ANTI-NOCICEPTIVE EFFECT OF *TERMINALIA CORIACEA* (ROXB.) WT. & ARN. LEAF METHANOLIC EXTRACT

Mohammed Safwan Ali Khan[1, 2 *], Mohd Wasimul Hasan[2], Mubeena Shereen[2], Tanveer Sultana[2], Irfana Mumtaz Dastagir[2], Arwa Jafar Ali[2], Shamim Qureshi, Syed Safiullah Ghori and Syed Ahmed Hussain[3]

[1]*Department of Biomedical Sciences, Faculty of Medicine & Health Sciences, University Putra Malaysia, UPM – Serdang 43400, Selangor Darul Ehsan, Malaysia.*

[2]*Department of Pharmacognosy and Phytochemistry, Anwarul Uloom College of Pharmacy, New Mallepally, Hyderabad 500001, Andhra Pradesh, India.*

[3]*Department of Pharmacology, Shadan College of Pharmacy, Peerancheru,, Hyderabad 500008, Andhra Pradesh, India.*

Summary

The present study was undertaken to perform preliminary phytochemical screening, acute toxicity and to evaluate anti-nociceptive potential of *Terminalia coriacea* Leaf Methanolic Extract (TCLME). The anti-nociceptive activity was assessed by a physical and a chemical method ie. Eddy's Hotplate & Acetic acid induced writhing models. The percentage yield was found to be 11.65% (45gm), preliminary phytochemical screening reveals the presence of alkaloids, amino acids, carbohydrates, condensed tannins, diterpenoids, flavonoids, glycosides, resins, saponins, steroids, triterpenoids and phenolic compounds. Doses upto 2000 mg/kg,b.w,p.o were found to be safe on acute toxicity testing in mice. In Eddy's Hotplate method, TCLME 250 mg/kg, b.w, p.o increased response time of mice significantly ($p<0.01$-0.001) similarly TCLME 500 mg/kg though inconsistently, increased response time significantly ($p<0.05$) whereas in acetic acid induced writhing model TCLME 250 mg/kg failed to exhibit significant effect and TCLME 500 mg/kg significantly ($p<0.01$) reduced writhes and the effect was quite similar to that of standard (Paracetamol 500 mg/kg). Thus from the above results, it can be concluded that TCLME exhibits significant anti-nociceptive potential at higher doses.

Keywords: *Terminalia coriacea* Leaf Methanolic Extract (TCLME), Preliminary Phytochemical Screening, Acute toxicity, Eddy's Hotplate method and Acetic acid induced writhing model.

Introduction

Pain is defined as an unpleasant sensation that can be either acute or chronic and that it is a consequence of complex neurochemical processes in the peripheral and central nervous system (CNS) [1]. It is usually localized to a part of the body and is often described in terms of a penetrating or tissue destructive process (e.g. stabbing, burning, twisting, tearing, squeezing) and or of a bodily or emotional reactions (e.g. terrifying, nauseating, sickening). Furthermore pain is anything that accompanies anxiety and the urge to escape or terminate it [2]. Pain is sensorial modality, which in many cases represents the only symptom for diagnosis of several diseases [3].

The aetiology is attributed to special sensory receptors (nociceptors) connected to primary afferent nerve fibres of different diameters that innervate majority of tissues and organs. These afferent primary fibres terminate in the dorsal horn of the spinal grey matter consisting of various neurotransmitters involved in pain modulation such as glutamate and γ-amino butyric acid (GABA). Small myelinated, Aδ fibres and unmyelinated C fibres are believed to be responsible for the transmission of painful stimuli which is far more complex and not completely understood. The most important parts of this process are wide dynamic range cells (glial cell) that project to thalamus and beyond into spinothalamic tract [4].

Non-Steroidal Anti –Inflammatory Drugs (NSAIDs) forms the mainstay for the treatment of pain [5]. In many cases, for example, with headache or mild to moderate arthritic pain, NSAIDs are effective, whereas neurogenic pain responds best to tricyclic anti-depressants or serotonin or norepineprine reuptake inhibitor (duloxetine) rather than NSAIDs or opioids. However, for severe or chronic malignant pain, opioids or NSAIDs are usually the drug of choice [1]. NSAIDs produce their effect through cyclo-oxygenase inhibition and are used widely to relieve pain, with or without inflammation, in people with acute and chronic musculoskeletal disorders [4]. In single doses, NSAIDs have analgesic activity comparable to that of paracetamol [6]. The potential benefits of treatment with NSAIDs must be weighed against the risk. NSAIDS are contraindicated in patients with known active peptic ulceration and should be used with caution in the elderly and in those with renal impairment or asthma [4]. The long term side effects associated with these synthetic drugs led man to explore the plant based products that may have fewer side effects for treating various diseases.

In recent times, focus on medicinal plant research has increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems. More than 13,000 plants have been studied during the last 5 year period. As a remedy, several herbal therapies are being used by man for the management of pain [3]. Throughout the history, man has used several form of therapy for the relief of pain; among them medicinal herbs have gained popularity because of its wide use and less side effects [7]. The treatment of rheumatic disorder is an area in which the practitioners of traditional medicine enjoy patronage and success [8]. Natural products in general and medicinal plants in particular, are believed to be an important source of new chemical substances with potential therapeutic efficacy. Taking into account, the most important analgesic prototypes (e.g. salicylic acid and morphine) were originally derived from the plant sources. The study of plant species traditionally used as pain killers should still be seen as a fruitful research strategy in the search of new analgesic drugs [9].

Traditional medicine practitioners use a variety of herbal preparations to treat various diseases involving pain. The search for new bioactive agents led to the screening for bioactive compounds in *Terminalia* species. *Terminalia* is a genus of large trees of the flowering plant family, Combretaceae, comprising around 200 species distributed in tropical regions of the world. This genus gets its name from Latin "Terminus", referring to the fact that the leaves appear at the very tips of the shoots. Trees of this genus are known especially as a source of secondary metabolites, e.g. cyclic triterpenes and their derivatives, flavonoids, tannins, and other aromatics. Some of these substances have antifungal, antibacterial, anti-cancer and hepatoprotective indications [10]. Leathery Murdah (*Terminalia coriacea*) belonging to family Combretaceae is found in dried and warmer parts of Andhra Pradesh and Tamil Nadu states of India. It is known as Tani in Telugu (the regional language). Traditionally the stem bark of the plant is used as cardiac stimulant and in treatment of atonic diarrhea & callous ulcer [11]. Literature survey reveals that anti-nociceptive activity of *Terminalia coriacea* leaves is not reported. Hence, the present investigation was undertaken.

Material and Methods

Collection of plant material

The leaves of *Terminalia coriacea* were collected from Chittoor district, Tirumala hills. The plant was identified and authenticated by Dr. Madhava Chetty (Assistant Professor, Sri Venkateshwara University). A specimen has been deposited in Herbarium bearing voucher number: 985.

Preparation of extract

The leaves were shade dried at room temperature. After seven days of drying the leaves were powered by grinding and sieving. The powdered leaves of *Terminalia coriacea* were extracted by maceration for 48 hours using methanol. The plant material and the solvents were taken in the ratio of 1:5. Extract was filtered and later dried by heat treatment on a hot plate. Percentage yield of the semisolid mass obtained was calculated and was stored at 4^{o}C. The extract was subjected to preliminary phytochemical screening to identify phytoconstituents using various chemical reagents [12, 13].

Experimental animals

Male Swiss albino mice weighing 25-35 gm were used for the experimental study. The animals were kept in polypropylene cages maintained under standard laboratory conditions. The animals were fed with standard pellet diet and had free access to clean drinking water. Groups of five mice were used in all the experiments. The experimental protocols were approved by the Institutional Animal Ethical Committee (IAEC).

Acute oral toxicity study

Acute toxicity test was performed in mice by staircase method. Mice were divided into four groups with five animals per dose. A safe oral dose of TCLME was determined by the procedure as described by the Organization of Economic Co-operation and Development (OECD) as per 423 guidelines [14]. The TCLME, at different doses starting from 250-2000mg/kg, was prepared by dissolving the extract in distilled water and the concentration was adjusted in such a way that it did not exceed 1ml/100gm body weight of experimental animals. The extract was then administered and animals were observed individually for behavioural changes, mortality and toxicity up to 48 hours with special supervision given during first 4 hours and thereafter periodically. Three different doses (125, 250, and 500mg/kg, p.o) of TCLME were later chosen for evaluation of anti-nociceptive property based on the acute toxicity testing.

Pharmacological Evaluation

Thermal stimulus-induced pain (Eddy's hot plate test) in mice

The test was carried out using Eddy's hot plate apparatus [15]. The temperature was set to 56 ± 1^0c. Mice were placed on the hot plate and the reaction time was recorded in seconds for paw licking or jump response, following the oral administrations at 0, 15, 30, 60, and 90 minutes.

Experimental design: The mice were divided into 5 groups (n=5) and treated with the respective solutions as given below.

Group I (Control): Saline solution (1% w/v, NaCl 2 ml/kg b.w, p.o).

Group II (Standard): Paracetamol (500mg/kg b.w, p.o).

Group III (Test-I): TCLME (125mg/kg b.w, p.o).

Group IV (Test-II): TCLME (250mg/kg b.w, p.o).

Group V (Test-III): TCLME (500mg/kg b.w, p.o).

Acetic acid induced writhing in mice

Animals of each group were injected 0.4ml of 1% acetic acid intraperitonially after subjecting them to various treatments [16]. The number of writhes (abdominal muscle contractions) and stretching of the hind limbs were counted for 20 min after 5 min of acetic acid injection. Percent inhibition was determined for each experimental group by the following formula:

$$\text{Percent inhibition} = (N\text{-}N^t/N)\ \chi\ 100$$

Where N is the average number of writhes per control group, and N^t is the average number of writhes per test group.

Experimental design: The mice were divided into 4 groups (n=5) and treated with the respective solutions as given below.

Group I (Control): Saline solution (1% w/v, NaCl 2 ml/kg b.w).

Group II (Standard): Paracetamol (500mg/kg b.w, p.o).

Group III (Test-I): TCLME (250mg/kg b.w, p.o).

Group IV (Test-II): TCLME (500mg/kg b.w, p.o).

Statistical analysis

The values are expressed as Mean ± Standard Error of Mean (SEM). $P<0.05$ was considered statistically significant. Data obtained was analyzed by Non-parametric one-way ANOVA (Kruskal-Wallis test) followed by Dunnett's multiple comparisons post-hoc test using Graphpad Instat version 3.10, 32 bit for windows, Graphpad software, San Diego, California, USA. www.graphpad.com.

Results

The yield of *Terminalia coriacea* leaf methanolic extract (TCLME) was found to be 45 gm (about 11.65%). The phytochemical screening reveals the presence of alkaloids, amino acids, carbohydrates, flavonoids, glycosides, resins, saponins, sterols, tannins, triterpenoids and phenolic compounds as shown in **Table 1**. Acute toxicity testing of TCLME indicated that the doses up to 2000 mg/kg were safe as there was no mortality and signs of toxicity. Hence three test doses (125, 250 and 500 mg/kg, p.o) in the range of $1/16^{th}$ – $1/4^{th}$ of observed maximum safe dose were selected for the evaluation of anti-nociceptive potential.

Eddy's hot plate induced thermal stimulation is normally employed to find out the involvement of central analgesic activity [17] and acetic acid-induced writhings are used for detecting both central and peripheral analgesia. Intraperitoneal administration of acetic acid releases prostaglandins and sympathomimetic system mediators like PGE_2 and $PGF_{2\alpha}$ and their levels were increased in the peritoneal fluid of the acetic acid induced mice [18] therefore, these methods were adopted. The results are shown in **Table 2 & 3** and illustrated in **Figure 1 & 2** respectively.

TCLME exhibited dose-dependent anti-nociceptive effect from dose 250-500mg/kg. All the test doses 125-500mg/kg of TCLME showed significant analgesic effect ($p<0.05$) at 15 minutes. However, TCLME 125mg/kg did not show any significant effect at other time intervals. The most important observation was the increase in latency time of 250mg/kg group which increased with the time from 15-90 minutes (p value ranging from 0.01-0.001). TCLME 500mg/kg significantly ($p<0.05$) increase the reaction time of mice inconsistently. The significant anti-nociceptive activity was recorded at 0, 15 and 60 minutes.

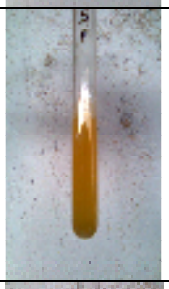
Further in acetic acid-induced writhing model, TCLME 500mg/kg reduced the number of writhes evoked by acetic acid in mice. The number of writhes in control group were 51 ± 4.88 (Mean ± SEM, n=5). The number of writhes reduced to 12 ± 5.26 ($p<0.01$) and 13 ± 0.73 ($p<0.01$) in TCLME 500mg/kg and standard drug (Paracetamol 500mg/kg) groups respectively. The calculated percentage inhibition of writhes was found to be 76.74% and 74.41% for TCLME 500 mg/kg and Paracetamol respectively. However, TCLME 250mg/kg showed 61.24% percentage inhibition as the data was non-parametric and on analysis with Kruskal-Wallis (non-parametric ANOVA), did not exhibit significant activity.

Table 1. Preliminary phytochemical screening of *Terminalia coriacea* leaf methanolic extract (TCLME).

S.No	CONSTITUENT	TEST	RESULT	OBSERVATION
1.	ALKALOIDS	i. Mayer's reagent	Positive	
		ii. Wagner's reagent	Positive	
		iii. Hager's reagent	Positive	
		iv. Tannic acid test	Positive	

2.	CARBOHYDRATES	i. Molisch test	Positive	
		ii. Barfoed's test	Positive	
		iii. Iodine test	Positive	
3.	REDUCING SUGARS	i. Fehlings test	Negative	
4.	PENTOSE	i. Bial's test	Negative	
5.	AMINO ACIDS	i. Millon's test	Positive	

6.	FLAVONOIDS	i. Shinoda test	Positive	
		ii. Alkaline reagent test	Positive	
		iii. Zinc-HCl test	Negative	
7.	SAPONINS	i. Froth formation test	Positive	
8.	TANNINS & PHENOLIC COMPOUNDS	i. $FeCl_3$ test	Positive	
		ii. Dil. HNO_3 test	Positive	

		iii. Gelatin test	Positive	
		iii. Chlorogenic acid	Positive	
9.	CONDENSED TANNINS	i. Vanillin test	Positive	
		ii. Lime water test	Positive	
10.	GLYCOSIDES	i. Keller-killani test	Positive	
11.	STEROLS & TRITERPENOIDS	i. Libermann-buchard test	Positive for steriods	

		ii. Salkowski test	Positive for steriods	
12.	DITERPENOIDS	i. Copper acetate test	Positive	
13.	PHLOBATANNINS	i. Aq. HCl acid test	Positive	
14.	RESINS	i. Acetone-water test	Positive	

Table 2. Results of Eddy's hot plate method.

Treatment	Dose (mg/kg)	Reaction time in seconds				
		0min	15min	30min	60min	90min
Control	0.2ml	4.2 ± 0.374	3.6 ± 0.509	4.4 ± 0.509	7 ± 0.707	9.6 ± 0.509
Standard	500	6.2 ± 0.374	9 ± 0.707*	9.2 ± 0.374**	8.6 ± 1.030	11.8 ± 0.734
TCLME	125	4.4 ± 1.030	9.4 ± 1.122*	7.6 ± 0.812	8.4 ± 0.509	11.6 ± 0.979
TCLME	250	9.4 ± 0.509**	8.8 ± 0.860*	8.8 ± 0.374*	12.6 ± 1.280**	19 ± 1.703***
TCLME	500	7.2 ± 3.450*	9.2 ± 0.860*	8.2 ± 0.583	11.4 ± 0.927*	12.6 ± 0.927

Values are Mean ± S.E.M. (n=5) Significance vs. control group: *P<0.05, **P<0.01, ***P<0.001

Figure 1. Effect of TCLME on Hot plate Induced pain in mice.

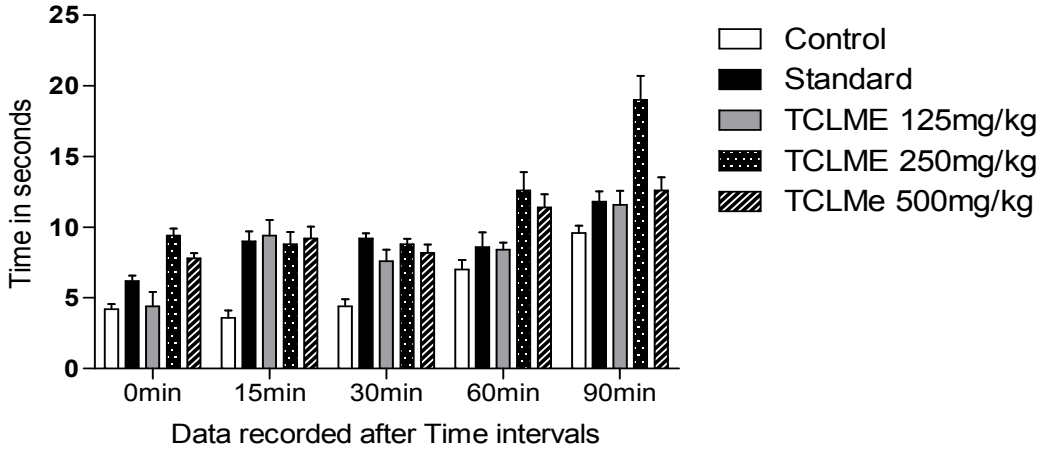
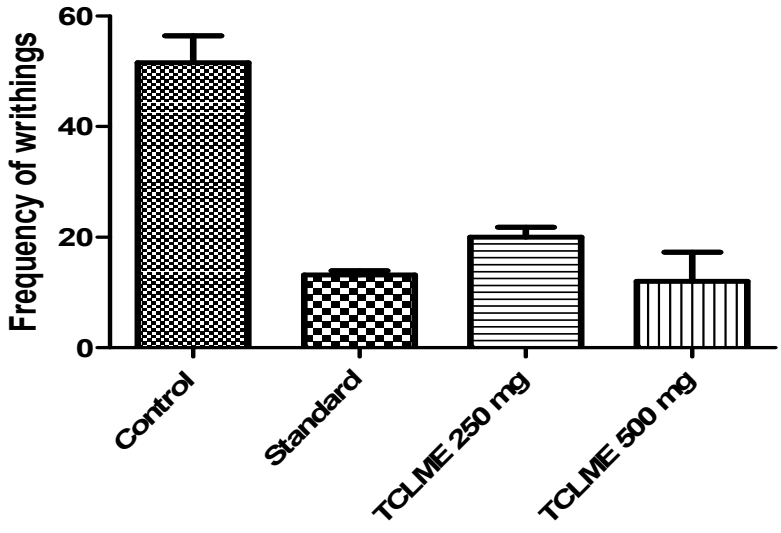


Table 3. Results of Acetic acid induced writhing model.

Treatment	Dose(mg/kg)	Mean no. of writhing ± S.E.M (20mins)	% inhibition
Control	0.2ml	51.6 ± 4.885	-----
Standard	500	13.2 ± 0.7348**	74.41%
TCLME 250 mg	250	20 ± 1.817	61.24%
TCLME 500 mg	500	12 ± 5.263**	76.74%

Values are Mean ± S.E.M. (n=5) Significance vs. control group: **P<0.01.

Figure 2. Effect of various treatments on Acetic acid induced writhing in mice.



Discussion

Since, TCLME inhibited pain both in physical and chemical models that involve both central and peripheral mechanism suggesting that it has not only anti-nociceptive but also anti-inflammatory activity. Preliminary phytochemical screening reveals the presence of flavonoids and hence the anti-nociceptive activity can be attributed to flavonoids. Certain flavonoids like quercetin exhibit anti-inflammatory response by inhibition of cyclooxygenase, 5-lipoxygenase pathway and even phospholipidase A_2 [19]. A study by Filho *et al.* reported on the anti-nociceptive effect of quercetin through a central mechanism [20]. Flavonoids may increase the amount of endogenous serotonin or may interact with 5-HT_2A [21] and 5-HT_3 receptors which may be involved in the mechanism of central analgesic activity [22]. Previously researchers reported the presence of several therapeutically valuable flavonoids in the leaves of allied species of *Terminalia* [23. This supports our assumption that flavonoids could be responsible agents for anti-nociceptive effect in *Terminalia* species.

Standard NSAIDs like aspirin offer relief from inflammatory pain by suppressing the formation of pain substances in the peripheral tissues, where prostaglandin and bradykinin were suggested to play an important role in the pain process. Prostaglandins elicit pain by direct stimulation of sensory nerve endings to other pain provoking stimuli [24]. TCLME might also suppress the formation or antagonize the action of these substances and exerts its peripheral analgesic activity in acetic acid induced writhing test. Thus, the present study provides preliminary data on analgesic activity of *Terminalia coriacea* leaves.

Conclusion

Our study showed that the TCLME produced significant analgesia and the effect could be due to both central and peripheral mechanisms by raising the threshold for pain. Peripherally acting analgesics act by blocking the generation of impulses at chemoreceptor site of pain, while centrally acting analgesics not only raise the threshold for pain and alter the physiological response to pain suppressing the patient's anxiety and apprehension [25].

From the results it could be concluded that the extract exhibited anti-nociceptive activity, especially at higher doses 250 & 500mg/kg. It can also be suggested that the anti-nociceptive mechanism of TCLME might be associated with the inhibition of prostaglandin synthesis, as observed for most non-steroidal drugs. It is important to point out that work is in progress to isolate and characterize the active compounds present in methanolic extract of *Terminalia coriacea* leaves.

Acknowledgements

The authors wish to thank Basharath Yar Khan for his help in preparing the manuscript.

References

1. Howland RD and Mycek MJ. 2006. Lippincott Williams and Wilkins. Illustrated review: Pharmacology. 3rd Ed. Wolters Kluwers health (India) Pvt. Ltd., New Delhi: 157-65.

2. Harrison's principles of internal medicine. 2005. 16th Edition. McGraw Hill medical Publishing division. 1:71-5.

3. Ahmadiani A, Fereidoni M, Semnanian S, Kamalinejad M, and Saremi S. 1998. Anti-nociceptive and anti-inflammatory effects of *Sambucus ebulus* rhizome extract in rats. J Ethnopharmacol. 61(3): 229-35.

4. Woolfrey S and Kapur D. 2007. Roger Walker and Cate Whittlesea. Clinical Pharmacy and Therapeutics. 4th Ed. Churchill Livingstone: 474-86.

5. Burke A, Smyth EM, Fitzgerald GA. 2006. Analgesic- antipyretic agents, Pharmacotherapy of gout. In: LL Brunton IS Lozo, Parker KL, Editors. Goodman GIlmans, The Pharmacological basis of Therapeutics. 11th Ed. New York: McGraw hill: 671-716.

6. Cashman J N. 1996. The Mechanism of Action of NSAIDs in Analgesia. Drugs 52; (supp. l5): 13-23.

7. Mate G.S, Naikwade N.S, Magdum C.S, Chowki A.A and Patil S.B. 2008. Evaluation of anti-nociceptive activity of *Cissus quadrangularis* on albino mice. Int J Green Pharm.: 118-21.

8. Akah P. A and Nwambie A. I. 1994. Evaluation of Nigerian traditional medicines: plants used for rheumatic disorder. J. Ethnopharmacol. 42: 179-82.

9. Shanmugasundaram P and Venkataraman S. 2005. Anti-nociceptive Activity of *Hygrophila auriculata* (Schum) Heine. Afr J Tradit Complement Altern Med. 2 (1): 62-69.

10. Mann A, Yahaya Y, Banso A and John F. May 2008. Phytochemical and antimicrobial activity of *Terminalia avicennioides* extracts against some bacteria pathogens associated with patients suffering from complicated respiratory tract diseases. J. Med. Plants Res. 2(5): 94-97.

11. Madhava Chetty K, Sivaji K, Tulsi Rao K 2008. Flowering plants of Chittoor District, Andhra Pradesh, India. 2nd Ed. Students Offset Printers and Publishers, Tirupathi : 125-26.

12. Khandelwal KR 2004. Practical Pharmacognosy. 12th Ed. Pune: Nirali prakashan.: 149-160.

13. Kokate. C.K., Purohit A.P., Gokhale S.B 2007. Pharmacognosy. 39th Ed. Pune: Nirali prakashan.: 108-109.

14. OECD guidelines for the testing of chemicals. 2010. Test no. 423: Acute oral toxicity – Acute toxic class method. 1(4): 1-14.

15. Eddy NB, Leimbach B. 1953. Synthetic analgesics II: Diathianyl and DithienyI butylamines. J. Pharmacia. 107: 385-93.

16. Purnima A, Koti BC, Tikare VP, Viswanathaswamy A, Thippeswamy A, Dabadi P. 2009. Evaluation of analgesic and antipyretic activities of *Centratherum anthelminticum* (L) kuntze seed. Ind. J. Pharm. Sci. 71: 461-64.

17. Paulino N, Dantas A.P, Bankova V, Longhi D.T, Scremin A, De Castro S.L and Calixto J.B. 2003. Bulgarian propolis induce analgesic an anti-inflammatory effect in mice and inhibits in vitro contraction of airway smooth muscle. J. Pharmacol. Sci.93: 307-13.

18. Deraedt R, Oughney J S, Delevakee F and Falhour M. 1980. Release of prostaglandin E and F in an algogenic reaction and its inhibition. Eur. J. Pharmacol.51: 17-24.

19. Williams C.A, Hoult J.R, Harborne J.B, Greenham J and Eagles J. 1995. A biologically active lipophilic flavonols from *Tanacetum parthenium*. Phytochem.38: 267-70.

20. Filho V.C, Santos A.S, Decmpos R.O.P, Miguel O.M and Yunes R.A *et al*. 1996. Chemicals and pharmacological studies of *Phyllatus caroliniensis* in mice. J. Pharm. Pharmacol. 48 (12): 1231-36.

21. Lee B.H, Jeong S.M, Lee J.H, Kim J.H and Yoon I.S *et al*. 2005. Quercetin inhibits the 5–hydroxytriptamine type 3 receptor-mediated ion current by interacting with pre-transmembrane domain I. Mol. Cells.20: 69-73.

22. Colpaert F.C, Tarayre J.P, Koek W, Pauwels P.J and Bardin L *et al*. 2002. Large-amplitude 5-HT1A receptor activation: A new mechanism of profound, central analgesia. Neuropharmacol. 43: 945-58.

23. Lin Y.L, Kuo Y.H, Shiao M.S, Chen C.C and Ou J.C. 2000.Flavonoid glycosides from *Terminalia catappa* L. j. Chin. Chem. Soc.47: 253-56.

24. Kanodia L, Das S. A. 2009. Comparative study of analgesic property of whole plant and fruit extracts of *Frageria vesca* in experimental animal models. Bangladesh J Pharmacol. 4: 35-38.

25. Shreedhara CS, Vaidya VP, Vagdevi HM, Latha KP, Muralikrishna KS, Krupanidhi AM. 2009. Screening of *Bauhinia purpurea* Linn. For analgesics and anti-inflammatory activities. Indian J Pharmacol.41: 75-79.

Address for Correspondence & Reprints – Mohammed Safwan Ali Khan M.Pharm (PhD), H.No: 6-3-786, Ameerpet, Hyderabad 500016, Andhra Pradesh, India.

E-mail: mohammedsafwanalikhan@yahoo.co.in & Mobile: 0091-8801339801.