Research Paper

International Journal of Pharmaceutical Sciences and Nanotechnology
Volume 10 • Issue 3 • May – June 2017
MS ID: IJPSN-2-14-17-VENKATESH

Mechanistic Evaluation of Antinociceptive Effects of Bioactive Guided Fractions of *Barleria prionitis*

Azmathunnisa Begum[1], Sama Venkatesh[1]*, Rajesh Bolleddu[1], Ravi Alvala[1] and D. Jaya Prakash[2]

[1]G. Pulla Reddy College of Pharmacy, Mehdipatnam, Hyderabad, 500028, Telangana, India, and [2]Faculty of Pharmacy, University College of Chemical Technology, Osmania University, Hyderabad, Telangana, India

Received February 10, 2017; accepted March 29, 2017

ABSTRACT

Barleria prionitis Linn.(Acanthaceae) is a prickly shrub and traditionally whole plant is used as anti-inflammatory, expectorant, analgesic, diuretic, antirheumaticand antidiabetic. This study was conducted to investigate the antinociceptive and CNS depressant activity of ethanolic extract and the fractions of *B. prionitis* in mice. Ethanol extract and its fractions were tested at a dose of 200 and 400 mg/kg. Ethanol, petroleum ether and chloroform fractions demonstrated significant antinociceptive activity at 400 mg/kg and significantly increased the latency in hot plate test and the action was antagonised by naloxone, indicating a potential opioid-like mechanism. In conclusion, the ethanol, pet ether and chloroform fractions of *B. prionitis* markedly demonstrated the antinociceptive action. The CNS depressant and good protective effect on pain stimuli suggest that the possible mechanisms appear to be due to involvement of opioid and/or peripheral receptors.

KEYWORDS: Antinociceptive activity; analgesia; morphine; naloxone; *Barleria prionitis*.

Introduction

The new chemical substances are the important source of medicinal plants that potentially have strong therapeutic effect. In developing countries most of the people dependent on traditional medicines and practices for their primary health care needs (Calixto, 2005).

Barleria prionitis Linn. belongs to the family-Acanthaceae is a prickly shrub 1.5 m high with simple opposite decussate leaves. The flower is yellow in colour and sessile. The plant is cultivated as hedge plants and is distributed throughout warmer parts of India (Gupta *et al.,* 2006). The major constituents found are acetyl barlerin and barlerin (Taneja *et al.*, 1975, Damtoft *et al.,* 1982) and other chemicals constituents present are 6-O-acetylshanzhiside methyl ester (El-Emary *et al.,* 1990), 6-O-cis-p-coumaroyl-8-O-acetylshanzhiside methyl ester and its trans isomer, shanzhiside methyl ester and α-amyrin, verbascoside (Chen*et al.,* 1998), β-sitosterol (Moitra *et al.*, 1970), stigmasterol-3-O-D-glucoside (El-Emary *et al.*, 1990). The plant is known to have anti-inflammatory, expectorant, analgesic, diuretic, antirheumatic (Pullaiah and Chandrasekhar, 2003, Kiritikar and Basu, 1996) and antidiabetic properties (Banfield, 1951). The plant extract also known to possess hepatoprotective activity (Singh *et.al.*, 1951), in respiratory infections (Chen *et al.*, 1998) and tuberculosis (Oomanchan, 1991).

In view of the reported analgesic properties of *Barleria prionitis,* the present study was conducted to evaluate antinociceptive effects of *B.prionitis* and the action mechanism on several experimental models in mice. The activity on CNS was also investigated to examine antinociceptive activity related to central depression action.

Materials and Methods

Preparation of Plant Material and Authentification

The aerial parts of *B. prionitis* were collected from Rajendra nagar, Hyderabad. The plant material was taxonomically identified and authenticated by Dr P.V Prasanna, Scientist and Taxonomist of Botanical Survey of India, Hyderabad, India. A voucher specimen (AUB-BPA-2013) has been preserved in our laboratory for future reference. The aerial parts were cut, air dried and grounded into powder.

Preparation of Plant Extract and its Fractions

The aerial parts of *B. prionitis* were dried under shade, powdered with a mechanical grinder and extracted with 80% aqueous ethyl alcohol by maceration for 5 days. The percentage yield of crude ethanolic extract is 10.71. The concentrated aqueous ethanolic extract is suspended in 500 ml of distilled water and is fractionated with petroleum ether (4 × 500 mL), chloroform (4 × 500 mL), ethyl acetate (4 × 500 mL) and n–butyl alcohol (4 × 500 mL). The percentage yields of petroleum ether, chloroform, ethyl acetate, n-butanol and

left over aqueous fractions of *B. prionitis* is 0.46, 0.65, 0.29, 0.64 and 6.86 respectively.

Chemicals

All the chemicals, reagents, solvents used were of analytical grade. Aspirin, morphine, diazepam and naloxone were purchased locally.

Animals

Male Swiss Albino mice, (25-30 g) were used for animal studies. The animals were grouped in clean polyacrylic cages and maintained under standard laboratory conditions of temperature (21 ± 2 ºC), relative humidity (50 – 5%), 12 h dark and 12 h light cycles and they were allowed free access to feed and water *ad libitum* during the quarantine period. The animals were fasted for 6h before experimentation, but had been allowed free access to water. The Institutional Animal Ethics Committee of G Pulla Reddy College of Pharmacy had approved the experimental protocols and approved all the procedures for investigating experimental pain in conscious animals (Zimmermann, 1983) and care of animals was taken according to CPCSEA guidelines. All extracts and drugs were administered orally as a fine suspension of 0.5% carboxy methyl cellulose (CMC).

Antinociceptive Activity

Acetic acid induced writhing test: The acetic acid induced abdominal writhing test was performed as described by Sigemond and Cadmus 1957, technique modified by Koster *et al.*, 1959 in pre-screened mice. Fasted normal mice were divided into 16 groups of 6 animals each. Group 1 served as control received vehicle CMC, group 2–11 received aqueous ethanolic extract, petroleum ether, chloroform, ethyl acetate and butanol fractions respectively at an oral dose of 200 and 400 mg/kg of each extract. Group 12 served as positive control and received acetyl salicylic acid at an oral dose of 100 mg/kg. In an attempt to investigate the participation of opioid system in antinociceptive effects of *B. prionitis*, a separate group of mice were pre-treated with non selective receptor antagonist, naloxone (5 mg/kg), which was injected 15 min before the administration of ethanol, petroleum ether and chloroform fractions (400 mg/kg, *po*) for groups 13, 14 and 15. After 30 min of extract/drug administration all the animals were given an i.p injection of 0.6% acetic acid (volume of injection is 0.1 mL/10 g) and number of writhes produced were recorded for 30 min. Group 16 animals received Naloxone (5 mg/kg) and aspirin (100 mg/kg).

Hot plate test: The method described by Eddys and Leimbach was employed (Eddy *et al.*, 1953) using Eddy's hot plate. The temperature of hot plate was maintained at 55±0.2 ^{0}C. The basal reaction time of all the animals towards thermal heat was recorded. Animals were placed in Perspex square of the heated surface; the animals which showed paw licking or jumping response within 5 seconds were selected for the study. The pre-screened fasted animals were divided into 16 groups of 10 animals each. Control animals were treated with CMC (group 1), groups 2–11 received aqueous ethanolic extract, petroleum ether, chloroform, ethyl acetate and butanol extracts at an oral test doses of 200 and 400 mg/kg. Group 12 served as positive control and treated with morphine (5 mg/kg, *po*). The opioid receptor antagonist naloxone (5 mg/kg) was also tested along with ethanol, petroleum ether and chloroform extract (400 mg/kg, p.o, group 13, 14 and 15) and morphine (5 mg/kg, p.o; group 16). All the substances were administered 30 min before the beginning of the experiment. The latency time was measured before and at 30, 60, 120 and 180 min after the administration of the extract. The latency period of 20 sec is defined as a cut off mark and measurement was terminated if the latency exceeded this latency period to avoid injury.

CNS depressant activity: The activity of *B. prionitis* on CNS was evaluated by performing assays of its effect on exploratory capacity (hole board test) and locomotory activity. In each experiment the mice were divided into 13 groups consisting of six animals in each group. The animals of group 1 were treated with vehicle and served as control. Aqueous ethanol, petroleum ether, chloroform, ethyl acetate and butanol extracts were administered to groups 2 to 11 animals at an oral dose of 200 and 400 mg/kg, as a fine CMC suspension. Group 12 and 13 served as positive control and orally received diazepam (2 mg/kg) and morphine (10 mg/kg).

Hole board test: Boisser and Siman 1964 employed hole board test. The apparatus consists of wooden box (40 × 40 cm) with 16 holes of 3 cm evenly distributed in 4 rows. The apparatus is elevated and animals were placed upon them. The animals were treated with test and standard substance and 30 min after each animal was placed in the centre of board and number of head pokes during 5 min were recorded.

Locomotory activity: The locomotor activity investigated by the method described by Kulkarni (Kulkarni, 1999) and Yadav (Yadav et al., 2008) using actophotometer (INCO photoactometer, Ambala, India). The animal was placed individually and a count was recorded when the beam of light falling on the photocell of the actophotometer is cut off by animals. The mice were treated with the drug extracts 30 min before and the basal activity score was recorded for 10 min.

Statistical analysis: All the values were expressed as mean ± SEM. Results were analysed statistically by using analysis of variance (ANOVA) followed by Dunnetts multiple comparison test. Values of $p<0.05$ were considered to be significant (Ghosh, 2005).

Results

Effect on acetic acid induced writhing test

Table 1 represents the inhibitory effects of ethanolic extract and fractions of *B. prionitis* in writhing test. Except butanolic and ethyl acetate fraction all other extracts have significantly ($p<0.01$) inhibited the writhings in mice at both test dose levels. The animals receiving a test dose of 400 mg/kg showed maximum

activity. Among all the extracts ethanol, petroleum ether and chloroform extracts has produce maximum inhibitory effect with 30.36, 59.40 and 33.70 percent protection when tested at 400 mg/kg. The administration of naloxone (5 mg/kg) along with ethanol, petroleum ether, chloroform and aspirin demonstrated significant ($p<0.01$) analgesic effects with percentage inhibition of 34.59, 60.48, 39.49 and 66.07. These results indicate that Naloxone has no effect on antinociceptive activity of *B. prionitis* and aspirin. None of the extracts inhibitory effects are comparable with aspirin activity.

Effect on the hot plate test

Table 2 shows the antinociceptive effects of orally administered extracts of *B.prionitis* assessed using the hot plate test. The ethanol, petroleum ether and chloroform extracts at 400 mg/kg, exhibited significant ($P < 0.001$) ability to prolong the latency of response to discomfort against thermal-induced nociception throughout the whole experiment. Overall, 5 mg/kg morphine demonstrated the most effective effect when compared to the all extracts at both doses used. Among all the extracts ethanol, petroleum ether and chloroform extracts have produced higher latency time at 120 and 90 min respectively. The course of analgesic action was initiated from the 30 min of experiment. The animals receive 400 mg/kg test dose has produced maximum antinociceptive properties. Ethyl acetate and butanol extract is not able to produce any significant analgesic property. At 90 min the mean latency time of ethanol, petroleum ether and chloroform extracts were 5.8 ± 0.48, 5.9 ± 0.45 and 7.1 ± 0.58 sec compared with 2.3 ± 0.21 and 19.2 ± 0.48 for control and morphine treated groups respectively. The analgesic effects of both extracts is not comparable with morphine activity during any course of time. Administration of naloxone (5 mg/kg) has reversed the analgesic properties induced by ethanol, petroleum ether and chloroform extracts. Similarly the effects produced by morphine (5 mg/kg) is significantly blocked by administration of naloxone.

Hole board test

Aqueous ethanolic, petroleum ether and chloroform extracts have significantly reduced the head dip responses when treated with a dose of 400 mg/kg. The results of hole board test was presented in Table 3. The action produced by petroleum ether extract (400 mg/kg) is significantly high when compare to standard during entire course of experiment.

Locomotory activity

The results of *B. prionitis* locomotor activity are shown in Table 4. On comparing the values of all extracts; ethanol, petroleum ether and chloroform fractions at a dose of 400 mg/kg have produced significant ($p< 0.01$) reduction in locomotor activity with percentage reduction of 53.76, 58.6 and 41.09 respectively. Among the two test dose levels animals receiving 400 mg/kg has produced maximum activity. Ethyl acetate and butanolic extracts did not produce any action compared with control. The effect of diazepam and morphine is more marked with 67.88 and 69.75 reduction in activity.

Discussion

The overall results of the present study indicated that the oral administration of ethanolic extract and petroleum ether, chloroform fractions at a dose of 400 mg/kg, produced significant analgesic activity in mice.

The writhing and hot plate test models provide different background of mechanism to test the efficacy of the extract. The hot plate method is specific central analgesic and on the other hand peripheral analgesic activity is determined by writhing method.

The extract (ethanol, petroleum ether and chloroform extracts of *B. Prionitis*) exhibited high significant central analgesic (hot plate test) and protective effect on chemical (acetic acid) stimuli at 400 mg/kg indicating that the extract possessed peripheral and central antinociceptive actions, which is the characteristic of opioid analgesics (i.e., morphine). Naloxone, a nonselective opioid antagonist attenuates the antinociceptive effects of various extracts of *B.prionitis* shown by the involvement of opioid receptors at peripheral and central levels

The acetic acid-induced abdominal constriction test is a sensitive procedure to screen peripheral analgesic activity of new agents/compounds. The results of table 1 shows that the ethanol, petroleum ether and chloroform extracts inhibited the acetic acid induced writhing with percentage protection of 30.36, 59.40 and 33.70. The underlying mechanism of nociception is upon injecting acetic acid it causes the liberation of endogenous substances such as histamine, substance P, serotonin, bradykinins and increased levels of PGE2 and $PGF_{2\alpha}$ as well as lipooxygenase products in the peritoneal fluid which stimulates the nociceptive neurons (that are sensitive to NSAID's and narcotics) resulting in pain (Witkin *et al.,* 1961 and Collier *et al.,* 1968). Increase levels of PGEs in the peritoneal fluid causes prolong irritation of peritoneal cavity resulting in enhancement of capillary permeability and release of glutamate and substance P occurs from afferent fiber terminals. The above mentioned facts suggests that the *B.prionitis* extracts may reduce acetic acid induced abdominal constriction by inhibiting COX and LOX in the peripheral tissues thus reducing PGE's synthesis and impedes pain transduction pathway in primary afferent nociceptor (Sani *et al.,* 2012).

The significant increased latency time was shown by petroleum ether and aqueous ethanolic extracts at 400mg/kg in hot plate test, when compare to other fractions. The possible mechanism of action of these extracts were determined by using naloxone, a selective opioid receptor antagonist which acts by antagonising the action of endogenous opioid involved in pain and stress (Faden, 1988). This model of nociception, is selective for centrally (opioids) but not peripherally (NSAID's) acting analgesic and predominantly involves a

spinal reflex targeting supraspinal nociceptive processing selectively. Katzung proposed that drugs acting on CNS inhibit pain impulse transmission by causing the release of endogenous peptide through periaqueductal gray matter (PAG) from where they are carried to the spinal cord (Katzung., 1995). From the results it was suggested that naloxone significantly reversed the antinociceptive effects of ethanol, petroleum ether and chloroform extracts (400 mg/kg) and morphine (10 mg/kg) against thermal induced nociception.

The positive results of antinociceptive study provoke us to investigate the effects of *B.prionitis* on CNS hole board test and locomotor activity in mice. Of all the extracts, significant dose dependent activity on CNS was shown by aqueous ethanol, petroleum ether and chloroform extracts. Although the underlying mechanism is not clear, it is certain that *B.prionitis* may have morphinomimetic properties from the point of CNS depressant and good protective effect on chemical and thermal stimuli.

Conclusions

In conclusions, the results of the writhing test clearly show that naloxone is unable to alter the *B.prionitis* induced antinociceptive effects. Hence, the observed antinociceptive activity might have resulted from the activation of peripheral and/or opioid receptors. The results of present investigation confirm the antinociceptive properties of *B. prionitis* and the reports of traditional practices were stands to be correct. However, the exact mechanism of action is not known at this stage and has to be established in various models.

Conflict of interest statement

The authors report no conflict of interest.

References

Banfield AF (1951). Anonymous, the Wealth of India, Raw materials, vol-2: B, Publications and information directorate, CSIR, New Delhi, India.

Boisser JR and Simon P (1964). Dissociation dedeux compasanates dansle compartment investigationde lasouris. *Arch Int Pharmacodyn* **147:** 372-388.

Calixto, J.B (2005). Twenty-five years of research on medicinal plants in Latin America: a personal view. Journal of Ethanopharmacology **100:** 131-134.

Chen JL, Blanc P, Stoddart CA, Bogan M, Rozhon EJ, Parkinson N, Ye Z, Cooper R, Balick M, Nanakorn W and Kernan MR (1998). New iridoids from the medicinal plant *Barleria prionitis* with potent activity against respiratory syncytial virus. *J Nat Prod* **61:** 1295-1297.

Collier HO, Dinneen LC, Johnson CA and Schneider C (1968). The abdominal constriction response and its suppression by analgesic drugs in the mouse. *Br J Pharmacol Chemother* **32:** 295-310.

Damtoft S, Jensen SR and Nielsen BJ (1982). Structural revision of barlerin and acetyl barlerin. *Tetrahedron Lett* **23:** 4155-4156.

Eddy NB and Leimbach D (1953). Synthetic analgesics. Dithienylbutenyl and Dithienyl butylamines. *J Pharmacol Exp Ther* **107:** 385-393.

El-Emary NA, Makboul MA, Abdel-Hafiz MA and Ahmed AS (1990). Phytochemical study of *Barleria cristata* L. and *Barleria prionitis* L. cultivated in Egypt. *Bull Pharmaceut Sci Assiut Univ* **13:** 65-72.

Faden AI (1988). Role of thyrotropin-releasing hormone and opiate receptor antagonists in limiting central nervous system injury. *Adv Neurol* **47:** 531-546.

Ghosh MN (2005). Fundamentals of esperimental pharmacology. 3rd ed. Calcutta: Hilton and company 218-29.

Gupta AK, Neeraj Tandon and Madhu Sharma (2006). Quality standards of Indian medicinal plants, editor. 1st Ed. New Delhi: Indian Council of Medical Research **4:** 36.

Katzung BG (1995). Basic and clinical pharmacology. 6th ed. Applton and Lange, Stanford, Conn, USA.

Kiritikar KR and Basu B.D (1996). Indian medicinal plants. **3:** 1887.

Koster R, Anderson M and de Beer EJ (1959). Acetic acid for analgesic screening, *Fed Proc* **18:** 412.

Kulkarni SK (1999). Hand book of experimental pharmacology 3rd ed New Delhi: Vallabh Prakashan 117-119.

Moitra SK, Ganguly AN, Chakravarti NN and Adhya RN (1970). Chemical investigation of *Barleria prionitis*. *Bull Calcutta Sch Trop Med* **8:** 7.

Oomanchan MM (1991). Ethno-botanical and conservation aspects of medicinal plants of Madhya Pradesh. *In J of Pure and Appli Sci* pp: 6.

Pullaiah T and Chandrasekhar Naidu K (2003). Antidiabetic plants in India and herbal based antidiabetic research. Regency publications, New Delhi pp: 99.

Sani Mohd MH, Zakaria ZA, Balan T, Teh LK and Salleh MZ (2012). Antinociceptive activity of methanol extract of Muntingia calabura leaves and the mechanisms of action involved. *Evidence based complementary and alternative medicine* pp: 1-10

Sigemond R and Cadmus RG. Analgesic activity of aqueous ethanolic extract of whole plant of *Leucas hirta* in Swiss Albino mice, *Proc Soc Exp Biol Med* **95:** 72.

Singh B, Chandan BK, Prabhakar A, Taneja SC, Singh J and Qazi GN (2005). Chemistry and hepatoprotective activity of an active fraction from *Barleria prionitis Linn.* in experimental animals. *Phytother Res* **18:** 391-404.

Taneja SC and Tiwari HP (1975). Structures of two new iridoids from *Barleria prionitis* Linn. *Tetrahedron Lett* **24:** 1995-1998.

Vat ZR, Filho VC, Yunes RA and Calixto JB (1996). Antinociceptive action of 2-(4-bromobenzoyl)-3-methyl-4,6-dimethoxy benzofuran, a novel xanthoxyline derivative on chemical and thermalmodels of nociception in mice. *J Pharmacol Exp Ther* **278:** 304-312.

Witkin LB, Heubner CF, Galdi F, O'keefe E, Spitaletta P and Plummer AJ (1961). Pharmacology of 2-amino-indane hydrochloride (Su-8629): A potent non-narcotic analgesic. *J Pharmacol Exp Ther* **133:** 400-408.

Yadav AV, Kawale LA and Nade VS (2008). Effect of *Morus alba* L. (mulberry) leaves on anxiety inmice. *Indian J Pharmaco* **140:** 32-36.

Zimmermann M (1983). Ethical guidelines for investigating experimental pain in conscious animals. *Pain* **16:** 109-110.

Address correspondence to: Dr. Sama Venkatesh, *Professor G. Pulla Reddy College of Pharmacy, Mehdipatnam, Hyderabad 500028, Telangana, India.*

Ph: 040-23517222; E-mail: venkateshsama@hotmail.com