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ANTI-INFLAMMATORY, ANALGESIC ACTIVITY AND ACUTE TOXICITY STUDY OF *CORDIA GHARAF* FRUIT EXTRACT

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ABSTRACT

The present research work deals with the analgesic and anti-inflammatory activity of *Cordia gharaf* fruit extract. The study involves determination of analgesic and anti-inflammatory activity by using acetic acid induced writhing model, tail flick method and Hot plate method respectively. The anti-inflammatory potential of *Cordia gharaf* was also evaluated with the help of Carrageenan induced rat paw edema model. From results of this study we came to conclusion that CGAFE has potential anti-inflammatory activity in Carrageenan induced rat paw model and support the claimed use of this plant in traditional system of medicine. *C.gharaf* also has analgesic activity which is both centrally and peripherally mediated. Further isolation, characterization and purification of active constituents and further experimentation would necessary to elucidate the exact mechanism of action of *C.gharaf* fruits.

INTRODUCTION

Cordia gharaf (Forsk.) Ehrenbq & Asch.(Boraginaceae)(Syn.*C.Rothii*.) is commonly called as Gondi, Gundi, Naruvili.In Ayurvedic the plant is considered as source of Laghushleshmataka. It is a small deciduous tree. Leaves are simple, hairy viscous, oblanceolate, rounded at apex; flowers white small in axillary and terminal cymes. Fruits are obovoid drupe, orange or scarlet when ripe. Flowering –fruiting February- June. It is widely occurs in major parts of India. In India it is mostly found in Maharashtra, Gujarat, Punjab, Sind, Rajputna, and Rajasthan. The bark of the plant having the properties astringent, bitter, demulcent and diuretic etc. While leaves possesses astringent, nutritive, aphrodisiac, cooling, demulcent, diuretic and used in the treatment of sore throat, diarrhoea, stomatitis, general debility, etc. Paste of leaves is applied on cuts, wounds and boils.

Fruits are astringent, antiseptic,cooling, diuretic, tonic, aphrodisiac, nutritive, demulcent and useful in leucorrhoea, spermatorrhoea, diabetes, thirst, anorexia, strangury, burning micturation, general debility etc. Root has abortifacient and anti-inflammatory activities. While whole plant has Antidiabetic and antileprotic activities. The decoction of the bark possesses astringent properties and is used as gargle. Ripe fruits contain sugars, mucilage, etc.Bark contains tannins. Leaves contain tannin, mucilage, etc (Kirtikar and Basu, Vaidya BG, 1965; Chunekar K & Pande G,1988; Watt JM & Breyer-Branwjick MG,1962; Thakar JJ, 1952). The reported phytoconstituents from plants are β -Sitosterol (from stem and leaf), hydrocarbon and n-hexaconasol (from leaf), D-galactose, D-fructose, D-xylose, L-rhamnose and D-galactouronic acid (from mucillage) (Vishwanathan N et al.1976).The reported work on the Pharmacognosy and biological activity (Cardiotonic activity) of alcoholic extract of *Cordia rothii* Roem.& Schultz bark.

C. gharaf is used traditionally in Ayurveda, and also various uses has been described but there was no scientific study about its pharmacological activities. Therefore, we decided the present study was designed to evaluate the acute toxicity study, preliminary Phytochemical screening and Anti-inflammatory and Analgesic activity of *Cordia gharaf* (Forsk.) Ehrenbq & Asch Fruit aqueous extract (CGFAE) in rats and mice using classical pharmacological methods like Carrageenan-induced rat paw edema, acetic acid writhing , hot plate and tail flick tests.

MATERIALS AND METHOD

Plant material:

Matured fruits of *Cordia gharaf* (Forsk.) Ehrenbq & Asch.(Boraginaceae) were collected from Solapur district during April 2009 of Maharashtra, India. The plant was authenticated by Mr. P.G.Diwakar, Deputy Director Botanical Survey of India, Pune. The voucher specimen was deposited in department Botanical Survey of India, Pune.

Preparation of aqueous extract of CG:

The fresh ripe fruits are subjected to maceration with distilled water at room temperature for 7 days with occasional shaking. The extract collected was filtered, evaporated (yield 12.5%w/w), and stored in vacuum desiccator. The crude extract was diluted with distilled water just before administration to animals.

Phytochemical screening:

The qualitative chemical tests of CGFAE were carried out using standard procedure (C.K.Kokate & Khandelwal), for the determination of presence of Carbohydrate, sterols, Coumarins, Flavonoids and Alkaloids.

Acute toxicity studies:

Acute toxicity study were tested in Swiss albino mice of either sex weighing 20-25 g according to Organization for Economic Co-operation and Development(OECD) guidelines No.425 (Ecobichon,1997).The animals received a *Cordia gharaf* (Forsk.) Ehrenbq & Asch. extract (3000mg/kg) and vehicle (water) by intragastric route and mortality rate and behavioral changes were observed for 48 h.

Animals:

Experiment were carried on Wistar albino rats(180-200g) of either sex/Swiss albino mice mice(20-25g) (n=6 per group) .Animals were kept in colony cages at 25±2°C,relative humidity 50-55% maintained under 12h light and dark cycle. The animals were fed with standard animal feed and water was applied ad libitum.Each animal was used only once. The animals were kept on fasting overnight prior to the experimentation and animals care and handling procedures were followed in accordance with International association for the study of pain guidelines for the use of animals in pain research (Zimmermann,1983) .

Anti-inflammatory activity:**Rat paws Carrageenan- induced edema**

The anti-inflammatory activity of CGAFE was evaluated in acute inflammation method described earlier (winter et al.1962). The extract was orally administered with the different doses of extract (200 and 250 mg/kg, p.o.). The paw volume of the rats were measured plethysmometrically immediately after administration of 0.1ml of 1% Carrageenan(Merck, Mumbai) solution (zero time) and 1, 2 and 3h after stimulus. The Carrageenan solution was suspended in distilled water and was injected into the sub plantar region of the right hind paw of each rat. Control rats received 2% gum acacia (10 ml/kg) suspension, while Indomethacin (10 mg/kg) was used as standard drug. Increase in linear paw circumference was taken as a measure of edema. The percentage of inhibition was calculated by using the formula,

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

Where, V_c = Average paw volume of control and

V_t = Average Paw volume of treated rats.

Analgesic activity:**Acetic acid -writhing in mice:**

The test was carried according to method described earlier (Collier et al, 1968) applied to mice of both sexes. In the writhing test, 0.8% (v/v) acetic acid was injected intraperitoneally (10ml/kg body weight) and assessed by the acetic acid abdominal constriction test (writhing test)—a chemical visceral pain model. Swiss albino's mice were selected 1 day prior to each test and were divided into groups of six mice each. one group. The control, positive control and experimental groups were given the vehicle, control drugs (Indomethacin 10mg/kg) or the extract (CGFAE (200 and 250mg/kg)), one hour prior to the administration of acetic acid .The mice were observed for the number of abdominal constrictions and stretching, counted over a period of 30 min. Antinociceptive activity was expressed as inhibition percent of the usual number of writhes observed in control animals. The percentages of inhibition were calculated according to the following formula:

$$\% \text{ inhibition} = \frac{((\text{number of writhes}) \text{ control} - (\text{number of writhes}) \text{ treated group}) \times 100}{(\text{number of writhes}) \text{ control.}}$$

Tail flick Method

The tail-flick test was done as described by D'Amour and Smith (1941), with modification. Male and female mice were treated with 200 and 250 mg/kg of CGFAE and 1mg/kg

Diclofenac Sodium as reference standard. The reaction time of these mice measured at 0 h, 30min, 60min, 120min and 180 min after treatment. The temperature of water bath 55°C and the time taken to flick the tail was recorded. The cut off time of 25 s was fixed to avoid the tissue damage.

Hot plate method:

This method was used to determine the analgesic activity according to method described by Eddy and Leimback (1953).The mice were treated with different doses of CGFAE (200 and 250mg/kg,p.o.).After 1h the animals were placed on hot plate temperature fixed at $55\pm 0.5^{\circ}\text{C}$.Reaction time(elevation of paws, paw licking etc) was measured at 0,30,60,120,and 180 min. Tramadol 5 mg/kg was used as reference drug .The maximum duration for estimation was 30 s.

Statistical analysis:

The statistical analysis of all the results were expressed as mean \pm S.E.M. and analyzed by one way analysis of variance (ANOVA) followed by Dunnett's t -test. $P<0.05$ was considered as statistically significant.

Drugs:

Carrageenan was purchased from Merck Pvt.Ltd, Indomethacin, Tramadol and Diclofenac sodium was purchased from Local market. The solvents and chemicals of analytical grade were used.

Results:

Phytochemical screening:

Phytochemical screening revealed the presence of Carbohydrates, Gums, Mucilage, amino acid, Proteins, flavonoids, steroids, tannins and phenolic compounds.

Acute toxicity studies:

The CGFAE was found to be non- toxic even up to the highest dose (3000 mg/kg b.w.) tested in Swiss albino mice for the period 48h and no morbidity and/or mortality were recorded. The observation of the entire dose does not show any toxic effect on mice.

Anti-inflammatory activity:

The aqueous extract of *Cordia gharaf* (200 and 250 mg/kg p.o.) showed dose dependent significant anti-inflammatory activity at all concentrations tested in Carrageenan induced rat paw edema model .The anti-inflammatory activity of Indomethacin was significantly higher than that of CGFAE at 3h.Percentage of inhibition on carragennan –induced rat paw edema is

presented in table no.1.The CGFAE at a dose 250 mg/kg shows significant antiinflammatory activity 44.23 % at 3h which is comparable to standard drug Indomethacin (10 mg/kg).

Table 1 Effect of *Cordia gharaf* (Forsk.) Ehrenbq & Asch Fruit aq. extract (CGFAE) on carrageenin-induced rat paw edema (n=6)

Treatment	Oral Dose(mg/kg)	Mean increase in paw volume in (ml)		Percentage inhibition
		0 hr	3 hr	
Control	----	0.2750±0.01118	0.4333±0.02108	----
Indomethacin	10	0.2167**±0.01054	0.2250**±0.01708	48.07
CGFAE	200	0.2667±0.01054	0.2917**±0.008333	32.68
CGFAE	250	0.2250*±0.01118	0.2417**±0.01537	44.23

*p < 0.05 vs. control, **p < 0.01 vs. control, Values are mean ± S.E.M., n=6, Dunnett's multiple comparison test.

Analgesic activity:

Acetic acid -writhing in mice:

The effect of *CGFAE* on abdominal acetic acid (0.6%v/v) induced writhes in mice (n=6) is summarised in Table 2. The extract showed a significant activity in this animal model. The result showed dose dependent analgesic activity i.e. increase in the dose of plant extract shown a greater inhibition. Indomethacin at a dose 10 mg/kg b.w.p.o. inhibited the abdominal constriction by 50.10 % when compared with control value (**p < 0.01). This test widely used for screening of analgesic activity (Alexandre-Moreira et.al., 1999).

Table 2 Analgesic effect of *Cordia gharaf* (Forsk.) Ehrenbq & Asch aq. Fruit extract (CGFAE) on abdominal acetic acid(0.6%) induced writhes in mice (n=6)

Treatment	Oral Dose(mg/kg)	Mean no. of writhes in 30 min	Percentage Inhibition
Control	----	83.50±1.204	----
Indomethacin	10	41.67**±3.148	50.10
CGFAE	200	64.17**±1.352	30.12
CGFAE	250	50.50**±1.607	39.52

**p < 0.01 vs. control.

Values are mean ± S.E.M., n=6, Dunnett's multiple comparison test.

Tail flick Method:

The treatment of *Cordia gharaf* (Forsk.) Ehrenbq & Asch aq. Fruit extract (CGFAE) on tail-flick test in mice (n=6) is summarised in Table 3. Tramadol 5 mg/kg showed significant increase in reaction time at 60 min..after treatment. However the treatment with CGFAE showed a significant and dose dependent increase in reaction time in mice at 60 min..The standard drug Tramadol (5mg/kg) increase reaction time 44.27% at 60 min. when compared with control (*p < 0.05) while plant extracts 250 mg/kg increase in reaction time to the extent of 68.69% at 60 min. when compared with control (**p < 0.01).The plant extract at 250 mg/kg test dose showed better significant analgesic activity than standard drug, Tramadol.

Table 4 Analgesic effect of *Cordia gharaf* (Forsk.) Ehrenbq & Asch aq. Fruit extract (CGFAE) on tail-flick test in mice (n=6)

Treatment	Oral Dose(mg/kg)	Reaction time in sec	Percentage Inhibition
Control	----	2.842±0.2456	----
Tramadol	5	5.100*±0.8001	44.27
CGFAE	200	3.792±0.5595	----
CGFAE	250	9.077**±0.5776	68.69

*p < 0.05 vs. control, **p < 0.01 vs. control.

Values are mean ± S.E.M., n=6, Dunnett's multiple comparison test.

Hot plate method:

The CGFAE significantly increased reaction time in hot plate method in dose dependent manner presented in Table 4. Reaction time was noted at 0, 30, and 60 min after the administration of the different doses of plant extract (200 and 250 mg/kg) vehicle, and standard. The plant extract (200 and 250 mg/kg) significantly increase reaction time to the extent of 62.60%, 71.57% at 60 min..respectively while standard drug Diclofenac sodium (10 mg/kg) increase the reaction time to the extent of 62.69% at 60 min when compared with control (**p < 0.01).In this model ,higher dose of the extract 250 mg/kg showed better analgesic activity than standard drug.

Table 3 Analgesic effect of *Cordia gharaf* (Forsk.) Ehrenbq & Asch aq. Fruit extract (CGFAE) on the latency of paw withdrawal in hot plate test in mice (n=6).

Treatment	Oral Dose(mg/kg)	Reaction time in sec	Percentage Inhibition
Control	----	3.295±0.006708	----
Diclofenac sodium	10	8.832**±0.3423	62.69
CGFAE	200	8.810**±0.1523	62.60
CGFAE	250	11.59**±0.5211	71.57

**p < 0.01 vs. control.

Values are mean ± S.E.M., n=6, Dunnett's multiple comparison test.

DISCUSSIONS

This is the first time study demonstrated for testing in vivo acute toxicity, the anti-inflammatory and analgesic activities from aqueous extract of *Cordia gharaf* (Forsk.) Ehrenbq & Asch..The purpose of this work was to evaluate the scientific basis for its traditional claim uses of *Cordia gharaf* (Forsk.) Ehrenbq & Asch.Based on LD₅₀ calculated,the acute administration doses of CGAFE (200 and 250 mg/kg) are eastimated for analgesic and antiinflammatory activity in various animal models.

The present data clearly showed that CGAFE significantly inhibited carageenin-induced paw oedema in rats.It produced a dose- dependant inhibition of carageenin oedema and the effect of 250 mg/kg dose shows comparable activity with that of Indomethacin (10mg/kg). Carageenin-induced oedema falls in the category of acute inflamation, which involves the synthesis or release of inflammatory mediators at the injured site which further cause pain and fever(Salmon M 1991, Damas J 1992, Penn GB 1963).

Analgesic activity was evaluated using thermal (hot plate test and tail flick test) and chemical stimuli(acetic acid induced writhing test).The analgesic activity shown in the hot plate and tail flick method shows that the activity is supraspinally mediated (Hough et al.,1999).From the above results it clear that *C.gharaf* is centrally acting.

Acetic acid induced writhing response in mice was used to examine central and peripheral analgesic action of *C.gharaf* .It was found that C.gharaf significantly inhibited the acetic induced writhing response in dose dependant manner.The abdominal constriction is related to the sensitisation of nociceptive receptors to prostaglandins.Analgesic activity of *c.gharaf* can be brought about by the PG synthesis inhibition or action of prostaglandins.

Preliminary phytochemical screening results suggested that the anti-inflammatory and analgesic activity of CGAFE may be due to the presence of phytochemicals such as Carbohydrates, Gums, Mucilage, amino acid, Proteins, flavonoids, steroids, tannins and phenolic compounds. Several Flavonoids isolated from medicinal plants have been reported to possess significant anti-inflammatory and analgesic effects (Gulnur et al., 2004; Bujbal et al., 2008).

CONCLUSIONS:

From results of this study we came to conclusion that CGAFE has potential anti-inflammatory activity in Carrageenan induced rat paw model and support the claimed use of this plant in traditional system of medicine. *C.gharaf* also has analgesic activity which is both centrally and peripherally mediated. Further isolation, characterization and purification of active constituents and further experimentation would necessary to elucidate the exact mechanism of action of *C.gharaf* fruits.

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