Original article

Onosma bracteatum wall: A Potent analgesic agent

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Abstrtact

Background: This study was aimed to find out the central and peripheral analgesic activity of hydro methanolic extract of aerial parts of *Onosma bracteatum.* ***Material and methods:*** The central and peripheral analgesic activity is evaluated by tail flick test and acetic acid induced writhing test at the doses of 50, 100, 250 and 500mg/kg body weight respectively in animal models. ***Results:*** The results obtained from Tail flick test revealed that *O. bracteatum* possesses potent analgesic effects by inducing significant increase in latency period in dose dependent manner at all doses at 1, 2 and 3 hours post feeding respectively. The maximum effect was observed at a dose of 500mg/kg i.e. 258.9% (p<0.05) at 3hrs post feeding. Diclofenac sodium (5mg/kg body weight) run as standard also increased the latency period continuously and highest activity was noted at 3hr i.e. 284.5% (p<0.05). Acetic acid induced writhing test also showed significant activity in a similar manner by *O. bracteatum* i.e 54% (p<0.05) at 500mg/kg while standard drug Diclofenic sodium (5mg/kg body weight) showed 45.9% (p<0.05) activity. ***Conclusion:*** It is concluded that *O. bracteatum* possesses significant central and peripheral analgesic activity in animal model.

Keywords: *O. bracteatum*; Tail flick test; Acetic acid induced writhing test; albino rats and mice

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Introduction

Considering the most health problems from a skinned knee to cancer there is involvement of some level of pain. Pain is a multidimensional unwanted sensory and emotional condition linked with tissue injury, occurred by mechanical (excess pressure that break the skin surface), thermal (activated by noxious heat or cold at various temperatures) or chemical stimuli (environmental irritants)[1]. To get relieve from discomfort due to pain, various medicines or pain killers are use known as analgesics. These medicines are acting through a variety of physiological mechanisms to provide relief from pain. Therefore medicines which are effective in nerve pain may have a different mechanism of action than those for arthritis. Analgesics can be easily acquired without prescription and sometimes referred to as natural painkillers or as a natural pain reliever that relieves pain without loss of consciousness. There are few factors behind self medication or prescription includes immediate relief from pain or discomfort, education, family, society, law, availability of drugs and exposure to advertisements[2,3]. These drugs provide an easy and effective way to manage pain

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and are generally safe, cost effective, non addictive and had cultural acceptability. According to WHO worldwide interest is developed towards herbal medicines and now a days the number of patient using herbal or alternative medicine for treatment of pain is increasing[4]. A large number of medicines or drugs of plant origin have been used since long time to treat various disorders[5,6]. Therefore necessary efforts to evaluate new medicinal plants are mandatory to develop cheap, effective and safe analgesics.

Onosma bracteatum wall (Boraginaceae) commonly known as Gaozaban or Sedge found in Asian countries including Pakistan[7]. It grows in dry or moist and sunny weather usually in rock crevices and widely known as rock garden plants[8]. *O. bracteatum* is reported to have valuable chemical constituents like carbohydrates, glycosides, flavonoids, tannins and phenolic compounds. It is use as key ingredient in a number of Unani and Ayurvedic formulations to treat number of disorders regarding human health[9,10]. Literature revealed that in spite of its vast reported pharmacological activities no scientific or systematic approach has been made to study its analgesic activity. Thus the present study was aimed to assess the analgesic effect of hydro methanolic extract of aerial parts of *O. bracteatum.*

Material and method

Plant material

The aerial parts of *O. bracteatum* was purchased from the local market of Karachi, Pakistan and identified by the botanist of PCSIR Laboratories Complex; Karachi, the specimen voucher no: OB0182014 was preserved in the herbarium of the Pharmacology section of PCSIR Laboratories Complex; Karachi.

Preparation of plant extract

The aerial parts of *O. bracteatum* (3.5kg) was chopped and cut into small pieces. It was then soaked in methanol:water (70:30) mixture at room temperature (23-25^{o}C) and capped in screw tight bottles for 1 week with occasional shaking. It was first filtered through muslin cloth and then through Whatman No.1 filters paper. This procedure was repeated thrice, filtrate was pooled in glass bottle and then evaporated on rotary evaporator under reduced pressure (-760 mmHg) at 35-40^{o}C. By this procedure a thick semi solid mass of crude extract of *O. bracteatum* was obtained which was stored in amber glass bottle and preserved at -4^{o}C.

Chemicals and drugs

Methanol of analytical grade, diclofenac sodium (Platinum Pakistan), Nacl and acetic acid (Merck, Germany).

Animal selection

Male swiss albino mice (20-30g) and male wistar rats (150-200g) were selected for study. The animals were reared at animal house of PCSIR Laboratories Complex; Karachi. The selected animals were kept separately for 2 weeks under strict observation with free access to food and water. Food was withdrawn 12 hrs before the start of experiment. Animals showing sluggish/abnormal movement or any sign of illness were excluded from the study. The methods and procedures were approved by ethical committee for use of experimental animals of PCSIR Laboratories Complex; Karachi.

Analgesic activity by tail flick latency period in rats

Tail flick method (analgesiometer UGO Basile, Italy) was used to assess the analgesic effects of *O. bracteatum* by previously reported method[11]. Animals (wistar rats) were divided into six groups (n=5). Group I, II, III and IV served as test groups and received doses of 50, 100, 250 and 500 mg/kg body weight (p.o.) of *O. bracteatum* respectively. Group V served as standard group and received diclofenac sodium 5mg/Kg (p.o.) body weight while Group VI serves as control group and received distill water only in the same volume by feeding cannula at same time. The intensity of beam of heat was adjusted at 5 ampere with the cut off time of 15 sec to avoid injury to the exposed part of rat tail. The results were noted initially at 0min (Tb) then at the intervals of 1, 2 and 3 hrs after drug administration (Ta). Percentage analgesic activity was calculated as per formula shown below

$$\% \text{ of analgesic activity} = \frac{Ta-Tb}{Tb} \times 100$$

Acetic acid-induced writhing in mice

Six groups of mice (n=5) were selected for acetic acid-induced writhing. Test Groups I, II, III and IV received *O. bracteatum* 50, 100, 250 and 500 mg/kg body weight dose (p.o.). Standard Group V received Diclofenac Na 5 mg/Kg body weight (p.o.) while control Group VI received distilled water only in the same volume by feeding cannula. After 30 minutes of test and standard drugs 1% v/v acetic acid solution (0.1 ml/10g) was used intraperitonealy to induced abdominal constriction. Each animal was placed individually into separate cage and numbers of writhing movements displayed during 5 to 20 min after acetic acid injection were noted and recorded. The activity was express in % inhibition of writhes produced by acetic acid

$$C\text{-}D/C \times 100$$

Where: C – is the average number of writhing for

control group and D – is the average number of writhing of test and standard drug treated animals[11].

Statistical analysis

All numerical data collected were expressed as the mean ± SD and statistically analyzed by using student *t* test. The *p* values at <0.05 level were considered significant.

Results

The results obtained from Tail flick test revealed that hydro methanolic extract of *O. bracteatum* possesses potent analgesic action by inducing significant increase in latency period in dose dependent manner i.e. 66.28%, 155.4% (p<0.05), 166.2% at 50mg/kg. 81.3% (p<0.05), 156% (p<0.05), 208% (p<0.05) at 100mg/kg. 90.7% (p<0.05), 163.1% (p<0.05), 225% (p<0.05) at 250mg/kg and 146.5% (p<0.05), 200% (p<0.05), 258.9% (p<0.05) at 500mg/kg at 1, 2 and 3 hours respectively. The standard drug diclofenac Na (5mg/kg) also exhibited continuous increase in latency period throughout the experiment and showed160.5% (p<0.05), 229.5% (p<0.05), and 284.5 (p<0.05) at 1, 2 and 3hr respectively (Table 1, Fig.1). The results of acetic acid-induced writhing test showed that the hydro methanolic extract of *O. bracteatum* exhibited significant analgesic activity at the dose of 500mg/kg body weight 54% inhibition (p<0.05) followed by 45% (p<0.05) at 250mg/kg body weight respectively. The test drug at 50 and 100mg/kg dose exhibited non significant activity 22.9% and 35.4% respectively The standard group treated with diclofenac sodium (5mg/kg) also exhibited significant reduction in the number of writhes 45.9% (p<0.05) as compared to control group (Table 2, Fig. 2).

Discussion

Pain is a protective mechanism of the body and can be defined as an acute discomfort which is possibly an alarm of some physical illness or disorder to the body[12]. The main objective of the treatment of pain is to remove or abolish the pain stimulus, but it is not always possible to do so, therefore analgesics are used for the symptomatic relieve of pain[13]. Globally self medication is increasing day by day. Drugs used for the treatment of self-diagnosed illness is called self-medication. Sarahoodi *et al.,*[3] reported that self-medication with pain killers is very common in Iranian students (76.6%). Like other developing countries it is also very common in Pakistan. The reason behind self-medication among Pakistan's population includes low economic status, lack of knowledge about adverse effects and easy accessibility without prescription[2]. It is well known that plants/herbs served as a potential source of alternative medicine and food for humans as well as for animals, therefore play an important role in our life. Natural medicines can be purchased in bulk or as refined pharmaceutical dosage (capsules, tablets, concentrated extracts, teas, tinctures decoctions) play important role in treatment of minor and major ailments. Therefore this study was designed to verify and validate the dual analgesic action (central and peripheral) of *O. bracteatum* by using two different tests Tail flick and acetic acid induced writhing test. The results obtained so for from the radiant heat tail flick test showed that the test drug has stress tolerance capacity by enhancing the latency period in dose dependent manner. The maximum potent analgesic effect of *O. bracteatum* was observed at 3hour after drug administration. It exhibited strong analgesic action by inducing significant increase in latency period in dose dependent manner at all doses i.e. 66.28%, 155.4% (p<0.05), 166.2% at 50mg/kg.

Table 1: Effect of methanolic extract of *Onosma bracteatum* on Tail flick latency period in rats

Treatment	Dose (mg/kg)	Tail flick latency (in sec) at time (hr)			
		0hr	1hr (% elongation)	2hr (% elongation)	3hr (% elongation)
Extract	50mg/kg	1.48±0.22	2.46±0.35 (66.2%)	3.78±0.81** (155.4%)	3.94±1.45 (166.2%)
Extract	100mg/kg	1.5±0.18	2.72±0.49* (81.3%)	3.84±0.75** (156%)	4.62±0.68*** (208%)
Extract	250mg/kg	1.52±0.22	2.9±0.65* (90.7%)	4±0.65*** (163.1%)	4.94±0.95*** (225%)
Extract	500mg/kg	1.46±0.32	3.6±0.65** (146.5%)	4.38±0.58*** (200%)	5.24±0.92*** (258.9%)
Diclofenac sodium	5 mg/kg	1.42±0.88	3.7±0.30*** (160.5%)	4.68±0.50*** (229.5%)	5.46±0.54*** (284.5%)
Control	–	1.5±0.31	2.08±0.24 (38.6%)	2.14±0.45 (42.6%)	2.44±0.54 (62.6%)

statically calculation by student t test value are found to be significant at p<0.05 (significant*), (very significant**), (extremely significant***)

81.3% (p<0.05), 156% (p<0.05), 208% (p<0.05) at 100mg/kg. 90.7% (p<0.05), 163.1% (p<0.05), 225% (p<0.05) at 250mg/kg and 146.5% (p<0.05), 200% (p<0.05), 258.9% (p<0.05) at 500mg/kg at 1, 2 and 3 hours respectively after administration. Diclofenac Na (standard drug) at 5mg/kg dose also showed continuously increased the latency period and exhibited maximum activity at 3hr after drug feeding i.e. 284.5% *($p<0.05$)* (Table 1, Fig.1).

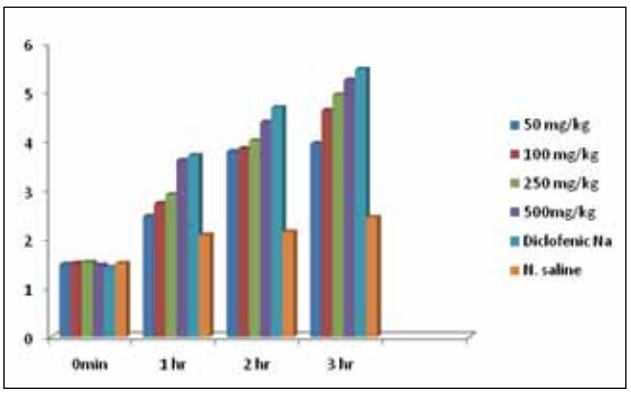

Fig. 1: Graph showing analgesic activity of test and standard drugs by tail flick test

Results of acetic acid-induced abdominal writhing test (visceral pain model) revealed that all applied doses had potent analgesic effects. According to our findings test drug *O. bracteatum* possesses significant analgesic effect at 500mg/kg body weight dose, by inducing 54% inhibition (p<0.05) followed by 45% (p<0.05) at 250mg/kg body weight respectively. The test drug at 50 and 100mg/kg dose do not exhibited significant activity 22.9% and 35.4% respectively. The standard drug diclofenac sodium (5mg/kg) also exhibited significant reduction in the number of writhes 45.9% (p<0.05) as compared to control group (Table 2, Fig. 2).

Table 2: Effect of methanolic extract of *O. bracteatum* on acetic acid induced writhing response in mice

Treatment Groups	Dose (mg/kg)	No. of writhing	Inhibition (%)
Extract	50	72.2±27.2	22.9%
Extract	100	60.4±30.5	35.4%
Extract	250	21.4±28	45%*
Extract	500	43±23.7	54%*
Diclofenac sodium	5	50.6±11.5	45.9%
Control	---	93.6±23.6	----

Data were expressed as Mean ± SEM (n=5) and *p value at* (p<0.05) was considered as significant*

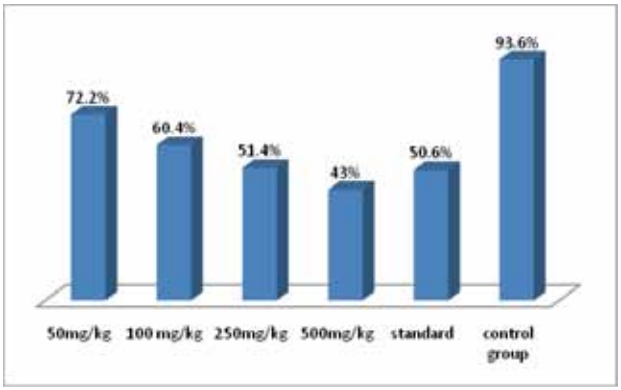

Fig. 2: Graph showing % of acetic acid-induced writhing inhibition effects of test and standard drugs

Acetic acid induced writhing assay is a mostly used simple screening model for the evaluation of peripheral antinociceptive activity. This chemical method is used to induce pain of peripheral origin by injecting the irritant chemical like acetic acid in animals intraperitonially. Acetic acid is an irritating agent that irritates the serous membrane of peritoneum to produce stereotype behavior in animals, characterized by abdominal stretching, extension of hind limbs, contraction of abdominal musculature and reduced motor activity. Due to pain and irritation the signals transmitted to central nervous system, cause release of mediators like prostaglandins PGE2 and PGF2α (responsible for pain sensation) in peritoneal fluids as well as lipoxygenase products which contributes to the increased sensitivity to nociceptors by enhancing inflammatory pain by increasing capillary permeability[14,15]. The significant pain reduction by test drug may be due to the presence of certain analgesic compounds acting with the prostaglandin pathways. Now it is well observed that peoples showed dissatisfaction towards conventional allopathic medicines because of some issues like effectiveness and/or safety, satisfaction with therapeutic outcome and the thinking that herbal medicines are inherently safe. Reason behind herbal medicines preference includes cultural and personal beliefs, philosophical views on life and health, as well as comparison of experiences between conventional healthcare professionals and complementary medicine practitioners by patients[16]. The bioactive compounds of plant origin are rising because of the drawback associated with synthetic medicines that, they can be even more harmful than the illness they claim to cure. For example non steroidal anti-inflammatory drugs (NSAIDs) are widely used as pain reliving as anti-inflammatory and for other health ailments but in chronic use may leads to GIT, renal, hepatic, CNS disorders and

dermatological unwanted effects[17,18]. While natural medicines are based upon natural substances that can improve health and alleviate illness and proved to be safe, better patient tolerance, relatively low cost and globally competitive. The numbers of chemical compounds present in plants is supposed to have better compatibility with the human body. Plants contain a large number of pharmacologically active ingredients and each herb has its own unique combination and medicinal properties. It is well reported that natural medicines had pain reliving ability because of their various chemical constituents (alkaloids, tannins, flavonoids, xanthone, coumarin, sterols, withaferin-A, andrographolide). These compounds inhibit synthesis prostaglandins (an enzyme involved in pain perception)[1]. From the above findings it can be assumed that the reason behind the potent analgesic action of aqueous methanolic extract of *O. bracteatum* even at low dose may be because of the presence of glycosides, tannins, phenolic compounds, carbohydrates and flavonoids. Further investigations are required to understand the precise mechanism of action of *O. bracteatum.*

Conclusion

Screening of hydro methanolic extract of *O. bracteatum* as an analgesic agent revealed that the extract produce potent analgesic effect which explains its common use in folk-lore system of treatment. Therefore on the basis of significant analgesic activity *O. bracteatum* may be used as a useful herbal remedy safely and effectively in pain disorders.

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