Research Article

Analgesic and anti-inflammatory activities of *Dichrostachys cinerea* (L.) Wight and Arn.

E. Susithra*, S. Jayakumari

ABSTRACT

Background: *Dichrostachys cinerea* (Family - Mimosaceae), known as "Viddathalai" in Tamil, is well-known plant used in the traditional system of medicines such as Ayurveda and Siddha. The traditional plant is commonly used by tribal of India and South Africa. The plant is claimed to possess more therapeutic medicinal uses such as anti-inflammatory, analgesic, antipyretic also used in rheumatism, urinary calculi, and in diarrhea, which is evident from traditional knowledge. **Methods:** Based on the literature review, the proposed study is intended to validate scientifically the folklore claims for its analgesic, anti-inflammatory, and antioxidant activity. Therein, an attempt has been made for screening the various inflammation-associated activities as per standard protocols to prove its efficacy on support of folklore uses. **Results:** Qualitative phytochemical analysis of leaf, stem bark, and root revealed that petroleum ether extract showed the presence of terpenoid and steroid. Chloroform extract has shown the presence of glycosides, the alcoholic extract showed the presence of tannin, flavonoids, and phenolic compounds. Aqueous extract showed positive test for saponin and tannins. The acute toxicity study of ethanolic extract of *D. cinerea* leaf (EDCL), ethanolic extract of *D. cinerea* bark (EDCB), and ethanolic extract of *D. cinerea* root (EDCR) carried out revealed that all the extracts were not toxic up to 2000 mg/kg. Hence, the tested extracts were found to be within the safe margin. The results of the different parameter carried out for 28 days in subacute toxicity studies of EDCL, EDCB, and EDCR did not reveal any toxic effect in the two dose levels tested by biochemical and histopathological examination and have wide safety margin. The test extracts, namely, leaf, stem bark, and root extract were found to show significant analgesic activity in both centrally and peripherally mediated analgesia. Anti-inflammatory screening indicates that stem bark, leaf, and root were effective in acute model, whereas stem bark is ineffective in chronic model. The results of antioxidant activity act as a prelude for anti-inflammatory study and these are attributed to the presence of phytoconstituents tested in our extracts. **Conclusion:** The data retrieved from the observations have been formulated into a diagnostic protocol of *Dichrostachys cinerea.* The promising biological activities will definitely lead to develop a new drug from the plant and also form the foundation for a future research work in bioactivity-guided isolation of active principles.

KEY WORDS: Analgesic, Anti-inflammatory, Antioxidant activity, Bark, *Dichrostachys cinerea,* Leaf, Root, Toxicity

INTRODUCTION

Plants play a major source in the treatment of diseases and constitute the backbone of all traditional medicinal practice and are a growing part of modern high-tech medicine. India has a rich heritage of usage of medicinal plants in Ayurveda, Siddha, and Unani system with a mention of about 45,000 plants.[1] A number of traditional medicines are dominating the practice of modern medicine; hence, the use of herbal drugs and folklore practice needs a scientific investigation.[2] According to the WHO traditional medicine strategy, 2002–2005, the developing countries use traditional medicine for their health care needs.[3] Herbal drugs are getting popularized in developed and developing countries because of its natural origin, lesser side effects, and low cost. Intense investigations have been carried out in the folklore medicine for finding out a new drug for arthritis, lithiasis, cancer, diabetes, and even for common cold.

The genus *Dichrostachys* belongs to *Mimosaceae* consisting of 12 species distributed all over tropical world.[4] *Dichrostachys cinerea* is wide spread in South India. The whole plant is astringent, stimulant, tonic,

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Department of Pharmacognosy, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies, Pallavaram, Chennai, Tamil Nadu, India

***Corresponding author:** E. Susithra, Department of Pharmacognosy, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies, Pallavaram, Chennai - 600 117, Tamil Nadu, India. Phone: +91-9445678234. E-mail: esusithra@gmail.com

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demulcent, antiscorbutic, and antidysentric.[5] Tender shoots are used in inflammatory conditions, diarrhea, arthritis, and pain.[6] Bark is used in piles, bloody stools, and diarrhea.[7] Root is astringent and cures sexually transmitted diseases,[8] rheumatism, used in urinary calculi, renal troubles, diseases of vagina, retention of urine, and pain in joints.[9] The aerial parts and leaves possess antibacterial activity.[10-12] The leaves also possess cyclooxygenase inhibitory activity for pain and inflammation[13] and muscle relaxant property.[14] The plant also possesses anti-snake venom property.[15]

Reports on phytochemical composition of the plant reveal the presence of sterols, flavonoids, poly phenols, etc. Leaves contain β-sitosterol, β-amyrin, imperatorin, marmesin, and esculetin with high phenolic content in young leaves.[16,17] Root contains β-amyrin, α-amyrin, β-sitosterol and stigmasterol,[18] friedelin 3-one, and octacosanol.[19] Stem bark contains friedelin, friedelin 3-one, β-amyrin,[20] and 3-o-acyl mesquitol.[21] Flowers were reported to contain quercetin.

EXPERIMENTAL

Materials

The plant *D. cinerea* (Mimosaceae) has been claimed in the literature to cure inflammations and gout. It is used for pain and eye infection by tribals of India. The plant is used for headache, toothache, stomach infection, and snakebite by tribal of South Africa. Hence, an attempt has been made to study analgesic and anti-inflammatory activities of the plant.

Preparation of the Extracts

The powdered leaf, stem bark, and root were extracted separately with ethanol by cold maceration. The extracts were filtered and distilled at temperature and finally dried using rotary vacuum flash evaporator to yield ethanolic extract of *D. cinerea* leaf (EDCL), ethanolic extract of *D. cinerea* bark (EDCB), and ethanolic extract of *D. cinerea* root (EDCR).

Methodology

Acute oral toxicity study was performed as per guidelines 423 of Organization for Economic Co-operation and Development (OECD), acute toxic class method.[22] The experimental protocol was approved by the Institutional Animal Ethics Committee IAEC Ref-no. 290/CPCSEA dated 12.12.00. Sub-acute toxicity study of EDCL, EDCB, and EDCR was carried out at two dose levels (200 mg/kg and 400 mg/kg, p.o.) for 28 days in albino rats as per the method.[23] Changes in hematological parameters such as red blood corpuscles, white blood corpuscles (WBC count), differential leukocytes count, and hemoglobin were analyzed. Furthermore, changes in the biochemical parameters such as serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, and alkaline phosphatase were analyzed by the reference method of International Federation of Clinical Chemistry. Total protein, total WBC count, creatinine, and bilirubin were analyzed using Span diagnostic kit. The vital organs such as liver, spleen, lungs, heart, kidney, and brain were weighed, and differences to control animals were noted. Any abnormalities or variations compared to control animals were also noted.

Similarly, the different extracts were screened for their analgesic, anti-inflammatory, and antioxidant potential by *in vitro* methods. The analgesic activity was performed by Eddy's hot plate method[24] and acetic acid-induced writhing methods.[25] The anti-inflammatory activity of EDCL, EDCB, and EDCR was evaluated by acute[26] and chronic model[27] (carrageenan-induced paw edema and cotton pellet granuloma model, respectively). The *in vitro* antioxidant study was done by nitric oxide[28] and 1,1-diphenyl-2-picrylhydrazyl (DPPH) methods.[29]

RESULTS AND DISCUSSION

The acute toxicity study of EDCL, EDCB, and EDCR carried out revealed that all the extracts were not toxic up to 2000 mg/kg. Hence, the tested extracts were found to be within the safe margin.

In hot plate method, EDCL showed a significant increase in basal reaction time from 30 min onward and effect was sustained till 120 min at both tested dose levels. While in case of EDCB and EDCR, low dose was ineffective, but at higher dose, 400 mg/kg significant increase in basal reaction time was observed from 30 min to 120 min. The effects were comparable with that of standard Table 1. In the hot plate test, the test extracts only at higher doses increased the pain threshold significantly. Such an effect is characteristic of central analgesic effect, while peripheral analgesic known to be inactive of this kind of stimuli.[30]

Acetic acid-induced writhing test is simple, sensitive, and predictive method to assess peripheral analgesic effect. The abdominal writhing due to acetic acid is related to the sensitization of local peritoneal receptors to prostaglandins. In this model, among the test extracts, EDCR shown maximum percentage protection of analgesia [Table 2]. All the tested extracts exerted good analgesic activity in acetic acid-induced writhing model, which may be probably by inhibiting the synthesis or action of prostaglandins indicating that extract possess central- and peripheral-mediated analgesic activity. Acetic acid-induced writhing test is simple, sensitive, and predictive method to assess peripheral analgesic effect. The abdominal writhing due to acetic acid is related to the sensitization of local

peritoneal receptors to prostaglandins. In this model, among the test extracts, EDCR shown maximum percentage protection of analgesia. All the tested extracts exerted good analgesic activity in acetic acid-induced writhing model, which may be probably by inhibiting the synthesis or action of prostaglandins indicating peripheral mechanism. The results of our present study suggest that extracts possess central- and peripheral-mediated analgesic activity.

In carrageenan-induced paw edema (acute model), EDCL (200 mg and 400 ng/kg dose level) exhibited a significant reduction in volume of paw edema from 1st h and effect sustained till 4th h, while in case of EDCB and EDCR, the reduction starts only at 2nd h and sustained till 4th h. The percentage inhibition of edema at 3rd h and 4th h was found to be 72.42% and 70.48% for indomethacin, 63.64% and 69.97% for EDCL, 42.42% and 47.47% for EDCB, and 61.21% and 61.83% for EDCR at 400 mg/kg dose level, respectively. The results are shown in Table 3. Carrageenan-induced hind paw edema is a standard experimental model in acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs. Carrageenan-induced edema is a biphasic response. The first phase is mediated through the release of histamine, serotonin, and kinin, whereas the second phase is related to the release of prostaglandin and slow reacting substances which peak at 3rd h. The results of the present study showed that the test extracts exhibited a marked inhibition of edema at 3rd h and 4th h at both dose levels, indicating that the extracts may probably act by inhibiting prostaglandin synthesis or release.

The results of the cotton pellet granuloma model indicate that EDCL at 400 mg/kg reduces granuloma formation significantly, whereas 200 mg/kg was not able to do so. EDCB at both the tested dose levels was not able to reduce granuloma formation

Table 1: Effect of EDCL, EDCB, and EDCR - Eddy's hot plate method

Group	Drug and dose	Pre-drug time	Time in seconds		
			30 min	60 min	120 min
Group I	1% w/v CMC (10 ml/kg) vehicle control	4.33±0.21	4.83±0.17	4.76±0.21	4.5±0.22
Group II	Pentazocine (5 mg/kg)	4.5±0.39	8.1±0.53**	10.5±0.74**	7.8±0.65**
Group III	EDCL (200 mg/kg)	4.16±0.31	7.83±0.21**	7.16±0.31**	6.50±0.35**
Group IV	EDCL (400 mg/kg)	4.12±0.07	6.17±0.12**	6.67±0.08**	7.83±0.19**
Group V	EDCB (200 mg/kg)	4.16±0.31	5.16±00.17^{NS}	4.66±0.21^{NS}	5.83±0.31^{NS}
Group VI	EDCB (400 mg/kg)	4.0±0.14	7.85±0.13**	8.33±0.08**	8.17±0.74**
Group VII	EDCR (200 mg/kg)	4.66±0.34	5.88±0.17^{NS}	5.66±0.21^{NS}	4.83±0.49^{NS}
Group VIII	EDCR (400 mg/kg)	4.34±0.13	6.67±0.13**	6.34±0.14**	7.37±0.13**

EDCL: Ethanolic extract of *Dichrostachys cinerea* leaf, EDCB: Ethanolic extract of *Dichrostachys cinerea* bark, EDCR: Ethanolic extract of *Dichrostachys cinerea* root, CMC: Carboxymethyl cellulose

Table 2: Effect of EDCL, EDCB, and EDCR by acetic acid-induced writhing response in mice

Group	Drug and dose	Number of writhings	% inhibition
I	1% w/v CMC (10 ml/kg) vehicle control	40.01±0.365	-
II	Aspirin (100 mg/kg)	8.83±0.477***	78.05
III	EDCL 200 mg/kg	31.16±0.833**	22.25
IV	EDCL 400 mg/kg	17.33±0.421***	56.75
V	EDCB 200 mg/kg	19.66±0.760***	51.05
VI	EDCB 400 mg/kg	16.50±0.223***	58.75
VII	EDCR 200 mg/kg	22.50±0.885**	43.75
VIII	EDCR 400 mg/kg	9.83±0.477***	75.50

EDCL: Ethanolic extract of *Dichrostachys cinerea* leaf, EDCB: Ethanolic extract of *Dichrostachys cinerea* bark, EDCR: Ethanolic extract of *Dichrostachys cinerea* root, CMC: Carboxymethyl cellulose

Table 3: Effect of EDCL, EDCB, and EDCR by carrageenan-induced paw edema method

Group	Drug and dose	Paw volume	% inhibition
I	1% w/v CMC (10 ml/kg) vehicle control	40.01±0.365	-
II	Indomethacin (10 mg/kg)	8.83±0.477***	78.05
III	EDCL 200 mg/kg	31.16±0.833**	22.25
IV	EDCL 400 mg/kg	17.33±0.421***	56.75
V	EDCB 200 mg/kg	19.66±0.760***	51.05
VI	EDCB 400 mg/kg	16.50±0.223***	58.75
VII	EDCR 200 mg/kg	22.50±0.885**	43.75
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significantly, while EDCR at both dose levels showed a significant reduction in granuloma weight with the percentage of 32.84 and 33.84, respectively, as shown in Table 4. Cotton pellet implants in rats induce a chronic proliferative inflammation. The cotton pellet granuloma method has been widely employed to assess the transudative, exudative, and proliferative components of chronic inflammation. This inflammation involves proliferation of macrophages, neutrophils, and fibroblast which are basic source of granuloma formation. Hence, decrease in the weight of granuloma in our present investigation by EDCL and EDCR indicates the proliferative phase was effectively suppressed.

All the tested extracts exhibited significant scavenging of nitric oxide radical. The IC_{50} values of EDCL, EDCB, EDCR, and Vitamin C were found to be 41.68, 63.30, 27.54, and 12.50 µg/mL, respectively [Figure 1]. Similarly, the alcoholic extracts of leaf, bark, and root were studied for its *in vitro* antioxidant activity by DPPH assay. Vitamin C was used as reference standard. All the tested extracts significantly scavenged DPPH radical. IC_{50} values of EDCL, EDCB, and EDCR were found to be 11.48 µg/mL, 10.96 µg/mL, and 10.86 µg/mL, respectively. The results are presented in Figure 2. The possible antioxidant property of *D. cinerea* was investigated because such actions may contribute to explain that the therapeutic effect in various systems. Besides, ethanolic extract of root, stem bark, and leaf of the plant was also found to contain higher concentration of total phenolic compounds. Hence, in our present study, we aim to evaluate antioxidant activity of extracts. Nitric oxide is a free radical produced in mammalian cells involved in the regulation of various physiological processes. However, excess production of nitric oxide is associated with several diseases. In the present study, nitrite produced by sodium nitroprusside was reduced by test extracts. This may be due to the antioxidant principles in the extract which compete with oxygen to react with nitric oxide, thereby inhibiting the generation of nitrite. The free radical scavenging activity of test extracts was evaluated based on the ability to quench the synthetic DPPH radical. This assay provides information on reactivity of test compounds with the stable-free radical. Our results clearly demonstrate that extracts were effective in scavenging DPPH radical.

CONCLUSION

The traditional plant, *D. cinerea* (L.) Wight and Arn. *Mimosaceae*, is commonly used by tribes of India and South Africa. The plant is claimed to possess medicinal uses such as anti-inflammatory, diuretic, used in rheumatism, urinary calculi, and in diarrhea which is evident from literature. The safety profile of the plant extract was studied according to OECD Guidelines 423. No abnormality was observed at the

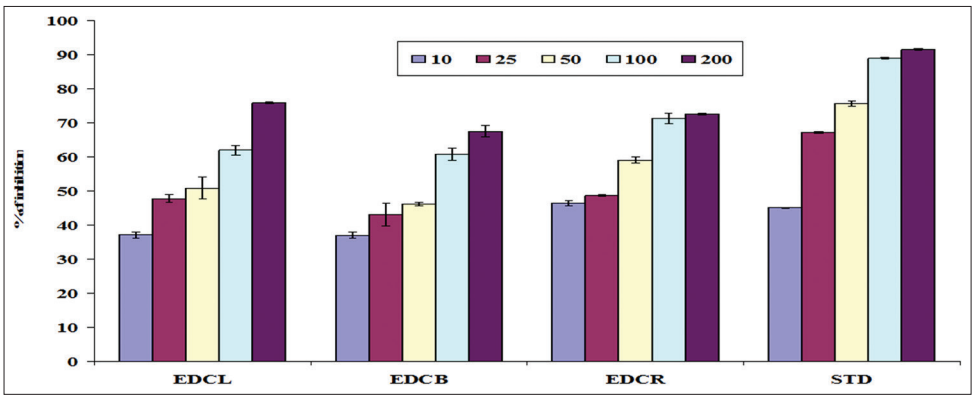

Figure 1: *In vitro* antioxidant activity: Nitric oxide method

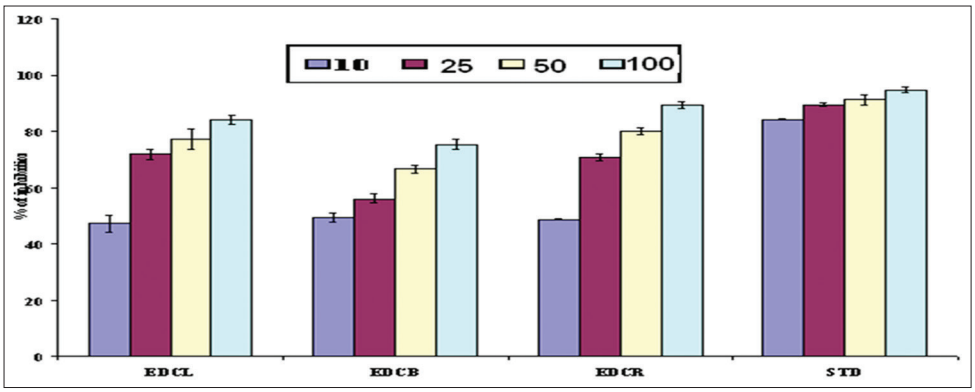

Figure 2: *In vitro* antioxidant activity: 1,1-diphenyl-2-picrylhydrazyl assay

Table 4: Effect of EDCL, EDCB and EDCR on cotton pellet induced granuloma

Group	Treatment and dose	Weight of granuloma (mg)	% inhibition
I	Vehicle control 1%w/v CMC (10 ml/kg)	148.16±11.49	-
II	Indomethacin (10 mg/kg)	89.66±5.81**	40.01
III	EDCL (200 mg/kg)	133.5±12.12	9.89
IV	EDCL (400 mg/kg)	93.16±6.23*	36.85
V	EDCB (200 mg/kg)	113.5±18.77	23.39
VI	EDCB (400 mg/kg)	112.0±5.73	24.40
VII	EDCR (200 mg/kg)	99.5±3.88*	32.84
VIII	EDCR (400 mg/kg)	98.01±8.67*	33.85

Values are expressed as mean±SEM, n=6. **P<0.01, *P<0.05, compared to vehicle control. EDCL: Ethanolic extract of D. cinerea leaf, EDCB: Ethanolic extract of D. cinerea stembark, EDCR: Ethanolic extract of D. cinerea root

initial dose of 2000 mg/kg. Leaf, stem bark, and root extract showed significant analgesic activity in both centrally and peripherally mediated analgesia. *In vitro* anti-inflammatory study indicated root was more effective than bark and leaf. As the extracts showed analgesic and anti-inflammatory activity screening, antioxidant properties of *D. cinerea* were investigated because such actions may contribute to explain the therapeutic efficacy in various systems.

Flavonoids and tannins are natural products which have shown to possess various biological activities related to antioxidant mechanism.[31] Leaf and root showed comparatively higher antioxidant efficacy which is reflected in few of the pharmacological activity carried out, whereas root was found to be more effective. The data retrieved from the observations have been formulated into a diagnostic protocol of *D. cinerea*. The study thus contributes to the best of our knowledge of scientific standardization of the drug of traditional claims. The promising biological activities can definitely lead to the development of a new drug from the plant and also forms the foundation for a future research work in bioactivity-guided isolation of active principles.

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REFERENCES

1. Hakim ZS, Santani DD, Gopal RK. Potential anti-diabetic agents from plant sources. J Nat Prod 1998;29:3-22.
2. Subramani SP, Jishtu V, Rawal RS. Plant diversity conservation needs. Botanica 2005;55:45-59.
3. Sharma R, Arora R. Herbal Drug Twenty First Century Perspective. New Delhi: Jaypee Brothers, Medical Publications (P) Ltd.; 2006. p. 11.
4. Mabberley DJ. The Plant-Book. Cambridge, New York: Cambridge University Press; 2005. p. 224-6.
5. Shanmugam NK. Dictionary of Medicinal Plants. Chennai: Kalaiselvi Publications; 1989. p. 768.
6. Vaidyaratanam PS. Indian Medicinal Plants-A Compendium of 500 Species. Vol. 1. Aryavaidyasala, Kottakkal: Orient Longman Limited.; 1994. p. 330-3.
7. Mudhaliyar KS. Gunapandam Herbal Division. Materia Medica. 3rd ed. Part 1. Mumbai: Popular Prakash; 1969. p. 637.
8. Kambizi L, Afolavon AJ. An Ethnobotanical study of plants used for the treatment of sexually transmitted diseases (Njovhera) in Guruve district Zimbabwe. J Ethno Pharm 2001;77:5-9.
9. Kirtikar KR, Basu BD. Indian Medicinal Plants. Vol. II. Allahabad: Lalit Mohan Basu; 1975. p. 902-12.
10. Almagboul AZ, Bashir AK, Salih A, Karim M, Fariyi A, Khalid SA. Antimicrobial activity of certain Sudanese plants used in folklore medicine, screening for antibacterial activity. Fitoterapia 1988;5:57-62.
11. Eisa MM, Almagboul AZ, Omer ME, Elegami AA. Antibacterial activity of *Dichrostachys cinerea*. Fitoterapia 2000;71:324-7.
12. Pitchai RP, Saraswathi A. Phytochemical characterization and antimicrobial activity of *Dichrostachys cinerea*. J Med Aromat Plant Sci 2000;22(4A):185-6.
13. McGaw LJ, Jager NK, Van Staden J. Prostaglandin synthesis inhibitory activity in Zulu, Xhosa and Sotho medicinal plants. Phytother Res 1996;11:113-7.
14. Anayo OB, Ugonna E. Preliminary pharmacological screening of *Dichrostachys cinerea* Linn. family Mimosaceae. Fip-World Congress (International Pharmaceutical Federation World Congress); 2002;62:135.
15. Mishal HB. Screening of antisnake venom activity of *Dichrostachys cinerea* W & A. J Nat Remedies 2002;2:92-5.
16. Ernst WH, Kuites AT, Nelissen HJ, Tolsma DJ. Seasonal variation in pheloics in several savanas tree species in Botswana. Acta Bot Neeri 1991;40:63-24.
17. Harinder PS, Beckar K. Isolation of tannins from leaves of some tree and shrubs and their properties. Agric Food Chem 1994;42:731-4.
18. Jain R, Saxena U. Aliphatics and triterpenes from the heartwood of *Dichrostachys cinerea*. J Indian Chem Soc 2003;80:656-8.
19. Krishna J, Sharma T. Chemical constituents of the root of *Dichrostachys cinerea* Macb and the stem bark and heart wood of *Acacia leucophloea* Willd. J. Indian Chem Soc 1977;54:649.
20. Krishna J, Sharma T. Triterpenoids and some other constituents from *Dichrostachys cinerea*. Phytochemistry 1974;13:2010-1.
21. Jagadeeshwar Rao R, Tiwari AK, Kumar US, Reddy SV, Ali AZ, Rao JM, *et al.* Novel 3-O-acyl mesquitol analogues as free-radical scavengers and enzyme inhibitors: Synthesis, biological evaluation and structure-activity relationship. Bioorg Med Chem Lett 2003;13:2777-80.
22. Ecobichon DJ. The Basis of Toxicity Testing. 2nd ed. New York: CRC Press; 1997. p. 43-9.
23. Domer FR. Animal experiments in Pharmacology. Springfield, Illinois USA: Charles C Thosmas Publishers; 1971. p. 71-5.
24. Jain NK, Singh A, Kulkarni SK. Analgesic, antiinflammatory and ulcerogenic activity of a zinc naproxen complex in mice and rats. Pharmacol Commun 1999;5:599-602.
25. Ghosh MN. Fundamentals of Experimental Pharmacology. 2nd ed. Calcutta: Scientific Book Agency; 1984. p. 191.
26. Winter CA, Risley EA, Nuss GW. Carrageenan induced edema in hind paw of rats as an assay for antiinflammatory drugs. Proc Soc Exp Biol Med 1962;111:544-7.
27. D'Arcy PD, Howard EM, Muggleton PW, Jowsend SB. The anti-inflammatory action of griseofulvin in experimental animals. J Pharmacol 1960;12:659.

28. Sreejayam N, Rao MN. Free radical scavenging activity of curcuminioids. Drug Res 1996;46:169.
29. Green LC, Wagner DA, Glogowski J, Skipper PL, Nok JS, Tannenbaum SR. Analysis of nitrate and (15N) nitrate. Anal Biochem 1982;126:131-8.
30. Durate JD, Nakamura M, Ferreira SH. Participation of the sympathetic system in acetic acid induced writhing in mice. Braz J Med Biol Res 1988;21:341-3.
31. McDonald S, Prenzler PD, Autolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. Food Chem 2001;73:73-84.

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