WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 2, 2400-2413. Research Article ISSN 2277– 7105

PHARMACOLOGICAL INVESTIGATION OF *BERBERIS ARISTATA* (BERBERIDACEAE) FOR ITS ANTIPYRETIC AND ANALGESIC ACTIVITY IN LABORATORY ANIMALS

Sushma Singh* and Sandeep K. Singh

Department of Pharmacology, School of Pharmacy, Babu Banarsi Das University, Lucknow (UP), India, 226028.

Article Received on
25 December 2021,

Revised on 14 Jan. 2022,
Accepted on 02 Feb. 2022

DOI: 10.20959/wjpr20222-23129

***Corresponding Author**
Sushma Singh
Department of Pharmacology, School of Pharmacy, Babu Banarsi Das University, Lucknow (UP), India, 226028

ABSTRACT

Aim: *B. aristata* also known as Indian Barberry or Tree turmeric belongs to the family Berberidaceae, mentioned in all the ancient ayurveda. In the present study antipyretic and analgesic activities of EEBA stem has been investigated. **Materials and methods**: Alkaloid (Berberine), tannin, flavonoid was isolated from the ethanolic extracts of stem of *Berberis aristata*. Phytochemical analysis was performed. Antipyretic activity was evaluated through yeast induced pyrexia while analgesic effect of EEBA was evaluated by hot plate test, acetic acid induced writhing, Tail flick test at a dose of 200 and 400 mg/kg orally. **Results**: The results of the antipyretic and analgesic study show that extract produced significant analgesia in the hot plate, tail flick and acetic acid induced writhing tests. The extract also inhibited the pyrexia induced by brewer's yeast. **Conclusion**: The results demonstrated significant response of EEBA for its antipyretic and analgesic activity. Phytochemical investigation of EEBA revealed the presence of alkaloid, flavonoids, tannins which may be responsible for antipyretic and antinociceptive activity of *B. aristata.*

KEYWORDS: *Berberis aristata*, Antipyretic, Analgesic, Brewer's yeast, Antinociceptive**.**

INTRODUCTION

Pain is defined by International association for the study of pain (IASP) as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. An analgesic is a drug that selectively relieves pain by acting in the CNS or peripherally pain mechanisms and does not alters consciousness.[1]

Fever or pyrexia is caused in response to infection, tissue damage, inflammation & other diseased condition.[2]

Berberis aristata also known as Indian Barberry or Tree turmeric belongs to the family Berberidaceae which mentioned in all the ancient ayurveda. It is characterized by an erect spiny shrub, glabrous herb, ranging between 2 and 3 meter in height with 10 to 20 cm stem diameter. It is native to northern Himalayan region at an elevation of 2000 to 3500.[3] Almost every part of this plant has some medicinal value. Its stem, root, bark and fruits are used in many ayurvedic preparations. Sothaghna or reduces inflammation or oedema. It is used for eye disease, Hence in severe conjunctivitis it is applied in the form of Rasanjan. In disease of mouth and throat it is used for gargling. *B.aristata* is used as appetiser, liver stimulant, and astringent, laxative. It is able to reduce blood sugar. Rasaut is an decoction of B. aristata leaves and is used to treat diarhoea, eye and ear infection, urinary tract infection.[4]

Pharmacological studies have proven the Antidiarrhoeal and Antispasmodic activity,[5] antioxidant and antimicrobial[6] anti-hyperglycemic activity[7] antidiabetic,[8] hepatoprotective activity,[9] hypnotic activity[10], anti-osteoporotic activity[11], anticancer activity,[12] antidepressant activity,[13] of *Berberis aristata*. Chemical constituents of *Berberis aristata* contains alkaloids, flavonoids, tannins, amino acids, terpenes, resins, phenols and reducing sugars as major compounds.[14] The present study was focussed on analgesic and antipyretic effects of ethanolic extract of *B. aristata* (EEBA) stem in animal models.

MATERIALS AND METHODS

Plant material

The stem of *Berberis aristata* was collected from Lucknow, during month of July 2014. The plant material was authenticated by NISCAIR, Delhi and voucher specimens were deposited for future reference. (Reference letter no.NISCAIR/RHMD/Consult/2013/2305/85). Plant material was air-dried in shade, reduced to fine powder, packed in tightly closed containers and stored for phytochemical and biological studies.

Chemicals

All the chemicals, reagents, solvents used were of analytical grade. Paracetamol (GlaxoSmith Kline Pharmaceuticals Limited Mumbai), Aspirin (USV Limited, Mumbai), Pentazocine (Ranbaxy Laboratories Limited, Ahmedabad). Paracetamol and Aspirin was suspended in

0.25% (w/v) carboxymethylcellulose sodium (CMC) and administered orally to animals. Brewer's yeast and acetic acid were diluted separately in normal saline.

Extraction

The collected plant material washed with water to remove dirt particles and then dried under shade and pulverized in a mechanical grinder. The powdered plant material was extracted with ethanol (95%) in a Soxhlet apparatus for 72 hours. The ethanolic extract was filtered and then concentrated on hot water bath till syrupy mass obtained.[15]

Animals

Albino wistar rats (100-200 g) of either sex were used in the study after approval of the Institutional Animal Ethics Committee (BBDNIIT/IAEC/037/2014). They were housed in four groups in polypropylene cages, fed with standard rodent diet and water *ad libitum*. At a temperature of 22±5°C, relative humidity 55±5% with 12 h light–dark cycle (08:00 to 20:00). Animals were allowed to acclimatize to the work area environment for one week prior to use.

Acute toxicity study

Animals were divided into 6 groups. The first group was control group and the remaining groups were experimental groups which received different doses (100, 200, 500, 1000, 2000 and 4000 mg/kg) of EEBA to study gross behavioral responses like hyperflexia, vocalization on touch, locomotor activity, autonomic responses such as tremors, convulsion, salivation, diarrhea, sleep, coma and observed after 24 hrs.[16]

Phytochemical screening

Powdered samples from the stem parts of *Berberis aristata* were subjected to preliminary phytochemical screening of alkaloids, flavonoids, tannins, anthraquinone glycosides.[17]

Pharmacological studies

Antipyretic activity

Rectal temperatures (TR) in rats were recorded by inserting a lubricated thermometer 2.8 cm into their rectum. Rats with initial rectal temperature 36–37.5 ◦C were chosen for these tests.[24] Hyperpyrexia was induced in rat by subcutaneous injection of 10 ml/kg of 15% w/v brewer's yeast suspension in 0.09% of saline in back below the nape of neck. After 18 hr, temperature of peak pyrexia was noted and doses of control (0.25% CMC 1 ml / kg), paracetamol (150 mg/kg b.w.), EEBA (200 and 400 mg/kg b.w.) administered orally.

Animals that did not show a minimum increase of 0.5 ◦C in temperature after yeast injection was discarded. The rectal temperature of each animal was recorded at 30 min intervals for 3hr.[18]

Antinociceptive activity

Hot plate method

Animals were divided into four groups of six animals each. Group I served as control (0.25% CMC, 1 ml/ kg/ p.o.). Group II received pentazocin at a dose of 5 mg/kg. Group III and IV received EEBA at a dose of 200 & 400 mg/kg orally. All the treatments were given 30 min before the thermal stimulus. Rats were placed individually on a thermostatically controlled hot plate maintained at 55±1 ◦C. The pain threshold is considered to be reached when the animals lift and lick their paws or attempt to jump out of the beaker. The nociceptive response was measured at 0, 30, 60, 90, 120 min.[19]

Acetic acid induced writhing

Mice were grouped into four (n=/6) and treated with 0.25 % CMC (1 ml/kg / p.o), acetylsalicyclic acid (150 mg / kg), EEBA (200 and 400 mg / kg). The animals were administered 0.75% (v/v) of an aqueous solution of acetic acid (1 ml/kg i.p.) 30 min after drug treatment and transferred to a smooth surface. The writhings induced by the acid, consisting of abdominal constrictions and hind limbs stretchings, were counted for 30 min immediately after acetic acid.[20]

Tail flick test

The radiant heat was focused on a blackened spot which is at 1–2 cm from the tip of the tail. Beam intensity was adjusted to give a tail-flick latency of 5–8 s in control animals. Acetylsalicylic acid (150 mg/kg p.o.) was used as reference standards. Measurement was terminated if the latency exceeded the end of time (15 s) to avoid tissue damage. The measurement started 30 min before drug administration to determine the baseline latency and then continued in interval of 30, 60, 90 and 150 min after drug administration.[21]

STATISTICAL ANALYSIS

Experimental results were expressed as means ± SEM. The data were analyzed by an analysis of variance, one way Anova followed by Tukey test.

RESULTS

Acute Toxicity study

The EEBA was found to be safe since no animal died even at the maximum dose of 4000 mg/kg body weight. The animals did not show any gross behavioral changes.

Phytochemical screening

The results of phytochemical analysis of *B. aristata* are presented in Table 1. Preliminary phytochemical screening of the plant revealed the presence of alkaloids, flavonoid, tannins, anthraquinone glycosides.

Table 1: Phytochemical analysis of stem of *Berberis aristata.*

S.N.	Test	Test/Reagent	Observation	Present/Absent (+)/(-)
1	Alkaloids	Dragendorff's test	Orange red precipitate	+
		Mayer's test	Cream colour precipitate	+
		Wagner's test	Reddish brown precipitate	+
2	Flavonoids	Lead acetate test	Yellow colour precipitate	+
		Shinoda test	Crimson red or occasionally green to blue colour	+
3	Tannins	Ferric chloride test	Greenish black colour	+
		Lead acetate test	White colour precipitate appear at the bottom of test tube	+
4	Anthraquinone glycosides	Modified Borntrager's test	Formation of rose pink colour in the ammonical layer	+

Yeast-induced hyperpyrexia in rat

Table 2 & figure 1, shows data related to the effect of EEBA on yeast-induced pyrexia at different time intervals. Administration of brewer's yeast to rats produced a significant increase in rectal temperature 18 hr after yeast injection. EEBA stem at a dose of 200 & 400 mg /kg caused a significant decrease in rectal temperature. The decrease in rectal temperature began at 30 min after the extract administration and continued over time (upto 120 min). The EEBA found to be less potent than PCM.

Table 2: Effect of EEBA stem extract on yeast-induced pyrexia in rats.

Treatments	Dose (mg/kg, p.o.)	Rectal temperature (◦C) at various time intervals					
		0 min	18 hr	30min	60 min	90 min	120 min
0.25 % CMC	1 ml / kg b.w.	36.43±0.07	39.37±0.05	39.30±0.03	38.00±0.10	37.69±0.12	37.86±0.15
PCM	150 mg/kg	36.55±0.10	36.45±0.03	37.60±0.02***	37.00±0.05***	36.84±0.08***	36.67±0.12***
EEBA	200 mg / kg b.w.	36.43±0.06	39.48±0.01	39.21±0.02*	37.60±0.12**	37.02±0.21**	37.34±0.10**
EEBA	400 mg / kg b.w.	36.45±0.08	39.49±0.03	39.19±0.01*	37.55±0.09*	36.86±0.12*	37.26±0.06*

All data were expressed in mean±SEM (n=6) by One way ANNOVA test followed by Tukey test

* Significantly different from the control group: $P < 0.05$.

** Significantly different from the control group: $P < 0.01$.

*** Significantly different from the control group: $P < 0.001$.

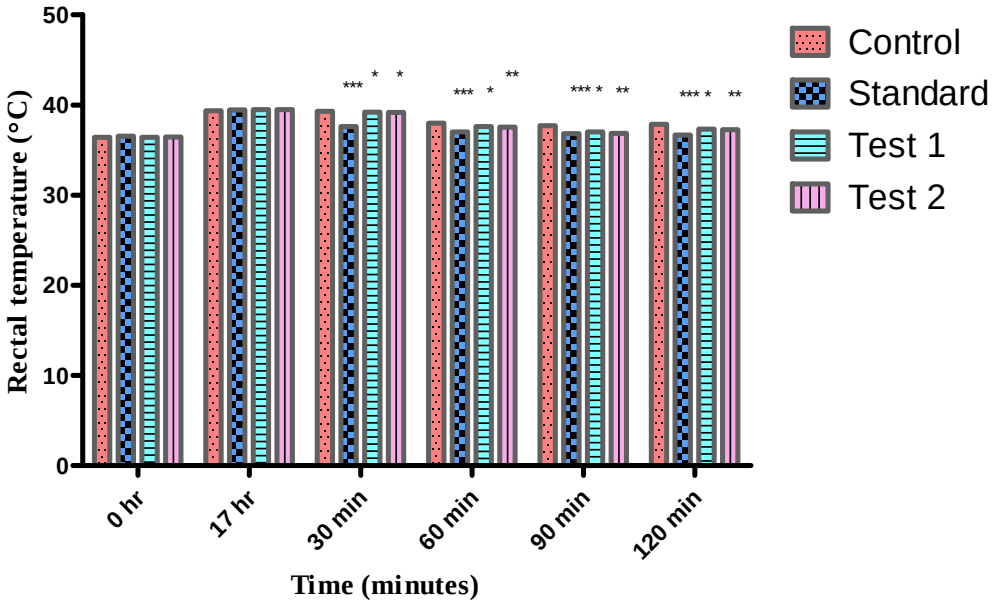

Figure 1: Effect of ethanolic extract of *Berberis aristata* on yeast-induced pyrexia in rats.

Hot plate method

The result is presented in Table 3 & figure 2, shows the time course of antinociception produced by extract of *Berberis aristata*. The animals pretreated with EEBA (200 mg/kg & 400 mg/kg) showed a dose dependent increase in latency of response in the hot plate method. The increase in the latency period was less than the effect caused by the standard drug pentazocine.

Table 3: Influence of EEBA extract on hotplate-induced pain

Treatments	Dose (mg/kg, p.o.)	Reaction time (s)					
		Pretreatment	0 min	30min	60min	90 min	120 min
0.25 % CMC	1 ml / kg b.w.	156±0.07	1.90±0.05	2.00±0.06	2.12±0.05	2.10±0.03	2.01±0.06
Pentazocine	5 mg / kg b.w.	1.77±0.07	2.54±0.07***	4.24±0.14***	6.00±0.28***	4.71±0.18***	4.21±0.21***
EEBA	200 mg / kg b.w.	1.78±0.08	2.19±0.07*	2.63±0.13*	3.14±0.26*	2.74±0.16*	2.62±0.14*
EEBA	400 mg / kg b.w.	1.79±0.04	2.29±0.06**	2.81±0.16**	3.40±0.19**	2.90±0.10**	2.80±0.15**

All data were expressed in mean±SEM (n=6) by One way ANNOVA test followed by Tukey test

* Significantly different from the control group: $P < 0.05$.

** Significantly different from the control group: $P < 0.01$.

*** Significantly different from the control group: $P < 0.001$.

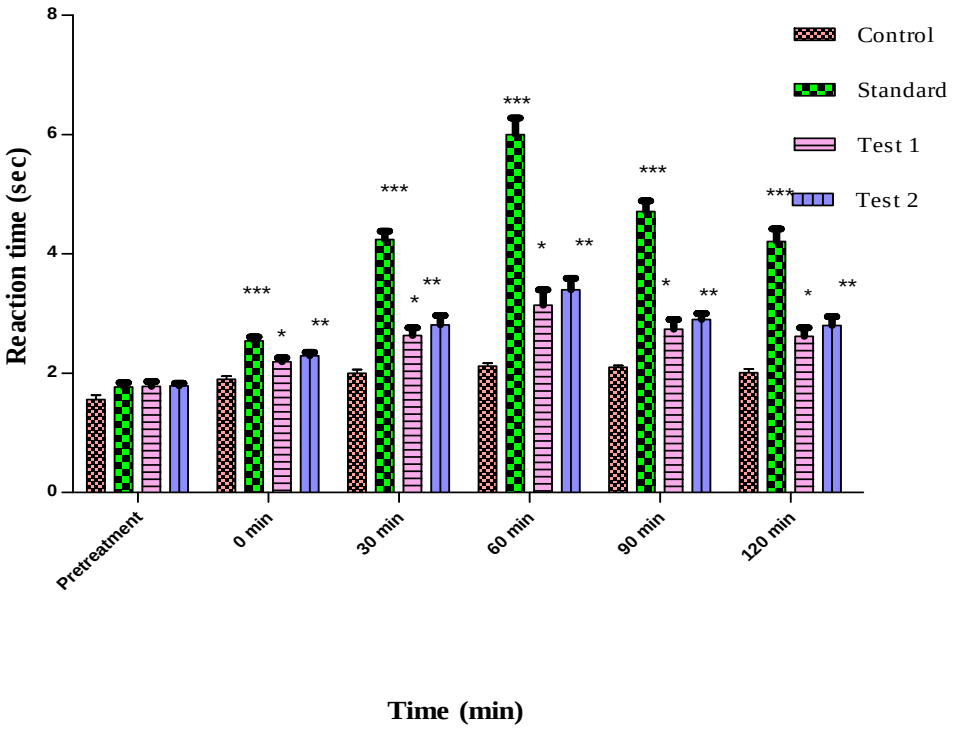

Figure 2: Effect of ethanolic extract of *Berberis aristata* on hot-plate method.

Acetic acid induced writhing method

The inhibitory effect of ethanolic extract and its fractions in writhing test is shown in Table 4 & figure 3. EEBA significantly ($p<0.05$) inhibited the acetic acid induced writhing response in a dose dependent manner at the dose of 200 mg/kg and 400 mg/kg. The inhibition elicited by the extract was however lower than that observed for aspirin at a dose of 150 mg /kg.

Table 4: Effect of on acetic acid induced writhing in mice.

Treatments	Dose (mg/kg, p.o.)	No. of writhes (per 20 mins)
0.3 % CMC	1 ml / kg b.w	22.17±1.06
Aspirin	150 mg/ kg b.w	14.00±0.61***
EEBA	200 mg / kg b.w	18.83±0.64*
EEBA	400 mg / kg b.w	17.50±0.62**

All data were expressed in mean±SEM (n=6) by One way ANNOVA test followed by Tukey test

* Significantly different from the control group: $P < 0.05$.

** Significantly different from the control group: $P < 0.01$.

*** Significantly different from the control group: $P < 0.001$.

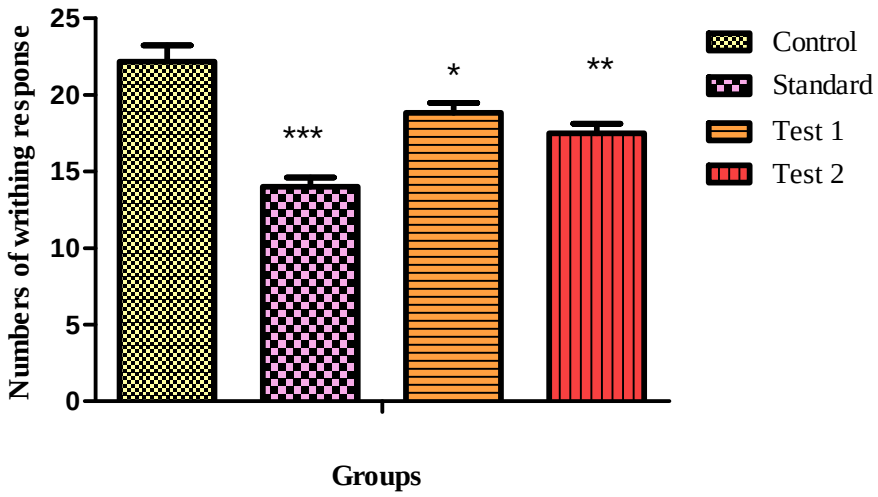

Figure 3: Effect of ethanolic extract of *Berberis aristata* on acetic acid induced writhing method.

Tail flick test

The result is presented in Table 5 & figure 4, shows the time course of antinociception produced by EEBA. The analgesic effect reflected in the tail flick test is dependent on centrally acting analgesics. EEBA significantly increased in the latency time in the tail flick test, indicating early phase analgesic activity.

Table 5: Antinociceptive activity of EEBA by the tail-flick test in mice.

Treatments	Dose (mg/kg, p.o.)	Reaction time (s)				
		Pretreatment	0 min	30min	90min	150 min
0.25 % CMC	1 ml / kg b.w.	1.24±0.02	1.30±0.02	1.56±0.04	1.63±0.05	1.40±0.04
Acetyl salicylic acid	150 mg / kg b.w	1.20±0.02	1.50±0.07*	3.54±0.07***	4.19±0.06***	3.34±0.06***
EEBA	200 mg / kg b.w.	1.21±0.01	1.36±0.04	1.84±0.07*	1.90±0.08*	1.63±0.05*
EEBA	400 mg / kg b.w.	1.22±0.03	1.40±0.05	1.92±0.08**	2.03±0.07**	1.71±0.05**

All data were expressed in mean±SEM (n=6) by One way ANNOVA test followed by Tukey test.

* Significantly different from the control group: $P < 0.05$.

** Significantly different from the control group: $P < 0.01$.

*** Significantly different from the control group: $P < 0.001$.

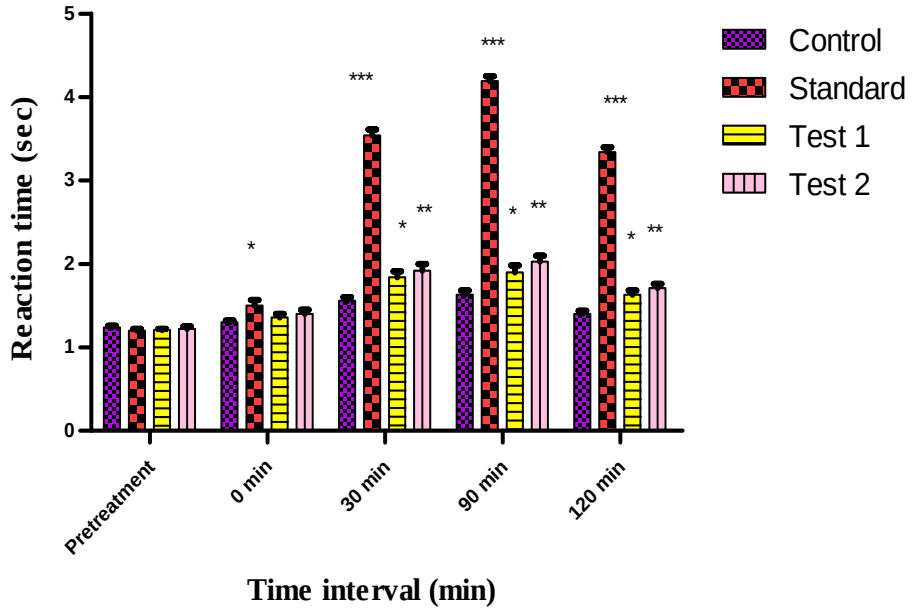

Figure 4: Effect of ethanolic extract of *Berberis aristata* on acetic acid induced writhing method.

DISCUSSION

The result showed that the ethanolic extract at the dose of 200 & 400 mg/kg produced significant response. According to the literature, there is no report on antipyretic and analgesic effects of *Berberis aristata*. The extract of *Berberis aristata* might contain active principles that exhibited inhibitory action on cyclooxygenase. The ethanolic extract of Berberis *aristata* shows significant reduction of body temperature in tested animals. Subcutaneous injection of yeast suspension induces pyrexia by increasing the synthesis of

prostaglandins which ultimately increases the body temperature and is a useful model for screening antipyretic effect of substances.[22]

The hot-plate test, which utilize thermal stimulus to induce pain, is frequently used to evaluate centrally mediated antinociceptive activity.[23] The central opoid pathway exerts its effect through the spinal and supra-spinal receptors.[24] The hot plate test predominately measures supraspinally organised responses. The paws of rats are very sensitive to heat at temperatures which are not damaging the skin. The responses are jumping, withdrawal of the paws and licking of the paws. The time until these responses occur is prolonged after administration of centrally acting analgesics.[25] In order to test the effects of EEBA on thermal stimuli, hot plate test was used and extract at a dose of 200 & 400 mg/kg orally significantly increased the nociception latency comparable to the control group.

Acetic acid is an irritating agent, which stimulates local peritoneal receptors to induce pain with characteristic abdominal constrictions when injected into the peritoneal cavity. Local irritation produced by an intraperitoneal injection triggers the release of a variety of mediators such as cyclooxygenase (COX), lipoxygenase (LOX) histamine, serotonin, prostaglandins especially PGI-2, substance P, bradykinins, as well as pro-inflammatory cytokines such as IL-1, IL-6, IL-8, and TNF-α . These substances activate the chemosensitive nociceptors that play role in the development of this type of inflammatory pain which is sensitive to non-steroidal anti inflammatory drugs like aspirin.[26]

The acetic acid writhing test is a standard sensitive test for both opioid and non-opioid analgesics,[27] and the most commonly used for screening peripheral analgesic.[28] Increased level of these mediators causes excitation of primary afferent nociceptors entering dorsal horn of the central nervous system.[29] The extract of *B. aristata* showed marked reduction in the abdominal constriction provoked by acetic acid in dose dependent manner. The antinociceptive effect of *B. aristata* in the acetic acid induced model, therefore suggests that *B. aristata* may be involved in the inhibition of COX, LOX and other inflammatory mediators resulting interrupted signal transduction in primary afferent nociceptors.

Tail flicking is predominantly a spinal reflex and is considered to be selectively sensitive to centrally acting analgesic compounds. In the tail-flick test, the thermal stimulation activates peripheral nociceptors, which leads to reflexive removal of the tail.[30] Consequentlly, one possible mechanism of antinociceptive activity of the extract of *B.aristata* could be due to

blockade of the effect or the release of endogenous substances (arachidonic acid metbolites) that excite pain nerve endings. The ethanolic extract of stem of *Berberis aristata* showed latency in tail flick time.

Phytochemical studies show that alkaloids, flavonoids, tannins, cardiac glycosides and phenols are present in this extract.

Analgesic and antipyretic effects of *Berberis aristata* properties were known to intertwine especially with the NSAIDs which act in a manner that the sensitization of pain receptors by prostaglandin at the inflammation site is inhibited.

CONCLUSION

The present study indicates that the ethanolic extract of *Berberis aristata (*EEBA*)* possessed a significant antipyretic activity and has both central and peripheral analgesic activity but standard drug is better in analgesic and antipyretic both responses than EEBA doses. The antipyretic and analgesic activity of EEBA was probably due to presence of alkaloids, flavonoids, tannins in which berberine alkaloid might play a key role for the activity which needs further molecular exploration.

ACKNOWLEDGEMENTS

I would like to express my sincere thanks to Prof. Rajiv Gupta, Dean, School of Pharmacy, BBD University, Lucknow, and U.P for encouragement and providing library facilities to access the journals.

REFERENCES

1. Singh SP, Sharma SK, Singh L, Goyal S, Gawad JKB. An Overview of NSAID's used in anti-Inflammatory and analgesic activity and prevention gastrointestinal damage. *J of Dr Dis and Th* 2013; 1: 41-51.
2. Annan K, Dickson RA, Sarpong K, Asare C, Amponsah K, Woode E. Antipyretic activity of *Polyalthia* (Annonaceae) on lipopolysaccharide induced fever in rats. *Journal of Medical and Biomedical Sciences,* 2013; 2: 8-12.
3. Bhardwaj A, Khera A, Malik YS, Kaushik M, Sharma M. Comparative evaluation of *Berberis aristata* extract as systemic administration and local application in management of chronic pharyngitis. *J of Pharma & Sci Inn,* 2013; 2: 61-65.

4. Gogte, 2000. Ayurvedic Pharmacolgy and Therapeutics uses of Medicinal plants. *Bhartiya Vidya Bhawan*, 1910-87.
5. Shamkuwar PB, Pawar DP. Antidiarrhoeal and Antispasmodic effect of *Berberis aristata*. *Inter J of Pharmacog and Phytochem Res*, 2013; 5: 24-26.
6. Lamichhane B, Adhikari S, Shrestha1 P, Shrestha BN. Study of phytochemical, antioxidant, antimicrobial and anticancer activity of *Berberis aristata*. *J of Trop Li Sci*, 2014; 4: 17-21.
7. Semwal BC, Gupta J, Singh S, Kumar Y, Giri M. Antihyperglycemic activity of root of *Berberis aristata* D.C. in alloxan-induced diabetic rats. *Int J of Gr Pharm*, 2014; 259-62.
8. Ahmad R, Srivastava S, Maurya R, Rajendran SM, Arya KR, Srivastava A. Mild antihyperglycaemic activity in *Eclipta alba*, *Berberis aristata*, *Betula utilis*, *Cedrus deodara*, *Myristica fragrans* and *Terminalia chebula*.. *In J of Sci and Tech* 2008; 1: 1-6.
9. Ajmire P. Effect of *Berberis aristata* DC. against Dimethylnitrosamine induced liver cirrhosis in rat model. *J of Phar Res*, 2011; 4: 4015-17.
10. Dehar N, Walia R, Ratol S. Potentiation of thiopentone sodium induced hypnosis by *Berberis aristata* in rodents. *As J of Pharm and Clini Res*, 2012; 5: 131-33.
11. Yogesh SH, Chandrashekhara VM, Kattib HB, Ganapatyd S, Raghavendrac SHK, Gowdae GK, Goplakhrishna B. Anti-osteoporotic activity of aqueous-methanol extract of *Berberis aristata* in ovariectomized rats. *J of Ethn*, 2011; 134: 334–38.
12. Mazumder PM, Das S, Das MK. Cytotoxic activity of methanolic extracts of *Berberis aristata* DC and *Hemidesmus indicus* R.Br. in MCF7 cell line. *J of Cu Ph Res*, 2010; 1: 12-15.
13. Kulkarni KS, Dhir A. On the mechanism of antidepressant-like action of berberine chloride. *Eu J of Pharmacol*, 2008; 589: 163–72.
14. Pareek A, Suthar M. Antidiabetic activity of extract of *Berberis aristata* root in streptozotocin induced diabetic rats. *Pharmacol online*, 2010; 2: 179-85.
15. Upwar N, Patel R, Waseem N, Mahobia NK. Hypoglycemic effect of methanolic extract of *Berberis aristata* DC stem on normal and streptozotocin induced diabetic rats. *Inter J of Phar and Ph Sci*, 2011; 3: 222-24.
16. Adikay S, Koganti B, Prasad K. Effect of decoction of root bark of *Berberis aristata* against cisplatin induced nephrotoxicity in rats. *Intern J of Ph and Pharml Sci*, 2008; 2 : 33-38.

17. Lamichhane B, Adhikari S, Shrestha1 P, Shrestha BN. Study of phytochemical, antioxidant, antimicrobial and anticancer activity of *Berberis aristata*. *J of Trop Li Sci*, 2014; 4: 17-21.
18. Habib M, Waheed I. Evaluation of anti-nociceptive, anti-inflammatory and antipyretic activities of *Artemisia scoparia* hydromethanolic extract. *J of Ethnopharmacol*, 2012; 145: 18–22.
19. Imam MZ, Nahar N., Akter S, Rana S 2012. Antinociceptive activity of methanol extract of flowers of *Impatiens balsamina*. *J of Ethnopharmacol*, 142: 804–10.
20. Kumar RS, Rajkapoor B, Peruma P 2013. Anti-inflammatory and anti-nociceptive activities of methanolic leafextract of *Indigofera cassioides* Rottl. Ex. DC. *J of Acute Dise*, 322-26.
21. Bahadir O, Citoglu GS, Smejkal K. Analgesic compounds from *Scorzonera latifolia* (Fisch. and Mey.) DC. *J of Ethnopharmacol*, 2010; 131:83–87.
22. Gupta M, Mazumder UK, Sambath KR, Gomathi P, Rajeshwar Y, Kakoti BB, Tamil S V. Antiinflammatory, analgesic and antipyretic effects of methanol extract from *Bauhinia racemosa* stem bark in animal models. *J of Ethnopharmacol*, 2005; 98: 263-73.
23. Vogel H., Vogel WH. Drug Discovery and Evaluation: Pharmacological Assays. Springer-Verlaag, Berlin, Heidelberg, 1997.
24. Soares PM, Lima SR, Mato SG, Andrade MM, Patrocinio MCA., *et al*. Antinociceptive activity of *Calotropis procera* latex in mice. *J of Ethnopharmacol* 2005., 99: 125-29.
25. Pinheiro B, Silva A, Souza G, Figueiredo J, Cunha F, Lahlou S *et al*. Chemical composition, antinociceptive and anti-inflammatory effects in rodents of the essential oil of *Peperomia serpens*. *J of Ethnopharmacol*, 2009; 188: 479-86.
26. Steranka LR, Dehaas CJ, Vavrek RJ, Stewart JM, Enna SJ, Synder SH. Antinociceptive effects of bradykinin antagonists. *Europ J of Pharmacol*, 2009; 136: 261-62.
27. Bentley GA, Newton SH, Starr J. Evidence for an action of morphine and the enkephalins on sensory nerve endings in the mouse peritoneum. *Bri J of Pharmacol*, 1981; 73: 325-32.
28. Ikeda Y, Ueno A, Naraba H, Oh-ishi S. Involvement of vanilloid recptor VR1 and prostanoids in the acid induced writhing responses of mice. *Life Sci*, 2001; 69: 2911-19.
29. Ramabadran K, Bansinath M, Turndorf H, Puig MM. Tail immersion test for evaluation of a nociceptive reaction in mice methodological consideration. *J of Ethnopharmacol Meth*, 1989; 21: 21-31.

30. Kuraishi Y, Harada Y, Aratani S, Satoh M, Takagi H. Separate involvement of the spinal noradrenergic and serotonergic systems in morphine analgesia-thee differences in mechanical and thermal analgesic tests. *Brain Res*, 1983; 273: 245-52.