Central and Peripheral Antinociceptive Activity of Terminalia Belerica Fruit Pulp Aqueous Extract in Swiss Albino Mice

Manohar V.R[1], Mohandas Rai[2,*], Chandrashekar. R[3], Smitha Sivan[4], Bharath Kumar D[5], Aishwarya Aravind[6]

[1]Professor, [2]Professor & HOD, [3]Assistant Professor, [4,5,6]PG/Tutor, Department of Pharmacology, A.J. Institute of Medical Sciences and Research Centre, Kuntikana, Mangalore, Karnataka, India

***Corresponding Author:**
E-mail: mohandassrai@gmail.com

Abstract

Background & Objectives: Pain is the most common disturbing symptom a person experiences in life. Many branded drugs are available in the market for relieving pain and moreover are sold over the counter but carries different adverse drug reactions. The objective of this study was to explore the role of Terminalia belerica fruit pulp aqueous extract in Swiss Albino mice.

Methods: Animals were divided randomly into control, standard and test groups consisting of six animals in each. Group I received the vehicle (1% gum acacia, 10 ml/kg) and served as the control, group II received Tramadol 20mg/kg and Diclofenac sodium 10mg/kg respectively which served as a standard for central and peripheral antinociceptive activity while group III, IV and V received the test drug Terminalia belerica fruit pulp aqueous extract (TBFPAE) in the doses of 9, 18 and 36mg/kg respectively. All the drugs were administered orally one hour prior to experiment and were evaluated for central analgesic activity by Eddy's hot plate method and peripheral analgesic activity by acetic acid induced writhing reflex in Swiss Albino mice.

Results: Our study has shown an increase in reaction time ($p<0.05$) in all three doses at 30 minutes interval as compared to the control by Eddy's hot plate method and also reduced the number of writhes ($p<0.05$) by acetic acid induced writhing reflex in Swiss Albino mice compared to the control.

Interpretation & Conclusions: Our study has shown central and peripheral antinociceptive activity of Terminalia belerica fruit pulp aqueous extract in swiss albino mice.

Keywords: Aqueous extract, Central antinociception, Fruit pulp, Peripheral antinociception, Terminalia belerica

Access this article online	
Quick Response Code:	Website: www.innovativepublication.com
	DOI: 10.5958/2393-9087.2016.00003.0

Introduction

Pain is derived from the Latin word 'poena' which means punishment. It has been defined by the International Association for 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[1]. Pain is classified into two types: Integumental pain and visceral pain. Integumental pain is superficial and related to skin, muscle and joints whereas visceral pain is deep seated and related to heart, kidney, stomach, gall bladder etc[2].

Analgesic is a drug that selectively relieves pain by acting centrally and or peripherally. Drugs used in the management of pain are Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) also known as non-opioid analgesics and the other being Opioid analgesics. NSAIDs are used to treat integumental pain and Opioid analgesics are used to treat visceral pain[3].

NSAIDs act by inhibiting prostaglandin synthesis and Opioid analgesics act by binding to the opioid receptors at spinal and supra-spinal level. Gastrointestinal disturbances in the form of ulcers and erosions are seen with NSAIDs while abuse potential and dependence is common with opioid analgesics. Hence, there is a need for newer anti-nociceptive molecule with minimal or no adverse effects[4,5].

Terminalia belerica also known as 'Bahera' belongs to the family Combretaceae. It is one among triphala, which is commonly used in Indian traditional medicine as herbal rasayana (meaning Path (ayana) and Juice (rasa) - rasayana – Elixir vitae (Juice of life sustainer) treatment. The phytochemical screening of the *Terminalia belerica* fruit pulp aqueous extract revealed the presence of several phytoconstituents such as tannins, saponins, flavanoids, glycosides etc[6].

The fruit pulp of *Terminalia belerica* are used as astringent, laxative, in fever, pain and also as a brain tonic in Indian system of medicine. They also exhibit bronchodilatory, antispasmodic, antiasthmatic, hepato-protective and spermicidal activities[6-8]. Various extracts of the fruit pulp has also shown to possess hypoglycemic, antimicrobial, hypolipidaemic and antioxidant activities[9-13]. There is lack of studies which substantiate the specific antinociceptive activity of *Terminalia belerica* fruit pulp. Hence the present study was undertaken to evaluate the central and peripheral antinociceptive activity of *Terminalia belerica* fruit pulp aqueous extract in Swiss Albino mice.

Materials and Methods

The experimental protocol was approved by the Institutional Animal Ethics Committee (Approval No. IAEC/02//2013/CPCSEA) dated 05/10/2013. The study was conducted according to Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA) guidelines. *Terminalia belerica* fruit was authenticated by Dr. Krishna Kumar G, Chairman, Department of Applied Botany, Mangalore University, Mangalore, Karnataka, India.

Procedure

About 1000 g of air dried crude powder of *Terminalia belerica* fruit pulp was extracted with water in Soxhlet extractor for 36 hours. It was dried and reduced under controlled pressure and temperature (40-50°C) using a rotator evaporator. The aqueous extract yielded a brownish mass weighing 145g. The yield obtained was 14.5% w/w with respect to dried powder.[14] Swiss Albino mice of either sex weighing 25-30g were obtained from animal house breeding stock of A.J. Institute of Medical Sciences and Research Centre. The animals were housed at 24±2°C with 12:12 hour light and dark cycle. They had free access to food and water *ad libitum*. The animals were acclimatized for a period of seven days before the study. On the day of the experiment, animals were divided randomly into control, standard and test groups consisting of six animals in each. Group I received the vehicle, 1% gum acacia (10 ml/kg) and served as the control, group II received Tramadol 20mg/kg and Diclofenac sodium 10mg/kg which served as a standard for central and peripheral antinociceptive activity respectively, while group III, IV and V received the test drug *Terminalia belerica* fruit pulp aqueous extract (TBFPAE) in doses of 9, 18 and 36mg/kg respectively per orally one hour prior to experiment. Eddy's hot plate method was used for the evaluation of central analgesic activity and writhing reflex was used for the evaluation of peripheral analgesic activity.

Eddy's Hot Plate Method

Eddy's hot plate maintained at 55±0.5°C was used for the study. Each mouse was placed on the pre heated plate and the reaction time was recorded at 0, 30, 60 and 90 minutes after the administration of the drugs. The animals were placed on the hot plate until reactions like paw licking and jumping were noted and the reaction time was recorded using a stop watch at specific time intervals[14].

Writhing test

Each animal was administered with 0.1ml (0.6%) of acetic acid intraperitonially and number of writhes in five minutes was counted. A writhe was considered when animal showed contraction of abdomen with simultaneous stretching of at least on hind limb[14].

Statistical Analysis

The observations are mean±SEM and all the data were analyzed using one way ANOVA followed by Dunnett's multiple comparison test. $p<0.05$ was considered as statistically significant.

Results

Our study has shown an increase in reaction time in all three doses at 30 minutes interval as compared to the control by Eddy's hot plate in Swiss Albino mice (Table 1 & Fig. 1). Similarly, all three doses has significantly reduced the number of writhing in Swiss Albino mice when compared to the control by Acetic acid induced writhing reflex in Swiss Albino mice (Table 2 & Fig. 2).

Table 1: Antinociceptive activity of *Terminalia belerica* fruit pulp aqueous extract by Eddy's hot plate method in Swiss Albino mice

Groups	0 minutes	30 minutes	60 minutes	90 minutes
Control (1% gum acacia) 10ml/kg, p, o	5±0.68*	5.33±0.42*	4.17±0.65*	3.50±0.50*
Standard (Tramadol 20mg/kg), p, o	3.67±0.66*	2.67±0.42**	3.17±0.70*	3.17±0.60*
TBFPAE 9mg/kg, p, o	3.67±0.33*	4.00±0.36**	4.67±0.61*	4.50±0.56*
TBFPAE 18mg/kg, p, o	3.50±0.42*	4.00±0.25**	4.00±0.44*	2.83±0.40*
TBFPAE 36mg/kg, p, o	4.83±0.54*	3.83±0.30**	4.17±0.16*	4.17±0.47*

Observations are mean±SEM. ANOVA followed by multiple comparison test. *p>0.05-Not significant, p<0.05-Significant. TBFPAE- *Terminalia belerica* fruit pulp Aqueous Extract, p.o-Per Oral

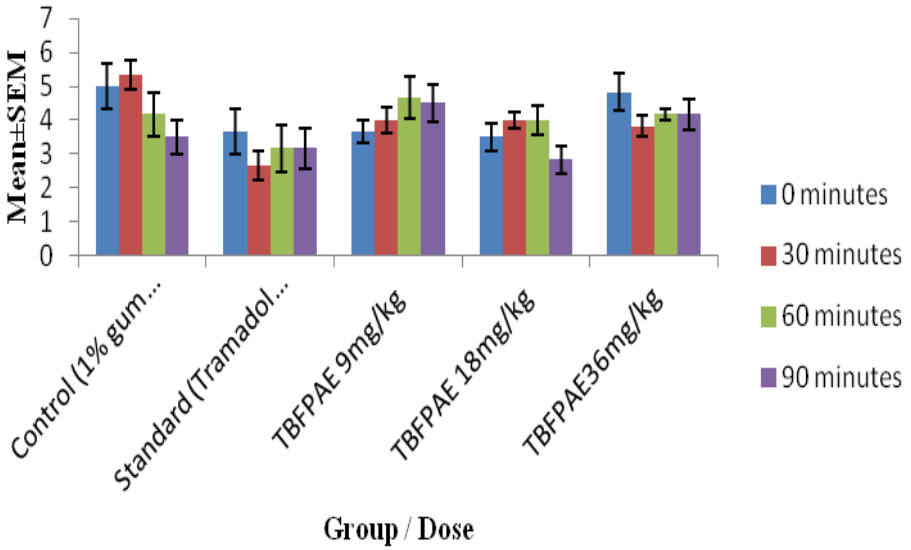

Fig. 1: Antinociceptive Activity of *Terminalia belerica* fruit pulp aqueous extract by Eddy's hot plate method in Swiss Albino mice

Table 2: Antinociceptive activity of *Terminalia belerica* fruit pulp aqueous extract by acetic acid induced writhing test in Swiss Albino mice

Group	No of writhing (mean±SEM)
Control (1% gum acacia) 10ml/kg, p, o	12.83±0.47*
Diclofenac sodium 10mg/kg, p, o	10.5±0.76**
TBFPAE 9mg/kg, p, o	9.33±0.42**
TBFPAE 18mg/kg, p, o	7.16±0.47**
TBFPAE 36mg/kg, p, o	5.66±0.55**

Observations are mean±SEM. ANOVA followed by multiple comparison test. *p>0.05-Not significant, **p<0.05-Significant. TBFPAE- *Terminalia belerica* fruit pulp Aqueous Extract, p.o-Per Oral

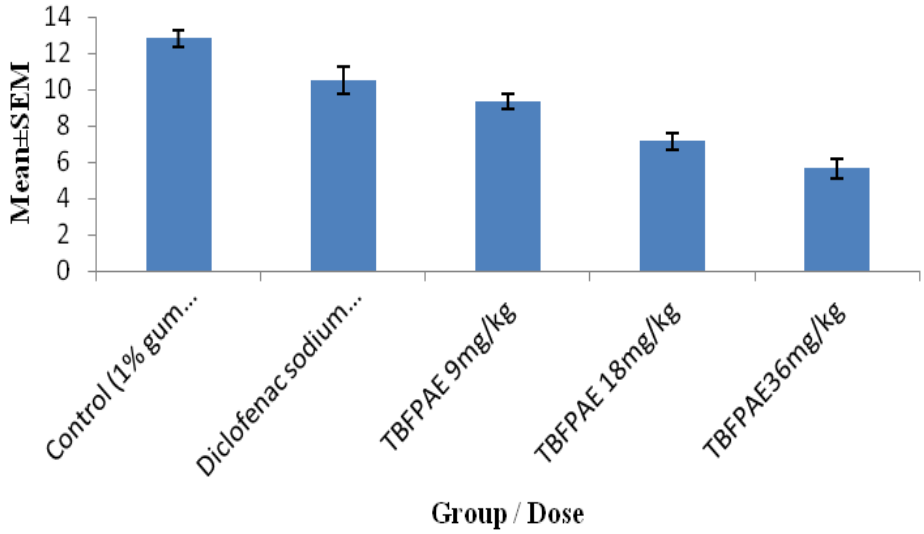

Fig. 2: Antinociceptive activity of *Terminalia belerica* fruit pulp aqueous extract by acetic acid induced writhing test in Swiss Albino mice

Discussion

The present study suggests that administration of TBFPAE in the dose of 9mg/kg, 18mg/kg and 36mg/kg significantly decreased the total number of writhes and exhibited peripheral analgesic activity. The acetic acid-induced writhing has been associated with an increased level of PGE_2 & $PGF_{2\alpha}$ in peritoneal fluids as well as lipoxygenase products. In addition to this, the flavonoids are known to inhibit prostaglandin synthase. Since prostaglandins are involved in pain perception and are inhibited by flavonoids, it could be suggested that reduced availability of prostaglandins by flavoniods present in TBFPAE might be responsible for its peripheral analgesic effect.

Similarly in our present study, there is significant increase in the reaction time to heat stimuli in all test groups by Eddy's hot plate method which strongly suggests the involvement of central analgesic mechanisms which may be elicited through opioid receptors.

Conclusion

TBFPAE has shown to possess central and peripheral analgesic activity in Swiss Albino mice. This would be of therapeutic value in relieving pain, an advantage over conventional antinociceptive agents which lacks either central or peripheral action with increased adverse effects. However further studies are required to evaluate the active principle responsible for its antinociceptive activity at the molecular level in different animal models.

Conflict of Interest: **None**

Source of Support: **Nil**

References

1. Barrett KE, Barman SM, Boitano S, Brooks HL, editors. Pain & Temperature. In: Ganong's Review of Medical Physiology. 23rd ed. New York: McGraw-Hill; 2010.p.168.
2. Usha. G, Pavani. B, Deepika. B, Tharun. T, Asish. B. Plants Possessing Potential Analgesic and Anti-inflammatory Activities: A Review. *Scholars Academic Journal of Pharmacy*. 2012;1(1):18-23.
3. Satoskar RS, Bhandarkar SD, Rege NN. Pharmacology and Pharmacotherapeutics. 21st edition. *Popular prakashan.*Mumbai.2009:p.176.
4. Jayanthi MK, Jyoti MB. Experimental animal studies on analgesic and anti-nociceptive activity of Allium sativum (Garlic) powder.*The Ind Jou of Res and Repo in Med Scien*.2012;2(1):1-6.
5. Abraham A, Mathew L, Samuel S. Pharmacognostic studies of the fruits of Terminalia bellirica (Gaertn.)Roxb. *Journal of Pharmacognosy and Phytochemistry*.2014; 3(2):45-52.
6. Trivedi V P, Nesamany S, Sharma V K. A Clinical Study of The Antiussive and Antiasthmatic effects of Vibhitakphal churna (*Terminalia belerica* Roxb.) in cases of Kapha swasa. *J Res Ayurveda Siddha.*1982;3(1):1-8.
7. Anand K K, Singh B, Saxena A K, Chandan B K, Gupta V N. Hepatoprotective studies of a fraction from the fruits of *Terminalia bellirica* Roxb. on experimental liver injury in rodents. *Phytother Res.*1994;8:287-292.
8. Setty B S, Kamboj V P, Garg H S, Khanna N M. Spermicidal potential of saponins isolated from Indian medicinal plants. *Contraception*.1976;14:571-578.
9. Bhutani K K, Kumar V, Kaur R, Sarin A N. Amoebicidal activity of some Indian medicinal plants. *Indian Drugs*. 1988; 25:p.220.
10. Ahamad I, Mehmood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties.*J Ethnopharmacol*. 1998;62:183-193.
11. Shiala H P, Udupa A L, Udupa S L.Preventive qactions of Terminalia bellirica in experimentally induced atherosclerosis. *Int J Cardiol*.1995;49:101-106.
12. Naik G H, Priyadarshini K I, Bhagirathi R G, Mishra B, Mishra K P, Banavalikar M M et al. In vitro antioxidant studies and free radical reactions of Triphala, an ayurvedic formulation and its constituents. *Phytother Res.*2005;19:582-586.
13. Manohar VR, Chandrashekar R, Rao SN. Phytochemical Analysis of *Terminalia belerica* Fruit Pulp Extracts. *WJPPS*.2012;4(1):1376-83.
14. Suresha RN, Amoghimath S, Vaibhavi PS, Shruthi SL, Jayanthi MK, Kalabarathi HL. Evaluation of Analgesic Activity of Perindopril in Albino Mice. *J Adv Pharm Technol Res.* 2014;5(3):129-33.