Available online on www.ijtpr.com
International Journal of Toxicological and Pharmacological Research 2013-14; 5(4): 74-78

ISSN: 0975-5160

Research Article

Anti-Inflammatory Activity of Ethanolic Extract of Cissus Pallida in Acute and Sub-Acute Models

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Available online: 1st December 2013

ABSTRACT
The present study is carried out to investigate the anti-inflammatory potential of Ethanolic extract of *Cissus Pallida* (EECP). Anti-inflammatory activity was evaluated by using Egg Albumin, Turpentine Oil & Formaldehyde as phlogistic agents. The animals were treated with doses 200mg/kg and 400mg/kg of extract and Diclofenac Sodium at a dose of 10mg/kg is used as a standard drug. The EECP showed a significant anti-inflammatory activity in a dose dependent manner in all the models when compared with the standard treatment. The extract (400mg/kg) exhibited maximum anti-inflammatory activity i.e., 67.46%, 68.75%, 69.43% (P<0.001) than the standard Diclofenac 64.50%, 65.28%, 64.77% in Egg albumin, Turpentine oil and Formaldehyde induced methods respectively. Based on the above results, we conclude that the EECP has significant anti-inflammatory activity and might prove efficacious for further design and development of agents with significant biological activity.

Keywords: *cissus pallida*, anti-inflammatory activity, phlogistic agents.

INTRODUCTION

Because of available drug failure to treat inflammatory diseases, many researchers have focused on the investigation of natural products as a source of new bioactive molecules. Inflammation is a primary physiologic defense mechanism that helps body to protect itself against infection, toxic chemicals, or noxious stimuli & allergens[1] which results in the liberation of endogenous mediators can elicit pain response even in small quantities[2]. Rheumatoid arthritis a ravaging disease is a major public health burden in about 1% of the population worldwide[3]. As the currently used drugs are associated with severe side effects, the urge to develop new chemical entities with potent biological activity from natural sources with lesser side effects has become mandatory.

Traditional medicine using plant extracts continue to provide health coverage for over 80% of the world's population especially in developing countries (WHO 2002)[4]. Many medicinal plants have been investigated for novel drugs or templates for the development of new therapeutic agents[5]. Various species from the genus *Cissus* have been reported to possess anti-inflammatory activity[6].

Cissus pallida (Telugu: Nallatige; Family: Vitaceae) is a woody climbing herb, tendrils are simple, opposite to leaves. It is mainly used as healer of bone fractures. The stem wood of *C. pallida* showed presence of stilbenes, triterpenoids and steroids[7].Stilbenes have been widely studied for anti-inflammatory and anti-cancer and estrogen receptor α agonist activity[6].Pallidol, a resveratrol dimer which is a phenolic derivative isolated from C*issus pallida*[8].In the present study anti-inflammatory potential of Ethanolic extract of *Cissus pallida* (EECP) was evaluated.

MATERIALS & METHODS

Collection of plant material & Extraction: The plant material was collected from Chitoor District, Andhra Pradesh, India. The plant authentication was done by Dr. K. Madhava Chetty, Dept. of Botany, Sri venkateswara University, Tirupathi, Chitoor District, Andhra Pradesh, India & the voucher was preserved. The plant material was thoroughly cleaned, shade dried at room temp. for 2-3 days & then pulverized to a coarse powder and shifted. 95% ethanol was added to coarsely powered (2kg) plant material & extracted by using soxhlet apparatus. The extract was concentrated by distillation under reduced pressure & evaporated to dryness.

Experimental animals: Healthy adult albino rats of wistar strains weighing 150-200gm of either sex were used in this study. The animals were kept properly in polypropylene cages under standard laboratory conditions (12/12hr light/dark cycle at 25±5^{0}c). The rats were fed a commercial diet & water ad libitum & were divided into 5 groups. The experimental protocol was approved by the Institutional Animal Ethical Committee (Approval no: 769/2011/CPCSEA).

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Table 1: Anti-inflammatory activity of EECP on Egg albumin induced paw edema

		Percentage inhibition of paw edema					
Group	Dose	0hr	1hr	3hr	6hr	12hr	24hr
Diclofenac	10mg/kg	2.78%	14.48%	25.0%	41.18%	55.07%	64.50%
EECP	200mg/kg	2.78%	10.53%	20.00%	38.66%	52.90%	61.54%
	400mg/kg	2.78%	18.42%	28.00%	42.86%	57.25%	67.46%

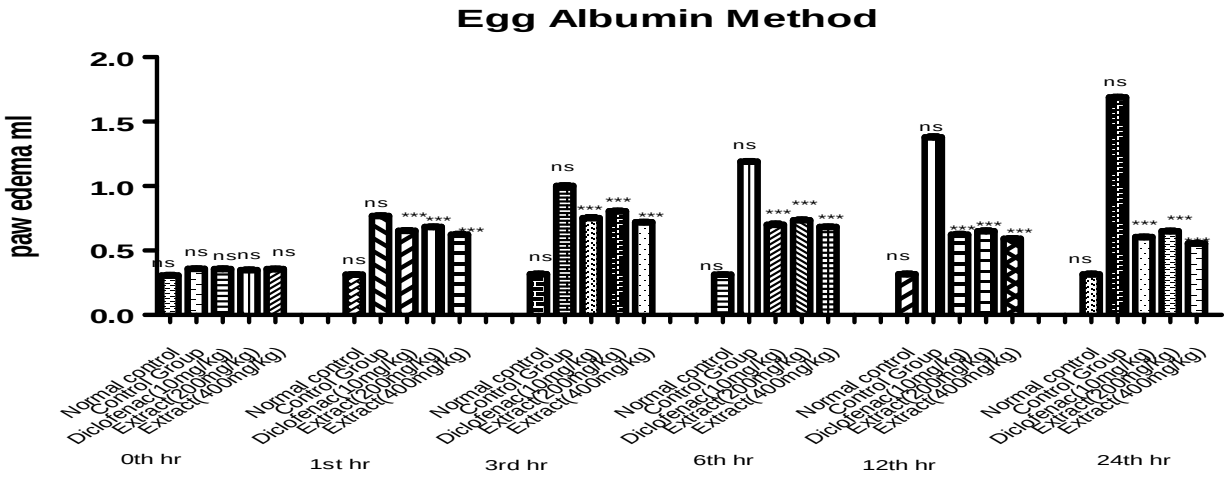
Table 2: Anti-inflammatory activity of EECP on Turpentine oil induced paw edema

		Percentage inhibition of paw edema			
Group	Dose	0day	1day	3day	7day
Diclofenac	10mg/kg	2.78%	33.34%	50.49%	65.28%
EECP	200mg/kg	2.78%	25.64%	35.90%	63.89%
	400mg/kg	2.78%	35.90%	53.40%	68.75%

Table 3: Anti-inflammatory activity of EECP on Formaldehyde induced paw edema

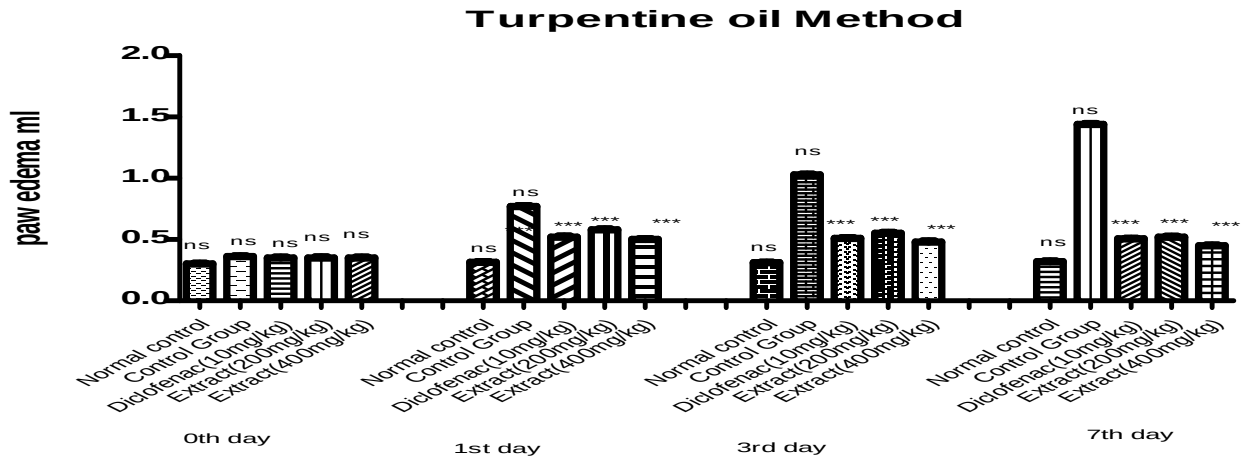
		Percentage inhibition of paw edema				
Group	Dose	0day	1day	3day	7day	10day
Diclofenac	10mg/kg	8.10%	28.21%	39.13%	54.25%	64.77%
EECP	200mg/kg	5.41%	23.08%	37.39%	50.98%	63.73%
	400mg/kg	5.41%	32.06%	43.48%	58.82%	69.43%

Figure 1: Effect of EECP on Egg albumin induced paw edema volume



All values are expressed as mean ± SEM, n=6, one way Analysis of variance (ANOVA) followed by Dunnett's multiple comparison test; $^{***}p<0.001$ as compared to control group; ns=non-significant.

Figure 2: Effect of EECP on Turpentine oil induced paw edema volume



All values are expressed as mean ± SEM, n=6, one way Analysis of variance (ANOVA) followed by Dunnett's multiple comparison test; $^{***}p<0.001$ as compared to control group; ns=non-significant.

Drugs & Chemicals: Diclofenac sodium(Gift sample from Empree medicaments Ltd. Karnataka), Turpentine oil, Formaldehyde, Egg albumin. All the chemicals used in this study were of analytical grade.

METHODOLOGY

Egg albumin induced rat paw edema: Five groups of adult rats (n=6) were used in this study. Animals were fasted over night with free access to water before the experiment. On the day of experiment, base line paw volume was recorded by using a plethysmometer (UGO Basile, 7140 Italy). Thereafter group-I (normal rats) received the vehicle (Distilled water 5ml/kg). Group-II (control rats) received the inducing agent & vehicle. Group-III (standard rats) received diclofenac sodium (10mg/kg) along with inducing agent. Group-IV & V received extract at doses of 200mg/kg & 400mg/kg respectively along with inducing agent. 1hr after administration of vehicle/drugs, edema was induced by administration of 0.1ml of fresh undiluted egg albumin solution into the subplantar region of right hind paw[9]. Paw volume of each rat from all groups was measured at 0, 1, 3, 6, 12 & 24hr after phlogistic agent administration. From the mean edema volume, the percent inhibition of edema was calculated by using following formula:

% Inhibition of edema = 100 (V_C-V_T/V_C)

Where, V_C = Mean paw edema volume of control group

V_T = Mean paw edema volume of treated group

Turpentine oil induced rat paw edema: Grouping of animals & drug treatments was same as above. 30min after administration of the vehicle/drug, edema was induced by administration of 0.05ml turpentine oil into the subplantar region of right hind paw of animal[10]. Paw volume of each rat from all groups was measured on 0, 1, 3 & 7th day after phlogistic agent administration. From the mean edema volume, the percent inhibition of edema was calculated.

Formaldehyde induced rat paw edema: Grouping of animals & drug treatments was same as above. Drugs/vehicles were administered for a duration of 10days. 30min after administration of the drug/vehicle, edema was induced by administration of 0.1ml of 2%v/v Formaldehyde into the subplantar region of right hind paw of all animals on days 1 and 3[10]. Increase in paw edema volume was measured on 0, 1, 3, 7 and 10th day, 30min after administration of the respective vehicle/drug. From the mean edema volume, the percent inhibition of edema was calculated.

STATISTICAL ANALYSIS

The statistical significance was measured by using one way analysis of variance (ANOVA) & followed by Dunnett's comparison test. All the data are presented as mean ± SEM & $p < 0.001$ was considered as significant.

RESULTS

The phlogistic agents induced inflammation was significantly inhibited by the treatment given when compared with the standard drug. EECP exhibited significant anti-inflammatory activity in a dose dependent manner.

Egg albumin induced paw edema: The effect of EECP on egg albumin induced paw edema was depicted in the table 1. The EECP at a dose of 400mg/kg showed significantly greater inhibitory activity (67.46%) against standard diclofenac sodium (64.50%).

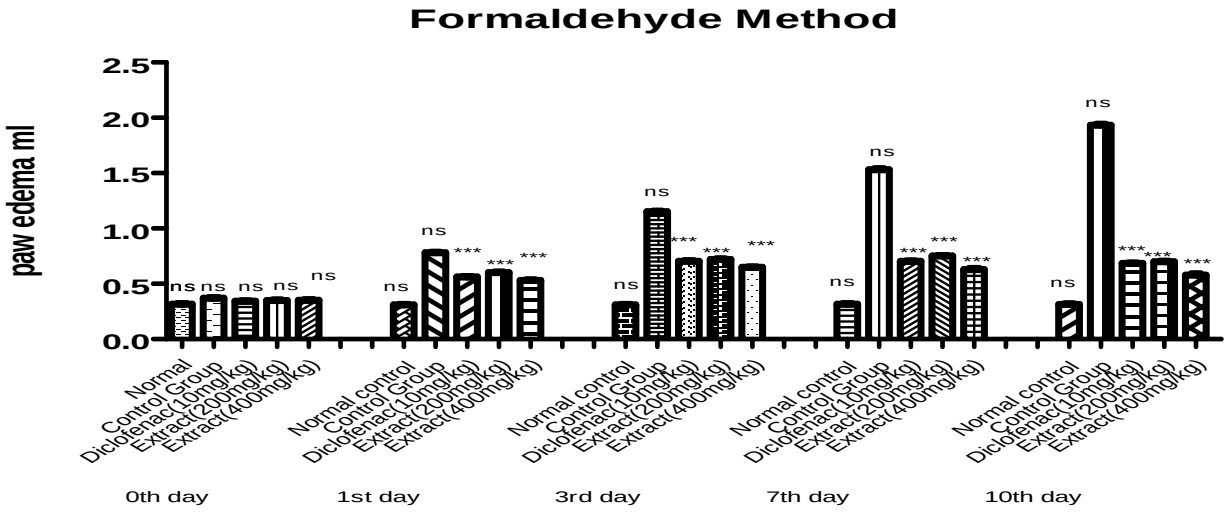
Turpentine oil induced paw edema: The inhibitory activity on turpentine oil induced paw edema are shown in table 2. The EECP at a dose of 400mg/kg showed inhibitory activity of 68.75% against standard (65.28%).

Formaldehyde induced paw edema: As shown in table 3 the EECP at a dose of 400mg/kg showed greater inhibitory activity (69.43%) against standard (64.77%).

DISCUSSION

The most widely used primary test to screen new anti-

Figure 3: Effect of EECP on Formaldehyde induced paw edema volume



*All values are expressed as mean ± SEM, n=6, one way Analysis of variance (ANOVA) followed by Dunnett's multiple comparison test; ***p<0.001 as compared to control group; ns=non-significant.*

inflammatory agent measures the ability of a substance to reduce local edema induced in the rat paw by a phlogistic agent[2]. In this study Egg albumin, Turpentine oil & Formaldehyde were used as irritants.

Egg albumin induced paw edema is similar to carrageenan induced acute inflammation & is a biphasic system. The early phage is due to release of histamine, serotonin & increase prostaglandins (PG) synthesis in the damaged tissue surroundings. The second phage is maintained by PG release & mediated by bradykinin, leukotriene's, polymorphonuclear cells[11]. The test formulation showed the better anti-inflammatory activity & this is may be due to the inhibition of release of histamine, serotonin, kinins & this also retarded the release of PG like substances[12].

Turpentine oil in the sub plantar region causes paw edema that was maintained throughout the observation period i.e., 7 days as it is subacute model due to the triphasic release of inflammatory mediators[10]. The initial phase is due to the release of histamine & serotonin, intermediate phase is due to kinin like substances and the late phase is due to cyclooxygenase & lipoxygenase products. EECP exhibited maximum inhibition of paw edema during the late phase of inflammation which suggest a prominent cyclooxygenase/lipoxygenase inhibitory activity.

Formaldehyde induced paw edema is one of the most suitable test procedure to evaluate the anti-inflammatory & anti-arthritic agents as it closely resembles human arthritis[13]. The paw swelling due to 2%v/v formaldehyde was maintained throughout the observation period i.e.10 days due to the release of histamine, serotonin, and PG. Histamine and PG are the key mediators in inflammatory hyperalgesia which are mediated through the activation of local pain receptors and nerve terminals producing hypersensitivity in the area of injury[14]. Inhibition of paw edema may be due to the ability of the EECP to inhibit histamine, serotonin & PG.

In all the experimental procedures, the results showed a significant inhibition of paw edema by EECP & standard drug when compared with the control. Further studies are required on isolation of potent chemical constituents of the plant & to investigate the mechanism of anti-inflammatory activity.

CONCLUSION

Interestingly, the test compound showed potential anti-inflammatory activity than standard Diclofenac sodium and justified the traditional use of *Cissus pallida* in the treatment of various types of pain & inflammation in all experimental models. Based on the above results, we conclude that the Ethanolic extract of *Cissus pallida* has significant anti-inflammatory activity and might prove efficacious for further design and development of agents with significant biological activities. Further, studies are required to elucidate the detail mechanism of action of these agents at molecular level to explore the therapeutic benefits.

ACKNOWLEDGEMENTS

The authors are thankful to AICTE & School of Pharmaceutical Sciences, JNTUK for providing the funds. We the authors are also thankful to SICRA management for providing necessary facilities to carry out the study.

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