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Evaluation of anti-inflammatory effect of *Calotropis gigantea* and *Tridax procumbens* on Wistar albino rats.

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Abstract
Calotropis gigantea Linn and *Tridax procumbens* Linn, are well documented medicinal plants distributed in Asia and Africa and are useful for many ailments. The anti-inflammatory activity of low dose of the aqueous extract of *T. procumbens* and ethanolic extract of C.gigantea were assessed on carrageenan induced paw edema and compared with standard drug Ibuprofen. The oral administration of 400mg/kg of *C.gigantea* and 300 mg/kg of *T. procumbens* were showed significant antiinflammatory activity more than that of 100mg/kg of Ibuprofen. This study also proved the greater anti-inflammatory action due to the combined effect of *C. gigantea* and *T. procumbens* with Ibuprofen than Ibuprofen alone. This may be due to the potentiation of its inhibitory effect on the synthesis and release of various inflammatory mediators.
Key words: Anti-inflammatory, *Calotropis gigantea*, *Tridax procumbens*, methanolic extract, Ibuprofen.

Introduction

Calotropis gigantea Linn. (Asclepiadaceae family) and *Tridax procumbens* Linn. (Compositeae family) are widely distributed in Asia and Africa [1,2]. Traditionally the milky juice of *C. gigantea* has been used as a violent purgative, gastrointestinal irritant and abortion inducer [3,4,5]. It has also been used in the treatment of earache, toothache, headache, sprain and stiff joints [6]. Similarly, *T.procumbens* Linn, is employed as indigenous medicine for a variety of ailments like jaundice, in bronchial catarrh, diarrhoea, dysentery, anti-fungal, anticoagulant and insect repellent [7, 8] . Reports are further available on its wound healing, hepatoprotective, immunomodulator, antimicrobial and antioxidant properties [9,10,11,12]. Keeping in view of the medicinal importance of these plants, the aim of the present study is to estimate the anti-inflammatory activity of *C. gigantean* and *T. procumbens* and the synergistic anti-inflammatory activity of *C. gigantea* and *T. procumbens* on standard drug Ibuprofen using carrageenan induced inflammation in rats.

Material and Methods

Plant materials and extraction:

Leaves of *Calotropis gigantea* and *Tridax procumbens* were collected from botanical garden of Bundelkhand university, Jhansi, India, in month of December. The materials were taxonomically identified and authenticated by National Botanical Research Institute, Lucknow, India and the vouchers of specimen were stored. The shed dried leaves of *C. gigantea* were extracted in 500ml of 50% ethanol using soxhlet apparatus [13]. The fresh leaves of *T. procumbens* were crushed with the help of mortar and pestle. Juice obtained was filtered through muslin cloth. The respective filtrates were evaporated by rotary evaporator (cryochiller) [9]. The water soluble portion of the ethanolic extracts of *C. gigantea* and the fresh leaves of *T. procumbens* were used for the investigation of their anti-inflammatory actions.

Animals:

The study used male and female albino rats weighing 130-170g. They were kept in polypropylene cages in centrally air condition room 12 hours light and 12 hours dark cycle. The animals had free access to water and food and were left to acclimatize at least one week before starting the experiment. The animals were distributed randomly six animals in each group. All experiments were carried out in accordance with the guideline of the CPCSEA.

Anti-inflammatory activity:

The test was used to determine the anti-inflammatory action of the extract by the paw edema method [14]. Paw edema was induced by a sub-plantar injection of 0.1 ml

of 1% carrageenan (in 5% gum acacia). The edema volume was determined using a Plethysmometer prior to and first, third and fifth hours after carrageenan injection. The animals were treated with the standard drug Ibuprofen at different dose levels (25, 50, 100 and 150 mg/kg. body weight). Other groups of animals were treated with the ethanolic extract of *C. gigantean* (400mg/kg. body weight) and the fresh leaves extract of *T. procumbens* (300mg/kg. body weight) along with each doses of Ibuprofen respectively. The test drugs were given one hour prior to carrageenan injection. The control group received saline only.

Statistical analysis:

Results were expressed as mean ± SEM. One-way ANOVA test was applied to evaluate the significant *p* values, where $p < 0.01$ was considered to be significant.

Results and Discussion

The anti-inflammatory activity of Ibuprofen, Ibuprofen along with *C. gigantea* and *T. Procumbens* using carrageenan-induced rat edema is shown in Table 1. The standard drug Ibuprofen presented a dose-dependent anti-inflammatory activity.

The oral administration of 400mg/kg of *C. gigantea* and 300mg/kg of *T. procumbens* along with different doses of Ibuprofen significantly reduced paw edema. In the first hour *C. gigantea* alone and with different doses of Ibuprofen shows 50.00%, 57.50%, 60.00%, 62.50%, and 67.50% inhibition of paw edema respectively. In the fifth hour *C. gigantea* alone and with different doses of Ibuprofen showed to exhibit 57.35%, 66.17%, 73.52%, 80.88% and 82.35% inhibition of paw edema respectively.

In the first hour *T. procumbens* alone and with different doses of Ibuprofen shows 55.00%, 60.00%, 62.50%, 70.00% and 75.00% rat paw edema inhibition respectively. In the fifth hour *T. procumbens* alone and with different doses of Ibuprofen shows 51.47%, 58.82%, 67.64%, 72.05% and 76.47% paw edema inhibition respectively.

From the present study it was observed that the water soluble portion of ethanolic extract of C. *gigantea* and the dried juice of *T. procumbens* in combination with Ibuprofen have greater anti-inflammatory activity than Ibuprofen alone.

Conclusion

The standard drug Ibuprofen presented a dose-dependent inhibition of inflammation activity at all concentrations after carrageenan injection and it is sensitive to clycooxygenase inhibitors. The water soluble portions of methanolic extract of *C. gigantea* and the dried juice of *T. procumbens* inhibited edema during all phase of inflammation. The comparative study showed that the *T. procumbens* has more synergistic anti-inflammatory activity in first and third hour than *C. gigantean*. In fifth hour *C. gigantea* showed more synergistic anti-inflammatory efficacy than *T. procumbens*. The extracts also increased the inhibition of edema along with standard drug. The local injection of Carrageenan induced inflammatory process in the rat involves three phases by several mediators released in ordinate sequence [14, 15]. An initial phase, during the first 1.5 h, is caused by the release of histamine and seratonin, second phase is mediated by bradykinine between 1.5 to 2.5 h and finally, third phase, the mediator of which is possibly to prostaglandin occurring between 2.5 to 6 h after the Carrageenan injection having maximum vascular response. It is well established that prostaglandin, by virtue of their activity as modulators of inflammatory responses, have a major role in inflammatory mechanism [17].

The inhibition of edema by the extracts of C. gigantea and T. procumbens starting from first hour and during all phases of inflammation suggested that it probably

Table 1: Effect of Ibuprofen, *Calotropis gigantea* and *Tridax procumbens* on carrageenan induced rat pedal edema.

Drug	Treatment (mg/Kg)	Mean increase in Paw edema volume (ml) and Inhibition (%) at		
		1 hour	3 hours	5 hours
Control	-	0.40±0.05 -	0.65±0.03 -	0.68±0.04 -
Ibuprofen	25	0.33±0.04^{ns} (17.50)	0.55±0.02^{ns} (15.38)	0.59±0.02^{ns} (13.23)
	50	0.30±0.03* (25.00)	0.41±0.03** (36.93)	0.53±0.04* (22.05)
	100	0.22±0.02** (45.00)	0.27±0.02** (58.46)	0.23±0.05** (66.17)
	150	0.15±0.03** (62.50)	0.18±0.04** (72.30)	0.17±0.02** (72.30)
C.gigantea + Ibuprofen	400	0.20±0.04** (50.00)	0.31±0.03** (52.30)	0.29±0.03** (57.35)
	400+25	0.17±0.02** (57.50)	0.24±0.04** (63.07)	0.23±0.03** (66.17)
	400+50	0.16±0.03** (60.00)	0.22±0.05** (66.15)	0.18±0.04** (73.52)
	400+100	0.15±0.04** (62.50)	0.17±0.02** (73.84)	0.13±0.03** (80.88)
	400+150	0.13±0.03** (67.50)	0.15±0.03** (76.92)	0.12±0.02** (82.35)
T.procambens + Ibuprofen	300	0.18±0.02** (55.00)	0.26±0.02** (60.00)	0.33±0.03** (51.47)
	300+25	0.16±0.03** (60.25)	0.22±0.03** (66.15)	0.28±0.02** (58.82)
	300+50	0.15±0.03** (62.50)	0.20±0.02** (69.23)	0.22±0.04** (67.64)
	300+100	0.12±0.04** (70.00)	0.13±0.04** (80.00)	0.19±0.02** (72.05)
	300+150	0.10±0.02** (75.00)	0.12±0.02** (81.53)	0.16±0.04** (76.47)

Test drug were orally administered 1 hour before carrageenan injection. The control group received saline only. Values represent the mean ± S.E.M. (N=6). Significantly different from the control group represents in * ($p<0.05$) and ** ($p<0.01$).

inhibited different aspects and chemical mediators of inflammation. The present study showed that supraadditive anti-inflammatory activity of C. gigantea and T. procumbens may be due to the potentiation of its inhibitory effect on the synthesis and release of various inflammatory mediators.

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