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Anti-Inflammatory Activity of *Calotropis gigantea* and *Tridax procumbens* on Carrageenin-Induced Paw Edema in Rats

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Abstract

The anti-inflammatory activities of extract of *Calotropis gigantea* R.Br. and *Tridax procumbens* Linn., were assessed on carrageenin-induced paw edema along with standard drug, Ibuprofen. The Ibuprofen significantly reduced paw edema at the dose of 200mg/Kg bw orally. The oral administration equi-effective dose (ED_{50}) of *C. gigantea* (600mg/Kg bw) and *T. procumbens* (400 mg/Kg bw) individually revealed about 20-35% more activity than the one rendered by administration of 50mg/Kg bw of Ibuprofen. The effect of *C. gigantea* and *T. procumbens* along with various dose regimen of Ibuprofen showed greater anti-inflammatory activities than the Ibuprofen alone.

Keywords : Ibuprofen, *Calotropis gigantea*, *Tridax procumbens* and Anti-inflammatory.

Introduction

Inflammation is a common reaction of the body to be insult cause by various biological and non-biological factors present in the environment. In our country there are a large number of people suffering of deprivation of even essential need, and the ‘urge to survive’ has prompt them to explore naturally available resources for therapeutic effects with respect to common ailments including inflammation. This has allowed to development of alternative traditional method of therapy. *Calotropis gigantea* R.Br. (Asclepiadaceae) and *T. procumbens* Linn. (Compositae), known as Arka and Jayanti in ayurveda, have been widely documented in the ayurvedic and traditional medical literature for various

therapeutics applications. C. *gigantea* has been used as a violent purgative, gastrointestinal irritant, inducing abortion and treatment of earache, toothache, headache, anxiety, sprain and stiff joints to cure pain[1-9]. It has been also reported as analgesic activity, anti-inflammatory and pregnancy interceptive activity[2, 10-12]. Similarly, *T. procumbens* has also been reported to be useful in various diseases like inflammation, hepatic disorder, wounds, microbial infections, reduce immunity and free radical generated diseases like arthrosclerasis, various neurological disorder[13-22]. Both of these plants are widely distributed in the planes of Asia and Africa and grow profusely in the wild[1,2].

Ibuprofen is an NSAID, which is believed to work through inhibition of cyclooxygenase (COX-1 and COX-2). It's analgesic, antipyretic and anti-inflammatory activities are achieved principally through COX-2 inhibition[23]. The toxic effects of an Ibuprofen are unlikely at doses below 100 mg/kg but can be severe above 400 mg/kg[24], however, large doses do not indicate that the clinical course is likely to be lethal[25].

Material and methods:

Materials:

Calotropis gigantea R.Br. and *Tridax procumbens* Linn. 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 stored. Carrageenin and Gum acacia were purchased from Sigma Biochemicals, Ibuprofen from Cipla and Ethanol purchases from E-Merk.

Extraction:

T. procumbens was extracted by simple maceration process. 300g of fresh leaves were ground with the help of mortar and pestle. Juice obtained was filtered through muslin cloth. The filtrate was evaporated by rotary evaporator (cryochiller). Similarly, 300g of shed-dried leaves of *C. gigantea* were extracted 500ml of 60% ethyl alcohol for 12 hour in using Soxhlet apparatus. The extraction was filtrated and dried by rotary evaporator.

Animals:

The study used male and female albino rats weighing 130-170g. They were kept in polypropylene cages in centrally air conditioned room at an ambient temperature of 25±1 ^{0}C and 12 h light and dark cycle. All animals were fed standard animal feed and tap water *ad libitum* and left to acclimatize at least for one week before beginning the experiment. All experiments were carried out in accordance with the guideline of the CPCSEA.

Induction of inflammation:

The test was used to determine the anti-inflammatory action of the extract by the method of Winter *et al.*,[26]. Paw edema was induced by an intradermal injection of carrageenin (5% in gum acacia). Carrageenin induced rat hind paw edema has been widely used for the discovery and evaluation of anti-inflammatory drugs; since the relative potency estimate obtained from most drugs tend to reflect clinical experience[26]. This suitable test also has frequently been used to access the anti-edematous effect of natural products[27]. The local injection of carrageenan induced inflammatory process in the rat involves three phases by several mediators released in ordinate sequence[28]. 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 carrageenin injection. The third phase appears to be the most interesting phase compared with the two earlier phases due to the maximum vascular response as determined by leucocytes migration to inflamed area[29]. It is well established that prostaglandin, by virtue of their activity, as modulators of inflammatory responses, have a major role in inflammatory mechanism.

Anti-inflammatory activity:

The edema volume was determined using a Plethysmometer prior to and first, third and fifth hours after carrageenin injection. The drug Ibuprofen and extract were diluted in saline and in distilled water respectively. The animal were distributed in different groups, each group were five animals. The test drugs were given orally one hour prior to carrageenin injection and control group received vehicle only. In first phase, only 25, 50, 100, 150 and 200mg/Kg bw of Ibuprofen were administrated to different group. In second phase 600mg/Kg (ED_{50}) of *C. gigantea* together with different dose regimen (as given above) of Ibuprofen administrated. Similarly, in third phase 400mg/Kg (ED_{50}) bw of *T. procumbens* along with the above dose regimen of Ibuprofen were used.

Results and Discussion:

The anti-inflammatory activity of orally administered Ibuprofen, Ibuprofen along with C. *gigantea* and *T. Procumbens* on carrageenin-induced rat hind paw edema is shown in figure 1a, b, c and d. Results were expressed as mean ± standard error of mean (SEM). One-way ANOVA test was applied to evaluate the significant *p* values and ≥ 0.05 was considered as significant. The 50% effective dose (ED_{50}), calculated as the dose required to 50% inhibition of the carrageenin-induced edema formation and it was ≈600mg/Kg bw of *C. gigantea* and ≈400mg/Kg bw of *T. procumbens.*

The standard drug Ibuprofen presented a dose-dependent anti-inflammatory activity after carrageenin injection. The Ibuprofen treatment dose of 200mg/Kg bw reduced the edema to 87.5% in

first, 64.71% in third and 50% in fifth hours respectively. While, 150 mg/Kg bw dose reduced it to 68.75% in first, 58.82% in third and 43.75% in fifth hours respectively (fig1a). Hence, the Ibuprofen seems to be sensitive to cyclooxygenase inhibitors. Cyclooxygenase has been employed to evaluate the effect of NSAID, which primarily inhibits the cyclooxygenase involved in prostaglandin synthesis. It has also been demonstrated that the suppression of carrageenin-induced hind paw edema after third hour correlates reasonably with therapeutic dose of clinically effective anti-inflammatory agents[28].

The extract of *C. gigantea* at 600mg/ Kg bw inhibits the edema upto 50% in first, 41.80% in third and 25% in fifth hours. While, *T. procumbens* at dose of 400mg/Kg bw inhibits upto 52.50% in first, 44.12% in third and 25% in fifth hours. The fact that the extract inhibited edema during all phases of inflammation suggested that it probably inhibited different aspects and chemical mediators of inflammation. The paw edema induced by carrageenin has been extensively studied in the assessment of the anti-inflammatory action of steroidal and non-steroidal drugs involving several chemical mediators such as histamine, serotonin, bradykinin and prostaglandins[30]. The administration of 600mg/ Kg bw of *C. gigantea* along with different doses of Ibuprofen

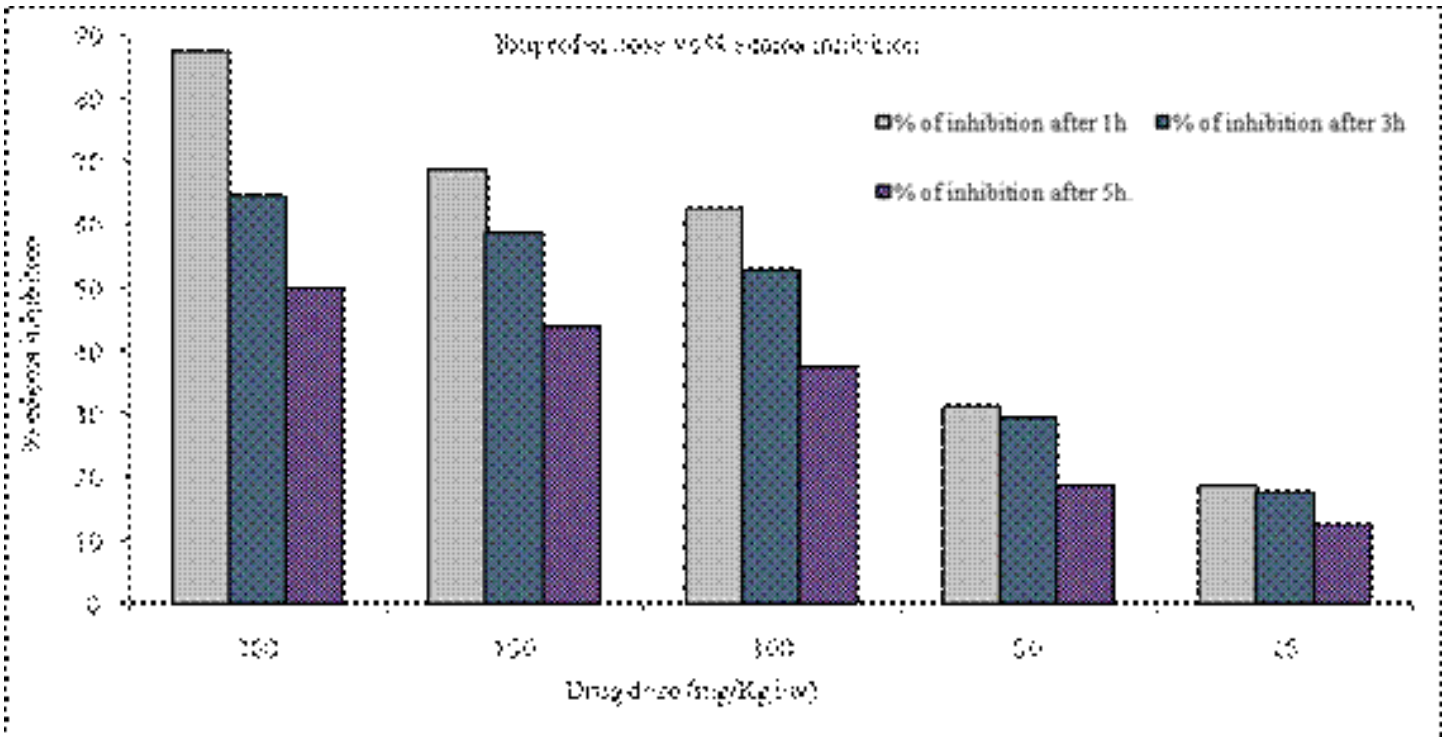

Figure: 1a, Inhibition of paw edema by *Ibuprofen* drug after 1st, 2nd and 3rd hours.

significantly reduced paw edema with respect to standard drug (Fig 1a,b, c,d). The dose of 100mg/Kg bw of Ibuprofen inhibited 62.50%, 52.94% and 37.50%, while, in combination with *C. gigantea* inhibited 75%, 70.59% and 62.5% in first, third and fifth hour respectively. The dose of 50mg/Kg bw of Ibuprofen inhibited 31.25%, 29.41% and 18.75% and in combination with *C. gigantea* the inhibition

increased from 56.25%, 47.06% and 43.75% in first, third and fifth hour respectively. Similarly, 400mg/Kg of *T. procumbens* in combination with dose of 100mg/Kg bw dose of Ibuprofen reduced the edema 75.0%, 70.59% and 62.5%, while with 50mg/Kg bw dose of Ibuprofen reduced 62.50%, 58.82% and 50.0% in first, third and fifth hour respectively.

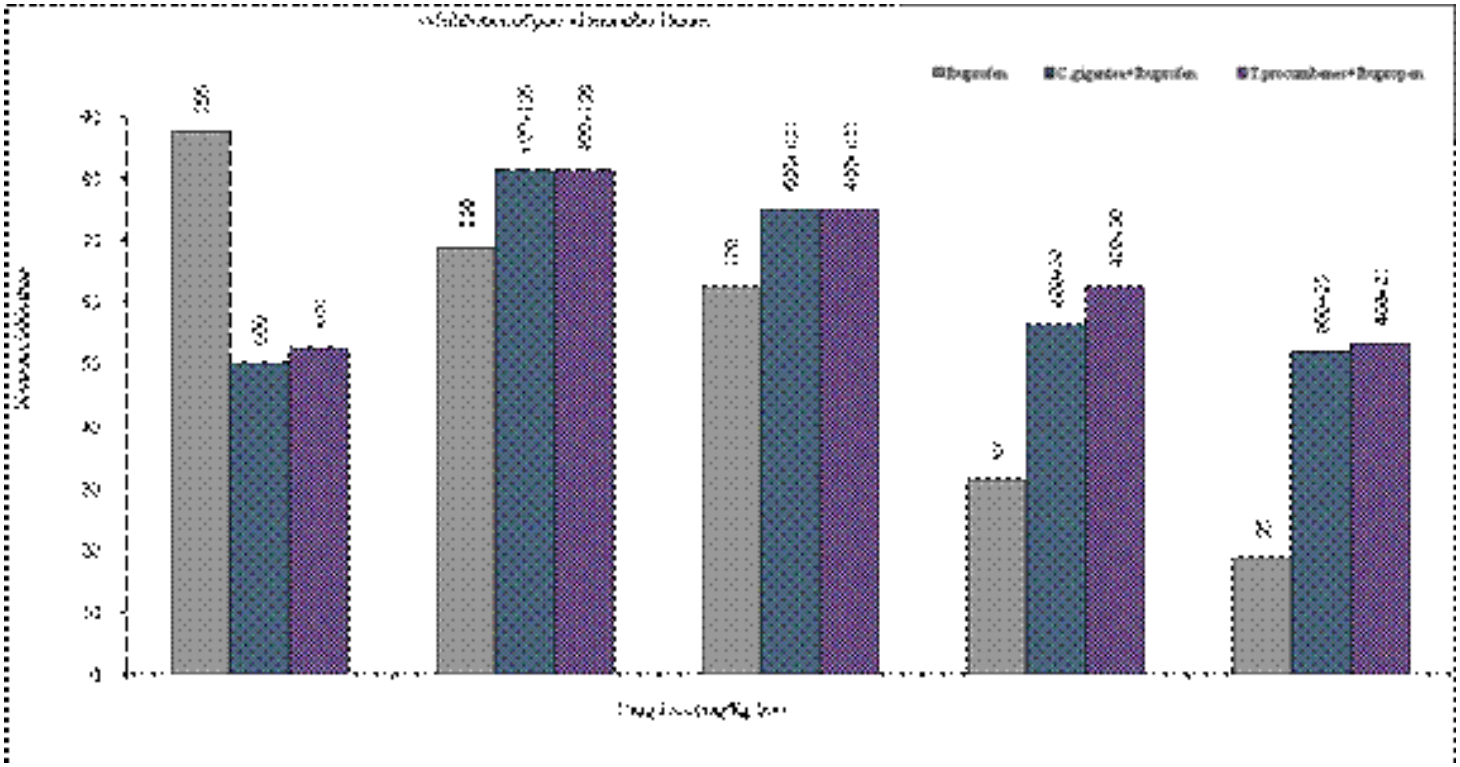

Figure: 1b, Inhibition of paw edema by *Calotropis gigantean* (600mg/Kg) and *Tridax procumbens* (400mg/Kg) together with Ibuprofen in 1st hour.

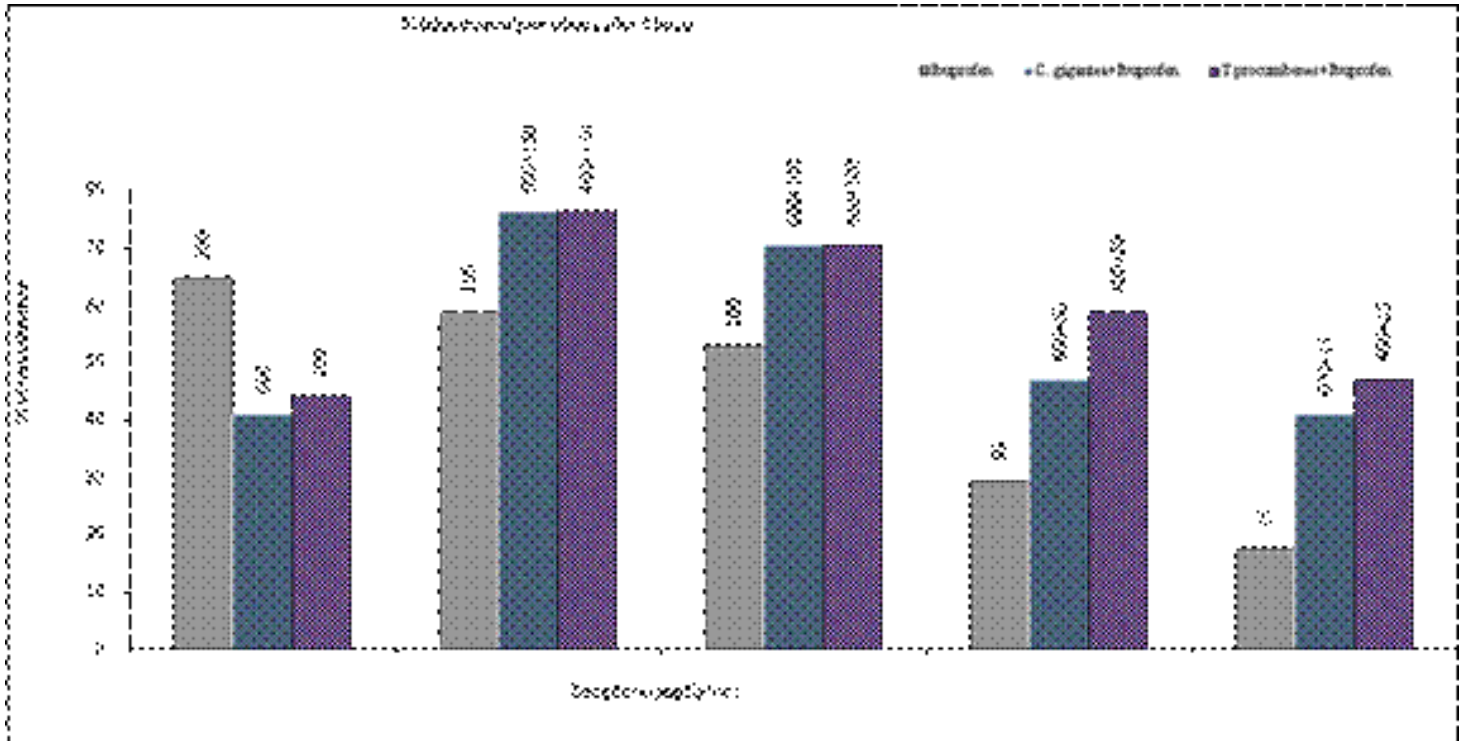

Figure: 1c, Inhibition of paw edema by *Calotropis gigantean* (600mg/Kg) and *Tridax procumbens* (400mg/Kg) together with Ibuprofen in 3rd hour.

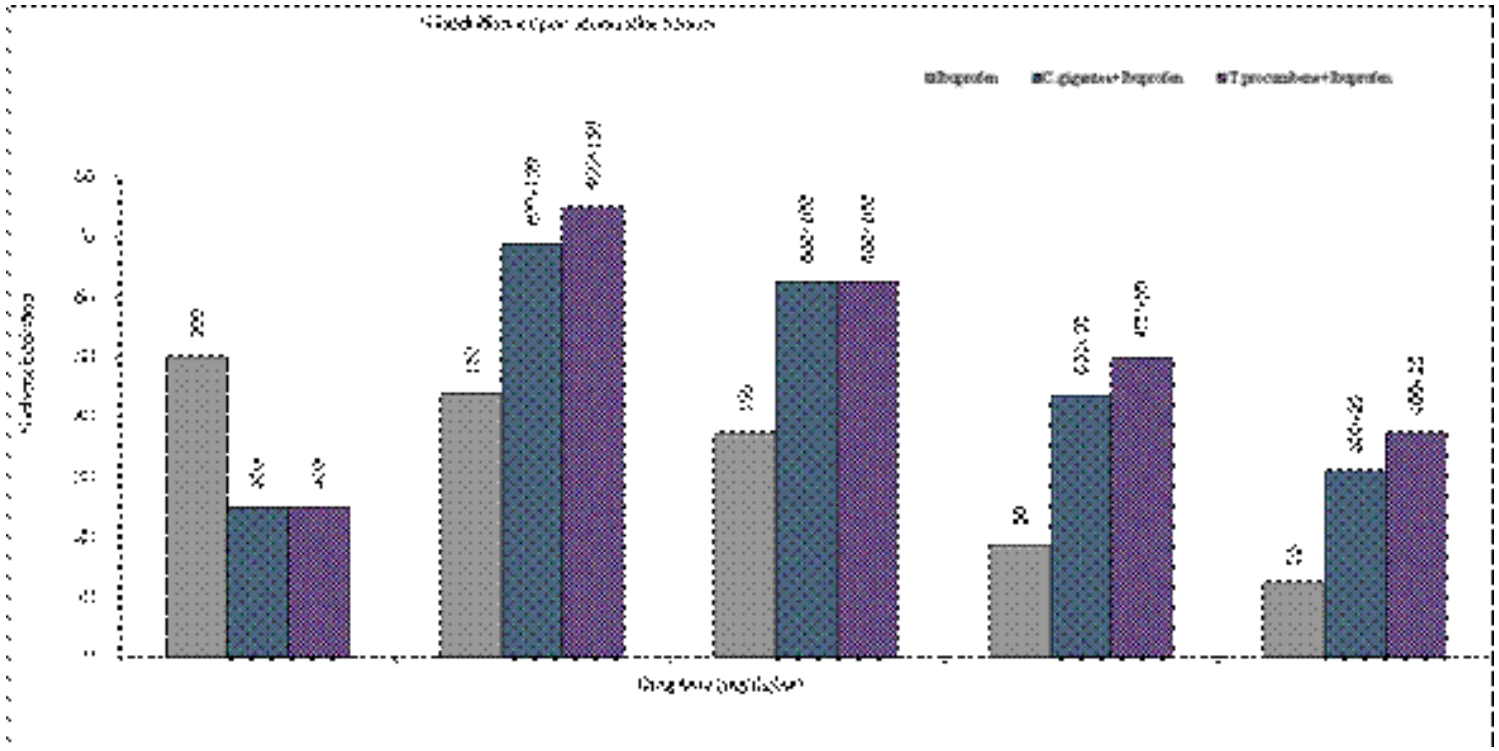

Figure: 1d, Inhibition of paw edema by *Calotropis gigantean* (600mg/Kg) and *Tridax procumbens* (400mg/Kg) together with Ibuprofen in 5th hour.

The comparative analysis shown that, *T. procumbens* have more anti-inflammatory activity than *C.*

gigantea at individual and together with Ibuprofen. It is therefore, suggested that the extract probably possesses anti-inflammatory activity and may inhibit the release or synthesis of various inflammatory mediators.

Conclusion

The standard drug Ibuprofen presented a dose-dependent anti-inflammatory activity at all the dose regimens selected for the treatment after the carrageenin injection. The Ibuprofen also seems to be sensitive to clycooxygenase inhibitors. The aqueous extract of *T. procumbens* and methanolic extract of *C. gigantea* inhibited edema during all phases of inflammation. The extracts also increased the inhibition of edema if treated with the standard drug Ibuprofen. The outcome of the experiment is on the expected, it probably inhibited the different aspects and chemical mediators of inflammation and has been used to evaluate the effect of non-steroidal anti-inflammatory agents.

Acknowledgment

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