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Antinociceptive Effect of Ethanolic Extract of *Codiaeum variegatum* Leaves in Mice

Md. Asrafuzzaman, Ambia Khatun, Md. Abdul Mannan*

Department of Pharmacy, Stamford University Bangladesh, Dhaka, Bangladesh
Email: rozenasrafuzzaman240@gmail.com, arin_ambia@yahoo.com, *manna.034@gmail.com

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Abstract

Background: *Codiaeum variegatum*, sometimes called garden croton, is a tropical plant in the Euphorbiaceae family. Historically used to cure various conditions, including intestinal infections, fever, ulcers, wounds, and gonorrhea. This work aimed to investigate the antinociceptive effects of ethanolic extract of *Codiaeum variegatum* leaves (EECV) in animal models. **Methods:** Five different pain models—the hot plate, tail immersion, acetic acid-induced writhing, formalin, and glutamate-induced nociception tests—were utilized to assess the antinociceptive activity in mice. The traditional drugs such as diclofenac sodium (10 mg/kg, i.p.) and morphine sulphate (5 mg/kg). EECV was administered orally at varying doses of 100, 200, and 300 mg/kg (0.1 mL/mouse), while the control group was given deionized water. **Results:** The current study found that all mouse models of heat- and chemical-induced pain had robust EECV reflections of their antinociceptive properties (*$p < 0.05$). In the hot plate and tail immersion tests, 200 and 300 mg/kg showed a significant (*$p < 0.05$) capacity to delay the reaction of latency to pain in contrast to thermally induced nociception. The levels of inhibition in the acetic acid-induced writhing test were 20.07%, 44.86%, and 59.87% for 100, 200, and 300 mg/kg doses, respectively. The extract prevented 18.89%, 44.88%, and 59.84% of licking during the early stages of formalin-induced nociception at varying doses of 100, 200, and 300 mg/kg. Compared to the control group, the extract exhibited higher licking rates throughout the late phase (28.78%, 48.48%, and 54.54%). The plant extract considerably (*$p < 0.05$) reduced the glutamate-induced nociception test. Compared to the control, it showed 13.07%, 52.30%, and 76.92% for 100, 200, and 300 mg/kg doses, respectively. **Conclusions:** The current finding offers a fresh perspective on the ethanolic extract of *Codiaeum variegatum* leaves' antinociceptive properties in mice. This plant's phytochemical analysis revealed the presence of triterpenoids, sterols, alkaloids, flavonoids, and general glycosides, all of which may have antinociceptive properties. More research on the mechanism of action and associated pharmacological

studies, such as *in vivo* analysis, medication formulation, and clinical trials, is strongly advised.

Keywords

Codiaeum variegatum, Extract, Phytochemistry, Antinociceptive

1. Background

An important aspect of creating potent medications is through the use of therapeutic herbs. Approximately 80% of people in underdeveloped nations still receive their main medical treatment from traditional medicine, which is mostly focused on plant and animal species. The demand for herbal medications is now high, and it is growing every day. Approximately 500 plants have been used medicinally in ancient texts, and about 800 species have been included in traditional medical systems. Herbal medications, often known as plant materials or herbals, heal wounds or ailments using whole plants or plant components [1]. A plant's ability to affect human physiological functions is attributed to the presence of a chemical compound. These substances fall into two groups: main and secondary metabolites. Metabolic processes produce secondary metabolites that are crucial to a plant's defense mechanism, whereas primary metabolites are required for a plant's growth and development. Alkaloids, carbohydrates, glycosides, steroids, flavonoids, coumarins, saponins, fatty acids, tannins, protein and amino acids, gum and mucilage, terpenoids, anthraquinone, and phenols are examples of compounds that are secondary metabolites. Research on these phytochemicals is crucial because they may be used to create new medications [2].

Garden croton, or *Codiaeum variegatum* (*C. variegatum*), is a plant belonging to the Euphorbiaceae family that grows in tropical regions such as Malaysia, Indonesia, Philippines, Thailand, Sri Lanka, India, and certain other Pacific Islands [3]. Used historically in tropical nations all over the world to cure gonorrhea, a sexually transmitted illness, using a prepared liquid extracted from the leaves. Bathing the patient in a green solution made of boiling leaves can help reduce fever. Direct application of sap is used to heal sores, while wounds are treated by preparing the root [4]. To relieve discomfort, apply a mixture of crushed *Codiaeum variegatum* leaves and mustard oil to the afflicted regions [5]. This plant's leaves and bark can be used to treat intestinal illnesses [6]. Moreover, this plant exhibits larvicidal properties against the mosquito that transmits dengue, chikungunya, and zika, Aedes aegypti [7]. Numerous features, including antioxidant [8], antilithiasis [9], anti-amoebic [10], anti-influenza [11], and anticonvulsant activity [12], as well as the absence of sub-chronic toxicity of up to 200 mg/kg [13], have been reported in a variety of experiments on different leaf extracts of *C. variegatum*. Additionally, several secondary metabolites were found in the screening of leaves for phytochemicals [3], including phenolic compounds, which were

widely recognized for their anti-inflammatory, immunomodulatory, and antioxidant qualities [14].

Alkaloids, anthraquinone, flavanoids, terpenes, steroids, phenol, saponins, tannins, phlobatannin, and cardenolide are also present in *Codiaeum variegatum*. In brian shrimp lethality bioassays, it shows strong cytotoxicities [15]. The free radical scavenging and antioxidant properties of phytochemicals, particularly polyphenols (flavonoids, tannins, phyenyl propanoids, phenolic acids, etc.), are well recognized. The scavenging capacity of phenolic compounds makes them well-known plant components. Polyphenolic substances have been shown to have inhibitory effects on the processes involved in carcinogenesis and mutagenesis. Additionally, in vitro research revealed that polyphenols could prevent the growth and formation of tumors by inducing cell cycle arrest and death in cancer cells or by functioning as prooxidants on cancer cells [16].

On the other hand, *C. variegatum* has been the subject of much research and is a highly valuable medicinal plant. In other words, this plant possesses a variety of therapeutic uses. Depending on the plant part and solvent utilized, the activity of different plant parts may vary due to the variable extracted chemicals and the uneven distribution of secondary metabolites. Consequently, the goal of the current investigation was to assess the ethanolic extract of *Codiaeum variegatum* leaves' antinociceptive potential in mice.

2. Methods

2.1. Plant Material and Extraction

Fresh *Codiaeum variegatum* leaves were gathered in March 2022 in Ramna Park, Dhaka, Bangladesh. The plant samples were subsequently identified and confirmed by Md. Asrafuzzaman of the Salar Khan Herbarium at Dhaka University, Bangladesh (Flora of Bangladesh). The Herbarium already has a voucher specimen number for future reference. The fresh leaves were crushed into a fine powder after being dark-dried for a week. 1200 ml of ethanol and 165.48 g of powdered dry leaves were macerated for three days at 25˚C ± 2˚C in a beaker with occasional stirring. Next, the extract was filtered using Whatman No. 1 filter paper and sterilized cotton filters. Next, the extract was filtered using Whatman No. 1 filter paper and sterilized cotton filters. The solvent was completely evaporated using a rotary evaporator (BC-R 201 Shanghai Biochemical Equipment Co. Ltd.), producing 29 g of extract. This extract was used in studies on acute toxicity and antinociceptive effects.

2.2. Animals

Swiss albino mice (20 - 30 g) were donated by the Animal Research Branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDR, B). Animals were kept in regular laboratory conditions (room temperature: 25˚C ± 2.0˚C, relative humidity: 55% - 65%, and 12-hour light/dark cycle) with food and

drink provided during the adaptation period. The animals had to spend two weeks getting acclimated to the lab environment before the research could begin. Mice were starved for the whole night before the trials began. The Ethical Principles and Guidelines for Scientific Experiments with Animals, published in 1995 by the Swiss Academy of Sciences and the Swiss Academy of Medical Sciences, governed the treatment of all experimental animals. The Stamford University Bangladesh Ethics Committee (SUB/IAEC/24) authorized all experimental procedures.

2.3. Drugs and Treatments

The control group received an oral dose of 0.1 mL/mouse of deionized water before the testing. The standard drug morphine in hot plate, tail immersion test, and acetic acid-induced writhing was given intraperitoneally (i.p.) to the positive control group at a dose of 5 mg/kg. 15 minutes before the experiments began, the same group received a dose of 10 mg/kg of diclofenac sodium in formalin, glutamate, and licking tests. EECV was administered orally at doses of 100, 200, and 300 mg/kg (b.w.) 30 minutes before to the experiments. To prepare each dosage of medicine and EECV, deionized water was utilized.

2.4. Phytochemical Screening

The crude ethanolic extract of *Codiaeum variegatum* (EECV) included alkaloids, flavonoids, glycosides, carbohydrates, steroids, tannin, reducing sugar, and saponin, as determined by accepted techniques [17].

2.5. Acute Toxicity Test

The presence of poisonous chemicals in the sap, which is created when the plant's above-ground sections sustain mechanical injury, is linked to *C. variegatum*'*s* dangerous characteristics. Conversely, the safety of other types may be associated with the variety of phytochemicals found in different cultivars or the lower extraction of toxic ingredients during decoction, the traditional way of making therapeutic recipes. In an acute toxicity assay, *C. variegatum* extract was administered to mice of both sexes at dosages of 100 - 4000 mg/kg b.w., respectively, and showed no adverse effects. This opinion is that *C. variegatum* can be utilized therapeutically in traditional healthcare settings without having any noticeable negative effects [18].

3. Antinociceptive Activity Test

3.1. Hot Plate Test

A favorite approach to examine possible centrally mediated analgesic effects was the hot plate test [19]. From the animals, five groups of five mice each were formed. The mice were placed on Eddy's hot plate, which was kept at 52˚C ± 1˚C. They were then given one of three treatments: morphine as a conventional drug (5 mg/kg, i.p.), a control (equivalent volume of deionized water, 0.1 mL/mouse,

p.o. or EECV (100, 200, and 300 mg/kg, p.o.). A 20-second time limit was maintained to preserve the tissue of the paws. Forepaw licking, paw withdrawal symptoms, or jumping were the reported reactions at 0, 30, 60, 90, and 120 minutes post-treatment. The percentage of the maximum potential impact (% MPE) was then calculated using the following formula:

% MPE = [(Post-drug latency) − (Pre-drug latency) / (Cut off time) − (Pre-drug latency)] × 100.

3.2. Tail Immersion Test

The tail immersion test is based on the finding that drugs that resemble morphine particularly prolong the typical reaction time of the mouse tail withdrawal reflex. This method was applied to evaluate the main mechanism behind the effectiveness of analgesics. The animals' agonizing reactions were brought on by the thermal incentive in this case—dipping their tail tips into hot water [20]. Divide the mice into the five groups, five mice each were employed. The technique called for submerging 1 to 2 cm of the tails of mice that had been pretreated with morphine (5 mg/kg, i.p.) or EECV (100, 200, and 300 mg/kg, p.o.) in warm water that was continuously maintained at 54˚C. The amount of time the tail took to deflect after it had sunk was observed. The mice's tail tissue was safeguarded by maintaining a 20-second delay interval. The latency length of the tail-withdrawal reflex was assessed at 0, 30, 60, 90, and 120 minutes following the administration of morphine and EECV. This was done to evaluate the potency of the analgesic. The % MPE was then calculated using the same formula as the hot plate test.

3.3. Acetic Acid-Induced Writhing Test

The peripheral analgesic effect of EECV in chemically induced pain was assessed using this test [21]. There were five animal groupings made (n = 5). On the other hand, writhing was brought on by injecting 0.6% acetic acid 15 minutes after drug administration and 30 minutes after oral dose of EECV. Diclofenac sodium, 10 mg/kg, intravenously; control; or EECV, 100, 200, or 300 mg/kg, orally. After the acetic acid injection, the mice were observed for five minutes, and the number of writhing animals was counted for thirty minutes [22]. The stomach squeezing, the body stretching, the trunk and pelvis twisting, and the limbs extending were all considered components of complete writhing. The analgesic activity was calculated as the percentage of inhibition of abdominal constriction.

3.4. Formalin Test

To use it as narration, Santos & Calixto and Santos *et al.* [23] [24] significantly altered the process. In all, there were five animal groups (n = 5). The control group received an oral dose of 0.1 mL/mouse of deionized water before the testing. Twenty microliters of 2.5% formalin (in deionized water, subplantar) were subcutaneously injected into the right hind paw of the mice one hour after EECV treatment (100, 200, and 300 mg/kg, p.o.) and fifteen minutes after the injection

of diclofenac sodium (10 mg/kg, i.p.). The length of time spent biting and licking the injected paw was recorded as a measure of pain response. Responses were observed for five minutes (first phase, neurogenic) and for fifteen to thirty minutes (second phase, inflammatory) following the injection of formalin. Antinociceptive activity was determined by calculating the percentage inhibition of licking time.

3.5. Glutamate-Induced Nociception

The method employed was comparable to the one previously detailed by Beirith *et al.* [25]. From the group of mice, five groups of five mice each were formed. The mice were given a volume of 20 L of glutamate solution (10 mol per paw) after being treated with EECV (100, 200, and 300 mg/kg, p.o.) for 30 min., followed by diclofenac sodium (10 mg/kg, i.p.) for 15 min. The control group received an oral dose of 0.1 mL/mouse of deionized water before the testing. After receiving a glutamate injection, the mice were observed on their own for fifteen minutes. It demonstrated nociception by licking its injected paw often.

3.6. Statistical Analysis

The results are displayed as mean SEM. The statistical analysis was performed using the SPSS 18.00 software and one-way analysis of variance (ANOVA), with Dunnett's post hoc test applied as needed. A significance level of ***$p < 0.001$ was used for the differences between the groups.

4. Results

4.1. Phytochemical Screening

Phytochemical analysis of *Codiaeum variegatum* crude extract revealed the presence of tannin, reducing sugar, flavonoids, glycosides, carbohydrates, steroids, and saponins (Table 1).

Table 1. *Codiaeum variegatum* ethanolic extract preliminary qualitative phytochemical screening (EECV).

Extract	EECV
Alkaloid	–
Flavonoid	+
Glycoside	+
Carbohydrate	+
Steroid	+
Tannin	+
Reducing Sugar	+
Saponin	+

EECV = Ethanolic Extract of *Codiaeum variegatum*; (+): Present; (–): Absent.

4.2. Acute Toxicity

Oral EECV therapy at doses ranging from 100 - 4000 mg/kg did not cause any death, allergic reactions, salivation, convulsions, tremors, diarrhea, or aberrant behavior throughout the observation period. Moreover, no macroscopic abnormalities or statistically significant changes were seen in any of the mice's key organs between the experimental and control groups.

4.3. Hot-Plate Test

Figure 1 and Table 2 demonstrate that EECV had a statistically significant antinociceptive effect (*$p < 0.05$) at the doses of 200 mg/kg and 300 mg/kg. Furthermore, the administration of morphine at a dose of 5 mg/kg showed a significant antinociceptive effect (**$p < 0.01$) in comparison to the control group (deionized water).

4.4. Tail Immersion Test

The results of the tail-immersion test indicated a significant antinociceptive effect (***$p < 0.001$) at doses of 100 mg/kg, 200 mg/kg, and 300 mg/kg in comparison to control. The antinociceptive effects of EECV at dosages of 100 mg/kg, 200 mg/kg, and 300 mg/kg were comparable to those of the reference drug (Figure 2 and Table 3). When compared to the control group that drank deionized water, morphine dramatically decreased pain (***$p < 0.001$).

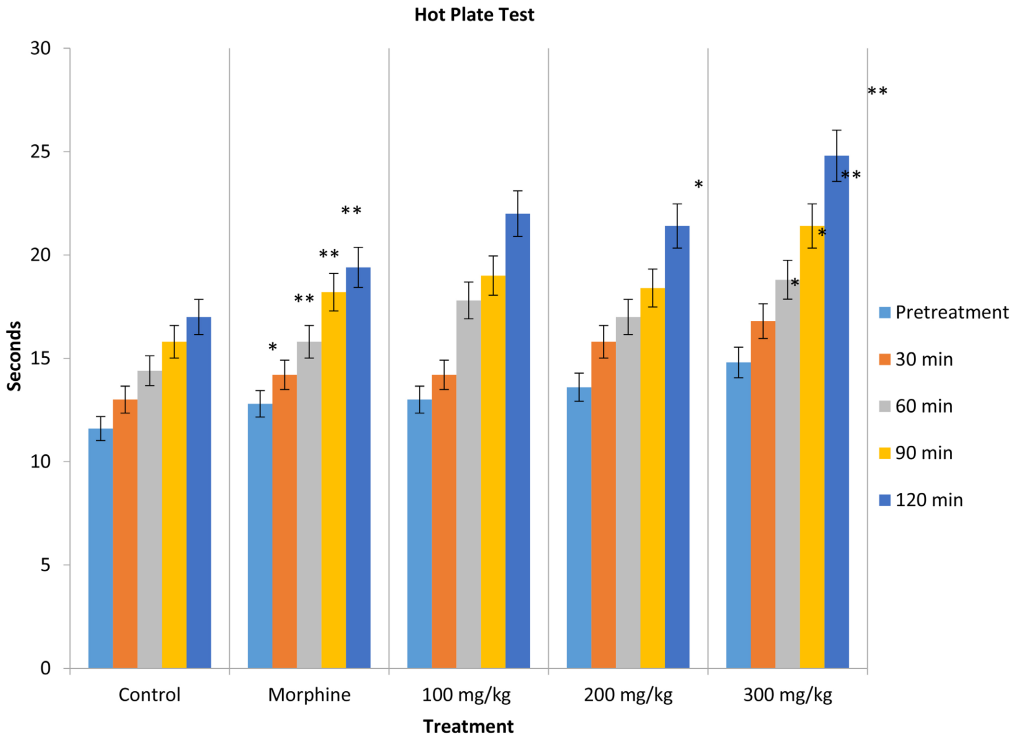

Figure 1. Antinociceptive effect of *Codiaeum variegatum* leaves extract and morphine in hot plate test. Values are presented as mean ± SEM (n = 5). **$p < 0.01$ compared with the control group (ANOVA followed by post hoc Dunnett's test).

Table 2. Antinociceptive effect of leaf extract of *Codiaeum variegatum* on hot plate test.

Treatment	Dose (mg/kg)	Latency of nociceptive response (in seconds)				
		0 min	30 min	60 min	90 min	120 min
Control	0.1 ml/mouse	11.60 ± 0.74	13.00 ± 0.70	14.40 ± 1.20	15.80 ± 1.88	17.00 ± 1.30
Standard (Morphine)	5	12.80 ± 1.15	14.20 ± 1.06**	15.80 ± 1.06*	18.20 ± 0.58**	19.40 ± 0.40**
EECV	100	13.00 ± 0.83	14.20 ± 1.39	17.80 ± 1.20	19.00 ± 0.44	22.00 ± 0.83
EECV	200	13.60 ± 0.67	15.80 ± 2.03	17.00 ± 1.54	18.40 ± 0.81*	21.40 ± 0.67*
EECV	300	14.80 ± 1.06	16.80 ± 1.06	18.80 ± 0.80*	21.40 ± 0.98**	24.80 ± 1.46**

Values are presented as mean ± SEM (n = 5). EECV = Ethanolic extract *of Codiaeum variegatum*; **p < 0.01 compared with the control group (Dunnett's test), *p < 0.05 compared with the control group (Dunnett's test).

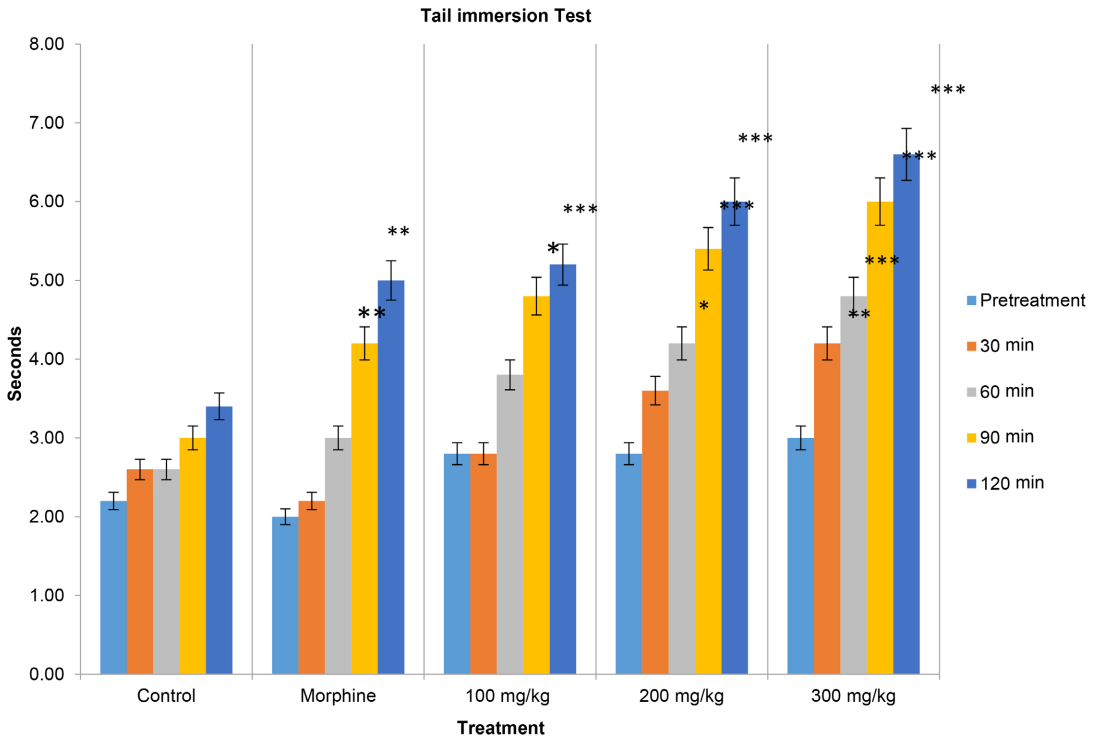

Figure 2. Antinociceptive effect of *Codiaeum variegatum* leaves extract and morphine in tail immersion test. Values are presented as mean ± SEM (n = 5). ***p < 0.001 compared with the control group (ANOVA followed by post hoc Dunnett's test).

Table 3. Effect of leaf extract of *Codiaeum variegatum* extract on tail immersion test.

Treatment	Dose	Response Time (in seconds)				
		0 min	30 min	60 min	90 min	120 min
Control	0.1 ml/mouse	2.20 ± 0.20	2.60 ± 0.40	2.60 ± 0.40	3.00 ± 0.31	3.40 ± 0.51
Standard	5 mg/kg	2.00 ± 0.00	2.20 ± 0.20**	3.00 ± 0.44**	4.20 ± 0.37***	5.00 ± 0.31***
EECV	100 mg/kg	2.80 ± 0.37	2.80 ± 0.58	3.80 ± 0.73	4.80 ± 0.24*	5.20 ± 0.37***
EECV	200 mg/kg	2.80 ± 0.37	3.60 ± 0.92*	4.20 ± 0.37***	5.40 ± 0.24***	6.00 ± 0.31***
EECV	300 mg/kg	3.00 ± 0.44	4.20 ± 0.37**	4.80 ± 0.37***	6.00 ± 0.44***	6.60 ± 0.51***

Values are presented as mean ± SEM (n = 5). EECV = Ethanolic extract of *Codiaeum variegatum*; ***p < 0.001 compared with the control group (Dunnett's test), **p < 0.01 compared with the control group (Dunnett's test), *p < 0.05 compared with the control group (Dunnett's test).

4.5. Acetic Acid-Induced Writhing Test

The effects of EECV therapy on the abdominal constriction test in mice are shown in Figure 3 and Table 4. When compared to the control group (Deionized water), it was demonstrated that EECV was able to significantly inhibit the nociceptive effects caused by acetic acid at doses of 100 mg/kg, 200 mg/kg, and 300 mg/kg, respectively (***p < 0.001). It was shown that different doses of diclofenac sodium may block constrictions to different degrees: 10 mg/kg, 20.07% EECV, 100 mg/kg, 44.86% EECV, 200 mg/kg, and 59.87% EECV, 300 mg/kg (Table 4).

Table 4. Effect of leaf extract of *Codiaeum variegatum* extract on acetic acid-induced abdominal writhing test.

Treatment	Dose (mg/kg)	Mean ± SEM	% of Inhibition
Control	0.1 ml/mouse	61.3 ± 0.90	0.00
Diclofenac Sodium	10	20.6 ± 0.57***	66.39
EECV	100	49.0 ± 1.63***	20.07
EECV	200	33.8 ± 1.84***	44.86
EECV	300	24.6 ± 1.13***	59.87

Values are expressed as Mean ± SEM (n = 5); EECV = Ethanolic extract *of Codiaeum variegatum*; ***p < 0.001 compared with the control group (Dunnett's test), **p < 0.01 compared with the control group (Dunnett's test).

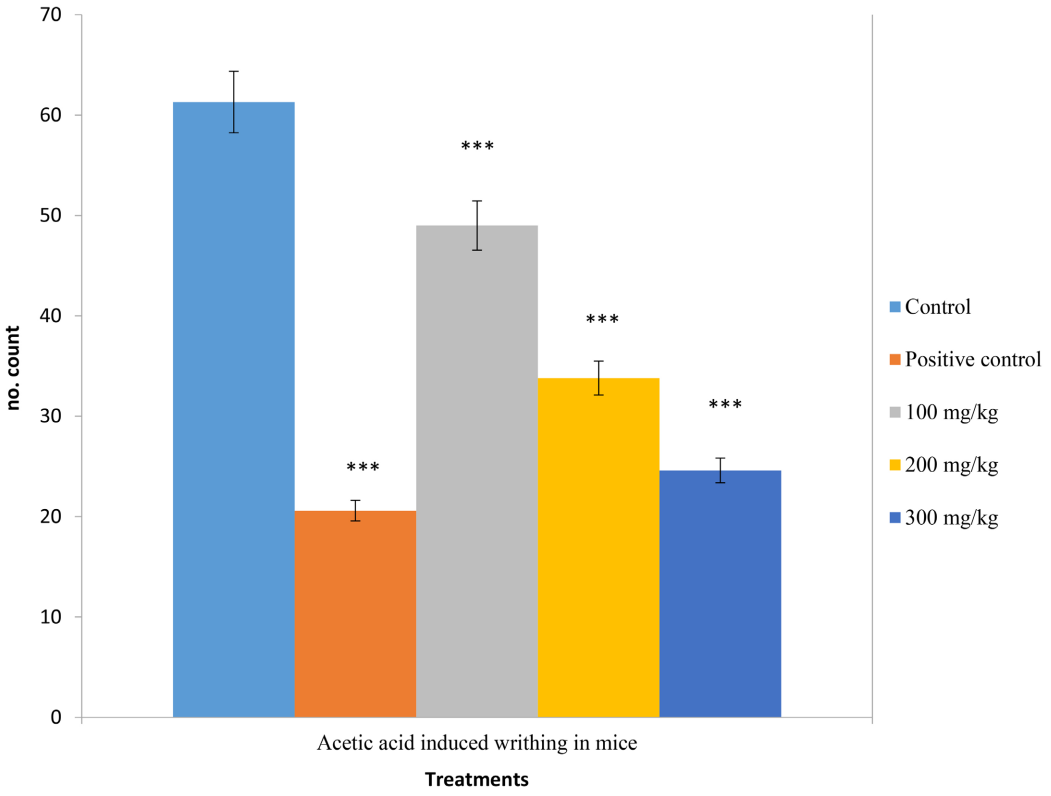

Figure 3. Antinociceptive effect of *Codiaeum variegatum* leaves extract in acetic acid-induced writhing. All values are presented as mean ± SEM (n = 5). ***p < 0.001 compared with the control group (ANOVA followed by post hoc Dunnett's test).

4.6. Formalin Test

In the formalin-induced licking test, EECV substantially decreased both the neurogenic (0 - 5 min) and inflammatory (15 - 30 min) phases, as well as formalin-induced nociception when compared to the control group (Deionized water) (**Figure 4** and **Table 5**). Nonetheless, the antinociceptive effect became more apparent in the second phase of this pain model. Diclofenac sodium (10 mg/kg, i.p.) significantly reduced formalin-induced nociception in all stages (***p < 0.001).

Table 5. Antinociceptive effects of *Codiaeum variegatum* leave extract in formalin-induced nociception.

Treatment	Dose (mg/kg)	Licking of the hind paw			
		Early phase	% of inhibition	Late phase	% of inhibition
Control	0.1 ml/mouse	25.40 ± 1.29	0.00	13.20 ± 0.49	0.00
Diclofenac sodium	10	09.40 ± 0.51***	62.99	04.20 ± 0.80***	68.18
EECV	100	20.60 ± 1.12*	18.89	09.40 ± 0.68***	28.78
EECV	200	14.00 ± 1.30*	44.88	06.80 ± 0.58***	48.48
EECV	300	10.20 ± 0.58*	59.84	06.00 ± 0.71***	54.54

Values are presented as mean ± SEM (n = 5). EECV = Ethanolic extract of *Codiaeum variegatum*; ***p < 0.001 compared with the control group (Dunnett's test), *p < 0.05 compared with the control group (Dunnett's test).

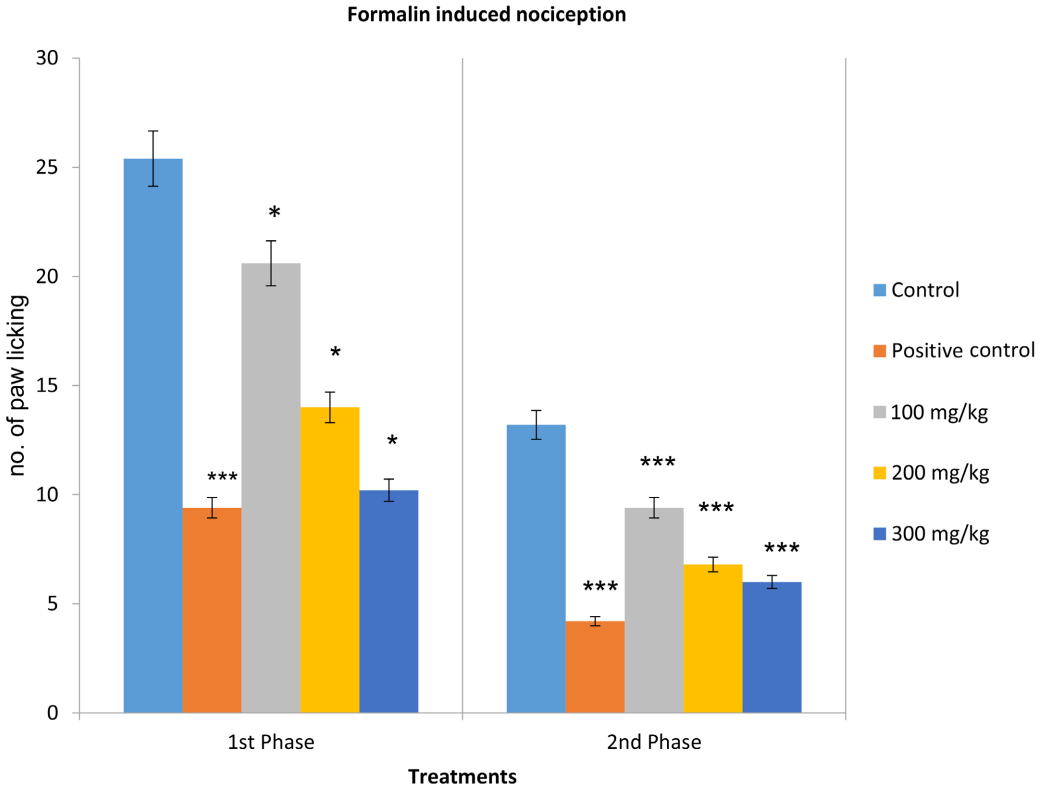

Figure 4. Antinociceptive effects of *Codiaeum variegatum* leave extract in formalin-induced nociception. Values are presented as mean ± SEM (n = 5). ***p < 0.001 compared with the control group (ANOVA followed by post hoc Dunnett's test).

4.7. Glutamate-Induced Nociception

An oral EECV antinociceptive effect was observed. At doses of 100 mg/kg, 200 mg/kg, and 300 mg/kg, it was shown that EECV effectively inhibited the glutamate-induced nociception test (Figure 5 and Table 6). Diclofenac sodium (10 mg/kg), a standard drug, inhibited licking 82.30% less than the control group. All treatments demonstrated significant antinociceptive activity as compared to the control group (deionized water) (***p < 0.001).

Table 6. Antinociceptive effects of *Codiaeum variegatum* leave extract in glutamate-induced nociception.

Treatment	Dose (mg/kg)	Licking time (s)	% of inhibition
Control	0.1 ml/mouse	26.00 ± 0.95	0.00
Diclofenac sodium	10	04.60 ± 0.51***	82.30
EECV	100	22.60 ± 1.08***	13.07
EECV	200	12.40 ± 0.81***	52.30
EECV	300	06.00 ± 0.71***	76.92

Values are presented as mean ± SEM (n = 5). EECV = Ethanolic extract of *Codiaeum variegatum*; ***p < 0.001 compared with the control group (Dunnett's test).

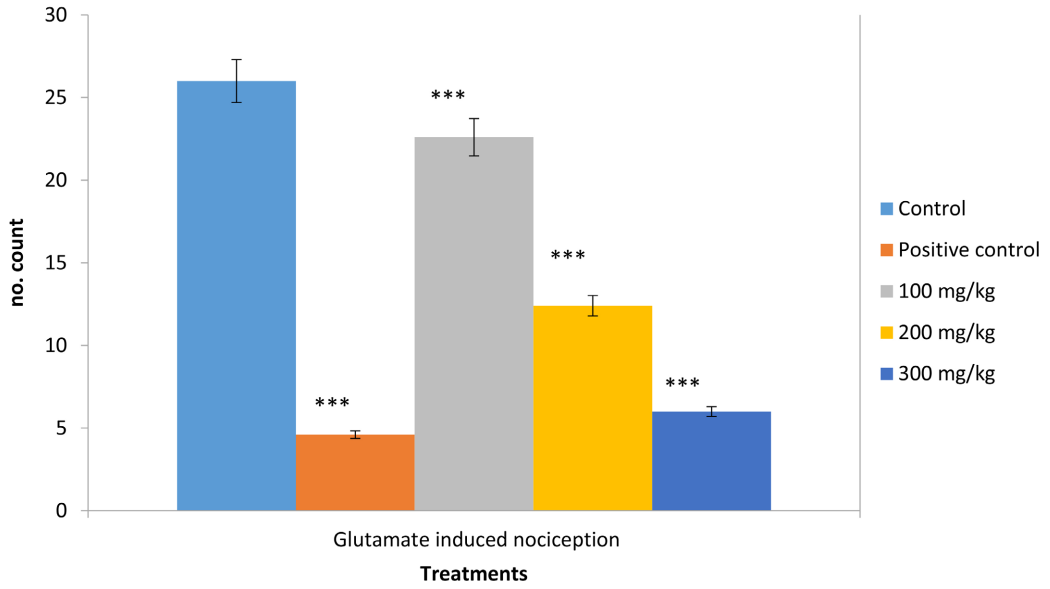

Figure 5. Antinociceptive effects of *Codiaeum variegatum* leave extract in glutamate-induced nociception test. All values are presented as mean ± SEM (n = 5). ***p < 0.001 compared with the control group (ANOVA followed by post hoc Dunnett's test).

5. Discussion

Pain is defined as an unpleasant emotional and sensory experience connected to potential tissue damage. As a result, physical and mental traits might contribute to pain [26]. The pain sense message is detected by nociceptors and is then sent to the brain by the pain sensory neuron and second row neurons through the spinal dorsal horn sensation guidance pathway. At different points throughout the brain stem, these nerves cross, go up to the spinal cord, and go down to the thalamus, especially at relaying nuclei. Third row neurons provide sensory pain fibers to the limbic system and other parts of the brain membrane from these nuclei. Long-term pain sufferers may have detrimental psychological effects, which is why there has long been a search for methods to lessen or even eliminate pain. Numerous attempts have also been made to elucidate the mechanisms behind pain and its management. Pain relief medications fall into two categories: synthetic and natural [27] [28].

The current work demonstrates that oral EECV treatment had antinociceptive effects in many pain models and reduced the threshold for heat and chemically induced pain. This suggests that EECV was not hazardous at the levels we used in our experiments, as no mortality, allergic reactions, salivation, convulsions, tremors, diarrhea, behavioral abnormalities, or physical alterations in important organs were observed in mice after receiving doses of EECV greater than the maximum experimental dosage. A well-validated paradigm for the detection of opioid analgesics and other analgesic drugs originating from the spinal cord is the nociceptive response to heat stimuli in mice, as demonstrated by the hot-plate test and tail immersion in hot water [29]-[31]. Acetic acid is a pain stimulus. Intraperitoneal acetic acid therapy induces localized inflammation by releasing free

arachidonic acid from tissue phospholipids through the activity of phospholipase A2 and other acyl hydrolases [32].

There are three primary processes by which arachidonic acid is converted into eicosanoids. Prostacyclines, thromboxanes, and prostaglandins are among the eicosanoids with ring structures that are synthesized via the cyclooxygenase route. The hydroxylated derivatives of straight-chain fatty acids known as leucotrienes, or HETE (hydroxy eicosatetraenoic acids) and HPETE (hydroperoxy eicosatetraenoic acids), are produced by the lipooxygenase pathway. Prostacyclines (PGI2) and prostaglandin-E, two secreted prostaglandins, are said to activate A-fibers and result in pain perception. When the A-fibers are activated, one experiences sharp, localized pain [33].

Analgesic activity was assessed by measuring the writhing effect induced by an injection of acetic acid and the prevention of the writhing effect produced by the test materials. Any drug that lowers the number of writhing people shows analgesia by inhibiting prostaglandin synthesis, which inhibits peripheral pain. This hypothesis supports the theories put forward by those authors, according to which acetic acid-induced writhing test procedures are useful instruments for evaluating analgesics with both peripheral and central effects [34] [35]. The EECV extract decreased the frequency of acetic acid-induced writhing in mice at higher doses. Diclofenac sodium was used as the positive control because it prevents prostaglandins from being produced. It reduces inflammation, edema, and discomfort associated with arthritis by blocking the production and release of prostaglandins [36]-[38]. Furthermore, in animal experiment models, diclofenac has been demonstrated to lessen inflammation induced by several phlogistic substances [39] [40]. It was plausible that this extract's antinociceptive property was mediated both centrally and peripherally given the antinociceptive activity shown by the EECV in the hot-plate, hot tail-flick, and acetic acid-induced writhing tests, as well as the responses of the positive control diclofenac.

Formalin induces pain in mouse paws via two distinct pathways. First, the early phase is brought on by both direct stimulation of sensory afferent fibers and activation of C-fibers and is characterized by neurogenic pain. This is the immediate aftermath of the formalin injection. Bradykinin and substance P are also involved in inducing nociception during this period. Second, the late phase, which lasts for 15 minutes after the formalin injection and is characterized by the activity of inflammatory mediators in peripheral tissues that produce pain and inflammation, including bradykinin, serotonin, prostaglandins (PGs), and histamine-like inflammatory mediators [41] [42]. Formalin-induced late phase pain is also influenced by functional changes in the spinal cord's dorsal horn [43]. The results of the formalin-induced paw-licking test (**Figure 4**) showed that both EECV and diclofenac significantly (***$p < 0.001$) reduced both phases of nociception. The late phase of the inhibition was dose-dependent and grew stronger. Aspirin and hydrocortisone are examples of peripheral analgesics that mostly reduce the late phase of formalin-induced paw-licking in mice, but opioids, or central analgesics, inhibit both stages [44]-[46]. Furthermore, as demonstrated by the paw-licking

deterrent in the late period and the acetic acid-induced writhing test, the inflammatory mediators were suppressed.

The glutamate-induced paw-licking nociception is driven by N-methyl-D-aspartate (NMDA) receptors; however, non-NMDA receptors (AMPA, Kainate) in the peripheral, supraspinal, and spinal areas also contribute to the generation of edema. In order to the transmission of pain signals from the peripheral nervous system to the central nervous system's dorsal horn, pro-inflammatory cytokines such as TNF-like glutamate release substance P and IL-1 are required. As part of the pro-inflammatory signals, nitric oxide synthase (NOS) and reactive oxygen species (ROS) also trigger the TNF-, IL-1, and IL-6 genes [47] [48]. EECV significantly (***$p < 0.001$) reduced glutamate-induced paw licking and edema (**Figure 5**). The results suggest that EECV has a role in both the inhibition of NMDA and non-NMDA receptors and the disruption of pro-inflammatory signals from ROS and NOS.

After screening for phytochemical contents, *Codiaeum variegatum* was discovered to be a good source of materials with medicinal potential, which may be further exploited to extract and synthesize contemporary medications. This research validates the need to identify and characterize the molecules with therapeutic activity. Because these compounds can be used to create new medications, research into these phytochemicals is essential. Alkaloids, tannins, phenolic compounds, glycosides, phytosterols, flavonoids, carbohydrates, and amino acids were found in this plant during preliminary phytochemical analysis [49]. These substances may be the source of the plant's analgesic and anti-inflammatory properties.

6. Conclusion

The current finding offers a fresh perspective on the ethanolic extract of *Codiaeum variegatum* leaves' antinociceptive properties in mice. This plant's phytochemical analysis revealed the presence of triterpenoids, sterols, alkaloids, flavonoids, and general glycosides, all of which may have antinociceptive properties. It is strongly advised that more research be done on the mechanism of action as well as associated pharmacological studies such as *in vivo* analysis, medication formulation, and clinical trials.

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Declaration of Interest

The paper's writing and content are solely the authors' responsibility.

References

[1] Kalyani, P. and Ratna Jyoti, D. (2013) Herbal Medicine—A Rational Approach in Health Care System. *International Journal of Herbal Medicine*, **1**, 86-89.

[2] Bijekar, S.R. and Gayatri, M.C. (2014) Phytochemical Profile of *Codiaeum variegatum* (L.) Bl. *International Journal of Pharmacology and Pharmaceutical Sciences*, **2**, 22-31.

[3] Ogunwenmo, K.O., Oluwatoni, A.I., Chukwudi, I., Edward, B.E. and Olatunji, A.O. (2007) Cultivars of *Codiaeum variegatum* (L.) Blume (Euphorbiaceae) Show Variability in Phytochemical and Cytological Characteristics. *African Journal of Biotechnology*, **6**, 2400-2405. https://doi.org/10.5897/ajb2007.000-2376

[4] Sangeetha, G., Mohan Krishna, L., Aruna, G., Sekar Babu, M. and Balammal, G. (2011) Study on Wound Healing Activity of Root of *Codiaeum variegatum*. *International Journal of Innovative Drug Discovery*, **1**, 19-23.

[5] Rahmatullah, M., Ferdausi, D., Mollik, A.H., Azam, N.K., Taufiq-UrRahman, M. and Jahan, R. (2009) Ethnomedicinal Survey of Bheramara Area in Kushtia District, Bangladesh. *American-Eurasian Journal of Sustainable Agriculture*, **3**, 534-541.

[6] Mfotie Njoya, E., Moundipa Fewou, P. and Niedermeyer, T.H.J. (2021) *Codiaeum variegatum* (L.) Rumph. Ex A. Juss. (Euphorbiaceae): An Overview of Its Botanical Diversity, Traditional Uses, Phytochemistry, Pharmacological Effects and Perspectives Towards Developing Its Plant-Based Products. *Journal of Ethnopharmacology*, **277**, Article ID: 114244. https://doi.org/10.1016/j.jep.2021.114244

[7] Saffoon, N., Uddin, R., Subhan, N., Hossain, H., Reza, H.M. and Alam, M.A. (2014) *In vitro* Anti-Oxidant Activity and HPLC-DAD System Based Phenolic Content Analysis of *Codiaeum variegatum* Found in Bangladesh. *Advanced Pharmaceutical Bulletin*, **2**, 533-541.

[8] Lorieza, M.M. (2022) Larvicidal Property of Sugar Apple (*Annona squamosa*) and San Fran-Cisco (*Codiaeum variegatum*) against Yellow Fever Mos Quito (*Aedes aegypti*). *Journal of Agriculture and Technology Management*, **25**, 249-252.

[9] Urmilesh, J., Singh, A. and Rajesh, J.O. (2016) *In-Vitro* Antilithiatic Effect of Ethanolic Extract of *Codiaeum variegatum* (L.) Blume. *Asian Journal of Pharmaceutical Technology & Innovation*, **4**, 95-102.

[10] Moundipa, P.F., Flore, K.G.M., Bilong Bilong, C.F. and Bruchhaus, I. (2005) *In vitro* Amoebicidal Activity of Some Medicinal Plants of the Bamun Region (Cameroon). *African Journal of Traditional, Complementary and Alternative Medicines*, **2**, 113-121. https://doi.org/10.4314/ajtcam.v2i2.31109

[11] Torres, M. and Ríos, A. (2008) Current View of the Immunopathogenesis in Inflammatory Bowel Disease and Its Implications for Therapy. *World Journal of Gastroenterology*, **14**, 1972-1980. https://doi.org/10.3748/wjg.14.1972

[12] Moshi, M.J. and Kagashe, G.A. (2007) A Study of the Effect of Extracts of *Codiaeum variegatum* (L.) A. Juss on Picrotoxin-Induced Convulsions in Mice. *Tanzania Medical Journal*, **19**, 391-392. https://doi.org/10.4314/tmj.v19i1.39192

[13] Mfotie Njoya, E., Eloff, J.N. and McGaw, L.J. (2018) Croton Gratissimus Leaf Extracts Inhibit Cancer Cell Growth by Inducing Caspase 3/7 Activation with Additional Anti-Inflammatory and Antioxidant Activities. *BMC Complementary and Alternative Medicine*, **18**, Article No. 305. https://doi.org/10.1186/s12906-018-2372-9

[14] Perche, O., Laumonnier, F., Baala, L., Ardourel, M.Y., Menuet, A., Robin, V., *et al.* (2010) Autisme, génétique et anomalies de la fonction synaptique. *Pathologie Biologie*, **58**, 381-386. https://doi.org/10.1016/j.patbio.2009.12.005

[15] Saffoon, N., Alam, M.A. and Uddin, G.M. (2011) Phytochemical and Cytotoxic Investigation of *Codiaeum variegatum* Linn. Leaf. *Stamford Journal of Pharmaceutical Sciences*, **3**, 51-53. https://doi.org/10.3329/sjps.v3i2.8039

[16] Dai, J. and Mumper, R.J. (2010) Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. *Molecules*, **15**, 7313-7352. https://doi.org/10.3390/molecules15107313

[17] Ghani, A. (2003) Medicinal Plants of Bangladesh with Chemical Constituents and Uses. 2nd Edition, Asiatic Society of Bangladesh, 274.

[18] Hossen, F.M., Asrafuzzaman, M. and Mannan, A.M. (2022) Evaluation of CNS Depressant Activity of Methanolic Extract of *Brownlowia tersa* Leaves in Swiss albino Mice. *Journal of Medicinal Plants Studies*, **10**, 150-155.

[19] Eddy, N.B. (1953) Leimbach D. Synthetic Analgesics: II. Dithienylbutenyl and Dithienyl-Butylamines. *The Journal of Pharmacology and Experimental Therapeutics*, **107**, 385-393.

[20] D'Amour, W.L. and Smith, D.L. (1941) A Method for Determining Loss of Pain Sensation. *The Journal of Pharmacology and Experimental Therapeutics*, **72**, 74-89.

[21] Wilson, C.O., Block, J.H. and Gisvold, O. (2004) Wilson and Gisbold's Textbook of Organic Medicinal and Pharmaceutical Chemistry. Wolters Kluwer Health.

[22] Sulaiman, M., Tengku Mohamad, T., Shaik Mossadeq, W., Moin, S., Yusof, M., Mokhtar, A., *et al.* (2009) Antinociceptive Activity of the Essential Oil of *Zingiber zerumbet*. *Planta Medica*, **76**, 107-112. https://doi.org/10.1055/s-0029-1185950

[23] Santos, A.R.S. and Calixto, J.B. (1997) Further Evidence for the Involvement of Tachykinin Receptor Subtypes in Formalin and Capsaicin Models of Pain in Mice. *Neuropeptides*, **31**, 381-389. https://doi.org/10.1016/s0143-4179(97)90075-5

[24] Santos, A.R.S., Miguel, O.G., Yunes, R.A. and Calixto, J.B. (1999) Antinociceptive Properties of the New Alkaloid, Cis-8, 10-di-N-Propyllobelidiol Hydrochloride Dihydrate Isolated from *Siphocampylus verticillatus*: Evidence for the Mechanism of Action. *Journal of Pharmacology and Experimental Therapeutics*, **289**, 417-426.

[25] Beirith, A., Santos, A.R.S. and Calixto, J.B. (2002) Mechanisms Underlying the Nociception and Paw Oedema Caused by Injection of Glutamate into the Mouse Paw. *Brain Research*, **924**, 219-228. https://doi.org/10.1016/s0006-8993(01)03240-1

[26] Fraser, G.L. and Gagnon, D.J. (2016) Pain and Analgesia. CCSAP, 8.

[27] Solati, K., Asadi-Samani, M. and Heidari-Soureshjani, S. (2017) Effects and Mechanisms of Medicinal Plants on Dopamine Reward System to Reduce Complications of Substance Abuse: A Systematic Review. *World Family Medicine Journal/Middle East Journal of Family Medicine*, **15**, 202-207. https://doi.org/10.5742/mewfm.2017.93162

[28] Chevalier, M.H. (1996) The Encyclopedia of Medicinal Plant. Dorling Kindersley, 171-182.

[29] Sewell, R. (1976) Antinociceptive Activity of Narcotic Agonist and Partial Agonist Analgesics and Other Agents in the Tail-Immersion Test in Mice and Rats. *Neuropharmacology*, **15**, 683-688. https://doi.org/10.1016/0028-3908(76)90037-x

[30] Owoyele, B., Olaleye, S., Oke, J. and Elegbe, R. (2010) Anti-Inflammatory and Analgesic Activities of Leaf Extracts of *Landolphia owariensis*. *African Journal of Biomedical Research*, **4**, 131-133. https://doi.org/10.4314/ajbr.v4i3.53896

[31] Adzu, B., Amos, S., Kapu, S.D. and Gamaniel, K.S. (2003) Anti-Inflammatory and Anti-Nociceptive Effects of *Sphaeranthus senegalensis*. *Journal of Ethnopharmacology*, **84**, 169-173. https://doi.org/10.1016/s0378-8741(02)00295-7

[32] Koster, R., Anderson, M. and De-Beer, E.J. (1959) Acetic Acid for Analgesic Screening. *Federation Proceedings*, **18**, 412-418.

[33] Rang, H.P. and Dale, M.M. (1983) Pharmacology. 2nd Edition, Churchill Livingstone, 706-711.

[34] Williamson, E.M., Okpako, D.T. and Evans, F.J. (1986) Pharmacological Methods in Phytotherapy Research. In: Williamson, E.M., Okpako, D.T. and Evans, F.J., Eds., *Selection*, *Preparation*, *Pharmacological Evaluation of Plant Materials*, John Wiley, 184-186.

[35] Silva, J., Abebe, W., Sousa, S.M., Duarte, V.G., Machado, M.I.L. and Matos, F.J.A. (2003) Analgesic and Anti-Inflammatory Effects of Essential Oils of Eucalyptus. *Journal of Ethnopharmacology*, **89**, 277-283. https://doi.org/10.1016/j.jep.2003.09.007

[36] Skoutakis, V.A., Carter, C.A., Mickle, T.R., Smith, V.H., Arkin, C.R., Alissandratos, J., *et al.* (1988) Review of Diclofenac and Evaluation of Its Place in Therapy as a Nonsteroidal Antiinflammatory Agent. *Drug Intelligence & Clinical Pharmacy*, **22**, 850-859. https://doi.org/10.1177/106002808802201102

[37] Todd, P.A. and Sorkin, E.M. (1988) Diclofenac Sodium: A Reappraisal of Its Pharmacodynamic and Pharmacokinetic Properties and Therapeutic Efficacy. *Drugs*, **35**, 244-285. https://doi.org/10.2165/00003495-198835030-00004

[38] Small, R.E. (1989) Drug Reviews: Diclofenac Sodium. *Clinical Pharmacology*, **8**, 545-558.

[39] Menassé, R., Hedwall, P.R., Kraetz, J., Pericin, C., Riesterer, L., Sallmann, A., *et al.* (1978) Pharmacological Properties of Diclofenac Sodium and Its Metabolites. *Scandinavian Journal of Rheumatology*, **7**, 5-16. https://doi.org/10.3109/03009747809097211

[40] Al-Tuwaijri, A.S. and Mustafa, A.A. (1992) Verapamil Enhances the Inhibitory Effect of Diclofenac on the Chemiluminescence of Human Polymorphonuclear Leukocytes and Carrageenan-Induced Rat's Paw Oedema. *International Journal of Immunopharmacology*, **14**, 83-91. https://doi.org/10.1016/0192-0561(92)90108-w

[41] Parada, C.A., Tambeli, C.H., Cunha, F.Q. and Ferreira, S.H. (2001) The Major Role of Peripheral Release of Histamine and 5-Hydroxytryptamine in Formalin-Induced Nociception. *Neuroscience*, **102**, 937-944. https://doi.org/10.1016/s0306-4522(00)00523-6

[42] Dalal, A., Tata, M., Allègre, G., Gekiere, F., Bons, N. and Albe-Fessard, D. (1999) Spontaneous Activity of Rat Dorsal Horn Cells in Spinal Segments of Sciatic Projection Following Transection of Sciatic Nerve or of Corresponding Dorsal Roots. *Neuroscience*, **94**, 217-228. https://doi.org/10.1016/s0306-4522(99)00328-0

[43] Hunskaar, S. and Hole, K. (1987) The Formalin Test in Mice: Dissociation between Inflammatory and Non-Inflammatory Pain. *Pain*, **30**, 103-114. https://doi.org/10.1016/0304-3959(87)90088-1

[44] Tjølsen, A., Berge, O., Hunskaar, S., Rosland, J.H. and Hole, K. (1992) The Formalin Test: An Evaluation of the Method. *Pain*, **51**, 5-17. https://doi.org/10.1016/0304-3959(92)90003-t

[45] Trongsakul, S., Panthong, A., Kanjanapothi, D. and Taesotikul, T. (2003) The Analgesic, Antipyretic and Anti-Inflammatory Activity of Diospyros Variegata Kruz. *Journal of Ethnopharmacology*, **85**, 221-225. https://doi.org/10.1016/s0378-8741(03)00020-5

[46] Beauparlant, P. and Hiscott, J. (1996) Biological and Biochemical Inhibitors of the NF-κB/Rel Proteins and Cytokine Synthesis. *Cytokine & Growth Factor Reviews*, **7**, 175-190. https://doi.org/10.1016/1359-6101(96)00020-2

[47] Ribas, C.M., Meotti, F.C., Nascimento, F.P., Jacques, A.V., Dafre, A.L., Rodrigues, A.L.S., *et al.* (2008) Antinociceptive Effect of the *Polygala sabulosa* Hydroalcoholic Extract in Mice: Evidence for the Involvement of Glutamatergic Receptors and Cytokine Pathways. *Basic & Clinical Pharmacology & Toxicology*, **103**, 43-47. https://doi.org/10.1111/j.1742-7843.2008.00245.x

[48] Presti, D. and Scott, J.E. (1994) Hyaluronan-Mediated Protective Effect against Cell Damage Caused by Enzymatically Produced Hydroxyl (OH·) Radicals Is Dependent on Hyaluronan Molecular Mass. *Cell Biochemistry and Function*, **12**, 281-288. https://doi.org/10.1002/cbf.290120409

[49] Balasubramanian, J., Sai Sugathri, K., Jothi, K., Nandhini, G.A. and Hariram, S. (2016) Analgesic and Anti-Inflammatory Activity of Novel (Fixed Dose) Topical Gel Containing Diclofenac Combination with Heparin. *World Journal of Pharmacy and Pharmaceutical Sciences*, **5**, 1077-1094.