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"EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF CLERODENDRUM INFORTUNATUM LINN- IN RATS"

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ABSTRACT

Clerodendrum infortunatum Linn. (Verbenaceae) is an important and widely used medicinal plant. Though variously used in Ayurveda, Unani and Homeopathy system of medicine in case of ailments like diarrhoea, skin disorders, venereal and scrofulous complaints, wounds, post-natal complications, as antihelminthic, external applications on tumours, etc., the plant needs thorough investigation for its specific medicinal activity. This study evaluates the acute anti-inflammatory effect of the ethanolic extract of the leaves of Clerodendrum infortunatum Linn.(EECI) in experimental animals. Acute toxicity test was done following the OECD guidelines. Following the Phytochemical analysis of the plant extract for specific plant constituents, EECI (100mg/kg, 200mg/kg, and 400mg/kg body weight b.w. p.o) was evaluated for acute anti-inflammatory activity against the carrageenan induced rat paw edema and the effects compared with reference drug aspirin (300mg/kg b.w). In acute inflammation, EECI produced significant reduction ($p<0.01$) at 5th hr in all the doses.This may be attributed to the inhibition of prostaglandin synthesis, phospholipase A2, and TNF alpha. Clerodendrum infortunatum Linn. as a commercial source of anti-inflammatory drug should be subjected to further research.

KEYWORDS: *Clerodendrum infortunatum, Anti-inflammatory; Carrageenan; Paw edema.*

INTRODUCTION

Inflammation is defined as the local response of living mammalian tissues to injury due to any agent. It is a body defense reaction in order to eliminate or limit the spread of injurious agent, followed by removal of the necrosed cells and tissues. The inflammatory reaction is characterized by blush, heat, tumor, pain and lost function.[1]

Depending upon the defense capacity of the host and duration of response, inflammation can be classified as acute and chronic.[2]

As presently available synthetic analgesic and anti-inflammatory drugs pose several health problems during their clinical use, search to develop new and more effective drugs with fewer side effects is necessary. The use of natural products is growing in the world especially in developing countries like India where over 75% of the population relies mainly on plants and plant extracts for healthcare.[3]

Clerodendrum infortunatum Linn. (Verbenaceae) is an important and widely used medicinal plant, reported to contain active bitter substance like clerodin, has been widely used as tonic and anthelmintic agent in the country sides of North India. Though, variously used in Ayurveda, Unani system of medicine and Homeopathy in case of ailments like diarrhoea, skin disorders, venereal and scrofulous complaints, wounds, post-natal complications, as external applications on tumours, etc., the plant needs thorough investigation for its specific medicinal activity. Traditionally, the plant is used as an antipyretic and antihelmentic. Leaves of the plant are prescribed for tumour, certain skin diseases and scorpion sting. The antioxidant, antimicrobial, anti-malaria, anthelmintic and analgesic activities of the plant have further created an upsurge in investigations on the plant.[4, 5]

The current study was therefore undertaken to evaluate the acute anti-inflammatory activity of ethanolic extract of Clerodendrum Infortunatum Linn in experimental animals.

MATERIALS AND METHODS

Materials used include

1. Carrageenan

Kappa type of cairngeenan was used to induce paw edema. Carrageenan (kappa) was obtained from TITAN BIOTECH LTD. MUMBAI, INDIA. 0.05ml of 1 % w/v carrageenan

was used to induce paw edema. 1 % w/v Carrageenan was prepared by dissolving 1 gm of carrageenan in 100 ml of normal saline.

2. Normal saline

It was used to prepare 1 % w/v carrageenan solution and also used as a drug in -control animals.

3. Leaves of Clerodendrum infortunatum Linn.

Fresh leaves of C. infortunatum Linn. were collected from AMRUTHA VANA CENTRE FOR HERBAL GARDENS AND LANDSCAPING SERVICES, GOVT. OF KARNATAKA, BANGALORE, INDIA.

4. Animals

All the animals were obtained from the ANIMAL HOUSE, DEPARTMENT OF PHARMACOLOGY, S N MEDICAL COLLEGE, BAGALKOT, KARNATAKA.

Wistar albino rats of either sex weighing 150-250g were selected for the experiment. Pregnant rats, animals with infection, animals with injuries, deformities were excluded from the study. 30 albino rats were used for the carrageenan induced paw edema test.

Swiss albino mice (15) of either sex weighing 25-30g were selected for the acute toxicity test. Pregnant animals, animals showing infections, injuries, and abnormalities were excluded from the study. All the animals were maintained at 12: 12 hr dark: light cycle, 25±2°C, and 35%-60% humidity. All the animals received standard laboratory diet (VRK NUTRITIONALS, PUNE) and water was provided ad libitum.

Equipments

1. Tuberculin syringe

Used to inject 1 % w/v carrageenan intradermally into the rat hind paw.

2. Plethysmograph

To measure the volume of the rat paw in the presence or absence of the test/ standard drug after inducing edema by l % w/v of carrageenan injection. It has U shaped glass tube with measuring scale on one side of the glass tube. Changes in the fluid level can be measured in the measuring scale.

3. Soxhlet apparatus

Consists of a Soxhlet extractor, used to obtain the Ethanolic extract of leaves of Clerodendrum infortunatum.

Methodology

1. Preparation of extract

The leaves of the Clerodendron infortunatum were dried under shade for a period of four weeks. The dried plant material was milled to a fine powder using the mechanical grinder. The powder plant material was extracted with absolute ethyl alcohol using Soxhlet extraction apparatus. Dried powder (300 g) was extracted in a Soxhlet extractor with ethanol for about 8-9 h at 45ºC. Extract was collected and dried using rotary flash evaporator at 40-45ºC and crude residue was collected. The solvent was completely removed under reduced pressure and semisolid mass was obtained. The yield was calculated as 30 g. The extract was stored in well closed glass container at 5ºC in refrigerator for further study.[5, 6, 7]

2. Acute oral toxicity study

It was done according to Organization for Economic Co-operation and Development (OECD) guidelines 425 (up and down procedure). All the five mice were administered 2000mg/kg of ethanolic extract of leaves of Clerodendrum infortunatum orally and observed continuously for a period of 14 days, every hourly for 24 hours, and every day for 14 days for its movements, grooming activity, exploring activity, writing reflex, eye movements, and convulsion etc.[8]

3. Phytochemical analysis

Qualitative Phytochemical Analysis of Plant Extracts: The leaf extracts were analyzed for flavonoids, alkaloids, glycosides, saponins, tannins, proteins and aminoacids, sterols and triterpenoids, carbohydrates, fixed oils, anthraquinone, steroids and resins.

1. Flavonoids: Alkaline reagent test: To the test solution, few drops of sodium hydroxide solution was to; formation of an intense yellow color, which turns to colorless on addition of few drops of dilute acid, indicates presence of Flavonoids.
2. Alkaloids: Tannic acid test: Alkaloids give buff color precipitate with 10% Tannic acid solution.
3. Glycosides: Keller killiani test: 0.4ml of glacial acetic acid containing a trace amount of ferric chloride was added to the extract and a small amount transferred to a small test tube.

Add carefully 0.5ml of concentrated sulphuric acid to the side of the test tube, blue color appears in the acetic acid layer.

4. Saponins: Froth Test: I ml solution of drug in water is placed in a semi-micro tube and shaken well and noted for a stable froth.

5. Tannins: Ferric chloride test: Test solution gives blue green color with ferric chloride.

6. Proteins and aminoacids: Millon's test: Test solution with 2ml of Millon's reagent (Mercuric nitrate in nitric acid containing traces of nitrous acid), white precipitate appears, which turns red upon gentle heating

7. Sterols and Triterpenoids: Libermann-Bachard test: Extract is treated with few drops of acetic anhydride, boiled and cooled. Conc. sulfuric acid is added from the sides of the test tube. Formation of a brown ring at the junction of two layers is seen. If the upper layer turns green indicates the presence of steroids and formation of deep red color indicates the presence of triterpenoids.

8. Carbohydrates: Benedict's test: Treat the test solution with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex). Boil on water bath. Reddish brown precipitate forms if reducing sugars are present.

10. Anthraquinones: Borntragers Test: About 0.5g of the extract was taken into a dry test tube and 5ml of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate was shaken with equal volume of l0% ammonia solution. A pink violet or red colour in the ammonical layer was observed for the presence of anthraquinone.

11. Resin: Five milliliter of distilled water was added to the extract and observed for turbidity.

12. Steroids: Two milliliter of acetic anhydride was added to 0.5g of extract and 2ml of H_2S0_4 was added along the sides of the test tube and the result was observed for red colour ring formation.[9, 10]

4. Antiinflammatory activity

Carrageenan induced paw edema: The test was carried out in healthy Wistar albino rats of either sex weighing 150-250 g. After 12 hr fasting 30 animals were randomly divided into 5 groups of 6 animals each. Group I received 0.5 ml of normal saline (control group), Group II received 300mg/kg of aspirin (standard group), Group III, IV and V received aqueous extract of leaves of C. infortunatum Linn. (Test groups). All the drugs were given orally. After l hr all the animals received 0.05ml of 1 % w/v carrageenan in normal saline solution intradermally in the left hind paw. Right paw served as the control for the same animal. The

paw edema volume was measured by using the plethysmograph by measuring fluid displacement at 1hr, 3hr, 5hr after carrageenan injection.[11]

Ethical approval for animal experiments has been obtained from "Ethical clearance committee" of the institution (S. Nijalingappa Medical College and Hanagal Sri Kumareshwar Hospital and Research Centre, Navanagar, Bagalkot-587102, Karnataka, India) **Statistical analysis:** All results are expressed as the mean ± SEM. The results were analyzed for statistical significance ($p < 0.05$, $p < 0.01$) by one-way (ANOVA) followed by Dunnett's test using computerized Graph Pad lnStat version 3.05. Graph pad software. U.S.A.

Figure.1: Clerodendrum infortunatum Linn

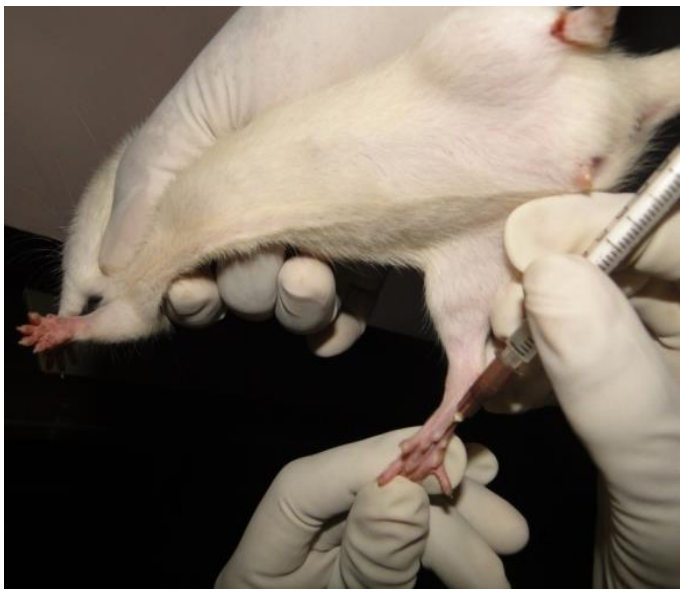


Figure 2: Rat paw injection with 1% w/v Carrageenan in normal saline

Figure 3: Rat showing paw oedema

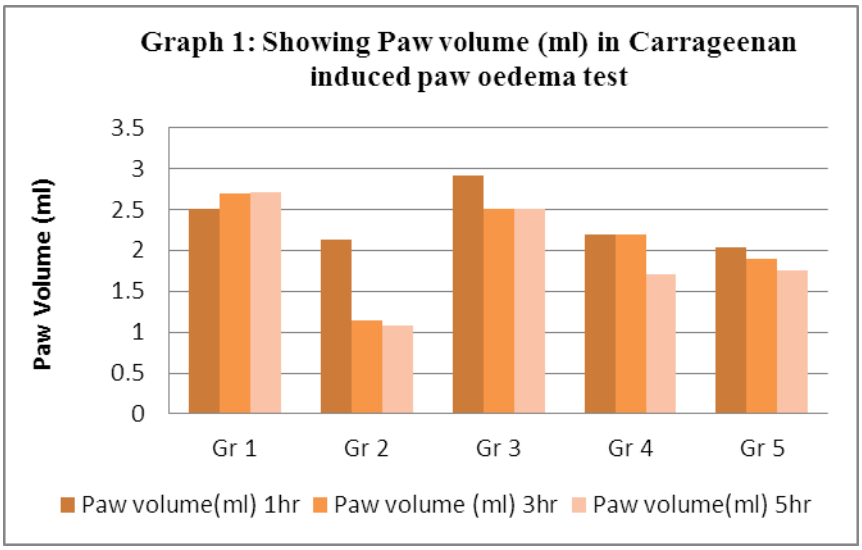
RESULTS

Carrageenan induced paw edema test for evaluating anti-inflammatory activity

Table 1: Paw volume (ml) in carrageenan induced paw edema test

Group	1hr M ± S	3 hr M ± S	5hr M ± S	Percentage of inhibition at 5 hr (%)
Control	2.5 ± 0.44	2.7 ± 0.41	2.7 ± 0.84	-
Standard (300 mg/kg)	2.13 ± 0.17	1.15 ± 0.19	1.08 ± 0.20	60.14
Test (100mg/kg)	2.91 ± 0.66	2.5 ± 0.4	2.5 ± 0.4	7.74
Test (200 mg/kg)	2.20 ± 0.24	2.20 ± 0.24	1.70 ± 0.27	37.2
Test (400 mg/kg)	2.08 ± 0.20	1.90 ± 0.37	1.75 ± 0.27	37.2
F	4.45	18.6	11.6	-
d*f*	4	4	4	-
P	0.007	0.0001	0.0001	-

M ± S: Mean ± Standard



DISCUSSION

Clerodendrum infortunatum is an important medicinal plant used in Indian folk medicine in the treatment of bronchitis, asthma, fever, diseases of the blood, inflammation, burning sensation and epilepsy. Pharmacological actions include Antimicrobial, antioxidant, analgesic, anticonvulsant and antipyretic activities.[12]

The extracts derived from leaves of C.infortunatum Linn. exhibited significant anti-inflammatory activity which was evaluated by using carrageenan induced paw edema.

Carrageenan-induced hind paw edema is the standard experimental model of acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover, the experimental model exhibits a high degree of reproducibility.[13,14]

Carrageenan-induced edema is a biphasic response. The first phase is mediated through the release of histamine, serotonin, and kinins whereas the second phase is related to the release of prostaglandin and slow reacting substances which peaks at third hour.[15, 16]

In this method the mean paw volume was found to be 2.71 $\pm$ 0.84 ml at 5^{th} hour in control group. Test drug at the dose of 200mg/kg body weight and 400 mg/kg body weight showed the percentage of inhibition of 37.2%. Percentage of inhibition of mean paw volume of test drug at the dose of 400mg/kg was less than that of the standard drug aspirin 300mg/kg body weight (60.14%).

Sudipta Das et al in 2010 demonstrated that the anti-inflammatory activity of Clerodendron infortunatum against carrageen induced paw edema was comparable to that of the of reference drug Phenylbutazone. The methanolic extract of Clerodendron infortunatum showed maximum inhibition of 49.64 and 65.63% at the dose of 250 and 500 mg/kg body wt. respectively after 3 hrs of the extract treatment against carrageenan induced paw edema whereas the reference drug produced 76.29% of inhibition at the dose 100 mg/kg body wt.[5]
The anti-inflammatory activity of this plant may be presence of the chemical constituents, tannins, saponin and flavonoid. Flavonoids are known to inhibit the enzyme prostaglandin synthesis, more specifically the endoperoxide and reported to produce anti-inflammatory effect.[5]

Preliminary phytochemical screening of the plant extract exhibited the presence of flavonoid, alkaloids, tannin, saponins, sterols, and fixed oils.

In earlier studies Clerodendrum infortunatum Linn. leaves on preliminary chemical analysis were found to contain saponin, clerodin (a bitter diterpene) 4, 6 and some enzymes. Leaves also contain a fixed oil which consists of Glycerides of Lenoleic, oleic, stearic and lignoceric acid.Luperol and β-sitosterol from roots.Clerosterol identified as 5, 25- sigmastadien_3β-ol, clerodolone as lup_20 (30)-en- 3β-diol-12-one and clerodone as 3β-hydroxylupan- 12-one and a steroidal glycoside from roots.[4]

Previous phytochemical investigation of the plant revealed the presence of alkyl sterols and 2,-(3, 4-dehydroxyphenyl) ethanol 1-O-α-2 rhamnopyranosyl-(1→3)-β-D-(4-O-caffeoyl) glycopyranoside (acteoside) in this plant.[17] The plant was also found to contain triterpenes, steroids and flavonoids.[4]

Various flavonoids, both glycosides and aglycones were previously reported as having potent anti-inflammatory and analgesic activity. It is suggested that some flavonoids blocks both cyclooxygenase and lipoxygenase pathway of the arachidonate cascade at high concentration, while at low concentration only lipoxygenase pathway is blocked.[17] Flavonoids also produces anti-inflammatory activity by inhibiting TNFα and phospholipase.[18] Also there are few reports on the role of tannins in analgesic and anti inflammatory activity.[19, 20] In the present study saponin might be attributed to the presence of anticonvulsant activity.

CONCLUSIONS

In conclusion, the extract from Clerodendrum infortunatum Linn. has been found to possess acute anti-inflammatory activity in experimental animals. The present study also substantiates the traditional use of C.infortumatum Linn. for the treatment of various inflammatory ailments. The plant can be recommended for the further studies to evaluate the chronic anti-inflammatory activity and to isolate the active ingredients

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REFERENCES

1. Heloina de S. Falcão, Igara O. Lima, Vanda L. dos Santos et al. Review of the plants with anti-inflammatory activity studied in Brazil. Brazilian Journal of Pharmacognosy., 2005; 15(4): 381-391.
2. Harsh Mohan. Inflammation and Healing. Textbook of Pathology(eds.6), New Delhi; Jaypee Publication: 2002, pp. 114-121.
3. Mishra S. Uma, Murthy P.N. and Parida K Sambit. Analgesic and anti-inflammatory activities of Indian medicinal plant Ziziphus xylopyrus stem barks in experimental animal models. Elixir Pharmacy., 2010: 44: 7265-7270.
4. Bhattacharjee Debayan, Das Arup et al. Clerodendrum Infortunatum Linn.: A Review. Journal of Advances in Pharmacy and healthcare Research., 2011; 1(3): 82-85.
5. Sudipta Das, Pallab K, Haldar, Goutam Pramanik and R.B. Suresh. Evaluation of Anti-Inflammatory Activity of Clerodendron infortunatum Linn. Extract in Rats. Global Journal of Pharmacology., 2010; 4(1): 48-50.
6. Sudipta Das, Pallab K, Haldar, Goutam Pramanik and R.B. Suresh. Anticonvulsant activity of methanolic extract of Clerodendron infortunatum Linn. in Swiss albino mice. Thai J. Pharm. Sci., 2010; 34: 129-133.
7. Gowda Girish, Bhosle Vaibhav et al. Evaluation of anticonvulsant activity of ethanolic leaves extract of Desmodium triflorum in mice. Rev. Bras. Farmacogn. Braz. J. Pharmacogn., 2012; 22(3): 649-656.
8. Maniyar Y, Bhixavathimath P. Antihyperglycemic and hypoglycemic activities of aqueous extract of Carica papaya Linn. Leaves in alloxan-induced diabetic rats. Journal of Ayurveda and Integrative Medicine., 2012; 3(2): 70-4.
9. Manjamalai A, Singh SS, Guruvayoorappa C, Grace VMB. Analysis of phytochemical constituents and anti- microbial activity of some medicinal plants in Tamilnadu, India. Global Journal of Biotechnology & Biochemistry., 2010; 5(2): 120-128.
10. De S, Dey YN, Ghosh AK. Phytochemical investigation and chromatographic evaluation of the different extracts of tuber of Amorphaphallus paeonifolius (Araceae). International Journal on Pharmaceutical and Biomedical Research., 2010; 1(5): 150-7.
11. Hong DD, Hein HM, Anh HT. Studies on the analgesic and anti-inflammatory activities of Sargassum Swartzii (Tumer) C. Agardh (Phaeophyta) and Ulva reticulate Forskkal (Chlorophyta) in experimental animal models. African Journal of Biotechnology., 2011; 10(12): 2308-14.

12. Bhattacharjee Debayan, Das Arup et al. Clerodendrum Infortunatum Linn. : A Review. JAPHR., 2011; l(3): 82-85.
13. Winter CA, .Risley EA, Nuss GW. Ca1Tageenan induced edema in hind paw of the rat as an assay for antiinflammatory drugs. Proc Soc Exp Biol Med., 1962; 111: 544-7.
14. Brahmbhatt MR, Patel JM, Patel VB, Saluja AK. Analgesic and Anti-inflammatory activity of leaves of Rivea hypocrateriformis. Journal of Pharmacognosy and Phytotherapy., 2010; 1(1): 1-3.
15. Vinegar R, Schreiber W, Hugo R. Biphasic development of carrageenin oedema in rats. J Pharmacol Exp Ther., 1969; 166: 96-103.
16. Chakraborty A, Devi RKB, Rita S, Sharatchandra K, Singh TI. Preliminary studies on antiinflammatory and analgesic activities of Spilanthes acmella in experimental animal models. Indian Journal of Pharmacology., 2004; 36(3): 140-50.
17. Pourmotabbed A, Farshchi A, Ghiasi G, Khatabi PM. Analgesic and Antiinflammatory Activity of Teucrium chamaedrys Leaves Aqueous Extract in Male Rats. Iranian Journal of Basic Medical Sciences., 2010; 13(3): 119-25.
18. Agnihotri A, Wakode S, Agnihatori A. An overview on anti-inflammatory properties & chemoprofile of plants used in traditional medicine. Indian Journal of Natural Product and Resources., 2010; 1(2): 150-167.
19. Goji ADT et al. A Study of the Anti-Inflammatory and Analgesic Activities of Aqueous Extract of Nauclea latifolia Leaves in Rodents. Asian Journal of Medical Sciences., 2010; 2(6): 244-7.
20. Farouk L, Laroubi A, Aboufatima A, Benharref A, Chait A. Evaluation of analgesic activity of alkaloid extract of Peganum harmala L.: A possible mechanism involved. J Ethnopharmacol., 2008; 115: 449-54.