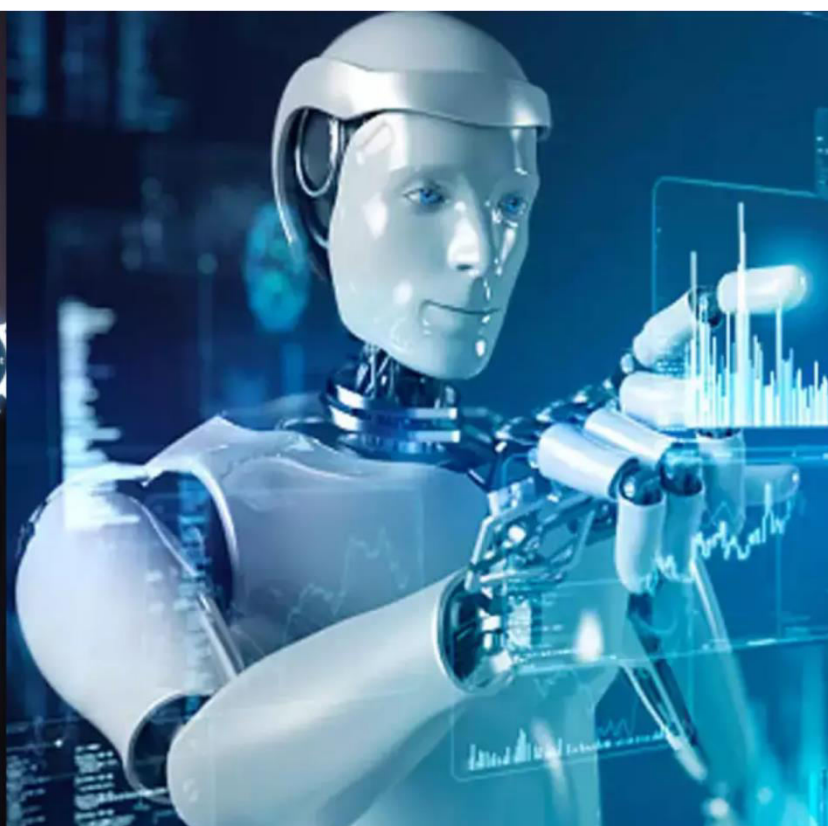




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ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF CLERODENDRUM INFROTUNATUM OF HYDROETHENOLOIC EXTRACT OF LEAF IN WISTAR ALBINO RAT

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ABSTRACT: This study examined the anti-inflammatory and analgesic effects of oral administration of 250 and 500 mg/kg of the hydro-ethanolic extract of *Clerodendrum Infortunatum* (Lamiaceae) in Wistar albino rats. The hot plate method and the carrageenan-induced paw oedema approach were used for this. Granuloma development was significantly inhibited in the ethanolic extract. When taken together, these findings show encouraging analgesic and anti-inflammatory effects against both acute and chronic pain as well as Pain and Inflammation and discomfort.

It was discovered that none of the dosages of the extract of *Clerodendrum Infortunatum* used for acute oral toxicity was non poisonous. Even at the doses used, *C. infortunatum* extract did not cause any mortalities. Carrageenan 0.1ml(1%w/v) subplantar injection in rats resulted in a time-dependent increase in paw thickness. At 0, 1, 2, and 3 hrs. after the injection of carrageenan, the thickness was measured. In the groups who received a vehicle injection, this rise started to be noticeable at 0min. and peaked at 120 minutes. The selected animals were lying on the hot plate at a stable temperature of 55°C as well as response time in(Second).The jumping of paw responses of each animal was noted at 0,30,60,90,and 120 minutes. However, treatment with 250 mg/kg and 500 mg/kg of extract and a prescribed dose of Diclofenac (10mg/kg) decreased the Pain and Pain and Inflammation caused by carrageenan considerably ($p \leq 0.05$) after 24 hour in all phases of the trial.

KEYWORDS: anti-inflammatory activity, Analgesic activity, Diclofenac, ethanolic extract, Carrageenan model, Hot plate model

I. INTRODUCTION

Inflammation:

A living, vascularized tissue local reaction to external and endogenous stimuli is inflammation. The words come from the Latin word "inflammare," which means to burn up. Fundamentally, redness serves two purposes: to contain tissue damage with to localise and eradicate the cause. As a result, Pain and Inflammation is a natural reactions to damage. Pain and Inflammation must not be viewed as an infection in and of itself, but slightly as a beneficial reaction to either aggression or certain diseases^[1]

Pain:

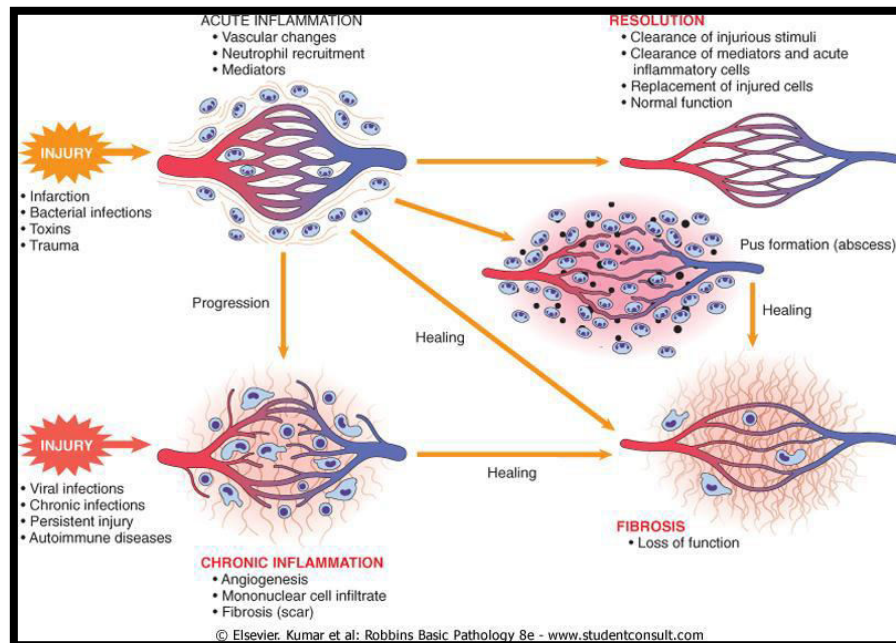
Pain is a distressing feeling often caused by intense or damaging stimuli. The International Association for the Study of Pain defines pain as "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage." Pain is the most common reason for physician consultation in most developed countries. It is a major symptom in many medical conditions, and can interfere with a person's quality of life and general functioning. People in pain experience impaired concentration, working memory, mental flexibility, problem solving and information processing speed, and are more likely to experience irritability, depression, and anxiety.

Classification:

Pain and Inflammation are classify depend on duration of abrasion and appearance into acute and chronic inflammation. As per the classification: acute and chronic.

A. Acute inflammation: Acute Pain and Inflammation can persist for minutes, hours, or even a few days, is an acute and early reaction to an inflammatory substance.^[2]

B. Chronic inflammation: It is distinguished by fluid and plasma protein exudation, as well as the migration of mostly neutrophilic, leucocytes to the place of damage.^[3]



II. PAIN AND INFLAMMATION SYMPTOMS

1. Redness:

Redness is develops in cellulitis and is caused by tiny blood vessels in injured tissue dilating.

2. Increase in blood flow

The increase in blood flow (hyperemia) brought on by localized vascular dilatation, which causes heat (calor).^[4]

3. Swelling:

Swelling is results from fluid buildup in the extra vascular space and is, in turn, brought on by an increase in vascular permeability.^[5]

4. Pain:

Pain is caused in part by pus being pressed into an abscess cavity and in part by tissues being stretched and damaged as a result of inflammatory edoema. Bradykinins, prostaglandins, and serotonin are a few molecules associated with acute Pain and Inflammation that be also known to cause pain.^[6]

5. Loss of function:

Pain inhibits to the inflamed region, and significant swelling may also render the tissue physically immobile.^[7]

Chemical mediators of inflammation:

The manifestations of Pain and Inflammation are caused by chemical mediators. Following is the order of inflammation: cell damage chemical agent's acute inflammation, which includes cellular and vascular processes. Mediators' sources: It is possible for cells or plasma to produce the chemical mediators of inflammation.^[8-9]

Table: chemical mediator of acute and chronic inflammation^[10]

Cellular mediator	Cells of origin	Function
Histamine	Mast cells, basophiles	Vascular leakage& platelets
Serotonin	Platelets	Vascular leakage
Lysosomal enzyme	Neutrophils	Tissue destruction
Prostaglandin	Leukocytes	ache, fever
Leucotriene	Leukocytes	LB4
Chemo-attractant	Lc4, Lcd4,& Le4	Broncho and vasoconstriction
Platelet activity factor	All leukocytes	Vasoconstriction
Nitric oxides	Macrophages	Leucocytes activation
Cytokines	Lymphocytes	Leucocytes activation

III. MATERIALS AND METHOD

Materials and methods:

Selection and Collection of plant materials:

The plant was select for the experiment on the base of its usual, seelection and collect from R. K. Pharmacy College, Kashipur, Surai, Sathion, Azamgarh, Uttar Pradesh.

Authentification of plant:

The herbarium file of plant profile was submitted in BHU Varanasi for authentication.

Preparation of extract:

Leaf of plant parts that had been powdered (500g) were defatted through water before being fully extracted through ethanol by using a soxhlet equipment at (40-50) °C for 72 hours. On Whatman No. 1 filter paper, the extract was filtered. To get a powdered material, the solvent was entirely evaporated under decreased pressure in a rotator vacuum evaporator. Vacuum desiccators were used to preserve the concentrated extract for later use.

Methods for phytochemical screening tests:

The occurrence of alkaloid, glycoside, flavonoid, tannin, anthraquinone, saponin, volatile oil, cynogenic glycoside, coumarins, sterols, and/or triterpenoids was determined using introduction phytochemical screening of the ethanolic extracts.

Qualitative tests:

Leaf extracts prepared in four different solvents (water, methanol, ethanol, ethyl acetate) were used for experimental purpose.

Test for flavonoids:

In this test we take 0.5ml leaf extract of CI was heated (80°-90°C) with 10ml of ethyl acetate on steam bath for three minutes. After that the mixture was filtered and 4ml of the filtrate was mixed with 1 ml of weak ammonia solution and then before adding few drop of sulphuric acid. It shows yellow color it indicates positive result.

Test for alkaloids:

The leaf extract (500µl) was treated with 5 ml of aqueous 1% HCl solution over a boiling water bath for 20 minutes. The solution was centrifuged at 3000 rpm for 10 minutes, and 1 mL of the supernatant was treated with a few drops of WAGNER'S reagent (2 g iodine and 6 g KI mixed in 100 mL of water).The reddish brown colour of the solution indicates a favourable result.

Test for phenol:

In this phenol test we take 0.5ml of leaf extract and was heated with 2ml of distilled water on water bath and then filter.10% of ferric chloride solution was add. If black color shows that it indicates positive results.

Test for anthraquinone:

In this test we should take 0.5ml of leaf extract was heated with 10ml of benzene and filtered. After that 5ml of 10% of ammonia solution was mixed and filtered if black color indicates then it shows positive results.

Test for terpenoids:

In this test we take 2ml of leaf extract and heated with 1ml of 2,4 dinitro phenyl hydrazine mixed in 100ml of 2M HCL. If the yellow orange color shows it indicates positive results.

Test for tannins:

We should take 0.5ml of leaf extract and was treated with 10ml of distilled water and filtered. After that 1M ferric chloride was diluted. If blue black precipitate are formed than it shows positive results.

Test for steroids:

In this test we take 1ml of leaf extract and treated with 2ml of acetic anhydride and on ice after that in this solution we should add 0.5ml of chloroform and 1ml of sulphuric acid. If reddish brown ring it shows positive results.

Test for saponins:

In this test we should take 0.5ml of leaf extract and cooked with 5ml of distilled water and filtered after that 2.5ml filter was add with 1.5ml distilled water. If the color is stable it shows positive results.

Test for carbohydrate:

In this carbohydrate test add few drop of molish reagent and add leaf extract and treated with sulphuric acid if purpal color rings are formed it shows positive results.

Test for glycosides:

In this test we should take 0.5ml of leaf extract which is dissolved in pyridine solution and add few drop of 20% sodium nitro preside and few drop of sodium hydroxide was mixed. if pink to red color it shows positive results.

Test for proteins:

In this test we should take 0.5ml of leaf extract mixed with 0.1ml of millions reagents. If brown color shows it indicates positive results.

Quantitative test:

In this test we should take 4 different solvent and leaf extract water, methanol, ethanol and ethyle acetate are used for the experiment .

Phenol test:

In phenol test 1ml of leaf extract was filled in a test tube, 2ml of distilled water and after that 0.5ml of folin reagent was added and after a few minutes 2ml of 20% sodium carbonate solution was mixed. And after that the tube was boiling in water bath and colled and then measured add 680 nm.

Flavonoids test:

In this test we should take 1ml of leaf extract with 4ml of distilled water and add 0.3ml of 5% sodium nitrate after 5 minutes add 0.3ml of 10% aluminium chloride added to this solution and follow by 2ml of sodium hydroxide and after that solution was mixed and the density was measured at 520 nm.

Experimental animals:

Wistar albino rats of both sex (wt. 120–180g) was used in the study. The animals were kept in a usual cycle of light and darkness. They were given the typical pellet meal and infinite contact to water. The week before the trial, the animals were used to their surroundings. After receiving permission from the Institutional Animal Ethical Committee (IAEC), the tests were conducted. The experiment was performed following animals ethics guidelines of “institutional animal’s ethics committee” (approval no-1384/PO/Re/S/10/CPCSEA)

Table 6.1 Grouping of experiment animals

Sr. No.	Group	No of animal
1.	Group I (Normal saline)	6
2	Group II (Negative control) Induced	6
3	Group III (Test I) 250mg/kg	6
4	Group IV (Test II) 500mg/kg	6
5	Group V (Positive control) Standard	6

Inflammatory activity in vivo:

Acute inflammatory studies:

The ability of *C. infortunatum* extracts to reduce Pain and Inflammation was assessed using the carrageenan- induced paw edema technique. Wistar albino rat of both sex were placed into five groups for the research (n=6). 1 hr before to the sub plantar injection of 0.10 ml of 1.0% w/v solution of the carrageenan in usual saline in the right hand paw of the rat, leaf extracts of *C. infortunatum* (250mg/kg and 500mg/kg) and Diclofenac (10.0 mg/kg) were given orally. At 0, 1, 2, 3 and 4 hrs following the injection, the plethysmographs were use to measures the paw's volume. Percent inhibition was computed using the mean paw volume.

The rats were divided into five groups as induced carragenan model.

Group I:Control – Normal saline

GroupII:Negative Control-Carrageenan(0.1ml of 1.0%w/v)

GroupIII:Positive control–Carrageenan+ Ethanolic leaf extract (250mg/kg) Group IV: Test – Carrageenan + Ethanolic leaf extract (500mg/kg)

GroupV:Test– Positive control– Carrageenan+ Diclofenac (10.0mg/kg)

The rats were divided into four groups by hot plate model

Group I:Control – Normal saline

GroupII: Standard drug Diclofenac (10mg /kg po)

Group III: Test + Ethanolic leaf extract (250mg/kg)

GroupIV:Test– Ethanolic leaf extrac (500mg /kg)

Grouping of experiment animals on hot plate model

Sr. No.	Group	No of animal
1.	Group I (Normal saline)	6
2.	Group III (Test I) 250mg/kg	6
3.	Group IV (Test II) 500mg/kg	6
4.	Group V (Positive control) Standard	6

IV. RESULTS AND DISCUSSION

Preliminary phytochemical investigation:

A receipt Voucher specimen no. Lamia. 2025/01 was deposited in the herbarium of BHU Varanasi for prospect indication. The plant was authenticated by professor N.K. Dubey, department of botany, BHU.

About 50g of ethanol extracted was adsorbed on silica gel (60-120 mesh) and fractionated using chloroform and ethanol. The recovered fractions were finally evaporated to give yields of 12.33% and ethanol. According to the preliminary qualitative analysis of *C. infortunatum*, alkaloids, flavonoids, glycosides, saponin, sterols, tannins, and terpenes are present. *C. infortunatum*'s ethanolic extract was discovered to have an 11.5% weight-to-weight phenolic content, indicating the presence of a variety of phenolic compounds including poly phenols, flavonoids, and phenolic acid. The quantity of flavonones in the ethanol extract of *C. infortunatum* was found to be 6.66% w/w from the calibration curve of naringenin and the concentration of flavonols in the extract of *C. infortunatum* was found to be 4.88% w/w from the calibration curve of quercetin. The total flavonoid content, calculated as the average of the results from these two techniques, was discovered to be 8.34% w/w.

Table: The preliminary phytochemical analysis findings are shown in below table.

Phytochemicals	Name of test	Observation	Results
Alkaloids	Wagner's test	Reddish brown colour	+
Glycosides	General test	Red to pink colour	+
Cardiac glycosides	Legal's test Baljet's test	Pink to red colour Yellow to orange	+ +
Terpenoids	General test	Yellow orange colour	+
Flavonoids	General test Specific test	Yellow colour Yellow colour	+ +
Steroids	Lieberman-burchard's test	Reddish brown ring	-
Tannins	FeCl ₃ test	Blue black PPT	+
Saponins	Frothing test	Formation of stable froth	+

4.2 Effect of ethanolic extract of *Clerodendrum Infortunatum* on Carrageenan-induced paw edema:

It was discovered that none of the dosages of the extract of *Clerodendrum Infortunatum* used for acute oral toxicity was non-poisonous. Even at the doses used, *C. infortunatum* extract did not cause any mortalities. Carrageenan sub-plantar injection in rats resulted in a time-dependent increase in paw thickness.

At 0, 1, 2, 3 and 4 hrs. after the injection of carrageenan, the thickness was measured. In the groups who received a vehicle injection, this rise started to be noticeable at 3 hours and peaked at 5 hours. However, treatment with 250 mg/kg and 500 mg/kg of extract and a prescribed dose of Diclofenac (10mg/kg) decreased the Pain and Inflammation caused by carrageenan considerably ($p \leq 0.05$) after 24 hours in all phases of the trial.

Table : Anti-inflammatory activity of *C. Infortunatum* on carrageenan induced rat paw edema.

Groups	Rat paw volume measured at different time intervals (hr)			
	0 hr	1 hr	2 hr	3 hr
Group I (Normal group)	0.74 ± 0.03*	0.74 ± 0.05*	0.74 ± 0.06*	0.74 ± 0.02*
Group II (Negative control)	0.75 ± 0.04	1.40 ± 0.05*	1.65 ± 0.07*	1.47 ± 0.02*
Group III (Test I)	0.74 ± 0.06*	1.1 ± 0.03*	0.95 ± 0.04*	0.86 ± 0.03*
Group IV (Test II)	0.73 ± 0.05*	0.97 ± 0.03*	0.89 ± 0.03*	0.81 ± 0.02*
Group V (Positive control)	0.74 ± 0.07*	0.94 ± 0.06*	0.86 ± 0.03*	0.78 ± 0.03*

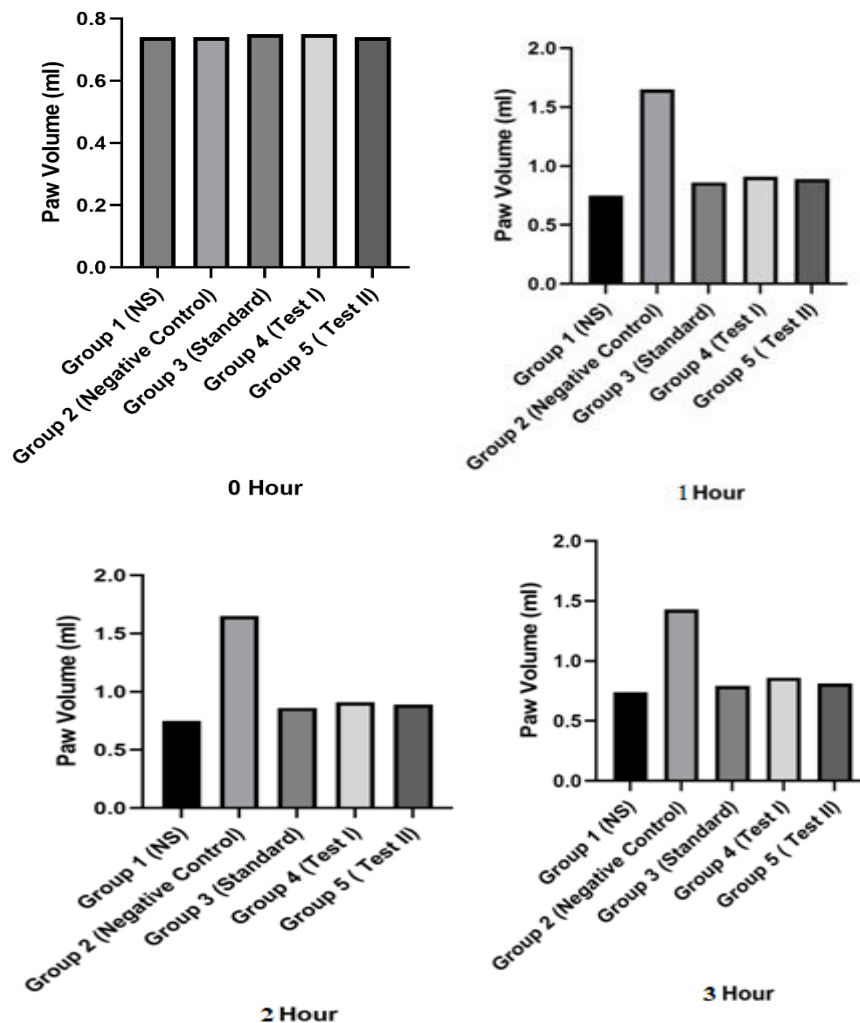
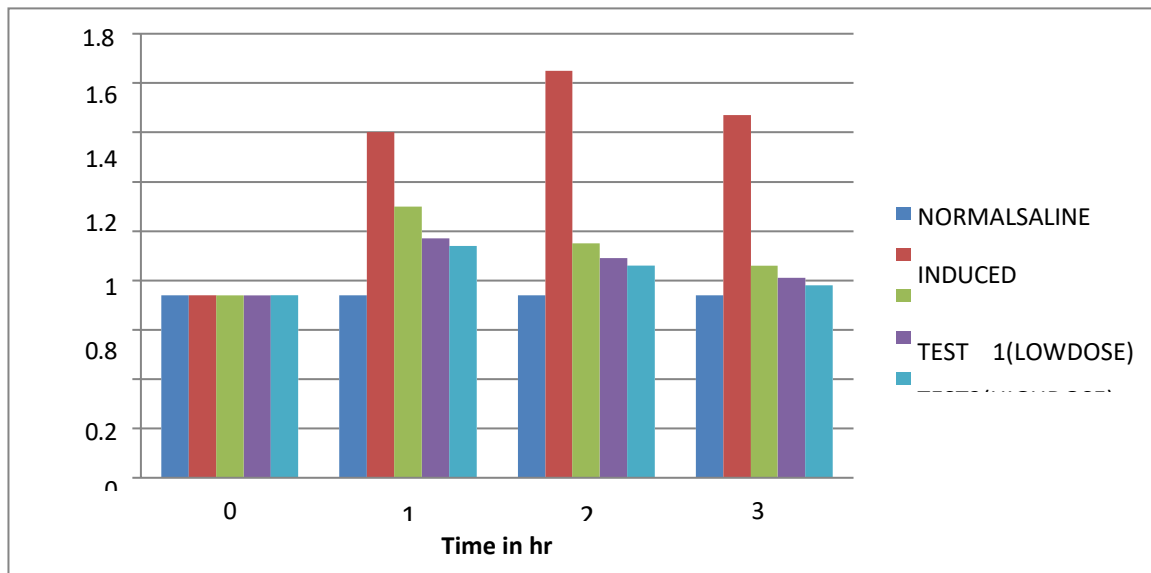
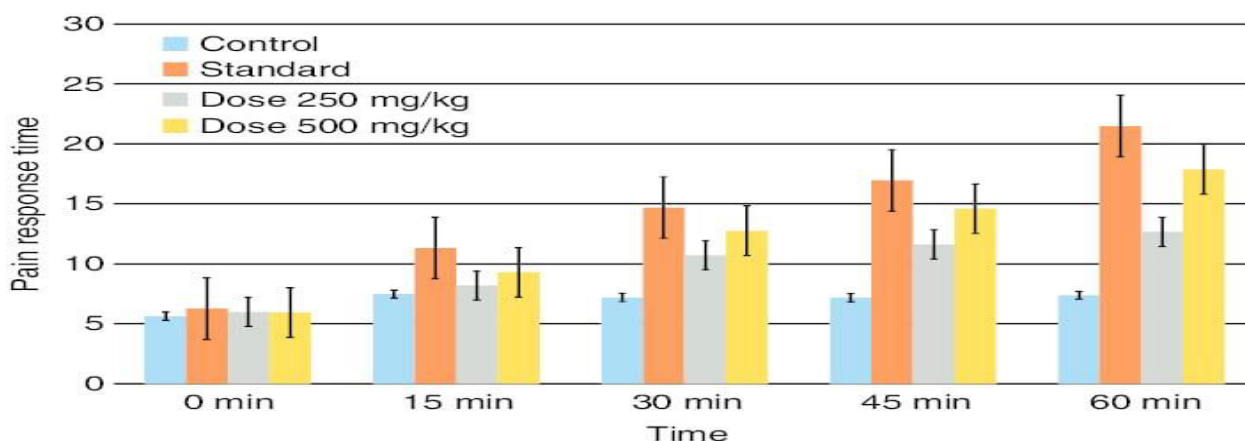


Figure: Rat paw volume measured at different time intervals (hr)



4.3 Effect of EECl in the hot-plate test

The study found that diclofenac sodium (10mg/kg.p.o) significantly improved reaction time in rats at all time intervals (30, 60, 90, and 120 minutes). EEAC (250mg/kg, p.o.) produced no significant effects at the various times examined. The EEAC (500mg/kg, p.o.) showed a substantial response time after 30 and 120 minutes of drug administration, but no meaningful reaction at 60 and 90 minutes. EEAC produced a significant outcome as compared to the control group, as seen in ⁶ table



4.4 Hot Plate response on rat

Treatment Group	Dose	Initial Reaction Time (Sec).	Reaction Time (sec).			
			15min	30min	60min	120min.
Normal Saline	10ml/kg.p.o.	3.34±0.33	4.33±0.21	5.16±0.30	5.50±0.42	5.50±0.56
Diclofenac sodium	10mg/kg.p.o.	3.66±0.33 ^{ns}	11.66±0.83 ^{***}	11.83±0.83 ^{***}	12.66±0.42 ^{***}	12.33±0.71 ^{***}
CI-test-I	250mg/kg p.o.	4.33±0.21 ^{ns}	4.66±0.84 ^{ns}	5.00±0.63 ^{ns}	5.16±0.70 ^{ns}	5.16±0.65 ^{ns}
CI-test-II	500mg/kg.p.o.	4.66±0.33 ^{ns}	7.16±0.87 [*]	7.50±0.76 ^{ns}	7.66±1.40 ^{ns}	8.50±0.99 [*]

One of the most accurate test methods for excluding anti-inflammatory and analgesic substances is the inhibition of carrageenan-induced Pain and Inflammation in rats. Histamine, 5-HT, and kinin release are responsible for the first phase of carrageenan-induced edoema, while prostaglandin release is responsible for the second phase. It was discovered that the triterpenoid saponin, was present in the alcoholic extracts of the leaves. Other triterpenoid, saponins have been shown to be beneficial in treating inflammatory disorders. Alkaloids and saponins are present in the extracts of *C. infortunatum* according to phytochemical screens. In summary, the studies have shown that the alcoholic extract of *C. infortunatum*'s leaf and may have anti-inflammatory and analgesic properties.

V. CONCLUSION

The findings of the present investigation showed that *Clerodendrum infortunatum* leaf extract had strong anti-inflammatory and analgesic effect. Its therapeutic properties are greatly enhanced by the presence of numerous kinds of phyto compounds, such as phenols, flavonoids, saponins, alkaloids, etc., which may be one of the causes for its use in treating a variety of disease. The goal of the current study was to scientifically back up the claims made by various tribes regarding the healing powers of *C. infortunatum* leaves on skin conditions as well as Pain and Inflammation. It also offers a starting point for additional research into the herb's potential as a medication condition.

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