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Evaluation of analgesic and anti-inflammatory activity of ethanolic extract *of chlorophytum tuberosum* dried roots

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Abstract
The present study aimed to expose the anti-inflammatory and analgesic activity of ethanolic extract of *Chlorophytum tuberosum* in animal models of anti-inflammatory and analgesic. The roots of *Chlorophytum tuberosum* are collected and shade dried. Coarse powder is made from these dried seeds and subjected to extraction in increasing polarities. Various extracts are prepared by using suitable solvents like ethanol. The extract showed presence of protein, carbohydrate and starch, gum, flavonoids, steroids, glycosides, tannin and phenolic compound and alkaloids and flavonoids. Ethanolic extract of roots of *Chlorophytum tuberosum* possess anti-inflammatory. Ethanolic extract of roots of *Chlorophytum tuberosum* possess analgesic activity. Ethanolic extract is more active in models employed in the study when compared with control and standard. Herbs are an integral part of nature. Plants contain natural substances that can promote health. This experiment employed three different doses of EECT extract for analgesic and anti-inflammatory screening namely minimum dose (250 mg), and maximum dose (500 mg). In the present study, ethanolic extract was studies for analgesic and anti-inflammatory activity by *in-vivo* method. This plant, which contains natural products such as flavonoids, steroids etc. have received considerable attention in recent years due to its diverse pharmacological properties including anti- inflammatory and analgesic activities. We propose that the active constituents such as flavonoids and steroids present in *Chlorophytum tuberosum* roots are responsible for the anti-inflammatory and analgesic activity. The present work suggests that it requires further studies to reveal the exact mechanisms of action responsible for the anti-inflammatory and analgesic activities of *Chlorophytum tuberosum* roots.

Keywords: *Chlorophytum tuberosum*, ethanolic extract, anti-inflammatory activity, analgesic activity, phytochemical screening

Introduction

India has very rich culture of medicinal herbs and spices, which includes about more than 2000 species and has a huge geographical area with high potential abilities for Ayurvedic, Unani, Siddha traditional medicines but only small portion have been studied chemically and pharmacologically for their potential medicinal value. [1]. Human beings have been using plants for the treatment of many diseases for thousands of years. [2]. According to the World Health Organization, most populations even today rely on traditional medicines for their physical health requirements, [3]. since they cannot afford the products of Western pharmaceutical industries. [4]. Herbal medicines are relatively safer and cheaper than synthetic. From their personal experience, people living in rural areas know that these traditional remedies are valuable sources of natural products to maintain human health, but they may not know the science behind these medicines but knew that some medicinal plants are highly effective only when used at therapeutic doses. [5]. People have been utilizing shrubbery for basic protective and curative well-being concern since era immemorial. Recently data hint that over more than 9,000 plants have recognized medicinal uses in diverse cultures and countries. [6]. Medicinal used plants have been used at the family by women enchanting care of their family, in rural area level by drug men or family shamans, and by the practitioners of traditional medicine systems such as Ayurveda, Chinese medicine, or the Japanese Kampo system. As per World Health Organization, nearly 80% of the entire world's inhabitants used traditional system of medicine. It has been provided by primary healthcare bases. [7]. If we audited prescription on recent system of medicine find that most probably plant synthetics active constituents has been used in treatment of various disease. [8]. India obtains, by dispersal quite than by deal, imperative curative genus excluding Cannabis and garlic from middle Asia, Aloe vera, opium poppy, Glycyrrhiza from the Mediterra nean, nutmeg from Southeast Asia; Thgonella foenum-graecum, Crocus sativus, Carum carvi (caraway) from Southwest Asia, Coriandrurn sativurn from the Mediterra nean and Southwest Asia from the Eurasian steppes; sativum from Tibet; and numerous other species. [9-11]. Inflammation, evidence of many diseases, is major concern for physicians throughout the world. Inflammation is often associated with pain and fever. Inflammation is a nonspecific defense response by the body to an injury in the tissue. It develops after a mechanical injury such as injury or blow to the skin. Inflammation is a complex phenomenon comprising of biochemical as well as immunological factors. in the reaction of inflammation, the local changes dominate. Connective tissue of the inflamed area shows vascular changes and the formation of exudates. The three single most important events in this process is accumulation of large number of phagocytic cells at the site of the inflammation. Tissue injury caused by introduction of a foreign antigen, trauma, or local exposure to certain chemicals triggers a complex process of inflammation. This may consist of a fluid stasis as well as the accumulation of several cellular and non-cellular elements of the immune response. Normal inflammatory response is essential to fight infectious diseases and is part of repair mechanism and removal of debris following tissue damage. Inflammation also causes disease due to damage of healthy tissue; this may occur if the response is over vigorous or persist longer than is necessary inflammatory response. [24].

Material and Methods

Collection of plant and extraction

The roots of plant *Chlorophytum tuberosum* were collected in the month of September 2018 from Local region of Indore (M.P.). The roots were washed with tap water and dried under the shade at room temperature. The ethanolic extracts of roots are prepared by the process of Soxhlet apparatus. Soxhlet is the process of extraction of a powder drug with a solvent. The collected roots were washed 2-3 times with tap water to remove adhering dust and allowed to dry in shade. The dried material was crushed to coarse powder with mechanical grinder. It was then passed through the 40 No. mesh sieve. The powder was stored in airtight container. A weighed quantity (150 gm) of the powder was subjected to continuous hot extraction in Soxhlet apparatus with ethanol as a solvent and extracted till the solvent became colorless. Extract was evaporated under reduced pressure using desiccator at a low temperature of 40-60°C until the extract turned syrupy and then this syrupy extract was transferred to an evaporating dish for drying on a water bath. [25]. Qualitative chemical tests were conducted for ethanolic extract of roots of *Chlorophytum tuberosum* to identify the various phytoconstituents. The phytochemical investigation showed presence of carbohydrates, proteins, steroids, amino acids, glycosides, tannin and phenolic compounds, flavonoids, alkaloids, and vitamins.

In vivo Pharmacological Investigation

Animal Grouping

Rats were randomly divided into five groups with each group consisting of six rats.

Group I	Control (Distilled Water)	2 ml/kg (*p.o*)
Group II	Ethanolic Extract of *Chlorophytum tuberosum* (EECT-250)	250 mg/kg
Group III V	Ethanolic Extract of *Chlorophytum tuberosum* (EECT-500)	500 mg/kg
Group IV	Standard drug (Diclofenac for analgesic activity)	10 mg/kg
	Standard drug (Indomethacin for anti- inflammatory activity)	20 mg/kg

1. Tail Flick method

A metal artery clip was applied to the root of animal's tail (1cm from the body) to induce pain. [21] A sensitivity test was carried out and animals that did not attempt to dislodge the clip within 15 sec. were discarded. Analgesic activity was evaluated 0, 30, 60, 90, and 120 min. after oral administration of the extracts and controls. An artery clip is placed at the root of tail and a positive analgesic response was indicated if animal attempt to dislodge the clip by biting the clip or tail within 5 sec. in any of the consecutive trials. The reaction time between application of the clip and response is noted by a stopwatch. The mean value was evaluated. [22]

2. Carrageenan induced rat paw oedema method

The anti-inflammatory activity of the test compounds was evaluated in Wistar rats employing this method. Anti-inflammatory activity of EECT in the dose of 125, 250 and 500 mg/kg was evaluated against Carrageenan induced paw edema model in rats. On the hind-paw oedema induced by sub plantar injection of 0.1ml Carrageenan (1% w/v) was injected into the sub plantar tissue of left hind paw of each rat. Swelling of carrageenan injected foot was measured. Animals were treated with test extract 1hour before the carrageenan injection. The linear paw circumference was then measured at 0, 30, 60, 90 and 120 min of the administration of phlogistic agent, using the Vernier calipers. The following formula was used to calculate percentage of inhibition [83]

$$\text{Inhibition (\%)} = \frac{Vc - Vt}{Vc} \times 100 \quad (1)$$

Where: Vc and Vt represent average paw volume of control and treated animals, respectively.

Statistical Analysis

The results are expressed as the means ± standard error of mean (SEM). Parametric data were compared to control group and were assessed by the method of one-way ANOVA followed by Dunnett's tests. Values $p<0.05$ was considered statistically significant.

Result and Discussion

Phytochemical Investigation

The Ethanolic extract of *Chlorophytum tuberosum* was subjected to preliminary Phytochemical screening for the presence of different Phytoconstituents such as alkaloids, saponins, glycosides, tannins, flavonoids, carbohydrates etc. The presence of Carbohydrate was confirmed by Fehling's test, Benedict test, Seliwanoff's test, Tolen's test. The presence of protein & amino acids was confirmed by Million's test & Wagner's test. The presence of steroids by Salkowski reaction, Libermannburchard reactions & Tannins by 5% FeCl3 solution whiles the Glycoside by Legal's test, Keller Killani test and Flavonoids by lead acetate test. As, the ethanolic extract show the presence of most of these compounds, these extracts were selected for the study.

Table 8.1: Phytochemical Analysis Roots of *chlorophytum tuberosum*

Phytochemicals	Observation
Carbohydrates	-
Proteins	+
Phytosterols	+
Glycosides	+
Tannins	-
Phenols	+
Flavonoids	+
Alkaloids	+
Volatile oil	-
Vitamines	+

1. Tail Flick Test

The effect of EECT extract on tail Flick test is shown in table. The extract at dose of 125 and 250 mg/kg dose not shown any significant, but at dose 500 mg/kg caused a significant inhibition of pain. However, diclofenac sodium 10 mg/kg was effective than ethanolic extract.

Table 1: Effect of EECT Extract on tail flick test

Treatment (mg/kg)	Latency (sec) ± SEM				
	0 min	30 min	60 min	90 min	120 min
Control	3.02 ± 0.14	3.12 ± 0.23	3.22 ± 0.16	3.40 ± 0.18	3.50 ± 0.17
EECT-250 mg/kg	3.20 ± 0.12 [a]	4.54 ± 0.90 [ns]	5.50 ± 0.84 [ns]	6.70 ± 0.74 [ns]	6.40 ± 1.10 [ns]
EECT-500 mg/kg	5.00 ± 0.16 [a]	6.90 ± 0.99 [b]	9.30 ± 1.24 [b]	10.54 ± 1.17 [a]	9.50 ± 0.62 [a]
Diclofenac 10 mg/kg	4.00 ± 0.24 [b]	8.99 ± 1.50 [c]	1.00 ± 1.74 [c]	3.50 ± 1.42 [b]	2.90 ± 0.84 [c]

Values are represented as means ± SEM (n=6 in each group) Data expressed by using one-way ANOVA followed by Dunnett's Test. p<0. 5.. [a]p<0.05, [b]p<0.01, [c]p<0.001 was considered as significant and n.s = non-significant as compared to control.

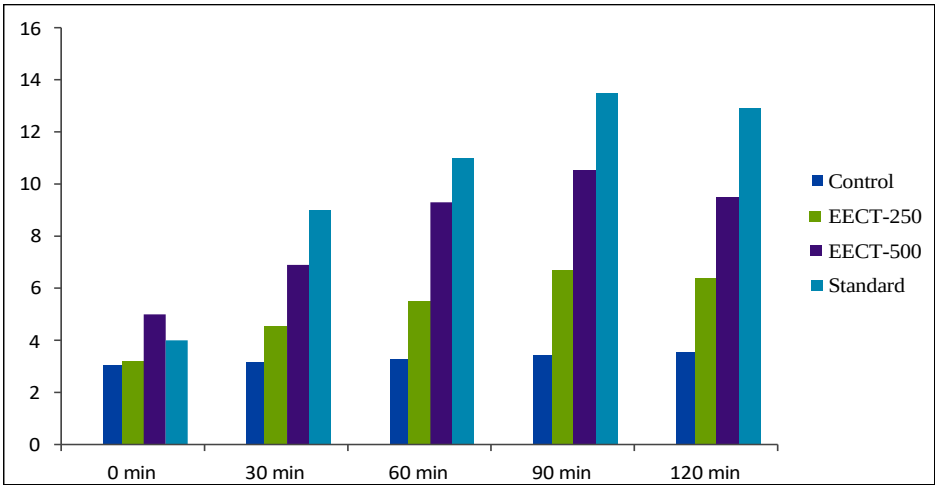

Fig 1: Effect of EECT Extract on tail flick test

2. Carrageenan Induced Paw Edema

The result represents in extract roots of EECT against carrageenan induced paw edema are given in below table.2

Table 2: Effect of extract of at 250 and 500 mg/kg doses against carrageenan induced paw edema in rat

Treatment (mg/kg)	Mean ± SEM MM					% Decrease
	0 min	30 min	60 min	90 min	120 min	
Control	3.35 ± 0.22	3.8± 0.15	4.2 ± 0.214	4.5 ± 0.167	5.2 ± 0.227	-
Extract- 250 mg/kg	3.5 ± 0.827 [b]	3.45 ± 0.799 [c]	3.1 ± 0.821 [b]	2.9 ± 0.822 [c]	2.5 ± 0.758 b	51.92%
Extract- 500 mg/kg	3.30 ± 0.22 [a]	3.55 ± 0.238 [c]	3.65 ± 0.262 ns	3.52 ± 0.238 ns	3.4 ± 0.254 a	34.23%
Indometha cin 20 mg/kg	3.45 ± 0.193 c	3.2 ± 0.170 c	2.9 ± 0.167 [c]	2.5 ± 0.134 [c]	2.2 ± 0.309c	57.69%

Values are represented as means ± SEM (n=6 in each group). Data expressed by using one-way ANOVA followed by p<0. 5.. [a]p<0.05, [b]p<0.01, [c]p<0.001 was considered as significant and n.s = non-significant as compared to control

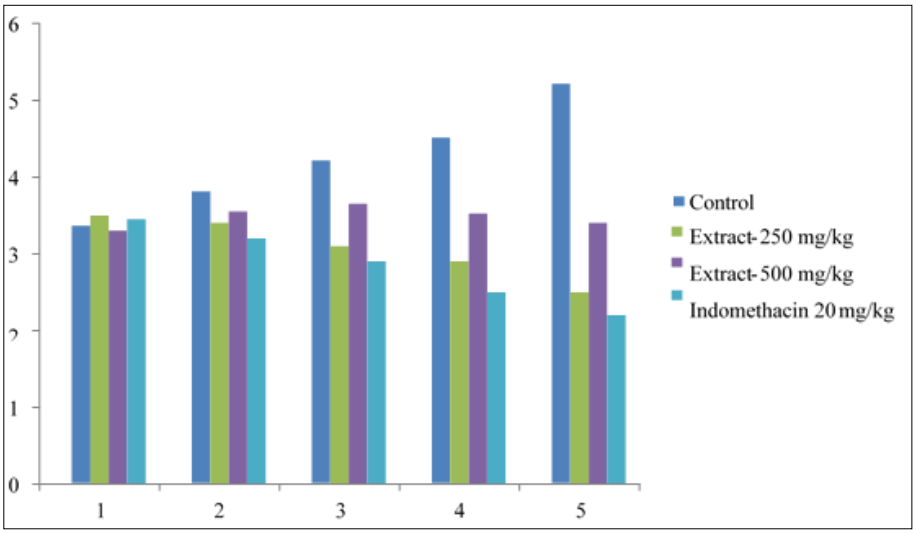

Fig 2: Anti-inflammatory activity of EECT extract on carrageenan induced paw edema

The result shown by extract of *EECT* against carrageenan induced paw edema are given in Table 6.3. Ethanolic extract at dose of 125 and 500 mg/kg showed non-significant reduction of paw oedema, but in ethanolic extract administered at a dose of 250 mg/kg, the paw volume was reduced by. The result demonstrated dose time related significant reduction by extract. Indomethacin 20 mg/kg similarly produced significant inhibitory effect of the paw

edema as compared to normal control group. The anti-inflammatory activity of the extract EECT against acute pedal oedema has been shown in Table 6.4 which showed significant anti-inflammatory activity and the results were comparable to that of control. It was observed that the ethanolic extract of EECT (250 mg/kg, *p.o.*) exhibits maximum anti-inflammatory activity against Carrageenan induced hind paw edema. The inhibition obtained with *C. tuberosum* was 51.92

Discussion

C. tuberosum is reported to contain chemical constituents which may exert analgesic and anti- inflammatory effect; however, till currently there were no data supporting the pharmacological properties of this plant. Therefore, the present investigation was designed to evaluate the use of C. tuberosum inpain and, inflammation. Ethanolic extracts of diverse polarities. Ethanolic extract of C. tuberosum were prepared and evaluated for their analgesic and anti-inflammatory activities. It is supposed that present anti-inflammatory medicine such as opioids and non-steroidal anti- inflammatory drugs are not useful in all cases because of their side effects and low potency. [86] As a result, investigating other alternatives became compulsory and essential. Novel anti-inflammatory agents might be revealed from medicinal plants containing a wide variety of phytoconstituents. Traditional medicine for the treatment of various diseases is becoming more popular. Many medicinal plants provide relief of symptoms comparable to that of conventional medicinal agents. This experiment employed three different doses of EECT extract for analgesic and anti-inflammatory screening namely minimum dose (250 mg), and maximum dose (500 mg). In the present study, ethanolic extract was studies for analgesic and anti-inflammatory activity by *in-vivo* method. Carrageenan has been widely used as a harmful agent able to induce experimental inflammation for the screening of compounds possessing anti-inflammatory activity. This phlogistic agent, when injected locally into the rat paw, produced a severe inflammatory reaction. [7] Carrageenan induced rat paw edema is a suitable *in vivo* model to predict the value of anti-inflammatory agents, which act by inhibiting the mediators of acute inflammation. [8] Carrageenan induced hind paw edema in rat is a biphasic event. The early phase of the inflammation is due to the release of histamine, serotonin and similar substances; and the later phase is associated with the activation of kinin-like substances, i.e., prostaglandins, proteases and lysosome. The ethanol extract of C. tuberosum inhibited the carrageenan induced rat paw edema formation, at both early and later phase. This result tends to suggest that the inhibitory effect of the extract on edema formation is probably due to the inhibition of the synthesis and/or release of the inflammatory mediators, especially the cyclooxygenase products. The carrageenan induced paw edema test is effectively controlled with the arachidonate cyclooxygenase (COX) inhibitors due to its COX-dependent mechanism, thus, it is suggested that the C. tuberosum may possess arachidonate COX inhibitory property.

Conclusion

C. tuberosum is reported to contain chemical constituents which may exert analgesic and anti- inflammatory effect; however, till currently there were no data supporting the pharmacological properties of this plant. Therefore, the present investigation was designed to evaluate the use of C. tuberosum inpain and, inflammation. Ethanolic extracts of diverse polarities. Ethanolic extract of C. tuberosum were prepared and evaluated for their analgesic and anti-inflammatory activities. It is supposed that present anti-inflammatory medicine such as opioids and non-steroidal anti- inflammatory drugs are not useful in all cases because of their side effects and low potency. [09] As a result, investigating other alternatives became compulsory and essential. Novel anti-inflammatory agents might be revealed from medicinal plants containing a wide variety of phytoconstituents. Traditional medicine for the treatment of various diseases is becoming more popular. Many medicinal plants provide relief of symptoms comparable to that of conventional medicinal agents. This experiment employed three different doses of EECT extract for analgesic and anti-inflammatory screening namely minimum dose (250 mg), and maximum dose (500 mg). In the present study, ethanolic extract was studies for analgesic and anti-inflammatory activity by *in-vivo* method. Carrageenan has been widely used as a harmful agent able to induce experimental inflammation for the screening of compounds possessing anti-inflammatory activity. This phlogistic agent, when injected locally into the rat paw, produced a severe inflammatory reaction [10]. Carrageenan induced rat paw edema is a suitable *in vivo* model to predict the value of anti-inflammatory agents, which act by inhibiting the mediators of acute inflammation. [11] Carrageenan induced hind paw edema in rat is a biphasic event. The early phase of the inflammation is due to the release of histamine, serotonin and similar substances; and the later phase is associated with the activation of kinin-like substances, i.e., prostaglandins, proteases and lysosome. The ethanol extract of C. tuberosum inhibited the carrageenan induced rat paw edema formation, at both early and later phase. This result tends to suggest that the inhibitory effect of the extract on edema formation is probably due to the inhibition of the synthesis and/or release of the inflammatory mediators, especially the cyclooxygenase products. The carrageenan induced paw edema test is effectively controlled with the arachidonate cyclooxygenase (COX) inhibitors due to its COX-dependent mechanism, thus, it is suggested that the C. tuberosum may possess arachidonate COX inhibitory property.

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