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ANTI-INFLAMMATORY AND ANALGESIC EFFECTS OF XANTHIUM INDICUM AND CHLORELLA VULGARIS IN ACUTE AND CHRONIC INFLAMMATORY MODELS

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ABSTRACT:
Medicinal plants and microalgae remain valuable sources for the discovery of anti-inflammatory and analgesic agents because of their diverse phytoconstituents. Xanthium indicum (Asteraceae) and Chlorella vulgaris, microalgae rich in bioactive proteins, polysaccharides, and pigments, have been traditionally associated with therapeutic potential; however, their comparative pharmacological profiles remain underexplored. The present study aimed to evaluate the anti-inflammatory and pain-relieving effects of X. indicum and C. vulgaris extracts using both acute and chronic experimental models. Swiss albino mice and Wistar rats are used as long-term animal models. Acute inflammation was induced using egg albumin and histamine-induced paw edema models, chronic inflammation was induced using a cotton pellet-induced granuloma model, and analgesic activity was evaluated using the acetic acid-induced writhing model. Extracts of X. indicum (100, 300, and 500 mg/kg) and C. vulgaris (50, 100, and 200 mg/kg) were administered orally and compared with standard drugs (ibuprofen and indomethacin). Both extracts produced significant, dose-dependent reductions in paw edema, granuloma weight, and writhing episodes ($p < 0.001$) compared to the control groups. X. indicum at 500 mg/kg and C. vulgaris at 300 mg/kg exhibited the most pronounced effects, approaching the standard drug efficacy. Cotton pellet granuloma inhibition reached 29.49% for X. indicum and 29.95% for C. vulgaris at their highest doses. Preliminary phytochemical screening revealed the presence of saponins, glycosides, and alkaloids in both extracts. These results support the potential development of X. indicum and C. vulgaris as alternative therapeutic agents for inflammatory illnesses by demonstrating their strong anti-inflammatory and analgesic potentials in both acute and chronic scenarios.

KEYWORDS: Chlorella vulgaris; Xanthium indicum; anti-inflammatory; analgesic; cotton pellet granuloma; oxidative stress

INTRODUCTION

Plants are a natural and traditional source of medicine in many regions of the world. Since ancient times, both doctors and ordinary people have used herbs to treat a wide range of human ailments [1]. In basic biological sciences, a large range of herbs, separately and in combination, have been thoroughly studied to assess their primary, supplemental, complementary, and synergistic effects on health and illness [2]. The public's interest in herbal goods is growing, and herbal therapy is currently the most widely used alternative [3]. Exploring traditional approaches to managing common ailments has been a subject of interest since ancient times. In modern society, a significant portion of both food and pharmaceuticals is derived from medicinal plants. Approximately 20,000 species across various plant families are recognized for their beneficial applications in these areas [4]. Living cells employ inflammation as a biological defence strategy to shield themselves from illnesses triggered by bacteria, fungi, viruses, physical factors, and compromised immune systems [5]. Common signs of inflammation include redness, pain, swelling, impaired cell function, and increased temperature. When the body experiences inflammation due to microbial infections, mechanical damage, or burns, human cells instinctively mount protective responses. The innate immune system employs both acute and chronic inflammation as natural defence strategies to preserve human health. Its primary goal is to deploy living cells to eradicate harmful agents and clear away damaged tissue, allowing the affected regions to heal effectively. The effectiveness of the inflammatory response is attributed to the generation of numerous mediators that manage the onset, development, continuation, regulation, and resolution of inflammatory processes. The genus Xanthium, belonging to the Family Asteraceae, comprises 25 species globally [6]. The Xanthium (koen) genus of flowering plants, which is indigenous to the Americas and Eastern Asia, is a member of the sunflower tribe of the flower family. However, widely distributed around the world. The leaves are in a spiral arrangement with sharply serrated borders. Some species, such as Xanthium spinosum, are very prickly due to long, thin spines at the base of their leaves. A wide range of aquatic life, including algae, plants, and animals, may be found in the seas, which comprise over 70% of the world. Microalgae are at the base of the aquatic food chain and provide oxygen to many aquatic organisms. Cyanobacteria are the oldest organisms on Earth, as they were the first to synthesize oxygen in the atmosphere [7].

Numerous microalgae have been employed for CO2 bio-fixation. CO2 fixation by microalgal species is more sustainable and ecologically more approachable than other CO2 removal methods. Furthermore, proteins, polysaccharides, PUFA, and photosynthetic pigments are abundant and useful materials found in these bacteria [8][9][10].

MATERIAL AND METHODS

Animal

The present study involved the use of Swiss albino mice and Wistar rats. These animals were housed in plastic cages, maintained at a consistent temperature of 22ºC and a relative humidity of 65%. They had unrestricted access to food and water. The care of the animals adhered to the standards and guidelines set by the Committee for the Purpose of Control and Supervision of the Experiments on Animals (CPCSEA). The IAEC, JSPMs, and Charak College of Pharmacy and Research in Wagholi, Pune, Maharashtra, India, approved the experimental protocols, (Protocol approval No. JSPM/CCOPR/IAEC/2019-20-1).

Drug and Chemicals

Ibuprofen (purchased from a local pharmacy), egg albumin, an aesthetic ether, Penicillin, Streptomycin, Indomethacin, Histamine, acetic acid, and aspirin were used in this study.

Egg albumin induced inflammation

To induce inflammation, 0.1 mL of egg albumin was injected into the subplantar region of the rats' right hind paw. The linear circumference of the paw was measured prior to the injection and at intervals of 0.5, 1, 2, 4, and 6 hours following the administration of the egg albumin [11].

Experimental design

Experimental design: 54 rats were randomly divided into seven groups of six animals each.
Group 1: saline-treated normal
Group 2: 0.1% egg albumin in a 1% saline solution
Group 3: standard (40 mg/kg of ibuprofen)
Groups 4 to 6 received 100, 300, and 500 mg/kg of Xanthium indicum extract, whereas Groups 7 to 9 received 50, 100, and 200 mg/kg of Chlorella vulgaris extract.

Cotton pellet-induced granuloma

Experimental design:
The animals were divided into nine groups, each consisting of six species. After the rats became unconscious, sterile cotton pellets measuring 10 ± 1 mg were inserted subcutaneously into the pelvic region on both sides of each rat.
Group I: Control
Group II: Standard
Group III to V: Starting from the day the cotton pellet was implanted, xanthium indicum extract was given orally at doses of 100, 300, and 500 mg/kg for a continuous period of seven days.
Group VI to VIII: Beginning on the day the cotton pellet was implanted, Chlorella vulgaris extract was given orally for a consecutive week at doses of 50, 100, and 200 mg/kg.

On the eighth day, the animals were euthanized, and the pellets along with granuloma tissues were meticulously removed and isolated from extraneous tissues. Initially, the wet pellets were weighed, then placed in an oven set at 60°C for 24 hours until their weight stabilized. Subsequently, the dried pellets were reweighed. An increase in the dry weight of the pellets was used to gauge the development of granulomas.
The following formula was used to calculate the protection.

$$\% \text{ Protection} = \frac{(\text{mean weight of granuloma in control} - \text{mean weight of granuloma in test group})}{\text{mean weight of granuloma in control group}} \times 100$$

Histamine-Induced Inflammation

Experimental design:
Rats were injected with 0.1 mL of 0.2% histamine in normal saline into the subplantar tissue of their right hind paw to induce inflammation. The test drugs were administered to the rats one hour prior to the induction of inflammation. The control group received 10 mL/kg of distilled water orally. The difference in paw volume between the control and 0.5, 1, 2, 3, and 4 hours after the inflammatory agent inhibition was administered was used to determine oedema [12].

Analgesic activity

Acetic acid induced writhing in mice

Experimental design:
Mice writhing due to acetic acid
Design of experiments:

For the present research, mice weighing between 25 and 30 grams were chosen, and they were split up into nine groups of six animals each [13][14].
Group I: Normal
Group II: Control
Group III: Standard
Group IV to VI: Oral administration of xanthium indicum extract at concentrations of 100, 300, and 500 mg/kg
Group VI to IX: Oral administration of Chlorella vulgaris extract at concentrations of 50, 100, and 200 mg/kg

A 1% v/v acetic acid solution was prepared using distilled water. Ibuprofen (dose: 100 mg/kg/10 ml) was dissolved in saline. Food was stopped 12 h before the medicine was administered and continued until the experiment was completed. The animals were numbered and weighed. Both the standard medication and the tests were given orally. After 60 minutes, an intraperitoneal injection of 1% acetic acid, at a dose of 0.1 ml per 10 g of body weight, led to writhing. During the 30-minute period when writhing episodes were recorded, movements such as back arching, torso elongation, and hind limb extension were noted. The following formula was used to calculate the percentage of inhibition:

$$\%\text{Inhibition}=\frac{(\text{mean of control group reading}-\text{mean of in test group reading})}{\text{mean of control group reading}}\times 100$$

Eddy's hot plate method
Albino mice exhibit heightened sensitivity in their paws to heat levels that do not harm the skin. The typical responses include jumping, withdrawing their paws, and licking them. A hot plate, which is available commercially, features a surface heated electrically. The temperature is maintained between 55 °C and 56 °C. This surface may be made of heated glass or a copper plate. A stopwatch was used to record the time taken for the animals to either lick or jump on the hot plate [14].
To assess analgesic efficacy, albino mice weighing 25-30 g were used, and six albino rats were housed in each group.

Experimental protocol
Nine groups, each consisting of six albino mice, were created.
Group I received the vehicle and consider as normal.
Group II served as the control group.
Group III animals received Diclofenac injections for the same duration at a dose of 10 mg/kg b.w.
Group IV to VI: Oral administration of Xanthium indicum extract at 100, 300, and 500 mg/kg Group VI to IX: Oral administration of the Chlorella vulgaris extract at concentrations of 50, 100, and 200 mg/kg
Food was removed 12 h before the drug was administered until the trial was completed. The animals were numbered and weighed. Both standard medications and tests were administered orally. The animals were placed on a hot plate after 60 min, and observations were made at 1, 2, 3, and 4 h.

RESULTS

Effect of Xanthium Indicum and Chlorella vulgaris on egg albumin-induced paw edema
In this study, Our study demonstrated that rats injected with egg albumin exhibited an increase in paw volume. A single dose of Xanthium Indicum, administered in response to egg albumin-induced paw inflammation, effectively mitigated the increase in paw volume. Notably, the group treated with 500 mg/kg of Xanthium Indicum showed significant improvement compared to other test groups. The 200 mg/kg CV dose had a more noticeable effect on carrageenan-induced paw inflammation. However, compared to the conventional medication shown in Figure 1, this effect was less effective.

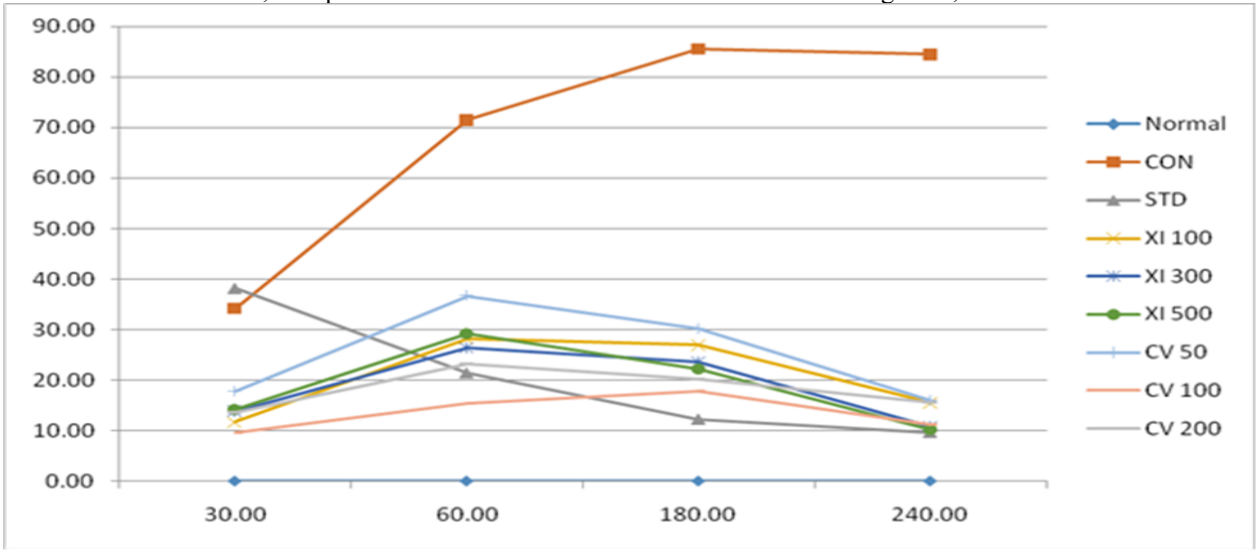

Figure 1: Effect of Xanthium Indicum and Chlorella vulgaris on egg albumin-induced paw edema

Effect of Xanthium Indicum and Chlorella vulgaris on Cotton pellet-induced granuloma

Granulomas and inflammation appeared in this cotton pellet granuloma animal model within a few days. Table 1 illustrates how XI and CV affect the proliferative phase of inflammation. Compared to the vehicle control group, XI and CV significantly reduced the formation of granulomas surrounding the pellet when tested for anti-inflammatory activity by lowering the wet and dry weights of the cotton pellet. Administering XI orally at dosages of 100, 300, and 500 mg/kg produced in dose dependent 100 % inhibition was 12.35% in wet weight of granuloma formation respectively, while the CV administration at doses 50, 100 and 200mg/kg, p.o resulted in dose dependent 100% inhibition was 14.82%. In case of dry weight of granuloma, XI produced a dose-dependent inhibition of 5.52, 20.73, and 29.49% at the doses used in the study. In addition, the CV-treated group displayed dose-dependent activity at 2.76, 17.51, and 29.95%. Indomethacin suppressed 40.09% of granulomas. The results of the model were significant, with $P < 0.001$ for wet weight inhibition and $P < 0.0002$ for dry weight inhibition of cotton pellet granuloma. As shown in Fig 2

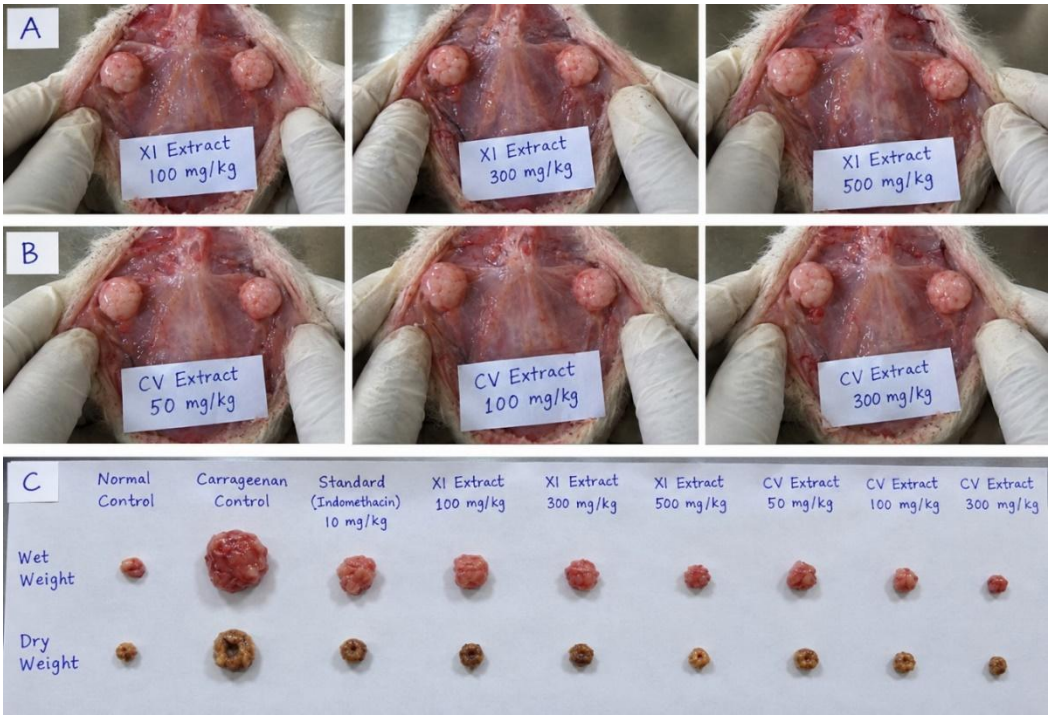

Fig 2. A) Animals exposed to cotton pellets during surgery, animals administered 100, 300, and 500 mg/kg of XI extract. B) After surgery, the animals were exposed to cotton pellets. Animals were administered 50, 100, and 200 mg/kg CV extract. and C) Group-specific photographs of wet and dry cotton pellets following surgical removal.

Table 1: Effect of Extract on Wet & Dry Weight of Granuloma in Cotton Pellet Granuloma in Mice

Groups	Wet weight of granuloma (mg)	Percentage Inhibition	Dry weight of granuloma (mg)	Percentage Inhibition
CON	170 ±2.198	00	54.25±1.652	00
STD	129.3±0.75	23.94118	32.5±0.645	40.09217
XI 100	168.8±1.109	0.705882	51.25±1.797	5.529954
XI 300	162.5±0.957	4.411765	43±1.08	20.73733
XI 500	149±2.273	12.35294	38.25±0.853	29.49309
CV 50	168±1.683	1.176471	52.75±1.25	2.764977
CV 100	158.3±1.931	6.882353	44.75±1.031	17.51152
CV 200	144.8	14.82353	38±0.9129	29.95392

Data was analyzed by One way ANOVA and represents as mean ± SEM, Dunnett's multiple comparison test,*P< 0.05, **P< 0.01 compared with control group.

Effect of Xanthium Indicum and Chlorella vulgaris on Histamine-Induced Inflammation

Figure 3 shows the anti-inflammatory effects of XI and CV against histamine-induced acute paw edema. The control group's paw inflammation peaked one hour after the histamine injection. Treatment with different doses of XI (100, 300, and 500 mg/kg, p.o.) and CV (50, 100, and 200 mg/kg) resulted in a statistically significant ($P < 0.001$) reduction in all phases of histamine-induced inflammation. XI 500 and CV 200 mg/kg, p. o., administered 1 h before the injection of histamine, showed a percentage increase in paw edema by 12.34 and 14.25 %, respectively, after 1 h of the injection of inflammatory stimulus. This result is similar to that observed for the same oral dose of the standard drug, which showed a 10.24% increase.

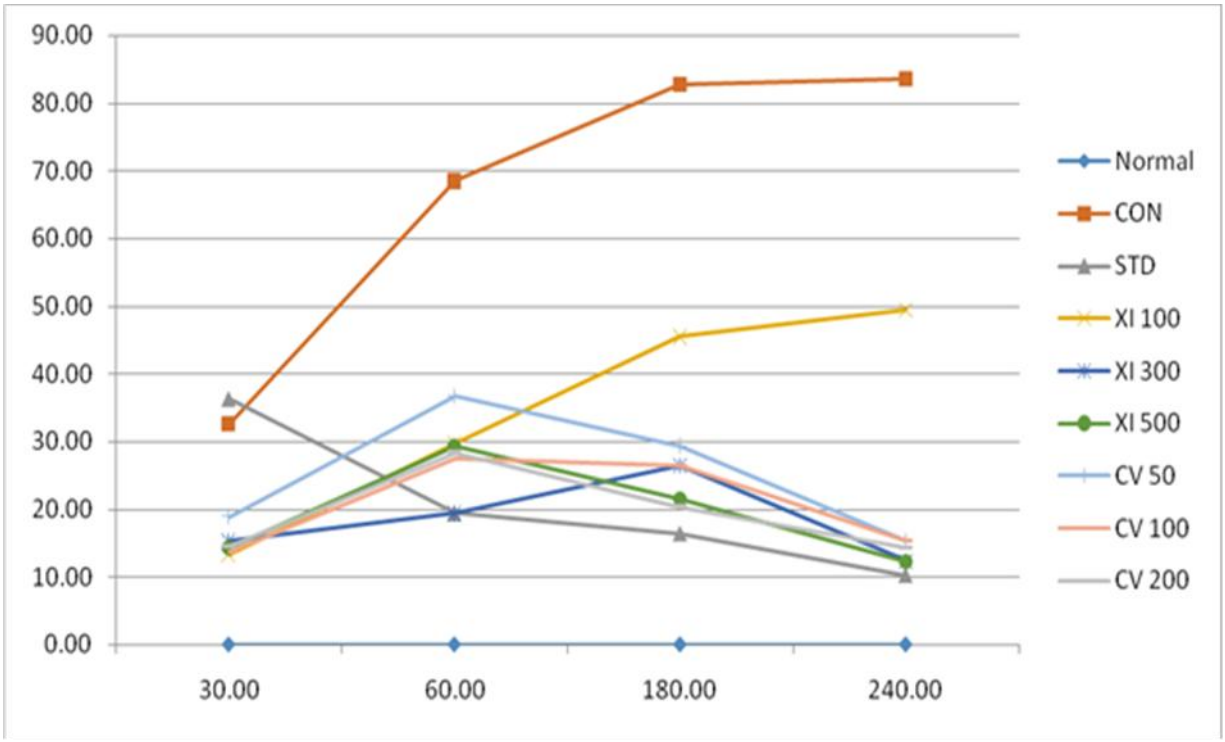

Figure 3: Chlorella vulgaris and Xanthium indicum's Impact on Histamine-Induced Inflammation

One-way Way ANOVA and Dunnet's test were used to analyze the data, and the results are shown in (mean ± SEM) with a p-value of 0.005 being deemed significant

Acetic acid induced writhing in mice

Mice writhing due to acetic acid administration. Figure 4 illustrates the impact of XI and CV on the pain behavior of the writhing response, which was displayed as cumulative abdominal stretching. Abdominal writhing caused by acetic acid was significantly ($P < 0.001$) and dose-dependently inhibited in mice treated with XI (100, 300, and 500 mg/kg, p.o.) and CV (50, 100, and 200 mg/kg). XI500 and CV200 mg/kg p.o. produced inhibition that was almost identical to that of a typical medication. XI and CV showed a considerable delay in the start of writhing at all tested doses and with the usual medication.

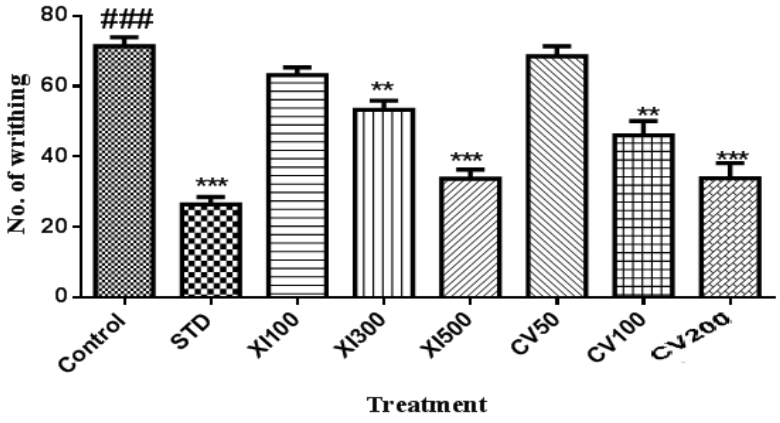

Figure 4: Effect of XI and CV on pain behavior of writhing response

Effect of X. indicum and C. vulgaris on Pain Inhibition Percentage in Hot Plate Test in Mice

Eddy's hot plate test was used to evaluate the analgesic efficacy of Xanthium indicum and Chlorella vulgaris, as shown in Figure No. 5. the percentage of pain inhibition at 1, 2, 3, and 4 h following oral treatment. The typical medication was diclofenac (10 mg/kg). Compared to the control group, both extracts showed considerable ($P < 0.01$) analgesic efficacy. The control group showed minimal pain inhibition throughout the experimental period (4.3 ± 0.14%, 3.7 ± 0.23%, 2.5 ± 0.24%, and 0.93 ± 0.21% at 1, 2, 3, and 4 h, respectively). Among the Chlorella vulgaris treatment groups, the analgesic effect increased with increasing dose. The 200 mg/kg dose produced the greatest response, showing 41 ± 0.68%, 61 ± 7.6%, 43 ± 0.47%, and 47 ± 0.71% pain inhibition at 1, 2, 3, and 4 h, respectively. Similarly, Xanthium indicum exhibits dose-dependent analgesic activity. At 1, 2, 3, and 4 h, the maximum dose (500 mg/kg) resulted in pain inhibition percentages of 42 ± 0.79%, 59 ± 6.9%, 49.1 ± 0.47%, and 47 ± 0.88%, respectively.

Group	**Pain Inhibition Percentage (Mean ± SEM)**			
	1 h	2 h	3 h	4 h
Control	4.3 ± 0.14	3.7 ± 0.23	2.5 ± 0.24	0.93 ± 0.21
Diclofenac (10 mg/kg, p.o.)	45 ± 1.4**	69 ± 2.9**	61 ± 1.8**	56 ± 1.5**
Chlorella vulgaris (50 mg/kg, p.o.)	27 ± 0.67**	45 ± 1.9**	40 ± 1.6**	32 ± 0.88**
Chlorella vulgaris (100 mg/kg, p.o.)	39 ± 0.45**	55 ± 0.35**	50 ± 0.64**	42 ± 0.40**
Chlorella vulgaris (200 mg/kg, p.o.)	41 ± 0.68**	61 ± 7.6**	43 ± 0.47**	47 ± 0.71**
Xanthium indicum (100 mg/kg, p.o.)	31 ± 0.76**	39 ± 1.3**	35 ± 1.2**	33 ± 0.68**
Xanthium indicum (300 mg/kg, p.o.)	38 ± 0.45**	50 ± 0.52**	44 ± 0.92**	42 ± 0.50**
Xanthium indicum (500 mg/kg, p.o.)	42 ± 0.79**	59 ± 6.9**	49.1 ± 0.47**	47 ± 0.88**

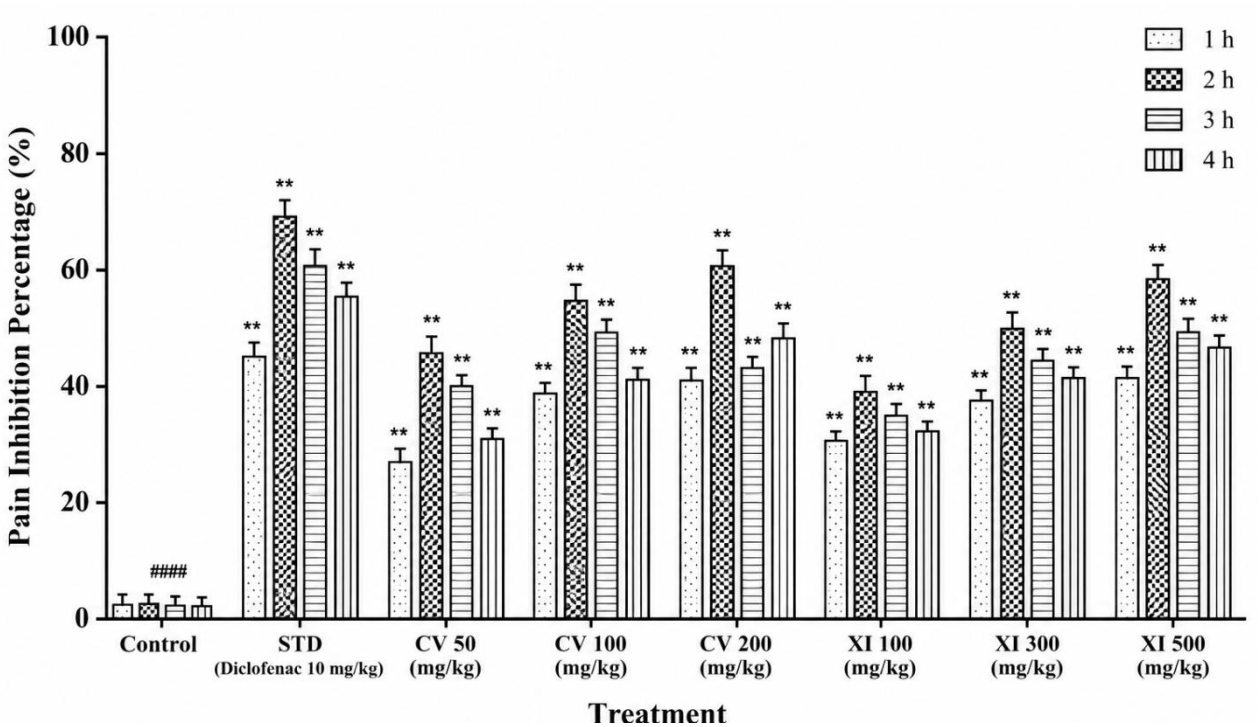

Figure 5: Eddy's hot plate method bar graph illustrating mean values for various treatment groups for analgesic activity

The values are expressed as mean ± SEM (n = 6). Dunnett's multiple comparison test was performed after one-way ANOVA. $P < 0.0001$ overall; *$P < 0.05$; **$P < 0.01$ compared to the control.

DISCUSSION AND CONCLUSION

To substantiate the medicinal uses of Xanthium indicum and Chlorella vulgaris, we investigated their potential to reduce inflammation and alleviate pain in rodents. This involved employing various experimental models to assess the extracts' effectiveness in rats and mice. The egg albumin-induced paw edema model demonstrated that both Xanthium indicum (XI) and Chlorella vulgaris (CV) significantly reduced acute inflammation in a dose-dependent manner by suppressing histamine, serotonin, and prostaglandin levels, which are examples of inflammatory mediators. The highest anti-inflammatory activity was observed with XI (500 mg/kg) and CV (200 mg/kg) administration. In the cotton pellet granuloma model, both extracts significantly decreased the wet and dry weights of granuloma tissue, indicating the inhibition of chronic inflammation, fibroblast proliferation, and collagen deposition. Although indomethacin produced greater inhibition, XI and CV exhibited promising anti-proliferative and anti-inflammatory effects. In the histamine-induced paw edema model, XI and CV effectively reduced vascular permeability and inflammatory exudation, suggesting possible antihistaminic activity and inhibition of early inflammatory signaling .The writhing test induced by acetic acid revealed that both extracts exhibited peripheral analgesic effects by inhibiting inflammatory mediators like prostaglandins. This was evidenced by a significant decrease in the number of abdominal writhes and a postponement in the onset of pain. XI and CV exhibited central analgesic action in the Eddy's hot plate test, significantly increasing the pain threshold in a dose-dependent manner. The combined results of the writhing and hot plate tests indicated that both extracts had notable peripheral and central analgesic effects. Overall, the results provide initial pharmacological evidence for the analgesic and anti-inflammatory properties of X. indicum and C. vulgaris. Nevertheless, comprehensive phytochemical characterization, extract standardization, toxicity assessment, and mechanism-oriented investigations are required to identify the active constituents, establish their molecular targets, and confirm their therapeutic safety. These results could serve as a basis for the development of standardized natural-product-based interventions for managing inflammatory conditions and associated pain.

Conflict Of Interest

The authors declare no conflicts of interest.

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