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Evaluation of the Combined Effect Onethanolic Extracts of *Pisoniagrandis* and *Cardiospermumhalicacabum* Inanti-Arthritic, Anti-Inflammatory and Analgesic Activities

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Abstract

In India, several plant species are utilized as a medicine for pain & arthritis condition. Various human disorders predominantly arthritic condition causes inflammation and pain as nonspecific symptoms. Pain is an uncommon mechanism which is caused by inflamed cells or tissues. Many plant-based medicines with analgesic and anti-inflammatory properties have been used for a long time with no negative side effects when comparing to synthetic drugs with same properties. The aim of this study is to evaluate the anti-inflammatory, anti-arthritic and analgesic properties of BHE which contain equal amount of *Pisoniagrandis* and *Cardiospermumhalicacabum* leaves with ethanolic extract extracts of *Pisoniagrandis* (EPG) and *Cardiospermumhalicacabum* (ECH). Analgesic activities of plant extract were measured by thermal methods such as hot plate, tail flick method along with chemical method of acetic acid writhing. The anti-arthritic and anti-inflammatory potential of BHE, EPG and ECH was evaluated by formalin induced acute non –immunological arthritis model as well as turpentine oil andcarrageenan induced paw edema in rats. The BHE showed a substantial ($p<0.01$) reduction in paw volume and edema when compared to EPG and ECH. The Hot plate, Tail flicking, and acetic acid writhing methods all showed the same effect, suggesting that BHE has potential central and peripheral analgesic activity.BHE, which is made up of two herbal plants, has anti-arthritic, anti-inflammatory, and analgesic properties that promote its traditional use by reducing the immunological and inflammatory reactions seen in most arthritic conditions.

Key words: Pain, Anti-Arthritic, Anti-Inflammatory, Analgesic activity, *Pisoniagrandis, Cardiospermumhalicacabum.*

Introduction

Inflammation is the body's complex biological response to harmful stimuli like pathogens, damaged cells, or physical or chemical irritants. It's a defense mechanism designed to eliminate the injurious stimuli from the body and induce the healing process. It entails the involvement of various cell types, each of which expresses and reacts to a different mediator in a very specific order.However,

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sustained inflammation can lead to various human disorders including arthritis, psoriasis, and inflammatory bowel disease. It is typically accompanied by pain, redness, swelling, heat, and other symptoms. Inflammation has been related to a number of diseases, including wounds, trauma, and swelling, as well as cancer, cardiovascular disease, arthritis, neurodegenerative disease, diabetes mellitus, and obesity, according to recent studies[1]. Arthritis is a group of diseases characterized by inflammation of the joints and surrounding tissues, which may causes pain, swelling, and stiffness in the joints, as well as cartilage and bone degradation and it occurs in two forms such as rheumatoid arthritis and osteoarthritis [2].It has been reported that production of pro-inflammatory mediators such as cytokines, prostaglandins, leukotrienes play key roles in the inflammation that occurs during the progression of arthritis and the changes in their inflammatory mediators production may be an effective treatment for inflammation and arthritis[3]. Pain is an abnormal sensation associated with tissue damage, such as injury, inflammation, or cancer, is called neuralgia[4]. In the central and peripheral nervous systems, pain transmission is a complex neurochemical mechanism[5]. Histamine, acetylcholine, bradykinin, prostaglandin, and 5-hydroxyl tryptamine are only a few of the naturally occurring compounds that can cause pain. It begins when nociceptors in the peripheral nervous system are activated, or when the peripheral or central nervous systems are weakened or malfunction[6]. The non-steroidal drugs used for the treatment of pain and inflammation may causes some disorders like ulceration, hemorrhage, kidney and liver dysfunction. Therefore plant-based medicines with analgesic and anti-inflammatory properties have been used for a long time with no negative side effects when comparing to synthetic drugs with same properties[7,8].

Several plant species are utilized as a medicine for various human diseases. However, they are often misused due to a lack of scientific support. As a result, a comprehensive pharmacological assessment and documentation of plants which are used in the herbal medicine is required[8]. *Pisonia grandis*, also known as 'Leechaikottaikeerai,' in tamil belongs to the Nyctaginaceae family and is found across India. *Pisonia grandis* has long been used as an antifungal and anti-rheumatic agent and *Pisonia grandis* leaves can be used to treat chronic rheumatism, wound healing, and as a vegetable[9]. In Asia and Africa, *Cardiospermumhalicacabum*, also known as the Balloon Plant or Love in a puff, is widely distributed. *Cardiospermumhalicacabum* is predominantly used as diuretic, emmenagogue, laxative, refrigerant, rubefacient, and stomachic. Rheumatism, nervous disorders, limb weakness, and snakebite are among the conditions for which *Cardiospermumhalicacabum* is recommended. Therefore, successful formulations must be created using indigenous medicinal plants in conjunction with appropriate

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pharmacological studies and clinical trials[10, 11]. Since *Pisoniagrandis* and *Cardiospermumhalicacabum* have a long history of use in the treatment of arthritis and have been clinically tested for their potency individually, the current research aims to determine their synergistic *invivo*anti-arthritic, anti-inflammatory and analgesic activity of the Bi-herbal ethanolic extract (BHE) which contain equal amount of leaves of *Pisoniagrandis*and *Cardiospermumhalicacabum* with ethanolic extract extracts of *Pisoniagrandis*(EPG) and *Cardiospermumhalicacabum* (ECH).

Materials and Methods

Collection of plant material

The leaves of *Pisoniagrandis*and *Cardiospermumhalicacabum* were obtained from the IMPCOPS, Chennai, India. Those were identified and authenticated by Dr. P. Jayaraman, Botanist, Plant Anatomy Research Centre (PARC), Chennai. The voucher specimen is available in the herbarium file of the PARC, Chennai, India. Chemicals reagents used in this study were purchased from SD Fine Chemicals Ltd., India and are of analytical grade.

Preparation of Plant extract

The leaves of *P. grandis*(500gms) and *C. halicacabum*(500gms) were shade dried and pulverized to a coarse powder. The extraction was carried out using 90% (v/v) ethanol by cold maceration process for consecutive three days which is followed by filtration. Initially the filtrate was subjected to evaporation under pressure to remove all solvent followed by freeze drying to eliminate water content which results in sample yield of 0.98 %(w/w). Similarly the EPG and ECH were also prepared separately. The EPG extract yield was 0.42 %(w/w) and ECH sample yield was 0.38%(w/w).The extracts were stored in refrigerator and used for the present investigations.

Animals

Adult albino male rats of Wister strain weighing 120 - 175 g and adult Albino mice were purchased from animal house in Vel's College of pharmacy, Chennai, India and used for pharmacological and toxicological studies. The inbred animals were maintained in ambient temperature with uniform 12:12-h light:dark cycle in propylene cages. They were fed with balanced rodent pellet diet got from Poultry Research Station, Nandanam, Chennai, India and tap water ad libitum was provided throughout the experimental period. In advance to start with the experimental studies, the animals were kept in the

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laboratory for nearly 7 days to make it to adapt for lab environment. The Animal Ethics Committee constituted for the purpose as per CPCSEA Guideline agreed the protocol.

Acute toxicity studies

Acute toxicity studies were organized using staircase method mentioned by Ghosh[12] for adult Albino mice with BHE, EPG and ECH extracts. Albino mice of either sex were chosen and confined to 7 groups having 6 animals each and were given the dose orally starting from 50mg/kg b.w. up to 2000mg/kg b.w. of BHE, EPG and ECH extracts dissolved in 0.5% aqueous Tween-80. The drug treated animals were observed carefully for its toxicity signs and mortality. LD_{50} doses were selected for evaluation of concomitant anti-arthritic, anti-inflammatory and analgesic activity. All the treated animals were regularly observed for the clinical symptoms and mortality for 14 days.

Determination of Acute non –Immunological Anti-arthritic activity

Formaldehyde induced arthritis in rats

The rats were induced to get arthritis using formaldehyde and it was examined via Kaithwas and Majumdar method [13]. For this experiment, the rats were divided in to five groups of six animals each.

Group I - control and received a suspension of 2ml/Kg CMC *p.o.* (2% v/v of CMC),

Group II, II and IV - animals received 200mg/Kg body weight of BHE, EPG and ECH respectively in 2ml/Kg of 2% CMC *p.o*and

Group V- animals received standard drug aspirin (100mg/Kg body weight of in 2ml of 2% CMC *p.o.*) for 10 days.

During the first day, 30 minutes after drug administration acute non immunological arthritis was induced by sub plantar injection of 0.1ml containing 2% formalin in 0.9% saline solution in the right hind foot and the same was repeated on day 3. Arthritis in mice was evaluated by measuring the mean increase in paw diameter for a period of 10 days using plethysmometer.

Determination of anti-inflammatory activity

Turpentine oil-induced joint edema in rats

Joint edema was induced in rats using turpentine oil and it was examined viaKaithwas and Majumdar[13]. For this experiment, the rats were divided in to five groups of six animals each.

Group I - control animals were given suspension of 2ml/Kg CMC *p.o.* (2% v/v of CMC)

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Group II-IV - animals received 200mg/Kg body weight of BHE, EPG and ECH respectively in 2ml/Kg of 2% CMC *p.o*only once.

Group V - animals received a standard drug aspirin(100mg/Kg body weight of in 2ml of 2% CMC *p.o.*).

Acute non-immunological inflammatory joint edema was produced by injecting 0.02ml of turpentine oil into the synovial cavity of the right knee joint, 30 min after the drug administration. Diameter of the joint was monitored at hourly intervals for 6 hrs using a plethysmometer. The paw volume increase percentage inhibition was calculated by the formula:

% Inhibition = (1 – (drug treated/negative control)) * 100

Carrageenan-induced Paw edema in rats

Paw edema was induced in rats via Carrageenan and it was studied by the method described by Venkatesaperumalet, al.[14]. For this experiment, the rats were divided in to five groups of six animals each.

Group I - control and received a suspension of 2ml/Kg CMC *p.o.* (2% v/v of CMC)

Group II-IV - animals received 200mg/Kg body weight of BHE, EPG and ECH respectively in 2ml/Kg of 2% CMC *p.o*for seven days.

Group V - animals received a standard drug aspirin (100mg/Kg body weight of in 2ml of 2% CMC *p.o.*) for seven days.

Acute inflammation was induced in all groups by injecting 0.1 ml of (1% w/v carrageenan in normal saline) into the sub-plantar region of the right hind paw of rats. On the seventh day, paw volume was measured1h prior to carrageenan injection using plethysmometer and every hour till 3 hr after carrageenan injection. Mean increase in the paw volume was measured and percentage inhibition was calculated.

Percentage of inhibition = 100 (1-Vt / Vc)

Where, Vc = volume of edema in control and Vt = volume of edema in test / standard compound.

Determination of Analgesic activity in mice

Eddy's hot plate method

For measuring response latencies to heat Eddy's hot-plate method was employed [15].

The animals were divided into five groups of 6 animals each,

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Group I - served as control in which animals were given a single administration of 2ml/kg vehicle (2% v/v of CMC) orally once.

Group II - Animals were treated with 1ml of 200mg/kg of BHE in vehicle (2% v/v of CMC) orally once.

Group III - Animals were treated with 1ml of 200mg/kg of EPG in vehicle (2% v/v of CMC) orally once.

Group IV - Animals were treated with 1ml of 200mg/kg of ECH in vehicle (2% v/v of CMC) orally once.

Group V - Animals served as positive control and were treated with 1ml of 100mg/kg of morphine in vehicle (2% v/v of CMC) orally.

Once the animals were treated with drug, after one hour they were kept on the hot plate at 55°C and their behavior towards pain stimulus in terms of paw licking or jumping were recorded. The response time was noted at 0, 30, 60, and 120 mins and a cut off reaction time of 15 secs is fixed.

Tail flick test

To assess the central analgesic activity of the plant material tail flick test is performed which measures the pain response in animals. Heat stress was applied to rat's tails and the effectiveness of analgesics is measured using Analgesiometer as described by Sahaet. al.[16]. The animals were divided into five groups of 6 animals each and were given dose schedule as the above experiment. By placing the tip of the tail (last 1-2 cm) the basal reaction time of the animals to radiant heat was calculated. The end point was calculated by flicking response (tail withdrawal from the heat).The animals which showed flicking response within 3-5 secs, were selected for the study. To prevent the tail being damaged a cut off period of 15 secs was fixed. At the time interval of 30, 60, 120 and 180 min after administration of drugs flicking response was calculated by tail flick apparatus.

Acetic acid induced writhing test

The peripheral analgesic activity of plant extracts were measured by the acetic acid induced writhing test in rats as described by Sahaet.al.[16].The animals were divided into five groups of 6 animals each.

Group I - served as control and received a suspension of 2ml/Kg CMC *p.o.* (2% v/v of CMC),

Group II-IV - animals received 200mg/Kg body weight of BHE, EPG and ECH respectively in 2ml/Kg of 2% CMC *p.o* and

Group V - animals received a standard drug diclofenac sodium (50mg/Kg body weight of in 2ml of 2% v/v of CMC) *p.o.*

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Followed by above animal grouping drug administration, 1% v/v aqueous acetic acid was administered intraperitonially to all the groups to produce writhing. Plant extracts were administered 30 minutes before injection of acetic acid. Individual animals after acetic acid injection were immediately kept inside the glass jar for observation for a period of 20 minutes. The time of onset of writhes was noted and the number of writhes in terms of trunk twist response, number of abnormal constrictions and extension of hind limbs were recorded. The mean writhing scores in control, standard (Diclofenac sodium), and test groups were calculated and the data represent the total numbers of writhes observed during 20 min and were expressed as writhing numbers.

Statistical analysis

The values reported are Mean±SE. The statistical analysis was carried out using analysis of variance (ANOVA) followed by Dunnet's't' test. The p values with < 0.05 were considered as significant.

Results

Acute toxicity studies

After administration of extracts at different dosage levels, no toxic symptoms were found up to maximum of 2000mg/kg *p.o.* according to OECD guideline 423. For further efficacy studies safe tolerable dose that is one tenth of dose was used. It was found that the maximum therapeutic dose level of BHE, EPG and ECH extracts were studied as 200mg/kg.

Determination of acute non Immunological Anti-arthritic activity

Formaldehyde induced arthritis in rats

Table-1 exhibits the efficiency of plant extracts and the standard drug aspirin on formaldehyde induced arthritis in rats. In the current study, the BHE significantly ($P<0.001$)quenched the joint edema while compared with their individual extracts of ECH and EPG between day 2 to day 10 post formaldehyde treatment.

Table-1- Effect of BHE, EPG and ECH extracts on formaldehyde induced Acute Immunological Arthritic model

Treatment	**Mean increase in paw diameter in (cm) and Inhibition (%) on**				
	2nd day	4th day	6th day	8th day	10th day
Group I CMC+ formalin treated (control)	0.40±0.07	0.73±0.06	0.82±0.10	0.90±0.03	1.19±0.18

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Group II BHE(200mg/kgb.w) + formalin treated	0.20±0.04a*** (50%)	0.30±0.05a*** (58.90%)	0.32±0.04a*** (60.97%)	0.35±0.23a*** (61.11%)	0.45±0.32a*** (62.18%)
Group III EPG(200mg/kgb.w) + formalin treated	0.25±0.05b** (37.50%)	0.40±0.07b** (45.20%)	0.42±0.07b** (48.75%)	0.43±0.10b** (52.22%)	0.55±0.12b** (53.78)
GroupIV ECH(200mg/kgb.w) + formalin treated	0.24±0.05c** (40%)	0.37±0.08c** (49.31%)	0.40±0.06c** (51.21%)	0.42±0.02c** (53.33%)	0.50±0.05c** (57.98%)
Group V Asprin (100mg/kgb.w) + formalin treated	0.11±0.03d*** (62.50%)	0.18±0.05d*** (75.34%)	0.20±0.07d*** (75.60%)	0.21±0.16d*** (76.66)	0.25±0.20d*** (78.99%)

Values are mean ± SEM from 6 animals in each group. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's `t' test. Comparison between a - Group I vs Group II,b – Group I vs Group III,c - Group I vs Group IV
and d- Group I vs Group V.P Values: *<0.05, ** <0.01, ***<0.001, ns – non significant.

Treatment with BHE exhibited 50, 58.90, 60.97, 61.11 and 62.18 percentage protection against edema, whereas EPG exhibited 37.50, 45.20,48.75, 52.22 and53.78 percent inhibition whereas ECH exhibited 40, 49.31, 51.21, 53.33and 57.98percent inhibition in edema respectively after 2,4,6,8 and 10^{th} days of treatment.The positive drug exhibited higher inhibition of 78.99% at the end of 10^{th} day treatment

Determination of anti-inflammatory activity

Turpentine oil-induced joint edema in rats

Table- 2 illustrates the efficiencies of BHE, EPH and ECG on joint edema in rats induced by turpentine oil. The BHE, amidst the three extracts displayed a highest reduction in swelling observed in the synovial cavity following the administration of turpentine oil. This was proved with 91.63% reduction in joint swelling when treated with BHE extract, whereas the ECH and EPG extracts revealed only 61.29% and 65.48% inhibition respectively at similar dosagefollowing 6hrs of treatment. The positive control aspirin exhibits 98.53% of inhibition in joint swelling.

Table-2- Effect of BHE, EPG and ECH extracts on Turpentine oil-induced joint edema in rats

Treatment	**Mean increase in paw diameter in (cm) and Inhibition (%) on**					
	1hr	2hr	3hr	4hr	5hr	6hr
Group I CMC+ turpentine oil	0.91±0.02	1.50±0.03	2.50±0.07	3.02±0.08	3.50.±0.13	4.78±0.21

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treated (control)						
Group II BHE(200mg/kg b.w) +turpentine oil treated	0.66±0.12*** (27.47%)	0.75±0.21a*** (50%)	1.40±0.19a*** (44%)	0.98±0.25a*** (67.54%)	0.47±0.17a*** (86.57%)	0.40±0.31a*** (91.63%)
Group III EPG(200mg/kg b.w)+ turpentine oil treated	0.80±0.17b** (12.08%)	0.87±0.15b** (42%)	1.44±0.11b** (42.40%)	1.70±0.25b** (43.70%)	1.64±0.17b** (53.14%)	1.85±0.69b** (61.29%)
Group IV ECH (200mg/kg b.w) + turpentine oil treated	0.81±0.10c** (10.98%)	0.95±0.15c** (36.66%)	1.54±0.19c** (38.40%)	1.61±0.17c** (46.68%)	1.49±0.31c** (57.42%)	1.65±0.30c** (65.48%)
Group V Asprin (100mg/kg b.w) + turpentine oil treated	0.40±0.43d*** (56.04%)	0.32±0.03d*** (78.66%)	0.23±0.03d (90.08)	0.12±0.17d*** (96.02%)	0.10±0.05d*** (97.14)	0.07±0.08d*** (98.53%)

Values are mean ± SEM from 6 animals in each group. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's `t' test. Comparison between a - Group I vs Group II,b – Group I vs Group III , c - Group I vs Group IV and d- Group I vs Group V.P Values: *<0.05, ** <0.01, ***<0.001, ns – non significant.

Carrageenan-induced Paw edema in rats

Table 3 clearly exhibits the acute inflammation in terms of paw edema of the right hind paws of control group within the first hour following the administration of carrageenan. On the other hand, the extracts of BHE, EPG, ECHand the reference drug aspirin displayed a significant (P<0.01) reduction in the paw edema of the rats present in group II, III, IV and V.

Table-3 Effect of BHE, EPG and ECH extracts on Carrageenan-induced Paw edema in rats

Treatment	**Mean increase in paw volume in (ml) and Inhibition (%) on**			
	0hr	**1hr**	**2hr**	**3hr**
Group I CMC+ carrageenan treated	0.10±0.09	0.42±0.06	0.68±0.08	0.85±0.09

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(control)				
Group II BHE(200mg/kg b.w) + carrageenan treated	0.08±0.14	0.15±0.02a*** (64.28%)	0.29±0.03a*** (57.35%)	0.36±0.03a*** (57.64%)
Group III EPG(200mg/kg b.w)+ carrageenan treated	0.08±0.05	0.20±0.06b** (52.38%)	0.45±0.07b** (33.82%)	0.48±0.09b** (43.52%)
Group IV ECH (200mg/kg b.w) +carrageenan treated	0.09 ±0.11	0.28±0.05c** (33.33%)	0.44 ±0.09c* (35.29%)	0.47±0.07c** (44.70%)
Group V Aspirin (100mg/kg b.w) +carrageenan treated	0.04±0.06	0.15±0.04d*** (64.28%)	0.22±0.03d*** (67.64%)	0.25±0.06d*** (70.58%)

Values are mean ± SEM from 6 animals in each group. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's `t' test. Comparison between a - Group I vs Group II,b – Group I vs Group III,c-Group I vs
Group IV and d- Group I vs Group V.P Values: *<0.05, ** <0.01, ***<0.001, ns – non significant

At the concentration of 200 mg/kg b.w. the BHE showed significant reduction (P<0.001) in paw edema than its individual extract EPG and ECH. The percentage inhibition of paw edema exhibited by BHE was similar to the reference drug aspirin which was used in the present investigation.

Determination of Analgesic activity in mice

Eddy's hot plate method

All the three experimental plant extracts were assessed for their anti–nociceptive activity through hot plate method,tail flick test and acetic acid induced writhing method of nociception in mice.

Table -4 express the result of hot plate testwith the analgesic activity of EPG, ECH and BHE extracts on the latency time of rats.

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Table-4 Analgesic activity of the of BHE, EPG and ECH Extracts on latency time of rats exposed to hot plate test.

Treatment	Pre drug reaction time in secs	**Reaction time in secs**			
		30	**60**	**120**	**180**
Group I CMC treated (control)	4.26±0.32	4.45±0.29	4.14±0.44	3.37±0.40	3.25±0.38
Group III BHE(200mg/kg b.w) + formalin treated	4.71±0.21	8.19±1.11a***	8.05±0.10a***	8.12±0.50a***	7.80±0.32a***
Group IV EPG(200mg/kg b.w) + formalin treated	4.34±0.29	5.41±1.16b*	5.32±0.21b*	5.27±0.53b*	5.16±0.31b*
Group V ECH (200mg/kg b.w) + formalin treated	4.31±1.22	4.59±1.07c*	4.91±0.32c*	4.93±0.54c*	4.70±1.63c*
Group VI Morphine (100 mg/kg) + formalin treated	4.43±0.21	10.12±1.08d***	9.97±0.73d***	9.63±0.21d***	9.32±0.63d***

Values are mean ± SEM from 6 animals in each group. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's `t' test. Comparison between a - Group I vs Group II,b – Group I vs Group III , c - Group I vs Group IV and d- Group I vs Group V.P Values: *<0.05, ** <0.01, ***<0.001, ns – non significant.

There was no significant difference in the mean pre drug reaction time between the different groups,but the administration of plant extracts increased the latency time of pain stimulus significantly(P<0.01) in the drug treated animals when comparable to normal control animals. The time taken by the drug treated animals to show the paw licking or jump response was found to be more in BHE treated animals when comparable (P<0.01) to the animals treated with its individual extracts EPG and ECH.

Tail flick test

In this method the basal reaction time to with draw the tail (flicking response) from radiant heat was taken as the end point. Table-5 exhibits the analgesic activity of EPG, ECH and BHE extracts on Tail flick response in mice. Matching the hot plate method, there was no significant difference in the mean pre drug basal reaction time between different groups.

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Table 5 Analgesic activity of BHE, EPG and ECH on response of rats exposed to Tail

Treatment	Predrug reaction time in secs	Reaction time in secs			
		30	60	120	180
Group I CMC treated (control)	6.26±0.53	6.23±0.22	6.22±0.53	6.27±0.66	6.22±0.97
Group III BHE+ Formalin treated	6.11±0.40	9.87±2.04a**	9.44±1.90a**	9.21±2.11a**	9.66±2.31a**
Group IV EPG+ Formalin treated	6.15±0.95	8.23±2.50b*	8.45±1.10b*	8.73±1.30b*	8.25±1.63b*
Group V ECH+ Formalin treated	6.33±0.55	8.73±1.50c*	8.44±1.51c*	8.26±1.56c*	8.07±1.11c*
Group VI Indomethacin + Formalin ttreated	6.21 ±0.27	10.34±1.10d***	10.01±2.20d***	10.80±3.01d***	12.12±2.60d***

Acetic acid induced writhing test

Once the irritants like acetic acid was administered into the peritoneal cavity of the experimental rats, pain was inducing immediately with a specific stretching actions called writhing behavior (contraction of abdomen, turning of trunk and extension of hind limb), the recognized model for chemically induced pain stimuli.The extracts of EPG, ECH and BHE were shown to minimize the so said induced writhing counts considerably (Table-6).

Table -6 Analgesic activity of BHE, EPG and ECH on acetic acid administration to produce writhing

Treatment	No. of writhing	% Inhibition of writhing
Group I CMC treated (control)	35.17±2.78	-
Group III BHE (200mg/kg b.w) + formalin treated	50.65±3.02a**	44.01
Group IV EPG(200mg/kg b.w) + formalin treated	45.05±3.01b*	28.09
Group V ECH (200mg/kg b.w) + formalin treated	46.58±3.12c*	32.76
Group VI Diclofenac sodium (50mg/kg) + formalin treated	52.34±4.31d***	48.82

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Values are mean ± SEM from 6 animals in each group. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's `t' test. Comparison between a - Group I vs Group II,b – Group I vs Group III c - Group I vs Group IV and d- Group I vs Group V.P Values: *<0.05, ** <0.01, ***<0.001, ns – non Significant.

Similar to the analgesic activity exhibited by the plant extracts in thermal methods the BHE exhibited the maximum inhibition writhing movements that is 44.01% when comparable (P<0.01)to their individual extracts ECG and ECH which showed the inhibition of 28.09% and 32.76% respectively at the same concentration.The standard drug diclofenac sodium exhibited 48.82s% inhibition of writhing movements in the present investigation.

Discussion

One of the most effective method for screening the anti-proliferative and anti-arthritic agents is formaldehyde-induced edema[17].Inflammation caused by formaldehyde occurs in two phases, Substance P is released in the early phase also known as neurogenic phase, while histamine, serotonin, bradykinin, and prostaglandins are released in the late phase also called as inflammatory phase, resulting in marked vasodilation and permeability. It has been reported that drugs that act on the central nervous system inhibit both phases, whereas drugs that act on the peripheral nervous systeminhibit late phase[18].The formalin-induced joint edema was inhibited by BHE, EPG and ECH was assessed in this present study. However, the BHE which contain equal amount of *Pisoniagrandis* and *Cardiospermumhalicacabum*leaves has shown higher inhibition informaldehydeinducedjoint edema when compared with ethanolic extract extracts of*Pisoniagrandis* (EPG) and *Cardiospermumhalicacabum* (ECH).

In the present study, all three plant extracts, BHE, EPG and ECH had shown strong anti-inflammatory activity against the acute inflammatory response induced by turpentine oil in the Knee joint of rats. A triphasic release of inflammatory mediators is observed in turpentine oil induced paw edema. Histamine and serotonin play a key role in the first phase, kinin-like substances involved in the second phase, and cycloxygenase and lipoxygenase products play a key role in the third phase[19].The turpentine induced edema wasinhibited by BHE, EPG and ECH. However, BHE, inhibited turpentine-induced edema starting from the first hour and across all stages of inflammation indicated that the bioactive substances present in the BHE cumulatively interact with each other and likely inhibited the production and release of inflammatory mediatorsat various stages.

Generally, inflammation has three phases, the first is caused by increased vascular permeability, the second by leucocyte infiltration, and the third by the formation of granulomas[20]. There are three different phases which are involved in the acute inflammatory responses induced by the carrageenan injection through the consecutive production of various mediators. The release of serotonin and histamine is involved in the first step (1.5 h), while bradykinin is involved in the second phase (1.5–2.5

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h). The third step, which occurs between 2.5 and 6 hours and is the most critical process in the inflammatory response, is known to be mediated by prostaglandins[21,22]. COX is the one of the key enzyme that produce prostaglandins and thromboxane from arachidonic acid, and Prostaglandins is known to be play an important role in the inflammatory mechanism. Inflammatory pathogenesis has been linked to abnormal COX-2 expression in diseases of the gastrointestinal tract, central nervous systems, ischemia, and lung inflammation and fibrosis[23,24]. The carrageenan -induced joint edema was inhibited by BHE, EPG and ECH was assessed in this present study. However, the BHE which contain equal amount of *Pisoniagrandis* and *Cardiospermumhalicacabum*leaves has shown higher inhibition informaldehydeinduced Paw edema when compared with ethanolic extract extracts of *Pisoniagrandis* (EPG) and *Cardiospermumhalicacabum* (ECH).

Anti-nociceptive drugs are generally categorized according to their mechanism of action on the central nervous system or the peripheral nervous system[25].The tail flick test was used to investigate the antinociceptive function of the spinal cord. The hot plate test, evaluates the latencies of animal nociceptive responses to thermal stimuli.However, the tail flick is primarily a spinal response, while the hot plate is predominantly supraspinal[26,27].In the present study,BHE which contain equal amount of *Pisoniagrandis* and *Cardiospermumhalicacabum*leaves increased mean basal latency time suggesting its central analgesic activitywhen compared with ethanolic extract extracts of *Pisoniagrandis* (EPG) and *Cardiospermumhalicacabum* (ECH).

Acetic acid induced writhing method was used to assess the peripheral analgesic property of BHE, EPG and ECH.It is well understood that acetic acid stimulates the neurons responsible for pain sensingby secreting endogenous pain mediator such as prostaglandin which are sensitive to anti-inflammatory drugs[28]. In the present study,BHE which contain equal amount of *Pisoniagrandis* and *Cardiospermumhalicacabum*leaves showed significant analgesic activity in acetic acid induced writhing method when compared with ethanolic extract extracts of *Pisoniagrandis* (EPG) and *Cardiospermumhalicacabum* (ECH)[29,30].

Narcotic analgesics inhibit both peripheral and central pain receptors, while nonsteroidal anti-inflammatory drugs only inhibited the peripheral pain.The plant extracts in the present study inhibited all forms of pain, implying that they work through both central and peripheral mechanisms.This is due to the presence of phenolic constituents in BHE extracts, which reduces nocicept ors stimulation and leads to the suppression of central pain mechanisms. Flavonoids are
believed to interfere with the cyclooxygenase system, interfering with arachidonic acid metabolism and i nhibiting the development of prostaglandins, which are also involved in pain[31,32].

Conclusion

Based on the findings of the study, we can conclude that our plant extract, BHE, which is made up of two herbal extracts EPG and ECH, has potential anti-arthritic, anti-inflammatory, and analgesic

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properties that are comparable to synthetic standard drugs like aspirin, morphine, and diclofenac.This could be due to the fact that various types of active concepts, each with a single or broad set of biological activities, could be useful adjuvants in the existing arsenal of pathological conditions.

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