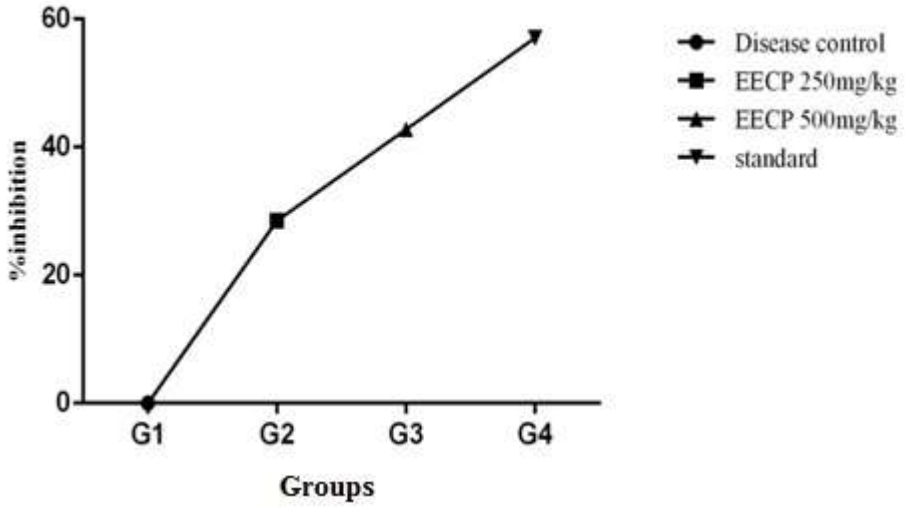
Formaldehyde induced rat paw edema

The results of anti-inflammatory activity of ethanol extract of *canthium perviflorum*fruit in formaldehyde induced paw edema is shown in the table**.** Injection of formaline subcutaneously into hind paw of rats produces localized inflammation. The administration of EECP-250mg/kg, EECP-500mg/kg and diclofenac-10mg/kg daily for 7 days successfully.EECP-250mg/kg and EECP-500mg/kg inhibited edema induced by formaline group showed maximum inhibition in paw edema (P<0.05 and P < 0.01 respectively). Diclofenac-10mg/kg group showed (57.1%, P <0.01) inhibition

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 paw thickness	% 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	0.9 ± 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	0.6 ± 0.56	0.3 ± 0.023	57.1**

Values are expressed as mean ± SEM. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's test. Comparison between Group I Group II, III.& IV *p<0.05;** p<0.01.



Effect Of Ethanolic Extract Of *canthium Perviflorum* on Formalin Induced Paw Edema

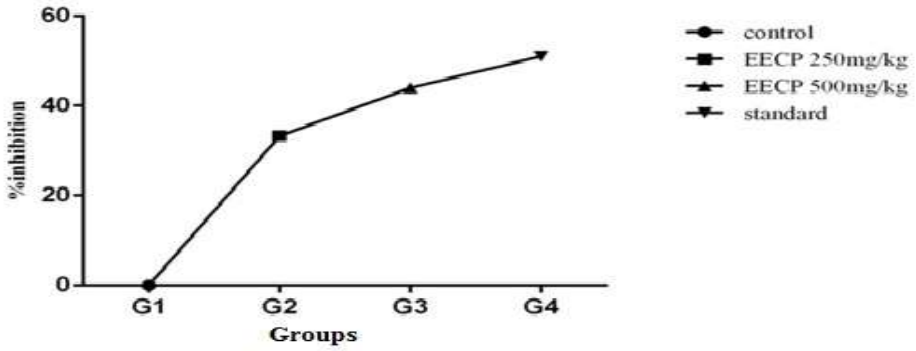
Cotton pellet induced granuloma in rats

The results of anti-inflammatory activity of methanol extract of *canthium Perviflorum* in cotton pellet induced granuloma is shown in table**.** EECP-250mg/kg andEECP-500mg/kg groups showed 33% and 44% inhibition in granuloma formation respectively as compared to control group, while standard diclofenac (10mg/kg) group showed significant decrease in granuloma formation (51%, P < 0.01).

Effect Of Ethanolic Extract Of *canthium Perviflorum* 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**

Values are expressed as mean ± SEM. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's test. Comparison between Group I Group II, III.& IV *p<0.05;** p<0.01.



Effect Of Ethanolic Extract Of*canthium Perviflorum* on Cotton pellet induced glanuloma.

ANALGESIC STUDIES

Effect of Ethanolic extract of *canthium Perviflorum* on Acetic-acid induced writhing method

Pre treatment of mice with extract *canthium Perviflorum* at dose 250 and 500 mg/kg produced a significant (p<0.001)reduction in wriths induced by 0.6%acetic acid.The percentage inhibition or percentage protection were found to be 58%, & 64% for extract at dose of 250 & 500mg/kg body weight respectively.However it exihibited dose dependent analgesic activity.

Table :5.7 Effect Of Ethanolic Extract Of *canthium Perviflorum* 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**

Values are expressed as mean ± SEM. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's test.*P<0.01, **P<0.001compared to disease control.

Eddy's hot plate method

The results of anti-inflammatory activity of methanol extract of ***canthium Perviflorum fruit*** inEddy's hot plate method is shown in table Animal produced paw licking & jumping in

canthium perviflorum 250/500 mg/kg group significantly inhibited (P<0.001) comapered to disease control.

Effect Of Ethanolic Extract Of *canthium Perviflorum* on Eddy's hot plate method in mice

Treatment	Basal reaction time				
	0min	30min	60 min	90 min	120 min
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**

Values are expressed as mean ± SEM. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's test. *P<0.01, **P<0.001compared to disease control.

DISCUSSION

Canthium is a genus of about 200 species found in tropical and subtropical regions. CP is used for the treatment of many diseases, such as epilepsy, stroke, abscess, and diabetes. Because the use of commercially available analgesic and anti-inflammatory drugs (opioids and nonsteroidalanti-inflammatory drugs) exerts different side effects, there is currently a strong interest indeveloping new therapeutic agents from natural products. Agents that inhibit differentmediators involved in the evolution of inflammatory processes are of great interest. Researchershave suggested investigating this class of macromolecules in animal models and evaluating their potential as biotechnological tools in the development of new pharmacological drugs in this study we explored *canthium perviflorum* in vivo models of anti inflammatory and analgesic activity.*C. perviflorun* produced antinociceptive and anti-inflammatory effects in models of nociception in mice (acetic acid-induced abdominal writhing, and hot plate test) and acute inflammation in rats(formalin induced paw edema cotton pellet induced granuloma tests.

In the present study, formaldehyde- and cotton pellet-induced inflammatory reactions.Tissue injury induces a cascade of cellular reactions in the lesion area, accompanied with the release of pro-inflammatory cytokines, such as TNF-a, IL-1b, IL-6, IL-,8 and other substances, which is then followed by subsequent,inflammatory reactions[54]. Prostaglandins such as PGE1 and PGE2, which are produced at elevated levels in inflamed tissues like rheumatoid synovium, increase local blood flow and potentiate the effects of mediators such as bradykinin that induce vasopermeability. Meanwhile, many studies suggested that activation

of PPARc with many classes of ligands (including telmisartan) may decrease production of the hypertrophic prostanoids PGE2, withconsequent amelioration or modulation of some component of inflammation. This is in agreement with the present results which revealed that CP treatment significantly prevented the formation of inflammatory edema due to challenge with formalin, and suppressed both exudates and granulation tissue formation as a result of subcutaneous cotton pellet implantation.

The ethanolic extract of*C. perviflorun* at dose of 500 mg/kg had showed reduction in the edema , faster rate of inhibition (48.5%), when compared with the control group of animals. But the animals treated with *C. perviflorun* 250 mg/ kg orally dosed showed moderate reduction in the edema and slower rate of inhibition (28.5%).

The inflammatory granuloma is a typical feature of acute inflammatory reaction. The cotton pellet-induced granuloma is widely used to assess the transudative and proliferative components of inflammation. In the present study, administration of *canthium perviflorum* extract (250 mg/kg and 500 mg/kg, p.o.) was found to inhibit the weight of cotton pellet in a dose dependent manner and the higher dose of the extract (500 mg/kg) exhibited inhibition of inflammation close to the inhibitory effect of diclofenac and better than the effect of the lower dose of the extract (250 mg/kg). In preview of this, results of present study demonstrated that leaf extract of *canthium perviflorum* has potential to inhibit the inflammation It has been reported that CP contains flavonoids and terpinoids . Many flavonoids are used in anti-inflammatory and analgesic agents in modern medicine. Preliminary phytochemical studies indicated the presence of gum resins of CP. Flavonoids and terpenoids isolated form plant extracts are known to produce anti-inflammatory and analgesic effects.

CONCLUSION

The findings of the present study have demonstrated that CP has potent anti-inflammatory and analgesic activity and justify its use in traditional medicine to treat inflammatory and painful conditions. The results also furnish evidence that the beneficial effects of this plant may be due to its free radical scavenging activity.

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