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***In Vitro* Anti-inflammatory Activity of *Exacum wightianum* Arn. (Gentianaceae)- An Endemic Medicinal Plant**

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ABSTRACT

The anti-inflammatory activity of ethanol, methanol and chloroform extracts of *Exacum wightianum*, was evaluated by carrageenan induced rat paw odema method. The dose effects of 200 mg/kg b.wt. of the ethanolic, methanolic and chloroform extracts of *E. wightianum* was more active than 100 mg/kg b.wt. and found to be statistically significant. In acute inflammation as produced by carrageenan 72.30% and 86.89% protection was observed in methanol extract of *E. wightianum*. These extracts did not show any sign of toxicity up to a dose of 1000 mg/kg.bw in rats. The results suggested that the methanolic extract of *E. wightianum* has exhibited an effective anti-inflammatory activity mediated via either by inhibition of cyclooxygenase cascade and by blocking the release of vasoactive substances like histamine, serotonin and kinins. The results also justified the use of this plant in traditional Indian medicine in the treatment of inflammation.

Keywords: *Exacum wightianum*, Carrageenan induced paw oedema, Methanol, Ethanol and Chloroform extract.

INTRODUCTION

Inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli. An uncontrolled and persistent inflammation may act as an etiologic factor for many of these chronic illnesses (Kumar *et al.*, 2004). Although it is a defense mechanism, the complex events and mediators involved the inflammatory reaction can induce, maintain or aggravate many diseases (Sosa *et al.*, 2002). Currently used synthetic anti-inflammatory drugs are associated with some severe side effects. Therefore, the development of potent anti-inflammatory drugs with fewer side effects is necessary from medicinal plants origin.

MATERIALS AND METHODS

The whole plant unadulterated powdered materials of *Exacum wightianum* was successively extracted with ethanol, methanol and chloroform in a soxhlet apparatus and concentrated to dryness. These extracts were made free of any solvent by distillation. All the extracts were subjected for acute toxicity studies and LD50 doses were determined for the pharmacological activity. These extracts were used as an emulsion in 5% suspension with gum acacia and administered orally at the dose of 100 and 200 mg/kg b.wt. The animals were grouped in cage in an air conditioned room at the temperature of 22±1°C with 12 hour light and dark cycle.

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Table 1. Effect of *Exacum wightianum* extracts on the Percentage inhibition of Carrageenan induced paw oedema

Treatment	Dose mg/kg	Oedema volume (ml)				% Inhibition after 180 min
		0 min	60 min	120 min	180 min	
CONTROL (Group-I)	Normal saline	39.63±2.16	85.11±4.15	103±2.33	123.31±9.33	-
Ethanol extract (Group-II)	100 mg/kg	35.08±1.57	74.20±3.60	94.12±3.18	105.46±5.43	14.68 %
	200 mg/kg	22.11±2.18*	39.73±4.05*	63.35±4.18*	74.77±3.58*	39.49 %
Methanol extract (Group-III)	100 mg/kg	28.32±1.95	38.43±2.18*	31.26±3.93*	34.24±2.87*	72.30 %
	200 mg/kg	27.02±1.84*	21.17±1.86**	17.47±2.69**	16.21±1.86**	86.89 %
Chloroform extract (Group-IV)	100 mg/kg	31.37±1.98*	71.37±2.67*	71.16±2.18*	54.41±1.69*	55.98 %
	200 mg/kg	29.31±1.89	53.43±2.68*	54.76±1.98*	47.33±1.90*	61.71 %
Indomethacin (Group-V)	10 mg/kg	25.71±1.69**	28.43±1.94*	49.11±1.69*	51.75±2.15**	58.13 %

Each Value is SEM ± 5 individual observations * P < 0.05; ** P<0.01 Compared paw oedema induced control vs drug treated rats

- Group I : Control rats given normal saline orally by using an Intra Gastric Catheter tube (IGC).
 Group II : Rats given ethanol EW extract at the dose of 100 and 200 mg/ Kg body weight by IGC
 Group III : Rats given methanol EW extract at the dose of 100 and 200 mg/ Kg body weight by IGC
 Group IV : Rats given chloroform EW extract at the dose of 100 and 200 mg/ Kg body weight by IGC
 Group V : Rats given Indomethacin at the dose of 10 mg/ Kg body weight by IGC

The animals were maintained with pellet diet and water *ad libitum*. They were further segregated in to various groups. This experiment was performed according to ethical guidelines for the investigation of experimental pain in conscious animals (659/02/a/CPCSEA). Stranded Intra Gastric Catheter tube (IGC) was used for oral drug administration.

Carrageenan-induced paw oedema in albino rats (Winter and Poster, 1957)

Animals were divided into 5 groups comprising five animals in each group. In all groups acute inflammation was produced by sub plantar injection of 0.1 ml freshly prepared 1% suspension of carrageenan in normal saline in the right hind paw of the rats and paw volume was measured plethysommetrically at 0 to 180 mins after carrageenan injection. All the animals were premedicated with indomethacin (10mg/kg b.wt.) orally two hour before infection. Mean increase in paw volume was measured and percentage was calculated for all the extracts. All the extracts were subjected for acute toxicity studies and 1/10th of the LD 50 dose was selected for pharmacological activity. Percentage inhibition of paw volume was calculated by the following formula

$$\% \text{ inhibition} = \frac{V_c - V_t}{V_c} \times 100$$

Where

Vt- means increase in paw volume in rats treated with test compounds.

Vc- means increase in paw volume in control group of rats.

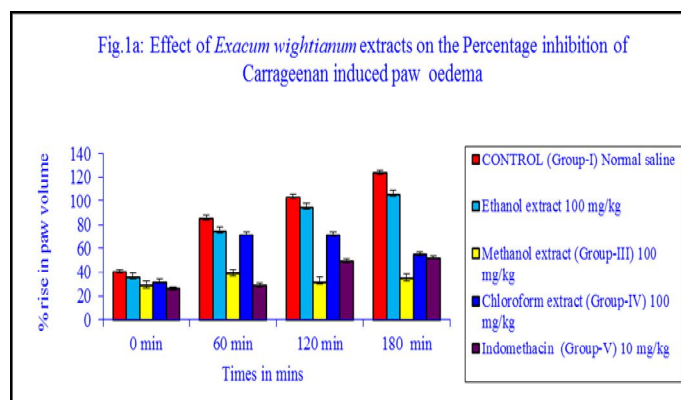
Statistical analysis

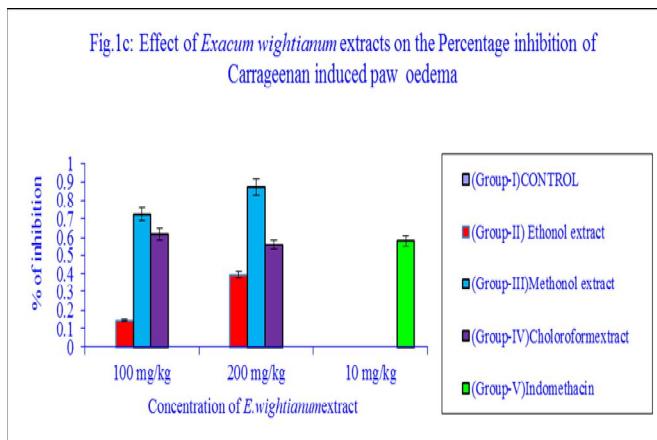
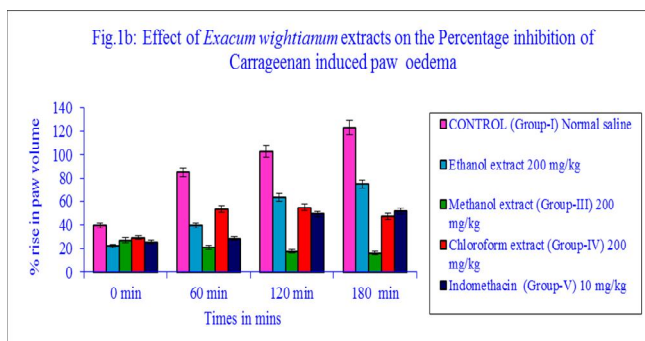
The mean paw volume was expressed in terms of mean ± SEM and evaluated for statistical significance by ANNOVA followed by Dunnett's test, P<0.05 was considered by statistically significant.

RESULT

E. wightianum was evaluated by carrageenan induced rat paw oedema method. The extracts were tested at two different dose

levels. The dose effect of 200 mg/kg b.wt. of the ethanolic, methanolic and chloroform extracts of *E. wightianum* was more active than 100 mg/kg b.wt. Which were found to be statistically significant. In the present study 100mg/kg b.wt. and 200mg/kg. b.wt of ethanolic, methanolic and chloroform extracts of *E. wightianum* significantly reduced the carageenan induced paw oedema inflammation as compared with that of the standard drug, indomethacin. This results indicated that the methanol extract with the dose of 200mg/kg.b.wt and the chloroform extract with the dose of 200 mg/kg b.wt showed a maximum anti-inflammatory activity as compared to the reference drug, indomethacin. The methanol extract with a dose of 200mg/kg b.wt produced 86.89% of inhibition and it is also high as compared to the reference drug. The ethanol extract with a dose of 200mg/kg b.wt. produced 39.49% of inhibition and was low as compared to the reference drug. The chloroform extract with two different doses of 100mg/kg b.wt. and 200mg/kg b.wt. showed only 55.98% and 61.71% inhibition respectively. It was lower as compared to the reference drug, whereas in case of ethanol, methanol and chloroform extracts of *E. wightianum* at the doses of 100 mg/kg b.wt. inhibited 14.68%, 72.30% and 61.71% respectively and it was higher inhibition per cent in case of methanol extract as compared to reference drug, Indomethacin which showed 58.14%. However, the ethanol and the methanol extract at the doses of 100mg/kg b.wt. showed 14.68% and 55.98% inhibition as compared to the control drug (Table 1 and Fig 1a, b and c).





DISCUSSION

The extracts *Exacum wightianum* were tested at two different dose levels (100 and 200 mg/kg b.wt.). The dose effect of 200 mg/kg b.wt. of the ethanolic, methanolic and chloroform extracts of *E. wightianum* was more active which was found to be statistically significant. In the present study, both concentrations (100mg/kg b.wt. and 200mg/kg. b.wt) of methanolic and chloroform extracts of *E. wightianum* significantly reduced the carrageenan induced paw oedema inflammation as compared with that of the standard drug, indomethacin. This result showed that the methanol and chloroform extracts have a maximum anti-inflammatory activity and it was compared to the reference drug, indomethacin. The methanolic extract inhibited high percentage of activity followed by chloroform and except ethanolic extract.

The inflammatory response is a common feature in many diseases and its control is of relevance in the treatment of these pathologies. There are several herbal drugs used for the anti-inflammatory activity. Plants exhibiting anti-inflammatory activity reveal that species of 96 genera belonging to 56 families have exhibited such potential (Chawla *et al.*, 1987 and Handa *et al.*, 1992). Keeping in view the growing significance of anti-inflammatory related herbal medicines in global market, the present anti-inflammatory study has been carried out on *Exacum wightianum* (Gentianaceae). Oedema, which develops after carrageenan inflammation, is a biphasic event. The initial phase is attributed to the release of histamine and Serotonin. The oedema maintained between the first and second phase is due to kinin like substances (vinegar *et al.*, 1969). The second phase is said to be

promoted by prostaglandin like substances. The second phase of oedema is sensitive to drugs like hydrocortisone, phenylbutazone and Indomethacin. (Winter *et al.*, 1962). The present results of carrageenan induced paw oedema model indicated dose dependent anti-inflammatory activity.

The efficacy of various extracts of *E. wightianum* used as an efficient therapeutic agent in acute anti-inflammatory conditions. The results of the study supported the traditional use of this plant in some inflammation and painful conditions which confirm the presence of active chemical compounds related to these activities. phytochemicals such as flavonoids, terpenoids, steroids and phenolic compounds expressed their anti-inflammatory activity at least in part by modulation of pro-inflammatory gene expression such as cyclooxygenase-2, inducible nitric oxide synthase and several pivotal cytokines. These are considered to be reasonable candidates for new anti-inflammatory drugs (Kim *et al.*, 2004). The plant *E. wightianum* also could have similar phytochemicals which support the Kim *et al.*, (2004) study. Numerous plants constituents have demonstrated anti-inflammatory properties. For example, numerous flavonoids compounds (gossypin, quercetin, gnapthalin) have been associated with anti-inflammatory and may have potential in the management of inflammatory disorders (Harbone and Baxter, 1993).

CONCLUSION

Indomethacin showed more or less uniform inhibition of edema in early intermediate and later phases. Methanolic extract showed also more or less significant inhibition of carrageenan induced paw edema in early phases while significant inhibition at later phases. Out of ethanol, methanol and Chloroform extracts from *E. wightianum*, methanol extract was more significant than the other extract in percentage inhibition of paw edema. Acute inflammation Induced by carrageenan results from cell damage, which provokes the production of endogenous mediators, such as, histamine, serotonin, prostaglandins, and bradykinin. It is well known that inhibition of edema induced by carrageenan in rats is one of the most suitable test procedures to screen anti-inflammatory agents as it closely resembles human arthritis. As some of the above extracts significantly inhibited this model of inflammation they can be thought to possess antiproliferative and antiarthritic activities similar to indomethacin, a cyclooxygenase inhibitor.

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