Journal of Complementary and Alternative Medical Research

5(2): 1-8, 2018; Article no.JOCAMR.40002
ISSN: 2456-6276

Evaluation of Analgesic and Anti-inflammatory Potentials of the Leaf and Root Extracts of *Vanda roxburghii* (Roxb)

Yesmin Begum[1*], Prosengit Kumer Sen[1], Israt Jahan Bulbul[1] and Fatema Nasrin[1]

[1]Department of Pharmacy, Southeast University, Banani, Dhaka-1213, Bangladesh.

Authors' contributions

This work was carried out in cooperation among all authors. Author YB designed, supervised the study and wrote the manuscript. Author PKS performed the experiments and analysed the data. Authors IJB and FN searched the literature and also supervised the experimental work. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JOCAMR/2018/40002
Editor(s):
(1) B. V. Suma, Assistant professor, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ramaiah University of Applied Sciences, Bangalore, India
Reviewers:
(1) Esraa Ashraf Ahmed ElHawary, Ain Shams University, Egypt.
(2) Thomas Heinbockel, Howard University College of Medicine, USA.
Complete Peer review History: http://www.sciencedomain.org/review-history/23902

Original Research Article

Received 16th January 2018
Accepted 27th March 2018
Published 30th March 2018

ABSTRACT

Aims: To evaluate the analgesic and anti-inflammatory potentials of the methanolic leaf and root extracts of *Vanda roxburghii* (LVR and RVR) belonging to the family Orchidaceae, a hill tract plant, traditionally used in rheumatism.
Study Design: The current study was carried out in vivo. LVR and RVR were divided into two concentrations, 50 mg/kg body weight and 100 mg/kg body weight and then subjected to different mice models to evaluate analgesic and anti-inflammatory potentials.
Place and Duration of Study: Department of Pharmacy, Southeast University, Banani, Dhaka-1213, Bangladesh, within a period of six month.
Methodology: Analgesic and anti-inflammatory activities of LVR and RVR (50 and 100 mg/kg) of *V. roxburghii* were evaluated in different mice models as acetic acid-induced writhing, formalin-induced paw licking and carrageenan-induced hind paw oedema models using diclofenac sodium as standard.

**Corresponding author: E-mail: yesumyta@gmail.com;*

Results: The results of the study showed that both LVR and RVR possess peripherally and centrally acting analgesic potential in mice model. In acetic acid induced writhing method, LVR (100 mg/kg) significantly (P<0.001) reduced pain sensation with 64.94% inhibition as compared to standard with 70.13% of inhibition. Correspondingly, LVR (100 mg/kg) also appreciably (p<0.01) reduced licking in early phase with 51.43% of inhibition and late phase (p<0.001) with 88.89% of inhibition as compared to standard. Furthermore, RVR also showed significant analgesic activity in both early and late phase at the dose of 50 mg/kg with 50.48% and 66.67% of inhibition. In the anti-inflammatory study, LVR and RVR (100 mg/kg) showed significant (P<0.01) activity after 3 hours of administration of plant extract against carrageenan-induced hind paw oedema with 67.14% and 61.37% of inhibition respectively as compared to standard 72.28% of inhibition.
Conclusion: The investigation revealed that the methanolic leaf and root extracts of *V. roxburghii* have both central and peripheral analgesic as well as anti-inflammatory potentials that would add a great medicinal value to develop it's ethnopharmacological study.

Keywords: Vanda roxburghii; analgesic; anti-inflammatory; carrageenan.

1. INTRODUCTION

The decreasing efficacy of synthetic drugs and the increasing contraindications make the usage of natural drugs contemporary again. Drugs presently used for the management of pain and inflammatory conditions are either narcotics (e.g., opioids), non –narcotics/ non steroidal anti-inflammatory drugs (NSAIDs) (e.g., salicylates) or corticosteroids (e.g., hydrocortisone). Non-steroidal anti-inflammatory drugs (NSAIDs) are still the most preferred medication for pain and inflammation which acts by blocking, preventing the transformation of arachidonic acid into prostaglandin. These synthetic agents have many adverse effects like ulcer and gastrointestinal bleeding [1]. Opioid analgesic's role in the long-term treatment of pain is controversial due to psychological dependence and side effects like sedation, dizziness, nausea, vomiting, constipation, physical dependence, tolerance and respiratory depression [2]. So the evaluation of using a natural medicinal source with fewer side effects was inevitable. *V. roxburghii* is an epiphytic herb from family Orchidaceae and distributed through Bangladesh & India [3]. Different parts of the plant are used in dyspepsia, bronchitis, inflammations, piles and nervous system disorder and also reported to cure rheumatism, bronchitis [4]. This herb has been reported to have aphrodisiac, analgesic, antibacterial, anticonvulsant, antidiabetic, antifungal, antioxidant, anti-inflammatory, antiulcer, cytotoxic, cholinesterase inhibitory, hepatoprotective and wound healing potentials [5-11]. Some important bioactive principles have been identified from this plant as alkaloids, glucosides, fatty acids, saponins, tannins, β-sitosterols, γ-sitosterols, fatty oils, resins, whereas roots contain tetracosyl ferrulate and β-sitosterol-D-glucoside [12]. The present study was aimed to evaluate and authenticate the analgesic and anti-inflammatory potentials of the methanolic leaf and root extracts of *V. roxburghii*, native to Bangladesh.

2. MATERIALS AND METHODS

2.1 Plant Material

The fresh leaves and roots of *V. roxburghii* were obtained from Sylhet, Bangladesh in January 2016 and then identified by Bangladesh National Herbarium with the accession number 73881. After identification, the herbarium specimen was submitted in the herbarium for future reference.

2.2 Preparation of Plant Extracts

After cleaning all the adhering dirt, the plant parts were shed dried for four days and then pulverized into a coarse powder using grinding machine. Each plant materials (200 gm) were then soaked for 14 days in 1l crude methanol, with occasional shaking. The extracts were then filtered through filter paper (Whatman filter paper number 45). At last, the filtrate was evaporated using a rotary evaporator at 45-55°C, which yield gummy concentrate of plant extract.

2.3 Experimental Animal

For this study, Swiss Albino mice (25-35 gm) of either sex were purchased from the International center for Diarrhea Disease and Research Bangladesh, icddr'b and housed in the animal house of Department of Pharmacy, Southeast University. The animals had free access to pellet feed and tap water ad libitum supplied by icddr'b. The animals were allowed to acclimatise to the environment condition (at 24.0±0°C temperature, 55-65% relative humidity and 12 hour light/12 hour dark cycle) for 7 days prior to the

experimental session. The animal experimental protocol was conducted in accordance with the guidelines titled as "Ethical Considerations for Animals" prepared by icddr'b.

2.4 Analgesic Activity

2.4.1 Acetic acid induced writhing test

The peripheral analgesic potential of *V. roxburghii* extracts were evaluated by counting total number of writhing following intra peritoneal administration of 0.7% acetic acid solution for 15 minutes starting after 5 minutes of administration [13]. Mice were treated with RVR (50 &100 mg/kg), LVR(50&100 mg/kg), Saline (10 ml/kg Negative control) and diclofenac sodium (10 mg/kg) prior to acetic acid administration. The number of writhing, constriction of abdominal muscles along with the stretching of hind limbs, were counted for all groups and percentage of inhibition of writhing response/ percentage increase in analgesia was calculated as follow.

% inhibition of abdominal writhing = (Wc-Wt) / Wc x 100

Wc=No. of writhing for control
Wt=No. of writhing for test animals

2.4.2 Formalin induced paw licking test

This test consists of two phases reflecting two different types of pain, neurogenic and inflammatory responses respectively [14]. In this method the animals were subjected to six groups (n=4) treated with saline (10 ml/kg .p.o), diclofenac sodium (10 mg/kg), 50 and 100 mg/kg of LVR and RVR extracts to evaluate analgesia by observing reaction time. After 30 minutes, paw licking was induced by the injection of 50 µL of 5% formalin into the dorsal surface of the mice and the duration of paw licking was measured from 0-5 minutes (early phase) and 20-30 minutes (late phase) after formalin injection by using a stop watch.

2.5 Anti Inflammatory Activity

2.5.1 Carragenan induced paw edema test

The assessment of anti-inflammatory potential was executed as described by winter at el. [15]. The animals were randomized into six different groups, each consisting of four mice and the normal paw volumes of all mice were measured. The Group I and II were treated with normal saline (10 ml/kg, i.p.) and standard diclofenac sodium (10 mg/kg, p.o.) respectively, while rest of the groups were treated with LVR and RVR extracts of different doses(G-III & IV, 50 and G - V &VI, 100 mg/kg, p.o.). After thirty minutes of the above treatment, carrageenan (1% 0.1ml) was administered into sub plantar tissue of right hind paw of each mice to induce edema and observed for six hours. The paw volume was measured by micrometer screw gauge before and at 1^{st}, 2^{nd}, 3^{rd} and 4^{th} after carrageenan administration. The percent inhibition of paw edema was calculated by using the following formulae:

Percent Inhibition = (Co-Ct)/Co × 100

Ct = volume of animal's paw after injection
Co= volume of animal's paw before injection

2.6 Statistical Analysis

All the values are articulated as mean ± SEM. The data were statistically analyzed by ANOVA followed by Dunnett's test with the Statistical Package for Social Sciences (SPSS 20.0, USA) program.

3. RESULTS

3.1 Analgesic Activity

3.1.1 Acetic acid induced writhing test

Acetic acid induced writhing method is one of the most applied test to evaluate peripherally acting analgesics. Both extracts RVR and LVR showed dose dependent reduction in writhing in mice model that had been presented in Table 1 (Fig. 1). Methanolic leaf extract of *V. roxburghii* (LVR -100 mg/kg) showed highly significant ($p<0.001$) analgesic potential with 64.94% as compared to standard with 70.13% of percentage reduction in writhing respectively. Root extract, RVR (100 mg/kg) also reduced writhing by 42.86%.

3.1.2 Formalin induced paw licking

Formalin test consists of two phases, early phase and late phase, presenting neurogenic and inflammatory pain respectively. LVR and RVR potentially reduced paw licking in both phases as shown in Table 2 (Fig. 2). LVR, at the dose of 100 mg/kg, showed the maximum inflammatory pain reduction ($P<0.001$) with 88.89% and neurogenic pain reduction with 51.43% of inhibition. On the other hand RVR, at 50 mg/kg reduced both neurogenic and inflammatory pains by 50.48% and 66.67% of inhibition respectively.

Table 1. Evaluation of Analgesic activity of LVR and RVR by Acetic acid induced writhing method

Treatment	No. of writhing	% of inhibition
Control	19.25±3.17	
Standard	5.75±0.94***	70.13
RVR 50 mg	16.75±1.65	12.99
RVR 100 mg	11±1.73**	42.86
LVR 50 mg	10.25±0.94**	46.75
LVR 100 mg	6.75±0.47**	64.94

*Each value represents Mean ± SEM, n=4.*P < 0.05, **P<0.01 & ***P<0.001 compared with control. Significance tests are done by using one-way-annova followed by Dunnett test*

3.2 Anti Inflammatory Potential

3.2.1 Carrageenan induced paw edema method

Treatment of carragenan induced mice model with LVR and RVR reduced paw edema dose dependently as presented in Table 3. All doses of LVR (50 &100 mg/kg) reduced paw edema significantly (P<0.01) at 3rd and 4th hr of the treatment with maximum 67.14% of inhibition. RVR (100 mg/kg) also showed potential anti inflammatory potential (P<0.01) at 3rd hour with 61.37% of inhibition.

4. DISCUSSION

Pain and inflammation underlie with each other. Inflammation is initiated upon tissue injury with a cascade of biochemical reactions that prime the nervous system for pain sensing. Long-term inflammation strengthens the adaptive changes in the nervous system and causes the sensation of pain to become inflated. Common OTC painkillers are very effective for reducing inflammation and pain but can cause anorexia, nausea, vomiting, epigastric pain, dyspepsia, constipation, diarrhea, gastritis, dark tarry stools, flatulence, severe GI effects include gastric ulceration with or without bleeding, peptic ulcer disease, or GI perforation. The present and future prospects of pain management would be focused to explore phytomedicines with minimal side effects. This study was based on the verification of the analgesic and anti-inflammatory potentials of leaf and root of *V. roxburghii*.

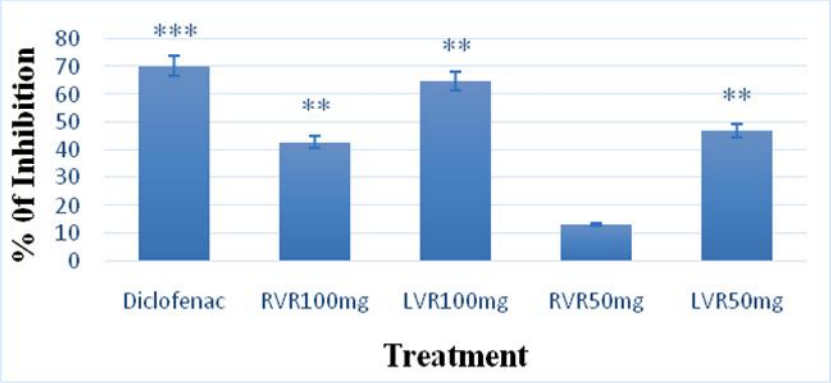

Fig. 1. Percentage of pain inhibition in acetic acid induced writhing method

Table 2. Evaluation of Analgesic activity of LVR and RVR by Formalin induced induced paw licking method

Treatment	Dose (mg/kg)	Duration of licking	% of inhibition	Duration of licking	% of inhibition
		Early Phase		Late Phase	
Control	0.1 ml/mice	26.25±1.377		13.5±1.190	
Standard	10	7.25±0.479***	72.38	0.25±0.25***	98.15
RVR	50	13±1.225***	50.48	4.5±1.443***	66.67
RVR	100	17.25±1.315*	34.29	4.25±2.658**	83
LVR	50	17±1.958*	35.24	9.25±1.493*	31.48
LVR	100	12.75±2.496**	51.43	1.5±0.866***	88.89

*Each value represents Mean ± SEM, n=4.*P < 0.05, **P<0.01 & ***P<0.001 compared with control*

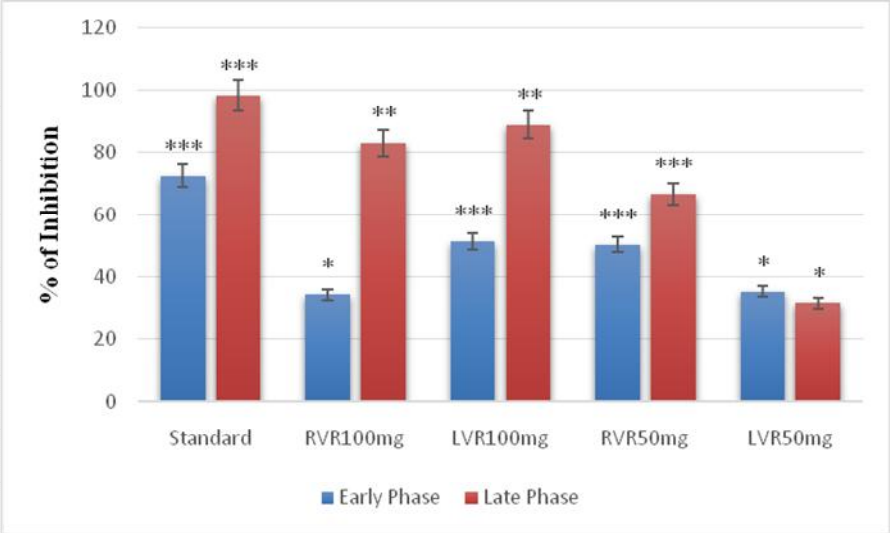

Fig. 2. Percentage of pain inhibition by formalin induced paw licking test

Table 3. Investigation of anti inflammatory potential of LVR and RVR by carrageenan induced paw edema test

Treatment	Mean increase in paw volume (cm)		% of inhibition	
	3 hr	4 hr	3 hr	4 hr
Control	1.31±0.025	1.21±0.043	-	-
Standard 10 mg	0.36±0.014**	0.36±0.011**	72.28	70.04
RVR 50 mg	0.70±0.075**	0.81±0.041**	48.89	42.8
RVR 100 mg	0.51±0.041**	0.56±0.07**	61.37	57.7
LVR 50 mg	0.47±0.029**	0.45±0.014**	64.25	63.2
LVR 100 mg	0.40±0.011**	0.42±0.037**	67.14	67.87

*Each value represents Mean ± SEM, n=4.*P < 0.05, **P<0.01 & ***P<0.001 compared with control*

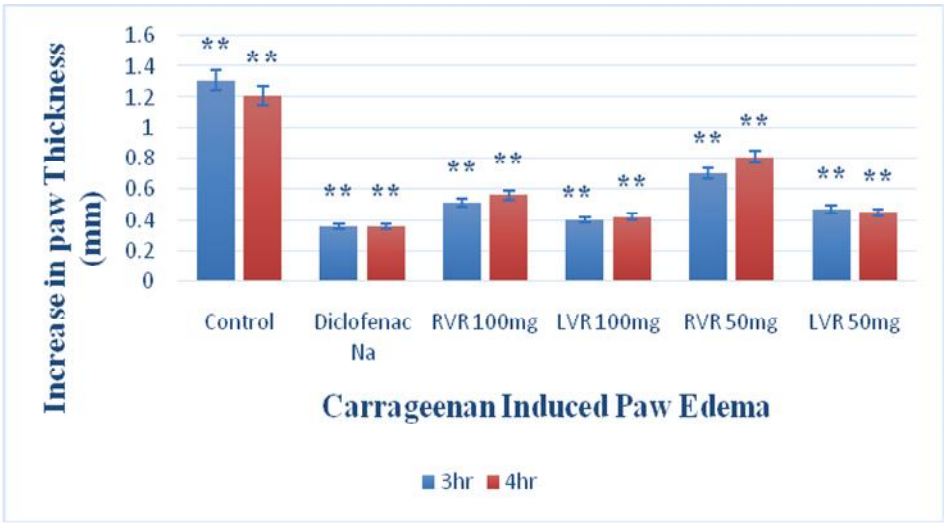

Fig. 3. Decrease of paw thickness at 3rd and 4th hour in carrageenan induced hind paw edema test

The results obtained from the experiments clearly imply that LVR and RVR possess noticeable analgesic effects both centrally and peripherally. Acetic acid induced writhing and formalin induced paw licking methods are well established tests to evaluate the analgesic activities.In acetic acid induced writhing method pain sensation in the experimental animal is caused by the arachidonic acid through cyclooxygenase pathway thus producing prostaglandin mainly PGE_2 and $PGF_{2\alpha}$ [16]. Both extracts showed statistically considerable dose dependent analgesic activities by effectively reducing the number of writhing in mice models. NSAIDs generate peripheral analgesic effect by blocking or inhibiting the cyclooxygenase and/or lipoxygenase [17]. Possibly LVR and RVR exhibited analgesic effect through this mechanism. Although it is a non-specific model to evaluate the peripheral anti-nociceptive activities but it is able to identify the abdominal constrictions through pain and with great sensitivity [18].

Formalin test is a great procedure to evaluate both central and peripheral pain within a single experiment. This method consists of two phases, first neurogenic phase in which pain is induced peripherally as an effect of formalin injection [19] followed by the second phase called inflammatory phase where inflammation resulting from kinins, leucocytes and cell injuries which promotes the release of some endogenous factors [20]. Both extracts significantly suppressed the licking activities throughout the experiment which indicates that V. *roxburghii* possesses high analgesic effects although the exact mechanism could not be verified.

Carrageenan induced paw edema is a preferable screening test to assess the anti-inflammatory potential. This is a biphasic method in which initial phase is observed through first hour of carrageenan injection where inflammation is induced by serotonin and histamine, while in the second phase inflammation is mediated by the release of arachidonic acid which converts into prostaglandins [21,22]. Several established NSAIDs are reported to block the prostaglandin synthesis in order to prevent inflammation [23]. Both extracts significantly reduced the paw volume throughout the experiment comparing with the standard drug Diclofenac sodium by inhibiting the cyclooxygenase of any other mediator listed above. Although the exact mechanism of action is unknown, the outcome of both samples are quite similar to the standard and sometimes even better.

5. CONCLUSION

From the ancient time medicinal plants are one of the most important and reliable source for safe medication. In this present study root & leaf extracts of *V. roxburghii were* subjected to analgesic and anti-inflammatory test to justify their traditional uses and most of the results are quite promising to establish the unknown bioactive entities. So further molecular and pharmacological study should be carried out on this orchid to find out the constituents responsible for these activities and to find new lead compounds.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The experimental protocols were approved by the Ethical Committee for Animal Care and Use at Department of Pharmacy, Southeast University (2016/007-12).

ACKNOWLEDGEMENTS

The authors are thankful to the department of pharmacy, Southeast University for providing the laboratory facilities to perform the above task. The authors are also grateful to Bangladesh National Herbarium (DACB), Mirpur, Dhaka to identify the plant.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Louis Kuritzky, George P. Samraj. Nonsteroidal anti-inflammatory drugs in the treatment of low back pain. J Pain Res. 2012;5:579–590.
2. Benyamin R, Trescot AM, Datta S, Buenventura R, Adlaka R, Sehgal N, Glaser SE, Vallejo R. Opioid complications and side effects. Pain Physician. 2008; 11(2 Suppl):S105-20.

3. Anisuzzaman Chowdhury M, Masudur Rahman M, Mohammed Riaz Hasan Chowdhury, Josim Uddin M, Mohammed Abu Sayeed, Aslam Hossain M. Antinociceptive and cytotoxic activities of an epiphytic medicinal orchid: *Vanda tessellate* Roxb.BMC Complement Altern Med. 2014;14:464.
4. Dilnawaz Pathan, Shirish Ambavade. Phytochemical and Pharmacognostical Evaluation of *Vanda roxburghii*. Research J. Pharm. and Tech. 2014;7(5):531-532.
5. Nayak BS, Suresh R, Rao AVC, Pillai GK, Davis EM, Ramkissoon V, McRae A. Evaluation of Wound Healing Activity of *Vanda roxburghii* R. Br (Orchidacea): A preclinical study in a rat model. The Inter. J. of L. Extre. Wounds. 2005;4(4): 200-4.
6. Md. Josim Uddin, Md. Masudur Rahman, Md. Abdullah-Al-Mamun, Golam Sadik. *Vanda roxburghii*: An experimental evaluation of antinociceptive properties of a traditional epiphytic medicinal orchid in animal models. BMC Complement Altern Med. 2015;15(1):305.
7. Dilnawaz Pathan, Shirish Ambavade. Phytochemical and pharmacognostical evaluation of *Vanda roxburghii*. Research J. Pharm. and Tech. 2014;7(5):531-532.
8. Md. Nasim Uddin, Rejina Afrin, Md. Josim Uddin, Md. Jalal Uddin, Alam AHMK, Aziz Abdur Rahman, Golam Sadik. *Vanda roxburghii* chloroform extract as a potential source of polyphenols with antioxidant and cholinesterase inhibitory activities: Identification of a strong phenolic antioxidant. BMC Complement Altern Med. 2015;15:195.
9. Shamsul Islam SM, Hasan Sayeed, Abrar Shahriyar SK, Afia Ferdous, Akherul Islam. Antioxidant, analgesic and cytotoxic activity of methanolic extract of vanda roxburghii root. Inter. J. of Pharm. Scie. and Research. 2016;7(7):2944-2950.
10. Dilnawaz Pathan, Shirish Kumar Ambavade. Investigation of anticonvulsant activity of *Vanda roxburghii*. J. of Pharm. and Phyto. 2014;2(6): 95-99
11. Mukhtar HM, Kalsi V. Therapeutic Potential of *Vanda roxburghii* Roxb.: A review. Inter. J. of Current Pharmaceut. Review and Research. 2017;8(3): 261-265.
12. Suresh Kumar PK, Subramoniam A, Pushpangadan P. Aphrodisiac activity of *Vanda tessellate* (Roxb.) hook. ex donextract in male mice. Indian J. of Pharmacol. 2000;32:300-304.
13. Musa YM, Haruna AK, Ilyas M, Yaro AH, Ahmadu AA, Usman H. Phytochemical, analgesic and anti-inflammatory effects of the ethyl acetate extract of the leaves of *Pseudocedrella Kotschyii.* Afr J Tradit Complement Altern Med. 2008;5(1):92–96.
14. Chandana Choudhury Barua, Jayanti Datta Roy, Bhaben Buragohain, Acheenta Gohain Barua, Prabodh Borah and Mangala Lahkar. Analgesic and anti-nociceptive activity of hydroethanolic extract of *Drymaria cordata* Willd. Indian J Pharmacol. 2011;43(2):121–125.
15. Winter CA, Risley EA, Nuss GW. Carrageenin-induced edema in hind paw of the rat as an assay for antiiflammatory drugs. ProcSoc Exp. Biol. Med. 1962;111: 544-7.
16. Bhesh Raj Sharma, Chul Min Park, Jong Won Choi, Dong Young Rhyu. Anti-nociceptive and anti-inflammatory effects of the methanolic extract of *Opuntia humifusa* stem. Avicenna J Phytomed. 2017;7(4):366–375.
17. Tayyaba Afsar, Mahammad Rashid Khan, Suhail Razak, ShafiUllah and Bushra Mirza. Antipyretic, anti-inflammatory and analgesic activity of *Acacia hydaspica* R. Parker and its phytochemical analysis. BMC Complement Altern Med. 2015;15: 136.
18. Collier HO, Dinneen LC, Johnson CA, Schneider C. The abdominal constriction response and its suppression by analgesic drugs in the mouse. Br J Pharmacol Chemother. 1968;32(2):295-310.
19. Shailly Gupta, Mathew Goerge, Manmohan Singhal, Ganesh N. Sharma, Vikas Garg. Leaves extract of *Murraya koenigii* Linn for anti-inflammatory and analgesic activity in animal models. J Adv Pharm Technol Res. 2010;1(1):68-77.
20. Arif Ullah HM, Sayera Zaman, Fatematuj Juhara, Lucky Akter, Syed Mohammed Tareq, Emranul Haque Masum, Rajib Bhattacharjee. Evaluation of antinociceptive, *in-vivo & in-vitro* anti-inflammatory activity of ethanolic extract of *Curcuma zedoaria* rhizome. BMC Complement Altern Med. 2014;14:346.

21. Vinegar R, Schreiber W, Hugo R. Biphasic development of carrageenin edema in rats. J of Pharmacol and Experiment Therapeutics. 1969;166(1):96-103.
22. Crunkhorn P, Meacock SC. Mediators of the inflammation induced in the rat paw by carrageenin. Br J Pharmacol. 1971;42(3): 392-402.
23. Vane JR, Botting RM. Mechanism of action of nonsteroidal anti-inflammatory drugs. Am J Med. 1998;104(3A):2S-8S.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/23902