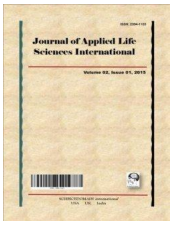

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Sedative, Anxiolytic, Antinociceptive, Anti-inflammatory and Antipyretic Effects of a Chloroform Extract from the Leaves of *Urena sinuata* in Rodents

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Authors' contributions

This work was carried out in collaboration between all authors. Authors TBE, TEM and SA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MMNU, AR and MSH managed the analyses of the study. Authors MNA and TRB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Context: *Urena sinuate* L. (Malvaceae) is a well-known medicinal plant and it is used in traditional medicine systems.
Objectives: The current study unravels the neuropharmacology, antinociceptive, anti-inflammatory and antipyretic effects of the chloroform extract of *Urena sinuata* leaves (CEUS) in rodents and also

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to determine the possible mechanism of antinociception which is involved in its acute toxicity and phytochemical studies.

Materials and Methods: Neuropharmacological activities of CEUS were performed by hole cross, open field test, elevated plus-maze test and Thiopental Na induced sleeping time test. For the analgesic activity of CEUS, different methods like hot plate test, acetic acid induced test, formalin-induced test, tail immersion test and glutamate-induced nociception were used. Additionally, a possible mechanism of nociception was identified by cyclic guanosine monophosphate (cGMP) and ATP-sensitive K+ channel pathway analysis. Carrageenan-induced rat paw edema and cotton pellet-induced granuloma test were conducted to detect anti-inflammatory activity and brewer's yeast induced pyrexia test for antipyretic activity. Before 60 min of subjection to the respective test, the extracts (200 and 400 mg/kg) were given orally.

Results: The obtained results showed that CEUS produced significantly ($p < 0.05$) neuropharmacological, anti-inflammatory and antipyretic activity with low or no toxicity. Moreover, in all the thermal and chemical-induced nociception models, antinociceptive response was exhibited. Furthermore, it involved cyclic guanosine monophosphate (cGMP) as well as ATP-sensitive K+ channel pathway mediated antinociceptive effect.

Conclusions: These data show that CEUS has significant neuropharmacological, anti-inflammatory and antipyretic effects that appear to have a relation with the inhibition of the glutamatergic system. Thus, the leaves of *Urena sinuata* could be used in the treatment of several types of inflammation in intestines and bladder.

Keywords: Urena sinuate; neuropharmacological; anti-inflammatory; antinociceptive activities; sleeping time; glutamate-induced nociception.

ABBREVIATIONS

CAM: Complementary and alternative medicine; ATP: Adenosine triphosphate; COX: Cyclooxygenase; NSAID: Non-steroidal anti-inflammatory drugs; cGMP: Cyclic guanosine monophosphate; PGE: Prostaglandins; ICDDR,B: International centre for diarrheal disease and research, Bangladesh; CEUS: Chloroform extract of Urenasinuata; h: Hour; min, minutes; sec: Second; kg: Kilogram; g: Gram; µg: Microgram; L: liter; ml, millilitre; µL: Micro liter; µg/ml: microgram per milliliter; mg/kg: Milligram per kilogram; %, percent; b.w.: body weight; ^{o}C: degree celsius; Å: Angstrom; rpm: Rotation per minute; cal: Calorie; kcal: kilocalorie; et al: et alliori (and others); w/w: Weight by weight; w/v: Weight by volume; v/v: Volume by volume; SEM: Standard error mean; NaCl: sodium chloride; p.o.: Oral administration; i.p.: Intra-peritoneal.

1. INTRODUCTION

In the last decade for the management of inflammation and anxiety, there has been a huge utilization of Complementary and Alternative Medicine (CAM) therapies [1-5]. In the perspective of developing countries, CAM therapy is cheaper than synthetic, patented drugs. Many people in these countries rely on CAMs for managing disorders like diabetes, cancer [6,7]. However, evidence regarding the efficacy of these therapies is not strong enough and their mechanism of action is often unclear [8]. As such in CAM therapy, people have been using medicinal herbs for therapeutic purposes over centuries. Use of these herbs for anti-nociceptive activity showed no adverse effect [9].

Urena sinuata (Borss) L. (Malvaceae) along with its other species like *U. lobata, U. morifolia, U. moricata, U. paradoxa, U. swartzii* is generally a wild shrubby plant used in folk medicine. In tropical and subtropical region across the globe, several species of this plant are found. Its roots are sweet, slightly cooling, anti-rheumatic and antipyretic. In Brazil it's decoction of the stem and root is used in the treatment of severe windy colic. For snakebites, sprains and bruises poultice prepared from the roots and leaves of this plant is used and also as emollient. The flowers are used in dry and inveterate coughs as an expectorant. In sore throat bronchitis, an infusion of this flower is used. People in India use the root for lumbago externally. For reproductive purposes, it is used in the Pacific, Trinidad and Tobago, China and India [10] for specific human problems of both the genders [11]. In Assam, most people use it as a diuretic and as an abortifacient. People of Philippine, sometimes use the root as an emollient and refrigerant; the leaves have effect on inflammation of the intestines and bladder. In

dysentery, enteritis, rheumatic pains, and tonsillitis people use a decoction of the dried root. However, many people think it as a medicinal plant, some as a weed, whereas its fiber (Aramina fiber) is used for various reasons in Madagascar, Nigeria, and Western Sudan, Central African Republic, Chad, Zaire and Gabon, which is believed to be termites and water resistant [12]. Nevertheless, it's biological activity information and the phytochemical information is yet to be completed. For that reason, the plant was chosen for further study of its bioactive potentials.

In this context, due to its therapeutic properties attributed to this medicinal plant by traditional medicine, The neuropharmacological, anti-inflammatory, antinociceptive and antipyretic effects of the chloroform extract obtained from the leaves of *Urena sinuata* (CEUS) were performed on *in vivo* and *in-vitro* experimental models. Furthermore, the COX enzyme inhibition mechanism involved in cyclic guanosine monophosphate (cGMP), as well as ATP-sensitive K+ channel pathway mediated antinociceptive effect, are also investigated.

2. MATERIALS AND METHODS

2.1 Ethical Statement

All experimental protocols were in accordance with Dhaka University Ethics Committee (approval number AE-DUEC 2012/118) on research in animals and internationally accepted principles for laboratory animal use and care [13].

2.2 Plant Material

The plant was collected from the Shitalakkha barrage side of Demra, Dhaka in May 2012. The samples of the plant were then identified by Bushra Khan, Principal Scientific Officer, Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh. The sample specimens have been stored in the national herbarium with the mentioned accession numbers (DACB: 37688). The plant leaves were thoroughly washed with water and dried in a hot air oven at room temperature 25 ± 1°C for 7 days in two days interval and at 40°C for the next 2 days.

2.3 Extraction of the Plant Material and Sample Preparation

After measuring (600g) of the dried and powdered) the sample soaked in 1500 ml of 99% chloroform (Merck KGaA, Germany) in clean, sterilized and flat-bottomed glass container.then it was sealed and maintained for 15 days with occasional stirring and agitation. The complete mixture then subjected to coarse filtration on a piece of clean, white sterilized cotton material and Whatman® filter paper. The extract obtained by evaporation using rotary evaporator (Bibby RE-200, Sterilin Ltd., UK) at 4 rpm and 65ºC temperature. It rendered a gummy concentrate of greenish black color. The gummy concentrate designated as a crude extract or chloroform extract. Then the crude chloroform extract dried by freeze dryer and preserved at +4°C (yield 13.56% w/w). The extracts and standard drug Diclofenac, diazepam and paracetamol were suspended in normal water using 1% Tween-80.

2.4 Chemicals and Reagents

Diclofenac sodium, paracetamol, and diazepam were purchased from Beximco Pharmaceuticals Ltd. Dhaka, Bangladesh. Carrageenan, Tween-80 and acetic acid, Glibenclamide, methylene blue were purchased from Sigma-Aldrich, Germany and Thiopental sodium from Merck, India Ltd.

2.5 Experimental Animals

For the experiment, Swiss albino mice (BALB/c) having a weight between (12-300g) of either sex were collected from animal house of International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR, B), Dhaka. The animals maintained under normal laboratory condition and held in standard polypropylene cages at room temperature of (30 ± 2ºC) and 60% to 65% relative humidity and provided with standard diet and water. Each group has five mice and to denote individual animal, they were marked as a group I, II, III, IV for test samples at the doses of 100 and 200 mg/kg body weight and a control and positive control group was also maintained for every test.

Albino Wistar rats (Rattusnorvigicus) of either sex weighing 120-150 g also used for the present study. They were bought from the animal house of Jahangirnagar University, Savar, Dhaka-1342, Bangladesh. They were kept in the animal house of North South University, Bashundhara, Dhaka-1229, Bangladesh for experimental purpose. The animals kept under controlled conditions of temperature 23 ± 2ºC, humidity 50 ± 5% and 12 hours light-dark cycles. All the animals were acclimatized seven days

before the study. The animals were randomized into experimental and control groups and kept individually in sanitized polypropylene cages containing sterile paddy husk as bedding. They had a free access to standard pellets as basal diet and water ad libitum. Animals were habituated to laboratory conditions for 48 hours prior to the experimental protocol to minimize if any of non-specific stress.

2.6 Acute Toxicity Test

Mice were divided into nine groups, each group having six animals. Group 1 was given 1% Tween-80 in water (2 ml per kg body weight). Remaining eight groups (Groups 2–9) were administered, respectively, 100, 200, 300, 600, 800, 1000, 2000 and 3000 mg of CEUS per kg body weight. All animals closely observed for the next 8 hours to observe any behavioral changes or mortality and kept under close observation for the next two weeks [14].

2.7 Phytochemical Screening

The crude chloroform extract of *U. sinuata* (CEUS) was qualitatively examined for the detection of carbohydrates, saponins, flavonoids, tannins, alkaloids, glycosides, reducing sugars, glucosides, proteins as well as steroids following standard procedures [15].

2.8 Neuropharmacological Activity

The neuropharmacological function of the chloroform extract of *U. sinuata* leaves (CEUS) was performed by hole cross, open field test, elevated plus-maze test, Thiopental Na induced sleeping time test. During every experiment, four groups of mice each containing 5 mice taken. Each group received a particular treatment: Group I: Control (1% v/v Tween-80 in water, 0.5 ml/mice), Group II: Positive control (diazepam 1 mg/kg body weight), Group III: Test sample 1 (CEUS at the dose of 200 mg/kg body weight), Group IV: Test sample 2 (CEUS at the dose of 400 mg/kg body weight).

2.9 Hole Cross Test

The hole cross test, as demonstrated by Subhan et al., 2008 [16] adopted for screening the sedative effect of the CEUS in mice. A wooden partition having a size of 30 × 20 × 14 cm was fixed in the middle of a cage. A hole (diameter 3 cm) made in the center of the cage at a height of 7.5 cm. After oral administration of the treatments, each mouse was immediately kept in any of the two chambers of the specified instrument. The number of passages through the hole from one chamber to another counted on 0, 30, 60, 90 and 120 min for a 3 min test period [16].

2.10 Open Field Test

This test was conducted to find out an angiogenic and anxiolytic activity under identical situations. Variety types of open field apparatus have been used to test the mice [17]. The effect of the CEUS on the spontaneous locomotor activity of the experimental animals evaluated by the method which was described by Gupta et al., [18] Twenty mice divided into four groups (n = 5). CEUS (200 and 400 mg/kg; p.o.) vehicle control (1% Tween-80 solution in water, 10 ml/kg; p.o.) and Diazepam (1 mg/kg, i.p.) were given to different groups. The floor of an open field of half square meter was divided into a series of squares each of which alternately colored black and white. The apparatus had a 40-cm height a wall. The number of squares passed by the animals was counted for 3min at 0, 30, 60, 90, 120 min during the study period.

2.11 Elevated Plus-maze Test

The apparatus was made of wood in elevated plus maze test, with two open and two closed arms across each other respectively which forms a plus-sign figure. The elevated plus maze (EPM; 30 cm × 6 cm × 6 cm, each arm) positioned 50 cm above the floor. After administration of the drug, each animal was placed at the center of the maze lining one of the closed arms. The number of open and closed arm entries, plus time spent in open and closed arms were counted for 5 min at 0, 30, 60, 90, 120 min after administration of the extract (200 and 400 mg/kg), diazepam (1 mg/kg) and vehicle (1% Tween-80 in water). The whole test was conducted in a sound-attenuated room [19]. Entry into an arm was defined as the point when the animal kept all four paws onto the arm.

2.12 Thiopental Sodium-induced Sleeping Time Test

For the experiment, the animals were divided into four groups randomly, each having 5 mice. The test groups were administered the CEUS at doses of 200 and 400 mg/kg body weight, while

the positive control treated with diazepam (1 mg/kg) as well as a control group with vehicle (1% Tween-80 in water). After thirty minutes Thiopental sodium (40 mg/kg) was given to each mouse to induce sleep. The animals were kept observing by placing them in separate chambers for the latent period (time counting from Thiopental administrations to loss of righting reflex) and duration of sleep i.e., the time between the loss and returning of righting reflex. The onset of sleep and total sleeping time were recorded for control, positive control as well as test groups [20].

2.13 Analgesic Activity

Analgesic activities of the chloroform extract of *U. sinuata* leaves (CEUS) were conducted by hot plate test, acetic acid induced test, formalin-induced test, tail immersion test and glutamate-induced nociception. During every experiment, four groups of mice each containing 5 mice taken. Each group was given a particular treatment as follows:

> Group I: Control (1% v/v Tween-80 in water, 10 ml/kg), Group II: Positive control (Diclofenac sodium 10 mg/kg body weight), Group III: Test sample I (CEUS at the dose of 200 mg/kg body weight), Group IV: Test sample II (CEUS at the dose of 400 mg/kg body weight).

2.14 Hot Plate Method

The paws of mice are very sensitive to temperature at 55 ± 0.5°C, which is not injurious to the skin. The animals were placed on Eddy's hot plate maintained at a temperature of 55 ± 0.5°C. A cutoff time of 30 sec [21] observed to avoid damage to the paw. Reaction time was recorded when animals beat thoroughly & conclusively their fore or hind paws or jumped at 0, 30, 60 and 90, 120, 180 and 240 min after oral administration of the samples [22]. Test samples were given to the animals of test groups at the doses of 200 and 400 mg/kg body weight. The positive control group received standard drug Diclofenac sodium at the dose of 10 mg/kg b.w. and water.

2.15 Acetic Acid-induced Writhing Test

The antinociceptive activity of the extract was examined using the acetic acid-induced writhing model in mice [23]. Test samples were given to the animals of test groups at the doses of 200 and 400 mg/kg body weight. The positive control group received standard drug Diclofenac sodium at the dose of 10 mg/kg body weight. Test samples were taken orally 30 min before intraperitoneal administration of 0.6% acetic acid and Diclofenac sodium was administered 15 min before injection of acetic acid. After 5 min, the mice were checked for specific contraction of the body referred to as 'Writhing' for the next 30 min.

2.16 Formalin-induced Nociception

Animals received 20 µL of 2.5% formalin solution (7% formaldehyde) made up of water and injected in the ventral surface of the right-hand paw through intra-planetary. Animals observed from 0 to 5 min (neurogenic phase) and 15-30 min inflammatory phase and the time spent licking the injected paw was recorded as indicative of nociception. The animals received CEUS at 100 and 200 mg/kg 1 h before, on the basis of a previous time response curve. Standard drug Diclofenac sodium was given to the positive control group at the dose of 10 mg/kg body weight [24,25].

2.17 Tail Immersion Test

For evaluating the central analgesic property, the tail immersion test was performed. This procedure based on the observation that morphine-like drugs extend the tail withdrawal time from hot water in mice [26]. One to two cm of the tail of the mice pre-treated with Diclofenac sodium or CEUS was immersed in the warm water keeping constant at 54 ± 0.5°C. The latency between tail immersion and deflection of tail recorded. A latency period of the 20s was maintained to avoid tail tissue damage in mice. The latency time of the tail-withdrawal response was taken as the index of antinociception and was recorded at 30, 60, 90 and 120 min after administration of the drug and extract. Then the % MPE (maximum possible effect) was calculated from the latency periods [27].

2.18 Glutamate-induced Nociception

After measuring 20 µl of glutamate (10 µM/paw) it was injected into the ventral surface of the right hind paw of the mice 30 min after CEUS treatment and 15 min after injection of Diclofenac sodium. Following glutamate injection, the mice were observed for 15 min. The number of licking of its injected paw was indicative of nociception [28]

2.19 Anti-inflammatory Activity

The anti-inflammatory activity of chloroform extract of *U. sinuata* leaves (CEUS) was carried out by Carrageenan-induced rat paw edema and Cotton pellet-induced granuloma test. During every experiment, four groups of mice each containing 5 rats were taken. Each group received a particular treatment:

Group I: Control (1% v/v Tween-80 in water, 10 ml/kg), Group II: Positive control (Diclofenac sodium 10 mg/kg body weight), Group III: Test sample I (CEUS at the dose of 200 mg/kg body weight), Group IV: Test sample II (CEUS at the dose of 400 mg/kg body weight).

2.20 Carrageenan-induced Rat Paw Edema

The rats were grouped into four groups (n = 5). The different groups treated orally with (200 and 400 mg/kg b.w.), Diclofenac sodium (10 mg/kg b.w.), and vehicle control (0.9% NaCl, 5 ml/kg b.w.). Administration of the extract and drugs was 30 min before the injection of 0.1 ml of 1% freshly prepared a suspension of carrageenan in normal water in the right hind paw subplantar of each rat. The paw volume was calculated initially and then at ½, 1, 2, 3 and 4 h after the carrageenan injection by using plethysmometer [29]. The anti-inflammatory effect of CEUS was calculated by the following equation:-

$$\text{Anti-inflammatory activity (\%)} = (1\text{-}D/C) \times 100$$

where D represents the percentage difference in paw volume after the introduction of drugs to the rats and C represents the percentage difference of volume in the control groups [23].

2.21 Cotton Pellet-induced Granuloma

Cotton pellets-induced granuloma in rats was examined in accordance with the method D'Arcy et al., 1960 [30]. The animals were divided into four groups of five animals in each group. The rats were anesthetized and 10 ± 1 mg of sterile cotton pellets were implanted into both sides of the groin region of each rat subcutaneously. Group, I denoted as control and received the vehicle (0.9% NaCl, 5 ml/kg b.w.). The extract CEUS at the concentration of 200 and 400 mg/kg b.w. administered orally to groups III and IV animals for seven consecutive days counting from the day of cotton pellet implantation. Group II animals received a dose of 10 mg/kg b.w of Diclofenac sodium for the same period. The animals were anesthetized and the pellets together with the granuloma tissues carefully separated and thus made free from extraneous tissues on the 8^{th} day. The wet pellets were weighed and then dried in an oven at 60°C for 24 hours to constant weight. After that, the dried pellets were weighed again. The increment in the dry weight of the pellets was taken as a measure of the formation of granuloma. The anti-proliferative effect of CEUS was then compared with control.

2.22 Antipyretic Activity

Anti-pyretic activity in albino rats was studied with fever induced by 15% brewer's yeast. Healthy Wistar strain albino rats of about 120-150g were taken. Before inducing pyrexia they fasted overnight with water ad libitum and animals allowed to be quiet in the cage for some time just before inducing pyrexia and then their basal rectal temperature wes measured using a clinical digital thermometer by insertion of thermometer to a depth of 1 inch into the rectum. Taking temperature, pyrexia was induced by injecting 15% w/v suspension of brewer's yeast in distilled water subcutaneously at a dose of 10 ml/kg body weight in the back below the neck nape. The site of injection was massaged to spread the suspension beneath the skin and the rats were reintroduced to their cage and then fed. After 18 h of brewer's yeast injection, the rise in rectal temperature was recorded. Only rats which were shown a rise in temperature of at least 0.6°C were used for the further experiment. After the drug administered, the temperature of all the rats in each group recorded at 1, 2, 3 and 4 h. The mean temperature was calculated for each group and was compared with the value of standard drug paracetamol [31,32].

Antipyretic activities of the chloroform extract of *U. sinuata* leaves (CEUS) were carried out by brewer's yeast inducing pyrexia method. During every experiment, 4 groups of mice each containing 5 rats taken. Each group received a particular treatment: Group I: Control (1% v/v Tween-80 in water, 10 ml/kg), Group II: Positive control (Paracetamol 150 mg/kg body weight), Group III: Test sample I (CEUS at the dose of 200 mg/kg body weight), Group IV: Test sample II (CEUS at the dose of 400 mg/kg body weight).

2.23 Analysis of the Possible Mechanism of Action of Ceus

2.23.1 Involvement of cyclic guanosine monophosphate (cGMP) pathway

To confirm possible relation of cGMP in the antinociceptive action caused by CEUS, the mice were pre-treated with methylene blue (20 mg/kg), a non-specific inhibitor of NO/guanylyl cyclase, intraperitoneally 15 min before taking CEUS. Then the nociceptive responses against 0.6% acetic acid injection were observed for 30 min, starting from 5 min after injection. The numbers of abdominal writhing were counted as an indication of pain behavior [33,34].

2.23.2 Involvement of ATP-sensitive K+ channel pathway

The possible role of K+ channel in the antinociceptive effect of CEUS found by conducting the method described in [33, 35]. The mice were pre-treated with Glibenclamide (10 mg/kg), an ATP-sensitive K+ channel inhibitor, intraperitoneally 15 min before administration of either vehicle or CEUS. The mice challenged with i.p. injection of 0.6% acetic acid, 30 min post-treatment. Introducing the injection of acetic acid, the animals were immediately placed in a chamber and the writhing number was recorded up to 30 min, starting from 5 min post-injection.

3. RESULTS

3.1 Phytochemical Screening

The presence of alkaloids, glycosides, carbohydrates, flavonoids, saponins, and tannins was confirmed by the phytochemical screening tests of the crude extract of *U. sinuata* leaves (Table 1).

3.2 Acute Toxicity Test

The current study confirmed that no sign of mortality, behavioral changes like sedation, excitability etc. or allergic manifestations was seen after oral administration of the CEUS at various doses and during the 8 h observation period after administration.

3.3 Neuropharmacology Activity

3.3.1 Hole cross test

In the hole cross test, CEUS at both doses (200 and 400 mg/kg body weight) demonstrated a reduction in locomotion activity by reducing the number of movement of test animal from one chamber to another in the cage at all the examination periods except the 3rd observation period (60 min) Fig. 1. An increased locomotion activity was observed in the mice by the extract the 3rd observation period, at a dose of 400 mg/kg body weight but statistically the result was insignificant. Treating with diazepam, the positive control exhibited decreasing locomotion activity at all the observation periods Fig. 1.

3.3.2 Open field test

At the dose levels of 200 and 400 mg/kg body weight, the CEUS confirmed a dose-dependent and significant ($p < 0.001$) decrease in locomotion in the test animals during the experiment period (Table 2). The depressant activities were similar to that of standard drug diazepam ($p < 0.001$).

Table 1. Qualitative phytochemical screening of chloroform extract of *U. sinuata*

Chemical constituent	Results of the crude chloroform extract of *U. sinuata*
Carbohydrates	**+**
Saponins	+
Flavonoids	+
Tannins	**+**
Alkaloids	+
Glycosides	+
Glucosides	-
Reducing sugars	-
Proteins	**-**
Steroids	**-**

+: Positive result; −: Negative result

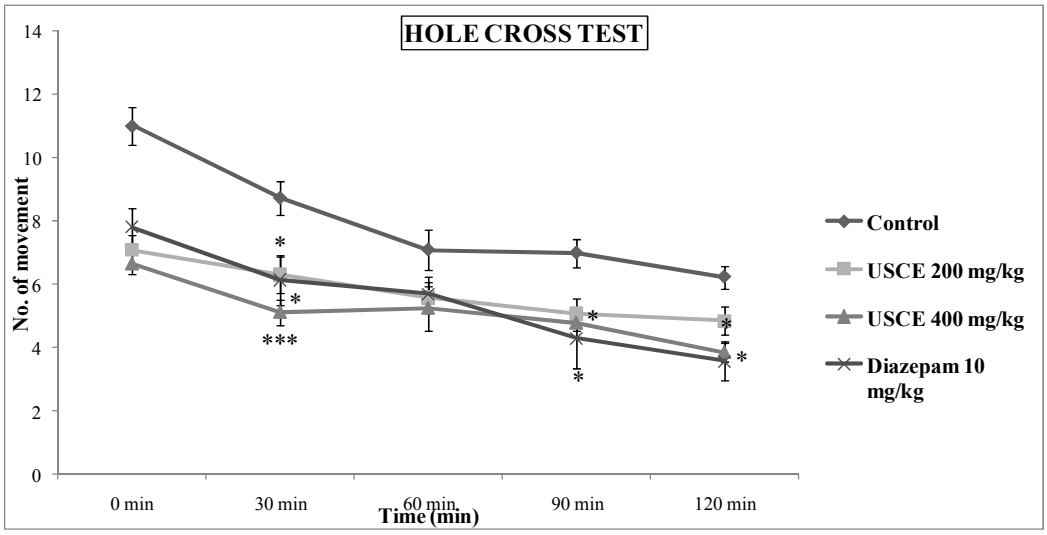

Fig. 1. Effect of chloroform extract of *U. sinuata* (CEUS) on exploratory behaviour (Hole cross test)

*Values are reported as mean ± SEM. for group of five animals. The data was analyzed by ANOVA followed by Dunnett's test. Asterisks indicated statistically significant values from control. *P<0.05, **P<0.01, ***P<0.001.*

Table 2. Effect of chloroform extract of *U. sinuata* (CEUS) on exploratory behaviour (Open field test)

Groups	Dose (p. o.) (mg/kg)	No. of movements			
		0 min	30 min	60 min	90 min
Control	0.5 ml/mice	278.21 ± 4.85	284 ± 3.69	290.04 ± 4.20	298.56 ± 5.60
Diazepam	1	130.45 ± 5.62	125.65 ± 1.23***	119.68 ± 3.45***	115.56 ± 4.21***
CEUS	200	155.79 ± 6.97	143.48 ± 2.65	141.58 ± 3.15	139.79 ± 5.26
CEUS	400	141.48 ± 8.12	135.58 ± 3.12	133.78 ± 4.12	122.84 ± 3.75

*Values are mean ± SEM; *** p<0.001 Dunnett's test as compared to control.*

3.3.3 Elevated plus maze test

The activities of mice model experimented in EPM test showed that CEUS at the dose of 400 mg/kg ($p < 0.001$), significantly increased the entry percentage of mice into the open arms and the percentage of spending time in the open arms of the EPM as shown in Table 3. The effects of treatment of mice at the dose of 200 mg/kg on open arm entries and time spent in open arms were dose-dependent. The number of closed arm entries and time spent in the closed arms reduced significantly in the extract treated groups which were worthy of comparison with the standard diazepam.

3.3.4 Thiopental sodium-induced sleeping time

In Thiopental Na induced hypnosis test, the extract at doses, 200 and 400 mg/kg demonstrated a significant reduction in the time of inception of sleep in a dose-dependent manner (Table 4). The effect of the extract (200 and 400 mg/kg) was similar to that of standard in the case of onset of sleep. Both doses of the extract increased the duration of sleeping time induced by Thiopental Na in test animals compared to controls Table 4.

3.4 Analgesic Activity

3.4.1 Hot plate method

Fig. 2 demonstrated the results of the hot plate test. CEUS amplified the latency time at doses of 200 and 400 mg/ kg after 60, 120, 180 and 240 minutes, respectively. The extract, at a dose of 400 mg/kg exhibited most significant at a time interval of 180 min.

3.4.2 Acetic acid-induced writhing test

Intraperitoneal administration of CEUS (200 and 400 mg/kg) had a dose-dependent

antinociceptive effect and reduced the number of writhing movements significantly which was induced by i.p. administration of the acetic acid compared with the control group ($p < 0.001$). The percentages of inhibition 55.97% were obtained at a dose of 400 mg/kg (CEUS) is most significant among all where Diclofenac sodium as positive control demonstrated with an inhibition of 45.90%. Nevertheless, at a dose of 200 mg/kg with inhibition 49.3% is got to be significant ($p < 0.01$) compared to the control group (Table 5).

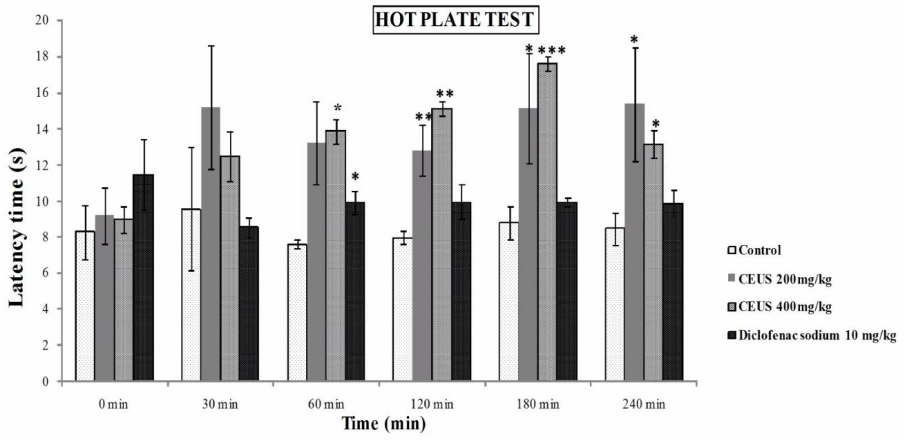

Fig. 2. Effect of chloroform extract of *U. sinuate* (CEUS) on hot plate test

*Values are reported as mean ± SEM. for group of five animals. The data was analyzed by ANOVA followed by Dunnett's test. Asterisks indicated statistically significant values from control. *P<0.05, **P<0.01, ***P<0.001.*

Table 3. Effect of chloroform extract of *U. sinuata* (CEUS) on EPM test during 5 min test session

Groups	% of number of entry into open arm	% of Time (in seconds) spent in open arm
Control	53.480 ± 2.011	50.780 ± 5.546
Diazepam	71.383 ± 1.5530***	77.450 ± 4.811**
CEUS 400 mg/kg	79.370 ± 2.557***	84.310 ± 3.830***
CEUS 200 mg/kg	63.170 ± 2.232*	65.420 ± 3.577

*Values are mean ± SEM; *p<0.05, **p<0.01, ***p<0.001, Dunnett's test as compared to control.*

Table 4. Effect of chloroform extract of *U. sinuata* (CEUS) on Thiopental sodium induced sleeping time

Groups	Onset of sleep (min)	Duration of sleep (min)
Control	39.20 ± 2.623	46.90 ± 0.459
Diazepam	12.98 ± 0.432***	147.26 ± 2.425**
CEUS 200 mg/kg	17.99 ± 1.512**	121.00 ± 7.631**
CEUS 400 mg/kg	11.44 ± 2.354**	180.00 ± 3.235**

*Values are mean ± SEM; **p<0.01, ***p<0.001, Dunnett's test as compared to control.*

Table 5. Effect of chloroform extract of *U. sinuata* (CEUS) on acetic acid induced writhing response in mice

Groups	Treatment	Dose	No. of writhing	Percent inhibition
Control	1% Tween-80 in water	10 ml/kg, p. o.	49.25 ± 2.594	-
Standard	Diclofenac Sodium	10 mg/kg, i, p.	21.3 ± 0.942*	45.9
Test	CEUS	200 mg/kg, p. o.	25.00 ± 1.414**	49.3
Test	CEUS	400 mg/kg, p. o.	17.23 ± 5.88***	55.97

*Values are mean ± SEM; *p<0.05, **p<0.01, ***p<0.001, Dunnett's test as compared to control.*

3.4.3 Formalin-induced nociception

Table 6 demonstrated the result of statistical evaluation of the effect of CEUS on formalin-induced writhing in mice. The results of the test exhibited that at a dose of 400 mg/kg CEUS showed very significant ($p < 0.001$) inhibition of writhing reflex in both two-phase with a inhibition of 37.39% at 1st phase and 65.98% at last phase, while the standard drug Diclofenac inhibition obtained to be significant 47.27% at a dose of 10 mg/kg body weight only in the last phase. The analgesic activity of the extract was noteworthy in comparison with the control animals.

3.4.4 Tail immersion test

The tail withdrawal reflex time following administration of CEUS at a dose level of 200 and 400 mg/kg b.w. showed almost same as raising the dose of the sample comparable to the reference drug (Table 7). Highly significant ($p < 0.001$) activity was found by the administration of CEUS at a dose of 400 mg/kg in 90 min, which was 4.94 ± 0.30.

3.4.5 Glutamate-induced nociception

In the glutamate-induced test, it was observed that CEUS significantly inhibited the noxious stimuli by a dose-dependent L-glutamic acid. These inhibitions are comparable to the Diclofenac sodium, the reference drug which creates 58.32% inhibition of the pain (Table 8). The 400 mg/kg created 88.68% inhibition of the nociception that is upper than the effect formed by the reference drug.

3.5 Anti-inflammatory Activity

3.5.1 Carrageenan-induced rat paw edema

The anti-inflammatory activity at test doses (200 and 400 mg/kg i.p.) of CEUS showed in Table 9 with the mean volume of the paw edema. In paw, the injection of the carrageenan produced inflammatory edema which increased gradually. The CEUS at the dose of 400mg/kg showed an anti-inflammatory activity that became satisfactory ($p < 0.001$) 3 h after the injection of carrageenan and was maintained all along the experiment with a maximum effect of %. The extract (200 mg/kg) also induced significant ($p < 0.001$) anti-inflammatory effect and the anti-inflammatory effect of Diclofenac sodium (10 mg/kg) was not higher than that of the extract as presented in Table 9.

3.5.2 Cotton pellet-induced granuloma

The effects of CEUS and Diclofenac sodium on the proliferative phase of inflammation are presented in Table 10. The weight of cotton pellets was significantly decreased with CEUS (200 and 400 mg/kg b.w.) compared to the vehicle-treated rats.

3.5.3 Antipyretic activity

The effect of CEUS on normal body temperature in rats is showed in Table 11. In this test, CEUS at a dose of 400 mg/kg body weight created a significant reduction in the body temperature up to 4 h. CEUS at this dose decreased temperature from 37.57 °C to 37.35 °C in 2nd hour ($p < 0.001$) and 37.16 °C ($p < 0.001$) in 3rd hour and lowest temperature 36.29 °C at the 4th hour.

3.6 Analysis of the Possible Mechanism of Action of CEUS

3.6.1 Involvement of the pathway of cyclic guanosine monophosphate (cGMP)

The current study found at the effects of 200 and 400 mg/kg CEUS and methylene blue (20 mg/kg) treatments. CEUS and methylene blue administration alone significantly inhibited abdominal writhing induced by acetic acid (Table 12). Together, methylene blue significantly ($p< 0.001$) developed CEUS (200 and 400 mg/kg) induced antinociception compared to the treatment of both CEUS and methylene blue alone.

Table 6. Antinociceptive profile of chloroform extract of *U. sinuata* (CEUS) assessed by the formalin test in mice

Groups	Dose (mg/kg)	Mean ± SEM (% writhing) 1st phase (0-5 min)	% inhibition 1st phase (0-5 min)	Mean ± SEM (%Writhing) last phase (20-30 min)	% inhibition last phase (20-30 min)
Control	-	14.33 ± 1.33	-	22.3 ± 1.838	-
Diclofenac Sodium	10	13.99 ± 1.0	2.31%	10.67 ± 1.67***	55.37%
CEUS	200	13.34 ± 0.764	6.897%	13.0 ± 2.66***	47.27%
CEUS	400	8.83 ± 1.187***	37.39%	9.5 ± 1.88***	65.98%

*Values are mean ± SEM; ***p<0.001, Dunnett's test as compared to control.*

3.6.2 Involvement of ATP-sensitive K+ channel pathway

The current learning looked at the effects of 200 and 400 mg/kg CEUS and Glibenclamide (10 mg/kg) treatments. It was found that only administration of Glibenclamide (10 mg/kg) did not change the abdominal writhing count during assessing through the injection of 0.6% acetic acid (Table 13). When given together, the antinociceptive activity of CEUS (200 mg/kg, 400 mg/kg) was noticeably reduced by Glibenclamide.

4. DISCUSSION

This current study reported the neuropharmacology, analgesic, anti-inflammatory and antipyretic potential of the chloroform extract of *U. sinuata* leaves (CEUS) along with its phytochemical and toxicity analysis. After treatment of different doses of control, diazepam, and CEUS, comparing with control group it was established that CEUS exerts a significant dose-dependent neuropharmacological activity observed by hole cross, open field test, elevated plus-maze test, Thiopental Na induced sleeping time test. Hole cross test was used for screening sedative effect. For hypnotic effect, we used Thiopental Na induced sleeping time test, open field, and EPM test were done for angiogenic and anxiolytic activity.

The presence of carbohydrates, free reducing sugars, alkaloids, combined reducing sugars, tannins, and saponins was confirmed by the phytochemical screening of the crude extract and the active fraction. The therapeutic effects of medicinal plant extracts used as traditional remedies have been accredited to a combination of active secondary metabolites [36]. Sedative properties, antagonistic effects of amphetamine as well as reduced spontaneous motor activity have been shown by saponins in study animals (Wagner et al., 1985). Moreover, tannins, flavonoids, alkaloids, sterols, terpenes, and resins in medicinal plants were found by Duke et al., 1992 [37] to have significant antinociceptive, sedative, and anti-psychotic effects.

Very few animal models are as familiar as The EPM for anxiety test [38,39]. Researchers have widely used EPM to monitor the effects of the drug [40,41] because of its use to natural conditions as well as stimuli to induce anxiety, such as a fear of brightness, open space and a new and the fear of balancing on a relatively narrow raised surface [42]. In EPM, immature mice normally prefer to spend much of their allotted time in the closed arms. This behavior seems to indicate an aversion towards open arms that is produced by fear of open spaces. Drugs that increase open arm exploration are believed as anxiolytics and the reverse holds true for anxiogenics [43]. In this learning, we experienced that the administration of different doses (200 and 400 mg/kg body weight) of CEUS generated an effect like anxiolytic in mice, as it improved open arm entries and the time spent in the open arms of the EPM as compared to the animals that are controlled.

Table 7. Effect of chloroform extract of *U. sinuata* (CEUS) on radiant heat tail immersion response in mice

Groups	Dose (mg/kg)	Response times (in min)				
		0 min	30 min	60 min	90 min	120 min
Control	10 ml/kg	2.87 ± 0.48	2.63 ± 0.44	2.65 ± 0.43	2.25 ± 0.43	2.55 ± 0.38
Diclofenac sodium	10	2.66 ± 0.56	5.35 ± 0.38***	6.27 ± 0.59**	4.79 ± 0.30***	3.47 ± 0.26
CEUS	200	2.71 ± 0.27	3.56 ± 0.16	4.62 ± 0.34*	3.38 ± 0.24*	2.94 ± 0.15
CEUS	400	2.66 ± 0.12	3.38 ± 0.16	4.3485 ± 0.29*	4.94 ± 0.30***	3.42 ± 0.34

*Values are mean ± SEM; * p<0.05, ** p<0.01, *** p<0.001, Dunnett's test as compared to control.*

Table 8. Effect of chloroform extract of *U. sinuata* (CEUS) on Glutamate-induced nociception response in mice

Groups	Dose (mg/kg)	Responses	
		Number of licking (Mean ± SEM)	% Inhibition
Control	10 ml/kg	167.20 ± 4.87	0
Diclofenac sodium	10	45.50 ± 1.09***	58.32
CEUS	200	51.00 ± 3.39***	69.73
CEUS	400	38.80 ± 3.85***	88.68

*Values are mean± SEM; *** p<0.001, Dunnett's test as compared to control.*

Table 9. Effect of chloroform extract of *U. sinuata* (CEUS), control and Diclofenac sodium (Diclofenac 10 mg/kg) on carrageenan-induced paw edema test

Groups	0 h	½ h	1 h	2 h	3 h	4 h	6 h
Control	0.5801 ± 0.0453	1.185 ± 0.1233	1.480 ± 0.021	1.485 ± 0.0300	1.576 ± 0.056	1.70 ± 0.0653	1.471 ± 0.0565
Diclofenac Sodium 10 mg/kg	0.7950 ± 0.0817	1.027 ± 0.0495	1.042 ± 0.0497**	1.087 ± 0.0402**	1.055 ± 0.0030***	1.330 ± 0.1084*	1.175 ± 0.1324**
CEUS 200 mg/kg	0.9500 ± 0.0337	1.074 ± 0.0857	1.144 ± 0.0822**	1.124 ± 0.0910**	1.007 ± 0.0480***	1.47 ± 0.0888	1.275 ± 0.0909
CEUS 400 mg/kg	0.8575 ± 0.0132	0.983 ± 0.0425	1.158 ± 0.0345*	1.281 ± 0.0483*	1.047 ± 0.0413***	1.555 ± 0.0858	1.395 ± 0.0696

*Values are mean ± SEM; *p<0.05, **p<0.01, ***p<0.001, Dunnett's test as compared to control.*

Table 10. Effect of chloroform extract of *U. sinuata* (CEUS), control and Diclofenac sodium (Diclofenac 10 mg/kg) on Cotton pellets-induced granuloma in rats

Groups	Dose (mg/kg)	Weight of cotton pellets (mg) (wet)	Percentage inhibition	Weight of cotton pellets (mg) (dry)	Percentage inhibition
Control (0.9% NaCl)	5 ml	163.17 ± 11.21	-	49.60 ± 3.1	-
Diclofenac Sodium	10	79.05 ± 6.36***	58.08	23.34 ± 2.47***	52.17
CEUS	200	102.63 ± 8.52*	44.51	32.36 ± 2.34	33.43
CEUS	400	88.45 ± 7.31**	53.52	25.99 ± 2.18*	47.66

*Values are mean ± SEM; *p<0.05, **p<0.01, ***p<0.001, Dunnett's test as compared to control.*

Table 11. Effect of chloroform extract of *U. sinuata* (CEUS) on Yeast-induced pyrexia in mice

Groups	Dose (mg/kg)	Rectal temperature (°C)					
		Initial rectal temperature before yeast injection	After administration of drug				
			0 h	1st h	2nd h	3rd h	4th h
Control	10 ml	37.46 ± 0.12	38.92 ± 0.17	38.72 ± 0.45	38.58 ± 0.11	38.48 ± 0.30	38. 38 ± 0.35
Paracetamol	150	37.06 ± 0.29	38.46 ± 0.14	37.60 ± 0.01**	37.88** ± 0.03	37.30 ± 0.02***	36.35 ± 0.08***
CEUS	200	36.58 ± 0.11	37.70 ± 0.13	37.43 ± 0.05**	37.38 ± 0.08**	37.19 ± 0.13***	36.76 ± 0.09***
CEUS	400	36.84 ± 0.09	37.57 ± 0.19	37.44 ± 0.12**	37.35 ± 0.09***	37.16 ± 0.04***	36.29 ± 0.13***

*Values are mean ± SEM; *p<0.05, **p<0.01, ***p<0.001, Dunnett's test as compared to control.*

Table 12. Effect of chloroform extract of *U. sinuata* (CEUS) on Involvement of cyclic guanosine monophosphate (cGMP) pathway

Treatment	Dose	No. of writhing	Percent inhibition
1% Tween-80 in water	1 ml/mice	69.50 ± 2.594	-
Methylene Blue	10 mg/kg, i. p.	58.25 ± 3.10	22.79
CEUS	200 mg/kg, p. o.	32.85 ± 2.45***	51.82
CEUS	400 mg/kg, p. o.	21.10 ± 0.90***	70.45

*Values are mean ± SEM; *** p<0.001, Dunnett's test as compared to control.*

Table 13. Effect of chloroform extract of *U. sinuata* (CEUS), control and Glibenclamide (Glibenclamide 10mg/kg) on Involvement of ATP-sensitive K+ channel pathway

Treatment	Dose	No. of writhing	Percent inhibition
1% Tween-80 in water	1 ml/mice	73.50 ± 1.89	-
Glibenclamide	10 mg/kg, i. p.	78.80 ± 2.48	-
CEUS	200 mg/kg, p. o.	40.89 ± 2.89***	45.88
CEUS	400 mg/kg, p. o.	18.23 ± 1.98***	77.83

*Values are mean ± SEM; *** p<0.001, Dunnett's test as compared to control.*

An increased locomotor activity is believed as an increase in watchfulness and a reduction in locomotor activity suggested sedative effect [44]. The current learning examined some neuropharmacological activities of CEUS. Central nervous system depressant activity has been found in the plant extract as showed by the reduction in open arm roaming in mice. Furthermore, locomotor activity study, as performed by hole cross and open field tests, presented that the frequency and the amplitude of movements were reduced by CEUS (200 mg/kg and 400 mg/kg). As locomotor activity exhibits the level of excitability of the CNS [45]. This reduction in spontaneous motor activity indicates to the sedative effect of the plant extracts [46].

The above result showed that crude chloroform extracts of *U. sinuata* leaves have strong sedative and hypnotic activity that mainly mediated by the GABA receptor complex in the central nervous system (CNS). GABA is one of the essential inhibitory neurotransmitters in the human CNS. An imbalance in the pathophysiology of convulsions, anxiety, and sleep between both excitatory and inhibitory neurotransmitters is indicated by undeniable evidence [47]. GABA receptor system is the major fast-acting inhibitory neurotransmitter system in the brain. To treat anxiety disorders and epilepsy it is believed as the main pharmacological target for many clinically used drugs. The GABA receptors are mainly heteromeric GABA-gated Cl-channels. The BZD place on GABA receptors controls the inhibitory effects of GABA [48]. Interacting with GABA receptor a barbiturate drug known as Thiopental generates sedative-hypnotic effect which increases the GABAergic transmission. On the other hand, excitatory glutamate receptors can be blocked by Thiopental. All these activities direct to reduce neuronal activity that endorses the following reference substances which show sedative action.

For the evaluation of centrally acting analgesics known to raise the pain threshold towards heat, the hot plate, and tail immersion methods are performed [49]. In short, the hot plate test determines the response, noxious stimulus having a close similarity to clinical pain. The complex response to a non-inflammatory, acute nociceptive input is determined by this test [50]. It is important that any agent causing a persistence of the hot plate latency by performing the test must be acting centrally [51]. In the hot plate test, the chloroform extract *U. sinuata* showed a latency of higher time than the control group in a dose-related manner. So, the extract is believed to have central nervous system activity.

The tail-immersion response is believed to be a spinally mediated reflex [52]. Moreover, Grumbach et al presented that the analgesic agents effectiveness in the tail flick pain model is very much correlated with human pain relief [53]. CEUS delayed the tail-immersion latency, supporting an enhance in the nociceptive threshold.

By the action of analgesic drugs, the formalin test is divided into the two phases. The Opiate that inhibits both phases equally, act centrally for

the most part. However, nonopiate analgesics, as an example dipyrone with both central and peripheral site of actions generates an analgesic effect in both phases of the formalin test, mainly in the 2nd phase, where the pain is blocked at lower doses than those necessary to be inhibited in the first phase. In the current investigation, the activity performed in the first phase of the formalin test, a phase in which, as confirmed by morphine, the action happens at the level of the central nervous system [54]. Moreover chloroform *U. sinuata* leaves extract generated a significant effect on the second phase at lower and higher doses but it exhibited significant effect only in a higher dose in the first phase.

The acetic acid-induced test, a well-known model that is widely used for the screening of analgesic or anti-inflammatory properties of novel agents [55] generally, used as a model to find out the peripheral antinociceptive effect of extracts or compounds. This model of nociception is suggested to signify the stimulation of peripheral mechanism as the administration of phlogogen guide to increase in the levels of cyclooxygenase (COX) and lipooxygenase (LOX) [56] and eventually guides to the discharge of endogenous nociceptive mediators such as prostanoids of the PGE2 and PGF2α types, histamine, cytokines, serotonin and eicosanoids and as well as other lipooxygenase products in peritoneal fluids that can induce sensitivity of various peripheral nociceptive neurons to NSAIDs within the peritoneal cavity [57,58].

The rise of PGEs levels in the peritoneal fluid associates to prolong irritation of the peritoneal cavity that increases capillary permeability [59,60], and the discharge of substance P and glutamate from peripheral afferent fiber terminals [61]. In this circumstance, the capability of CEUS to ease the acetic-acid-induced abdominal constriction test indicates that the extract's antinociceptive ability to inhibit COX as well as LOX in the peripheral tissues leading to reduce in PGEs synthesis and obstruction of the pain transduction in primary afferent nociceptor. As it could not be performed to demand the involvement of peripheral or central mechanisms in the CEUS antinociception, this test also has been used as a nonspecific test [62] This model is believed to have poor specificity because certain drugs such as muscle relaxants, and can lessen the number of abdominal constriction too [63]. However, further studies performing other nociceptive models are needed before having the last conclusion on the antinociception mechanisms of CEUS or other antinociceptive agents could be generated.

For establishing the function of the glutamatergic system in the modulation of CEUS antinociception, we subjected the extract to a test well known as glutamate-induced paw licking test. Glutamate is a vital excitatory neurotransmitter in the CNS [64], and it is found by many reports that the glutamate and glutamatergic receptors (both ionotropic and metabotropic glutamate receptors) are essential in the peripheral, spinal, and supraspinal nociceptive neurotransmission [65,66] which are highly mediated by both N-methyl-D-aspartate (NMDA) and non-NMDA receptors and by the release of NO and NO-related substances [28]. On the contrary, it has been found that NMDA receptor antagonists obstruct the spread of pain sensation and to minimize the hyperexcitability of spinal cord neurons triggered by C-fiber stimulation [67,68]. In addition, it has been found that the activation of glutamate receptors to support to the maintenance of peripheral nociceptive processes that are connected with inflammatory, but not physiological pain [69], which is concurrent with report that introduction of glutamate receptor inhibitor inhibited the inflammatory, but not neurogenic phases of the formalin-induced test [70]. According to our findings, the glutamatergic system involved in the modulation of CEUS antinociception.

The current learning also considered the probable contribution of cGMP pathway on the antinociceptive activity of CEUS. The cellular level of cGMP influences physiological functions such as pain and analgesia that is regulated by the action of sGC mediated by nitric oxide (NO) [34]. It has been found that the activation of protein kinases and phosphodiesterases may direct the action of cGMP [71]. The activation or deactivation of nociresponsive neurons is regulated by the availability of cGMP whereas the action of soluble guanylyl cyclase (sGC) and as well as the rate of degradation by cGMP-specific phosphodiesterase regulates the concentration of intracellular cGMP. For examining the possible attachment of cGMP in CEUS induced antinociception, methylene blue (MB), a guanylyl cyclase and/or NO inhibitor was administered before inducing nociception of acetic acid with intraperitoneal injection. The result shows that the pre-treatment with methylene blue significantly reduced the nociception caused by acetic acid and also

improved the antinociceptive effect exhibited by CEUS.

It has been found that MB enhances antinociceptive activity by inhibiting peripheral NO and sGC resulting NO interference (Abacioğlu et al., 2000). In acetic acid-induced writhing model in mice pre-treatment with MB and subsequent administration of CEUS at all doses increased antinociceptive activity than the groups where only CEUS were administered, So it is clear that the antinociceptive effect of CEUS involves the NO-cGMP pathway.

Our current study also demonstrated that Glibenclamide, an ATP-sensitive K+ channel antagonist, partly reversed the antinociceptive activity shown by CEUS. Previous studies reported specific blockade of ATP-sensitive K+ channel by Glibenclamide while that was not affecting other types of K+ channel like Ca^{2+} activated and voltage-gated K+ channels. The results of this learning might show the involvement of ATP-sensitive K+ channel opening and subsequent efflux of K+ ion and membrane repolarization and/or hyperpolarization by CEUS which minimize the membrane excitability [72].

Carrageenan-induced inflammation was found having two phases i.e. early phase (up to 2 hours) and late phase (1-6 h). Significantly severe inflammation was related to early phase while late phase was found to have a slow rise in the volume of paw edema. The initial phase accredited to the action of mediators like histamine, serotonin, and bradykinin on vascular permeability [73]. The late phase edema was presented to be a result of overproduction of prostaglandins [74]. In the early phase of inflammation, the result of pre-treatment of CEUS (at all the doses) is effective which has been found for releasing of histamine and serotonin primarily. A hypothesis can be prepared based on this that the extract may be exhibiting its effect through inhibition of histamine release.

Chronic inflammation is the reaction evolving if the acute response is insufficient to take out the pro-inflammatory agents [75]. Very few methods are as appropriate as the Cotton pellet implantation method for examining the efficacy of drugs against the proliferative stage of inflammation [76]. The cotton pellet granuloma method has been extensively used for determining the exudative, transudative and proliferative component and is a usual characteristic of established chronic inflammatory reaction. The wet weight of the granuloma, as well as the dry weight, are influenced by fluid absorbed by the pellet that links well with the amount of granulomatous tissue produced, Monocyte infiltration and fibroblast proliferation take place in chronic inflammation. The proliferation of small vessels or granuloma spread this proliferation. The size of the granuloma is minimized by NSAIDs, which results from the cellular reaction by blocking granulocyte inflammation/infiltration which impedes production of collagen fibers and suppressing mucopolysaccharides [77]. In the current study, the CEUS 400 mg/kg presented a reasonable significant reduction in the wet as well as the dry weight of the cotton palette granuloma which states that chronic administration of the drug minimizes the proliferation of fibroblast and fluid gathering in chronic inflammation in a weak manner.

5. CONCLUSION

The current study exhibits that neuropharmacological, antipyretic, anti-inflammatory and antinociceptive activities have been shown by CEUS. The antinociceptive effect of CEUS also concerned cGMP pathway and ATP sensitive K+ channel pathway. Most importantly crude extract of this plant did not show any toxicity to animals and rich of potent phytochemicals yet more experiments are needed to realize the mechanisms of action of CEUS clearly and testing of potential compounds which are isolated to find out the active agents that can function as a potential lead for better drug development.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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