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Research Article

ISOLATION OF CONSTITUENT FROM *CLINACANTHUS siamensis* AND ITS ANTI-INFLAMMATORY STUDIES

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ABSTRACT

The present study includes the anti inflammatory and antiarthritic studies of the compound isolated from the methanolic extracts of *Clinacanthus siamensis*. *Clinacanthus siamensis* is a medium sized perennial shrub grows up to 2m in height belonging to the family Acanthaceae. The structure of the isolated compound was confirmed by spectral analysis and the compound isolated may be 4,5-dinonyl-1,3-dioxolane. The compound showed significant anti-inflammatory activity in Carrageenan induced paw edema model and the maximum inhibition was to the extent of 37.98% at 180 minutes of administration ($p < 0.001$). The compound at 0.5 mg/kg doses produced a significant inhibition in formalin induced arthritis and the effect produced was 22.17 % ($P < 0.05$). From the present study it can be concluded that the compound isolated from methanolic fraction of *Clinacanthus siamensis* has a potential anti-inflammatory and antiarthritic activity.

Keywords: *Clinacanthus siamensis*, Isolation, Anti-inflammatory activity, Antiarthritic activity.

INTRODUCTION

Inflammation is an evidence of many diseases, is a major concern for physicians throughout the world. Prolonged use of anti inflammatory agents has been associated with gastrointestinal irritation. In India many ayurvedic practitioners are using various indigenous plants for the treatment of different types of arthritic and inflammatory conditions.[1] Inflammation is the immediate defensive mechanism or reaction to an injury, which may be caused by infection, chemical or physical agents. It involves pain, heat, redness , swelling and loss of function on affected part. Several bioactive molecules with potent anti-inflammatory activity are isolated from plants like *Piper longum, Aloe vera, Tridax procumbens* etc. The major limitations of currently used synthetic anti-inflammatory agents are gastrointestinal irritation and ulceration. Natural products devoid of these side effects will be promising group for treating inflammatory disorders.[2]

There is a huge potential for screening the biodiversity of plants and microbes using a variety of advanced techniques, as many of them have not been studied. It is also clear that as the purification, identification and testing technology improves, more and more natural products will become of interest in pharmaceutical industry. The increasing cost of energy and chemical raw materials, combined with the environmental concerns associated with conventional pharmaceutical manufacturing will make plants even more compatible in the future. *Clinacanthus siamensis* is a medium sized perennial shrub grows up to 2m in height belonging to the family Acanthaceae. It is distributed throughout the Western Ghats growing as an under shrub. The leaves, root and stem are used traditionally as Poison bites, inflammation, traumatic edema and swelling due to poison stings. The study includes the anti inflammatory and antiarthritic studies of the compound isolated from the methanolic extracts of *Clinacanthus siamensis*.

MATERIALS AND METHODS

Preparation of the Extracts and Phytochemical Screening[3,4]

The fresh plant were collected from Wayanad and it was authenticated at department of botany, Sir Sayed college Taliparamba, Kerala. The whole plant was dried and powdered and was subjected to successive extraction with n-hexane, chloroform, methanol, ethyl acetate and water and the crude extracts were subjected to preliminary phytochemical screening.

Isolation of constituent from methanolic extract of *Clinacanthus siamensis*[5,6]

The methanolic extract (40g) was subjected to column chromatography on silica gel (60-120 mesh, 500 gram). The extract was eluted from the column using a step wise gradient elution of hexane : ethyl acetate and finally washed with 100% methanol. A total of 20 fractions were collected and evaluated on TLC using solvent systems of hexane : ethyl acetate (9.5 : 0.5) in different polarity ratios. The fractions showing similar TLC profiles were combined to obtain seven pooled fractions. The fraction two and three were mixed and was further chromatographed on to silica gel (230-400 mesh) column and eluted with hexane and ethyl acetate (9.5:0.5) to give compound A.

Acute toxicity studies[7,8]

Healthy albino mice of either sex weighing 20-30gm were used for the study. The starting dose level of the extracts was 2000mg/kg body weight. The animals were starved overnight. After dosing, the animals were closely observed for first 4 hours for any abnormal activity and intermittently for the next 24 hours. The number of animals dead was noted after 24 hours.

Invivo anti-inflammatory studies[9,10]

Healthy adult wistar albino rats of either sex weighing 150-200gm were used for the study and the protocol was approved by Institutional animal ethical committee (CPCSEA No.282/CADD/27/2011). The invivo anti-inflammatory activity was studied for the compound isolated from the methanolic extract by Carrageenan induced paw edema model in rats. Both hind paws of each animal were marked at the tibiotarsal junction and measured the volume of paw up to the marked position. Inflammation was induced by injecting carrageenan solution (0.1ml of 1 % w/v) into the plantar region of the right hind paw. The left paw was used as reference for comparison of inflammation. The standard group was administered with Indomethacin 10 mg/kg body weight,(p.o) and the control group receives 1% CMC of 10 ml/ kg body weight**.** The paw volumes were measured before and three hours after Carrageenan administration by volume displacement method. The animal studies have been done as per OECD guidelines 423.

Antiarthritic activity[12,13]

Healthy adult wistar albino rats of either sex weighing 150-200gm were divided in to three groups of three animals. The control group receives 1% CMC, 10ml/kg,(p.o) and the standard receives Aspirin 5mg/kg body weight(p.o). Arthritis was induced in rats by using 2% w/v of formalin solution. The inflammation was produced by sub aponeurotic injection of 0.1 ml of the 2% w/v formalin solution in the left hind paw of the rats on the first and third day. Drugs and 1% CMC was given for 10 days and the paw volumes of all the animal groups were measured by vernier calipers at 0and 10 days after the injection of formalin solution.

RESULTS AND DISCUSSION

The results of the phytochemical screening of the extracts are summarized in table no 1. The methanolic extract showed the presence of maximum constituents therefore the methanolic extract was selected for the isolation of phytoconstituents.

Analysis of compound isolated from *Clinacanthus siamensis*

The compound isolated from methanolic extract showed a R_f value: 0.85 [Solvent system: n-hexane: ethyl acetate (9.5:0.5)] and a melting point of 112.72-113.0 ^{oc}. The chemical test of the isolated compound showed the presence of fixed oil. The absorption bands in the I R spectrum at 1259 $cm^{-1}$and 1013 cm^{-1}shows the presence of an ether function in the compound. The ^{13}C NMR spectrum showed two signals in the down field region at ẟ 63.06 and ẟ 96.2. The signalδ 96.2 is indicative of a carbon atom in between two oxygen atom. The signal at ẟ 63.06 suggests the carbon atom attached to ether oxygen atoms. The signal atδ 14.21 is for a methyl group and the other signals at δ 22.75, δ 25.82, δ 29.43, δ 29.51, δ 29.77, δ 31.99, δ 32.87 strongly indicate the presence of long chain alkyl group.

^{1}H NMR data shows the signal atδ 0.84 is for a methyl group; δ 1.21 is to the long chain methylene groups, the triplet at δ 3.61 is due to hydrogen atoms adjacent to ethereal oxygen atom, the down field signal at δ 7.3 is due to the methylene group in between two oxygen

atom. The other multiplet signals at δ 2.3 and δ 1.8 are due to β and α methylene protons to the ether oxygen atom.

The molecular ion peak was found to be 325.95. Based on the above factors the proposed structure may be 4,5-dinonyl-1,3-dioxolane. The structure of the compound is given in figure 1.Acute toxicity studies

The LD_{50} of methanolic extract of *Clinacanthus siamensis* was found to be more than 2000 mg/ Kg. In gross behavioral studies, no characteristic behavioral changes have been noticed on oral administration of methanolic extract of *Clinacanthus siamensis.*

Invivo anti-inflammatory studies

The maximum effect of isolated compound was produced at a dose of 0.5mg/Kg at 180 minutes of oral administration. The maximum inhibition was to the extent of 37.98% and the extract showed a dose dependent inhibitory effect. The effect of compound was found to be significant when compared with control by Bonferroni comparison test ($p < 0.001$). The results are summarized in table 2.

Antiarthritic activity

The effect of the compound on formalin induced arthritis at 0.5 mg/ kg was calculated as percentage reduction in thickness of the joint . The compound at 0.5 mg/kg doses produced a significant inhibition in formalin induced arthritis and the effect produced was 22.17 % ($P < 0.05$). Aspirin, which served as standard produced 72.65 % inhibition, which was highly significant ($P< 0.001$). The statistical analysis was done with control by Bonferroni comparison test and the results are summarized in table 3.

CONCLUSION

The compound isolated from the methanolic extract of *Clinacanthus siamensis* showed significant anti-inflammatory and antiarthritic activity. The structure of the isolated compound was confirmed by spectral analysis and the compound isolated may be 4,5-dinonyl-1,3-dioxolane. The compound showed significant anti-inflammatory activity in Carrageenan induced paw edema model. The compound significantly inhibited the formalin induced arthritis in rats, supporting the use of the plant in arthritis. From the present study it can be concluded that the compound isolated from methanolic fraction of *Clinacanthus siamensis* has a potential anti-inflammatory and antiarthritic activity.

Table 1: Phytochemical screening of the extracts

S.No.	Chemical Test	n-hexane Extract	Chloroform Extract	Ethyl acetate Extract	Methanolic Extract	Water Extract
1	Tests for Steroids	+	+	_	+	_
2	Tests for Triterpenes	+	+	_	_	_
3	Tests for Saponins	_	_	+	+	_
4	Tests for Alkaloids	_	_	_	+	_
5	Tests for Carbohydrates	_	_	_	+	+
6	Test for Reducing sugars	_	_	_	+	+
7	Tests for Tannins and phenolic compounds	_	_	+	+	+
8	Tests for Flavanoids	_	_	+	+	+
9	Test for Glycoside	_	_	_	_	_
10	Test for Fixed oils and fats	+	+	_	+	_
11	Test for Proteins and Amino acids	_	_	_	_	_

+ Present
-Absent

Table 2: Anti-inflammatory activity of compound isolated from *Clinacanthus siamensis*

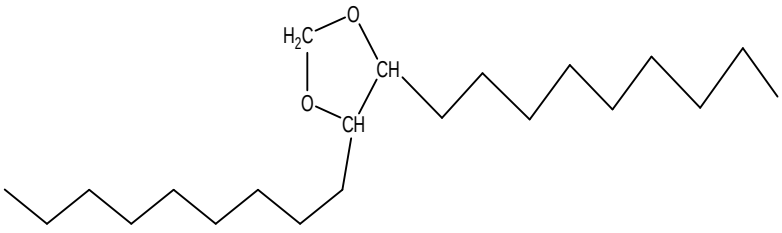
Treatment	Time in minutes	Paw volume, mean ± S.E	% Inhibition
Control 1 % CMC, 10ml/Kg (P.O)	0	---	---
	60	0.213± 0.0305	---
	120	0.420± 0.0721	---
	180	0.693± 0.0808	---
	240	0.7467± 0.0230	---
Standard Indomethacin 10 mg/ Kg. (P.0)	0	---	---
	60	0.0933± 0.0416*	56.26
	120	0.1400±0.040***	66.67
	180	0.216±0.0152***	68.74
	240	0.320±0.020***	57.15
Compound A 0.5mg/kg, (P.O)	0	---	---
	60	0.140±0.040 ^{ns}	34.37
	120	0.270±0.02646**	35.72
	180	0.4300±0.0793***	37.98
	240	0.4933±0.0230***	33.94

Values are Mean±SEM, n=3, *** - highly Significant (p-value < 0.001), ** - very significant (p-value < 0.01), * - significant(p-value < 0.05), ns- nonsignificant (p-value >0.05) significant compared to control group, ANOVA by Bonferroni.

Table 3: Antiarthritic activity of compound isolated from *Clinacanthus siamensis*

Group	Treatment	Difference in thickness (Mean ±SE)	%Inhibition
Control	1% CMC10ml/kg(P.O)	3.157±0.3202	-
Standard	Aspirin, 5mg/kg (P.O)	0.8633±0.2312***	72.65
Test	Compound A (0.5mg/kg)	2.457±0.3338*	22.17

Values are Mean±SEM, n=3, *** - highly Significant (p-value < 0.001), * - Significant (p-value < 0.05), significant compared to control group, ANOVA by Bonferroni.



4,5-DINONYL-1,3-DIOXOLANE

Fig. 1: Structure of compound A

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