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TO STUDY THE TOPICAL ANALGESIC ACTIVITY OF HYDROETHANOLIC EXTRACT OF LEAVES OF CODIAEUM VARIEGATUM IN ANIMAL MODEL

Pharmacology

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ABSTRACT

The present study was aimed at evaluation of topical analgesic activity of the leaves of Codiaeum variegatum. All the animals were divided into four groups (n=5).Group 1 received Simple ointment topically only, Group 2 received the standard drug Methyl salicylate ointment 30 % topically, Group 3 received HEECV 2% w/w topically and Group 4 received HEECV 4% w/w topically. The first test to assess the topical analgesic acitivity was by Formalin induced paw licking test. Values are expressed as mean ± SEM. Statistical differences in mean were analyzed using one way ANOVA (analysis of variance) followed by Dunnett's test. $p<0.05$ was considered significant. The second test to assess the topical analgesic activity was by Hot Plate Analgesiometer. The data were expressed as mean ± SEM. Difference between the experimental groups were statistically evaluated by one way analysis of variance (ANOVA) followed by Dunnett's test. The level of significance was at $p < 0.05$.

KEYWORDS

Codiaeum variegatum, leaves, topical analgesic activity.

INTRODUCTION

Pain is described as an unpleasant sensory and emotional event associated with possible tissue damage. Thus, pain is attributable to both emotional and physical characteristics.[1]

Analgesics act by simply elevating a patient's threshold of pain. It is commonly believed that aspirin and related nonsteroidal anti-inflammatory drugs (NSAID) act peripherally via interacting with the prostaglandin synthesis and narcotic analgesics act centrally, i.e, within the range of the central nervous system.[2]

Codiaeum variegatum ("garden croton" or "variegated croton") is native to southern India, Sri Lanka, Indonesia, Malaysia, and the western Pacific Ocean islands, is found growing in open forests and scrub[3].It is an an ornamental shrub, 1-6 m tall with variable leaf shapes ranging from elliptical to oval to elongated shapes, size and colour[4]. Leaves are thick, leathery, lustrous, arranged alternately. The inflorescenes are elongated racemes 8–30 cm long, with male and female flowers on different inflorescences. The male flowers are white with five small petals with 20–30 stamens and the female flowers are yellowish, with no petals. The fruit is a 3 lobed-capsule of 9 mm diameter, containing three 6mm seeds which are black in colour. The stem contains a milky sap that discharges from cut stems.[3] The flowers are monoecious and borne in racemes. Flowers and fruit may not mature on some forms (cultivars), whereas others may bear flowers and fruit throughout the year.[4] It has a global distribution throughout the world in Pacific Islands, Malaya, China, Bangladesh.. In India it is distributed in Assam, Uttar Pradesh, West Bengal, Maharashtra(Pune), Karnataka(Mysore), Tamil Nadu (all Districts).[5]

The leaves extracts of crotons are known to have numerous medicinal properties including purgative, sedative, antifungal, anti inflammatory, antiamoebic and anticancerous activities. It is also used to treat irregular menstruation and for wound healing.

Medicinal value of a plant is due to the existence of some chemical substance that generates a physiological action on the human body. These chemicals are categorized as, primary and secondary metabolites. Primary metabolites are those which are needed for growth and development of plant and secondary metabolites are the byproducts of metabolic processes and play an important role in defense system. Secondary metabolites are substances such as alkaloids, carbohydrates, glycosides, steroids, flavonoids, coumarins, saponins, fatty acids, tannins, protein and amino acids, gum and mucilage, terpenoids, anthroquinones and phenols. Study of these phytochemicals are important as they can be utilised to synthesize future drugs.[3]

Traditional Uses:

Traditionally used in the treatment of venereal disease gonorrhea with a preparation liquid pressed from the leaves in tropical countries world-wide. Fever may be relieved by bathing the patient in a green solution of boiled leaves. Sores are treated with a direct application of sap, and a preparation of the root is used to treat wounds[6]. The crushed leaves of *Codiaeum variegatum* are mixed with mustard oil and applied to affected areas as remedy for pain.[7]

THE PLANT:[8]

Kingdom : Plantae
Order : Malpighiales
Family : Euphorbiaceae
Genus : *Codiaeum*
Species : *variegatum*

Fig 1.

AIMS AND OBJECTIVE: To study the topical analgesic activity of hydroethanolic extract of leaves of *Codiaeum variegatum* in animal model

MATERIALS AND METHODS:

MATERIALS:

Preparation of Hydroethanolic extract [9]: Fresh leaves of *Codiaeum variegatum* were collected and were washed thoroughly with tap water and dried at room temperature for 3 weeks. The dried samples were grinded and packed in airtight ziplocks for further use. The 50% hydroethanolic extraction of the plant leaves were carried out by suspending 50 g of the dried powder in 500 ml of 50% ethanol (50:50 v/v). The extraction was carried out by cold maceration for 24 hrs on a shaker at room temperature. The extract obtained was filtered through cotton wool and concentrated using a rotary evaporator and the it was freeze-dried to obtain the *C. variegatum* hydroethanolic leaf extract.

Acute Dermal Toxicity Studies

This study was executed on rats. The skin of the animal, each about 500 mm^2, was shaved at three different sites on the dorsal side. The 1st area was considered as control, to which vehicle was applied. 2nd area was applied with HEECV 2% w/w and the 3rd area treated with HEECV 4% w/w. Four hours later, the skin was observed for signs of inflammation. [6]

Preparation of topical formulation:
Two ointment formulations were prepared, with different concentration of the extract ,viz. 2% (w/w) ointment, where 2 g of extract was incorporated in 100 g of simple ointment base, 4% (w/w) ointment where, 4g of extracts of the root were incorporated in 100g of simple ointment base B.P. Methyl salicylate ointment 30 % procured from Cadila Pharmaceuticals, Bangalore, India, was used as standard drug for studying the topical analgesic activity of the extract in different animal models.[6]

Animals:
Healthy Wistar Rats weighing 180-200g and ages between 2-3 months of age, were used for the study. Each group consists of 5 animals each. The experimental procedure was approved by the Institutional Animal Ethical Committee of Assam Medical College and Hospital, Dibrugarh, Assam.

Group 1: Simple ointment treated control group

Group 2: Animals treated with Standard Methyl salicylate ointment 30 %

Group 3: Animals treated with HEECV 2% w/w (2g extract in 100g simple ointment) (Hydroethanolic extract ointment of *Codiaeum variegatum* low dose 2% w/w)

Group 4: Animals treated with HEECV 4% w/w (4g extract in 100g simple ointment) (Hydroethanolic extract ointment of *Codiaeum variegatum* high dose 4% w/w[6])

METHODS:
Topical Analgesic activity:[10]
Formalin induced paw licking test:
- Right paw is taken as the control and left paw is taken as test.
- 0.3g of ointment containing 2% of HEECV was applied by gentle rubbing 50 times to the dorsal surface of the left hind paw with the index finger.
- Rats of the control group received only the ointment base.
- Methyl salicylate ointment 30 % applied in the same way was used as standard.
- Fifteen minutes later, the analgesic activity was tested using formalin test described by Dubuisson and Dennis.
- Fifty micro liters of 2.5% formalin was injected into the dorsal surface of the left hind paw.
- The rat was observed for 30 mins after the injection of formalin, and the time spent in paw lickings during the 30 mins observation period was noted down.
- The first 10 min post formalin injection is known as the early phase and the period between 15 and 30 min is known as the late phase.

Statistical analysis
- Values are expressed as mean ± SEM.
- Statistical differences in mean were analyzed using one way ANOVA (analysis of variance) followed by Dunnett's test.
- $p<0.05$ was considered significant.

Analgesic Effect Of The Topical Preparation Of *Codiaeum Variegatum* On The Formalin Induced Paw Licking:
TABLE 1

Treatment	Total time spent in paw lickings (secs)		Total time spent in paw licking (secs)	
	0-10 min	% Inhibition	15-30 min	% Inhibition
Control	159.2±0.37^{bcd}	--	323.0±19.51^{bcd}	--
Standard Methyl salicylate ointment 30 %	41.80±0.58^{acd}	73.74	173.40±0.60^{acd}	46.31
HEECV-2%	107.60±0.67^{abd}	32.41	226.20±0.58^{abd}	29.96
HEECV-4%	99.60±0.67^{abc}	37.40	204.20±0.37^{abc}	36.78

Fig 2. Formalin paw licking test (0-10 mins) EARLY PHASE

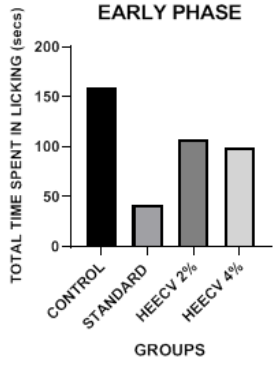
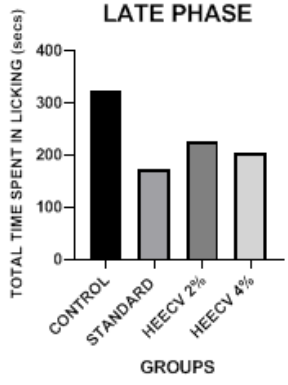


Fig 3 Formalin paw licking test (15-30 mins)



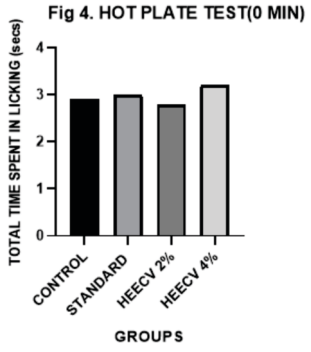
Topical analgesic activity:
Hot plate test:[11,12]
- The rats were divided into 4 groups and each group consisted from five animals.
- The pain reaction time was noted down pretreatment for each animal and was taken as a basal threshold.
- Group 1 was taken as a control and only ointment was applied topically on fore and hind limb.
- Group 2 was taken as a standard and 30% Methylsalicylate ointment was applied topically on fore and hind limb.
- Group 3 and 4 received 2% and 4% HEECV ointment respectively via topical application on fore and hind limb.
- The onset and duration of analgesic effect of each group were determined in rats by using Hot-Plate test.
- Rats were placed on a hotplate maintained at 55±1˚C.
- The reaction time is that time between placing the animals on the hot-plate andholding, jumping, licking of the fore or hind paws.
- A cut off time of 30 seconds is followed to avoid any thermal injury to the paws.
- The latency reaction time was recorded again after 3 min. and (10, 20, 30, 40, 50, 60 min.) following topical application of control, standard and in treated groups by using hotplate test.
- The prolongations of the reaction times of the groups were compared with the values of the control and was used to evaluate topical analgesic effects of *C.variegatum.*
- **% of increase in reaction time :** time after drug – time before drug/time before drug. · Cut off time: 30 seconds.

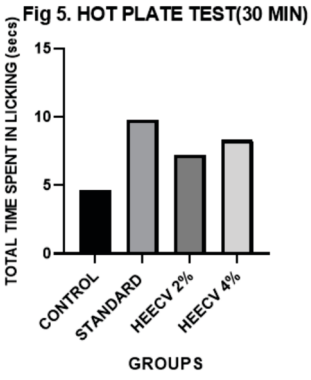
Statistical analysis:
- The data were expressed as mean ± SD
- Difference between the experimental groups were statistically evaluated by one way analysis of variance (ANOVA) followed by Dunnett's test.
- The level of significance was at $p < 0.05$.

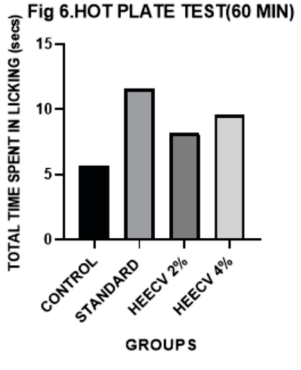
TABLE 2:Analgesic effect of the Topical preparation of *Codiaeum variegatum* on Hot Plate analgesiometer

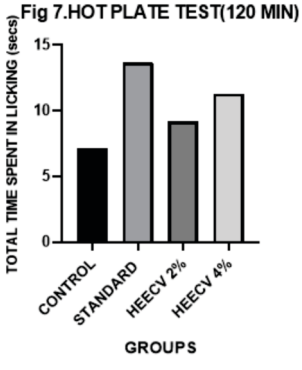
Treatment	Reaction time of licking 0min	Reaction time of licking 30min	Reaction time of licking 60min	Reaction time of licking 120min
Control	2.90±0.18	4.60±0.18^{bcd}	5.70±0.12^{bcd}	7.10±0.18^{bcd}
Standard Methylsalicylate ointment 30 %	3.00±0.15	9.80±0.33^{acd}	11.60±0.18^{acd}	13.70±0.20^{acd}
HEECV-2%	2.80±0.20	7.20±0.20^{abd}	8.20±0.12^{abd}	9.20±0.12^{abd}
HEECV-4%	3.20±0.12	8.30±0.12^{abc}	9.60±0.18^{abc}	11.30±0.12^{abc}

Values are expressed as mean±S.E.M One way Anova followed by Dunnett's multiple comparison test is done between the groups. p < 0.05 is considered significant. [a]p<0.05 when compared with group Control [b]p<0.05 when compared with group Standard [c]p<0.05 when compared with group HEECV 2% [d]p<0.05 when compared with group HEECV 4%









PHYTOCHEMICAL ANALYSIS:[9]

Phytochemical analysis of HEECV was evaluated according to standard methods as per (Trease and Evans, 1989; Sofowora, 1993; Harborne, 1998).

RESULTS:

The phytochemical analysis of HEECV extract revealed the presence of general glycosides, tannins, alkaloids, flavonoids, sterols, triterpenoids.[9]

TOPICAL ANALGESIC ACTIVITY:

Formalin-induced hind paw licking test:

The results of topically administered HEECV on the formalin induced hind paw licking are presented in Table 1, Fig 2, Fig 3.

In this model, HEECV 2% and 4%, showed a significant ($p < 0.05$) reduction in the licking time suggesting analgesic activity in the early phase (0-5 min). HEECV 4% showed a significant ($p < 0.05$) decrease in the duration of paw licking when compared HEECV 2% with HEECV 4% showing dose dependent effect.

The standard drug, Methylsalicylate 30% ointment also significantly ($p < 0.05$) reduced the paw licking duration in the early phase (0-5 min).

Also, HEECV 2% and 4%, showed a significant ($p < 0.05$) reduction in the licking time suggesting analgesic activity in the late phase (15-30 min). HEECV 4% showed a significant ($p < 0.05$) decrease in the duration of paw licking when compared HEECV 2% with HEECV 4% showing dose dependent effect.

The standard drug, Methylsalicylate 30% ointment also significantly ($p < 0.05$) reduced the paw licking duration in the early phase (15-30 min).

Hot Plate method test:

The results of the reaction time following the topical administration of different doses of HEECV 2% and 4% is presented in Table 2, Fig 4, Fig 5, Fig 6, Fig 7.

HEECV 4% produced a significant ($p < 0.05$) increase in the mean reaction time throughout the observation period, i.e., at 0 min, 30 min, 60 min and 120 min, compared to the control and HEECV 2% dose.

The standard drug Methylsalicylate 30% ointment also caused significant ($p < 0.05$) increase in the mean reaction time throughout the observation period, as compared to the control group.

DISCUSSION:

The present study was carried out to study the topical analgesic activity of the hydroethanolic extract of the leaves of *Codiaeum variegatum*.

In this study a total of 20 Wistar albino rats were selected to undergo the topical analgesic activity experiment. Two doses of the HEECV of 2% and 4% were selected.

The formalin induced paw licking test is considered as a sensitive and reliable model for screening the analgesic activity of many analgesic medications. The formalin test is known to produce a prominent biphasic responses, thus various analgesics may act differently in the early and late phases of this test. Thus, this test is useful to distinguish the various possible mechanisms of analgesia.[11]

In this model, HEECV 2% and 4%, showed a significant ($p < 0.05$) reduction in the licking time suggesting analgesic activity in the early phase (0-5 min). HEECV 4% showed a significant ($p < 0.05$) decrease in the duration of paw licking when compared HEECV 2% with HEECV 4% showing dose dependent effect.

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The standard drug, Methylsalicylate 30% ointment also significantly ($p < 0.05$) reduced the paw licking duration in the early phase (15-30 min).

From the above results, we can conclude that the topical preparation containing 2% and 4 % w/w of HEECV possesses good topical analgesic effect.

The hot plate test is an ideal test for distinguishing centrally acting analgesics from peripheral acting analgesics.[13] In the hot plate model, pain withdrawal reaction towards thermal stimuli in mice is a well-approved experiment for identification of opiate analgesics as well as various other types of analgesics medications from spinal origin[14] Centrally acting analgesics lengthens the duration of response whereas the peripherally acting analgesics such as acetylsalicylic acid (e.g. aspirin) or phenylacetic acid categories (e.g. diclofenac) do not affect these responses.[13]

In this model, HEECV 4% produced a significant ($p < 0.05$) increase in the mean reaction time throughout the observation period, i.e., at 0 min, 30 min, 60 min and 120 min, compared to the control and HEECV 2% dose.

The standard drug Methylsalicylate 30% ointment also caused significant ($p < 0.05$) increase in the mean reaction time throughout the observation period, as compared to the control group.

From the above results, we can conclude that the topical preparation containing 2% and 4 % w/w of HEECV possesses good topical analgesic effect.

Phytochemical investigation of this plant showed the presence of general glycosides, tannins, alkaloids, flavonoids, sterols, triterpenoids.

The probable mechanism of action of analgesia which may be involved and the phytochemicals responsible for the effect are discussed here under.

Pain is described as an unpleasant sensory and emotional event associated with possible tissue damage. Thus, pain is attributable to both emotional and physical characteristics.[1]

Analgesics act by simply elevating a patient's threshold of pain. It is commonly believed that aspirin and related nonsteroidal anti-inflammatory drugs (NSAID) act peripherally via interacting with the prostaglandin synthesis and narcotic analgesics act centrally, i.e, within the range of the central nervous system.[2]

Non-steroidal anti inflammatory drugs (NSAIDs) are extensively used as a remedy for fever, acute and chronic arthritis etc. Their mechanism of action is by inhibition of cyclooxygenase (COX) which decreases the release of prostaglandins (Pgs), and other inflammatory and pain mediators. PGs are known to be gastroprotective, as they promote the mucosal secretion of bicarbonates and decrease the gastric acid secretion.[15]

The extract probably follows this mechanism for analgesic effect. Methyl salicylate which is also an NSAID was used as standard drug in the formalin induced paw licking test and hot plate test.

However, the systemic use of NSAIDS may result in severe upper gastrointestinal ulcers and other side effects. Topical formulations have additional advantages over the conventional formulations. They are considered more efficacious and less harmful than conventional formulations due to the bilayer composition and structure. In the topical formulations, drug carriers are used for appropriate localization and penetration of the drug through the skin to promote the local actions and reduce the systemic action.[15]

Nowadays, transdermal drug delivery systems have have been developed to promote local but prolonged action of many drugs like anesthetics and analgesics. Commonly, NSAIDs are integrated in topical formulations to help deliver drugs by using skin as the route for drug entry. The topical formulations of drugs have certain advantages of drug direct delivery at the desired site of action, extending the period of drug delivery and increased local concentration of the drug at the desired site of action. Clinically, several non-steroidal anti-inflammatory drugs (NSAID) have been used, but due to side effects like gastro intestinal disorders, the use of these drugs is sometimes limited.

Several efforts have been undertaken to avoid these adverse effects.[15]

Earlier studies on chemicals and their pharmacology reveals that plants containing phytochemicals like alkaloids, tannins, phenolic compounds, glycosides, phytosterols, flavonoid, carbohydrates and amino acids may play a part for exhibiting topical analgesic effects.[16]

In a study conducted by Shylaja.H, K.Lakshman, Nabanita kar, Vikas maurya, G.L. Viswanatha to evaluate the analgesic and anti-inflammatory activity of topical preparation of *Lantana camara* leaves, was carried out using topical formulations containing 1%, 2%, 4%, 6% and 8% of alcoholic extract of *Lantana camara*(AELC) using formalin induced paw licking test in mice using Methyl salicylate as a standard. Preliminary phytochemical investigation of this plant showed the presence of alkaloids, tannins, phenolic compounds, glycosides, phytosterols, flavonoid, carbohydrates and amino acids which might be in part responsible for analgesic effects.[10]

A study done by Patil RA, Langade PM, Dighade PB, Hiray YA showed that petroleum ether extract of *Murraya koenigii L*. leaves (PMK) in mice via acetic acid-induced writhing method, hot plate method and tail immersion method exhibited antinociceptive activity. The extract revealed the presence of alkaloids, triterpenoids, and flavonoids in *Murraya koenigii* may be responsible for analgesic.[17]

A study carried out by Barua CC, Roy JD, Buragohain B, Barua AG, Borah P, Lahkar M. showed that hydroethanolic extract of *Drymaria cordata willd*. displayed analgesic and antinociceptive activity via models viz. acetic acid induced writhing model (female mice), Eddy's hot plate (mice) and tail flick model (rat) for analgesic study and formalin-induced paw licking model (mice) were used for anti-nociceptive study. The hydroethanolic extract was found to contain steroids, triterpenes, diterpenes and tannins which might be responsible for the significant analgesic activity.[14]

In a study conducted by Nitin Kharat, Shylaja.H, G.L.Viswanatha, K.Lakshman for evaluating preparation of methanolic root extracts of four different concentrations of *Ichnocarpus Frutescens* (L.). i.e IF 1%, IF 2%, IF 4% and IF 6%. It was found that the IF 6 % has showed significant analgesic effect by reducing the no. of paw lickings in formalin induced paw licking test in rats. The analgesic effect of the IF 6% was comparable with standard 30% Methyl salicylate ointment and thus could be used for the treatment of acute pain conditions. Preliminary phytochemical investigation of this plant revealed the presence of alkaloids, tannins, phenolic compounds, glycosides, phytosterols, flavonoid, carbohydrates and amino acids which might be responsible for the analgesic and anti-inflammatory effects.[16]

As, it can be seen from the above studies that components like alkaloids, tannins, phenolic compounds, glycosides, phytosterols, flavonoid, carbohydrates and amino acids possess topical analgesic effects.[16]

Phytochemical analysis of my plant also revealed the presence of these components, suggesting the potential topical analgesic activity of my plant may be due to the presence of these phytochemicals.

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CONCLUSION:
From these overall results, we can conclude that the topical preparation containing 4% w/w of hydroethanolic extract of leaves of *Codiaeum variegatum* possesses significant topical analgesic effect, which can be useful for the treatment of acute localized painful conditions.

Phytochemical investigation of this plant showed the presence of general glycosides, tannins, alkaloids, flavonoids, sterols, triterpenoids which might be responsible for analgesic effects. analgesic and anti-inflammatory activity of topical

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