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Research Article

EVALUATION OF ANALGESIC ACTIVITY OF ETHANOLIC EXTRACT OF *CLEODENDRUM SERRATUM* LINN LEAVES IN RATS

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Abstract: Ethanolic extract of *Clerodendrum serratum* Linn.,leaves (EECS) were evaluated for analgesic activity using Tail flick test and Acetic acid induced Writhing test. EECS at 250 mg/kg and 500 mg/kg were treated for 7 days and compared with diclofenc sod. 10 mg/kg single dose administered on 7th day before one hour of taking result. EECS at 250 mg/kg and EECS 500 mg/kg treated group showed highest significant ($p<0.001$) increase in latency time in tail flick test and protected no of writhing at 25.27% and 32.74% respectable. Both were comparable with standard diclofenc sod. 10 mg/kg. in both test models. The results of the present study support the folklore use of this plant in pain.

Key words: *Clerodendrum serratum* Linn., Analgesic activity, Tail flick test, Acetic acid induced Writhing test.

INTRODUCTION

In present time, plants are used to be an important source of new chemical substances with potential therapeutic effects. The research into plants with alleged folkloric use as analgesic activity should therefore be viewed as a fruitful and logical research strategy in the search for new analgesic drug.

Assam is enriched with plant diversity and several plants have been used traditionally by Assamese people for therapeutic potentials. *Clerodendrum serratum* of family Verbenaceae which is known as Nagol bhanga in Assamese is use traditionally for different pharmacological activities. [1] The root of the plant has reported to have analgesic and anti-inflammatory activity[2], antibacterial[3], hepatoprotective[4], anti-oxidant[5], anti-cancer[6] and anti-arthritic activity[7].

The plant leaves contain major active constituents like polyphenolics (hydrolysable tannins and flavonoids), terpenoids, saponins and flavanoids.[5, 7] However, there is no systemic report has been published on analgesic activity of this plant leaves to prove its folkloric use. The present study aimed to evaluate analgesic activity of *Clerodendrum serratum* Linn leaves.

MATERIAL AND METHODS

Plant material

Fresh leaves of the plant *Clerodendrum serratum* Linn were collected from Kamrup district of Assam. The plant material was taxonomically identified by Prof. A. P. Das, Taxonomy and Environmental Biology Laboratory, Department of Botany, University of North Bengal. A voucher specimen (A/N-9480 dated.19.12.2007) is submitted to Taxonomy and Environmental Biology Laboratory of University of North Bengal for future reference. The leaves were dried under shade and then powdered with a mechanical grinder and stored in airtight container. The dried powder material of the leaves was defatted with petroleum ether (60-80) and subsequently extracted with ethanol in a soxhlet apparatus at 55^{0}C and extract was concentrated and stored in refrigerator till use. The percentage yield was 11.4%.

Preliminary Phytochemical analysis

The plant extract screened for the presence of various phytochemical constituents i.e. steroids, alkaloids, tannins, flavonoids, glycosides, etc by employing standard screening tests.[8]

Experimental Animals

Healthy adult male Wister rats weighing (150-200gm) were selected for the studies. Rats were housed in polypropylene cages (3 animals per cage), maintained under standard laboratory conditions (i.e. 12:12hr light and dark sequence; at an ambient temperature of 25 +2 ^{o}C).The animals were fed with standard pellet diet and water *ad-libitum).* Before performing the experiment the ethical clearance was obtained.

Acute oral toxicity studies

Acute oral toxicity study was carried out for ethanolic extract of *Clerodendrum serratum* Linn leaves using Acute Toxic Class Method as described in OECD (Organization of Economic Co-operation and Development) Guidelines No. 423.[9] The extract was found non toxic when studied with starting dose of 2000 mg/kg. and studied for 14 days.

Experimental design

The adult male Wister rats Rats were divided into 4 groups of 6 each and studied for analgesic activity. The ethanolic extract of *Cleodendrum serratum* linn leaves (EECS) were made suspended in 1% Carboxy methyl cellulose and treated for 7 days and on 7th day standard drug diclofenac sodium in 3mg/kg is given 1 hr before study. The volume administered was 1ml/100 gm body weight and given by using intra-gastric feeding needle.

Group I: Control; (Vehicle, 1% CMC, P.O) for 7 days)

GroupII: Test Low dose, (EECS 250 mg/kg in 1% CMC, P.O) for 7days.

Group III Test High dose, (EECS 500 mg/kg in 1% CMC, P.O) for 7days.

Group IV: Standard, (Diclofenec sod., 10 mg/kg, P.O in 1% CMC) 1 hr before study on 7th day.

Tail flick Test

Centrally acting Analgesic activity was assessed by tail flick model using analgesiometer.[10-11] The instrument has a nichrome wire, which would be heated to the required temperature (55^{0}c) and maintained by means of heat regulators. The strength of the current passing through the naked nichrome wire was kept constant at 4 Amps. The rat was kept in a rat holder with only the tail portion protruding out. The tail was placed on the platform in such a way that the middle portion of the tail remained just above the hot wire but without touching it. The latency period (reaction time) was noted when the animal responded with a sudden and characteristic flick or tail lifting. A cut off time of 15 sec was planned to avoid any tissue damage in the animal. The reaction time for each group was measured at 30, 60, 90 and 120 minutes using analgesiometer on the 7th day after drug/extract administration.

Acetic acid induced writhing Test

Perpherally acting Analgesic activity was evaluated by Acetic acid induced writhing method.[12-13] The animals are administered acetic acid (0.6%, 1ml/100g) intraperitoneally. After 60minutes of the administration of test and standard drug. Rats are placed individually into glass chamber and number of writhes are counted for 30minutes. A writhe is indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb. The percent inhibition was calculated.

Statistical Analysis

The data were expressed as mean ± standard error mean (SEM). The data were analyzed by using Graph pad software version5 by one way analysis of variance (ANOVA). The test was followed by multiple Dunnett's 't'-test, p values less than 0.05 were considered as significance.

RESULT AND DISCUSSION

In this study analgesic activity of ethanolic extract of *Cleodendrum serratum* linn leaves was evaluated by Tail flick method and Acetic acid induced writhing test.

The phytochemical analysis of ethanolic extract of *Cleodendrum serratum* linn leaves revealed the presence of alkaloids, steroids, flavanoids, carbohydrate, tannins, phenols etc. In the acute toxicity assay no deaths were observed or no stereotypical symptoms associated with toxicity, such as convulsion, ataxy, diarrhoea or increased diuresis thus the median lethal dose (LD50) was determined to be higher than the dose tested i.e. 2.0 g/ kg b.w.

Effect of EECS on Tail flick test in rats

Effect of ethanolic extract of *Cleodendrum serratum* linn leaves (EECS) at 250 mg/kg, 500 mg/kg and Dicofenec sod. 10 mg/kg and control vehicle were studied on Tail flick test in rats. The result were given in **Table-1** and shown in **Fig-1.**

The result showed significant ($p<0.05$) increase in latency time in EECS 250 mg/kg treated and EECS 500 mg/kg treated group showed highest significant activity ($p<0.001$) and these maintains for longer period of time. Where as, standard drug diclofenc sod. 10 mg/kg showed most significant ($p<0.05$) activity at 60 min and reduced after 90 min.

Table-1: Analgesic activity of ethanolic extract of *Cleodendrum serratum* linn leaves in Tail flick test in rats

Treated groups	Latency time (sec)			
	3O min	60 min	90 min	120 min
Control, 1 % CMC	2.34±0.12	2.33±0.17	2.36±0.13	2.34±0.13
EECS 250 mg/kg	3.60 ±0.15 *	3.71±0.22*	3.77±0.22*	3.77±0.22*
EECS 500 mg/kg	4.76±0.01*	4.83±0.20**	4.89±0.20***	4.87±0.20***
Diclofenac sod. 10 mg/kg	2.86±0.02ns	4.18±0.16*	4.08±0.18*	3.58±0.15*

Values are in Mean ± S.E.M (n=6); ns -Non Significant, *p<0.05, **p<0.01, ***p<0.001
when all test groups compared with Control using One way ANOVA followed by Dunnet's "t" test.

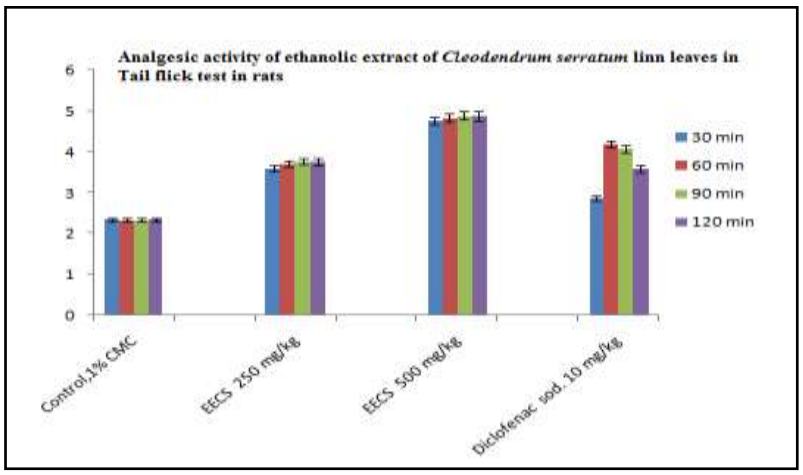

Fig-1: Analgesic activity of ethanolic extract of *Cleodendrum serratum* linn leaves in Tail flick test in rats

Effect of EECS on Acetic acid induced writhing Test in rats

Effect of ethanolic extract of *Cleodendrum serratum* linn leaves (EECS) at 250 mg/kg, 500 mg/kg and Dicofenec sod. 10 mg/kg and control vehicle were studied on acetic acid induced writhing in rats. The result were given in Table-2 and shown in Figure-2.

The result showed significant (p<0.01) reduction in no of writhes in EECS 250 mg/kg treated group and % percentage protection was 25.27% and EECS 500 mg/kg treated group showed significant reduction(p<0.001) and % percentage protection was 32.74% . Where as, standard drug diclofenc sod. 10 mg/kg showed most prominent reduction (p<0.001) and % protection was 69.94%.

Table-2: Analgesic activity of ethanolic extract of *Cleodendrum serratum* linn leaves in Acetic acid induced writhing in rats

Treatment	No. of Writhing	% Protection
Control, 1 % CMC	17.17± 1.50	-
EECS 250 mg/kg	12.83± 0.85**	25.27%
EECS 500 mg/kg	11.60± 0.85***	32.74%
Diclofenac sod. 10 mg/kg	5.16 ± 0.48***	69.94%

Values are in Mean ± S.E.M (n=6); ns -Non Significant, *p<0.05, **p<0.01, ***p<0.001
when all test groups compared with Control using One way ANOVA followed by Dunnet's "t" test.

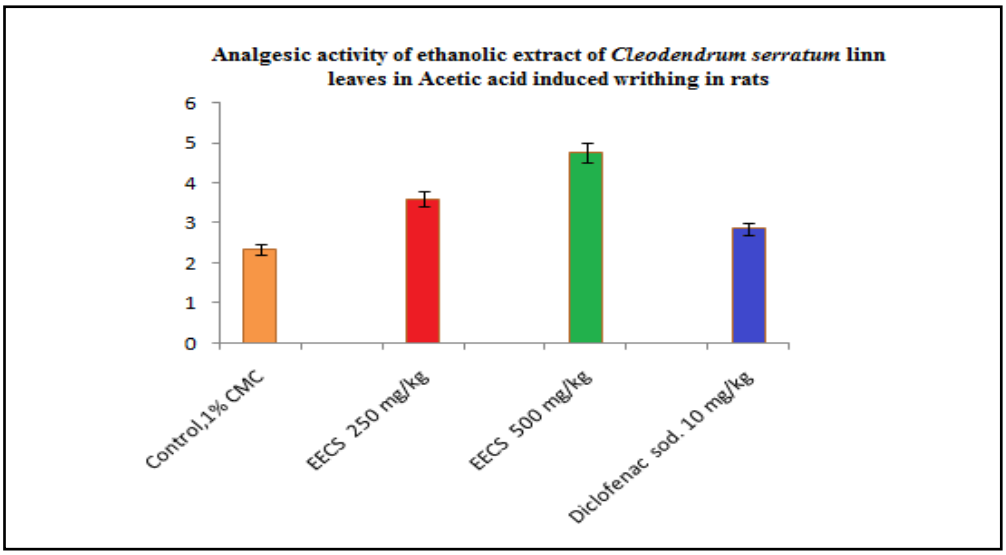

Fig-2: Analgesic activity of ethanolic extract of *Cleodendrum serratum* linn leaves in Acetic acid induced writhing in rats

Drugs that act centrally inhibit pain produced by thermal stimuli[14]. In our present study ethanolic extract of *Cleodendrum serratum* linn leaves (EECS) at both dose level showed significant analgesic activity. Although, this model is specific for centrally inhibited pain but in the present study, diclofenac also inhibited the pain produced by tail flick method where there are evidences that support that NSAID's also inhibit the pain induced by thermal stimuli[15].

In acetic acid induced writhing method, acetic acid causes an increase in peritoneal fluid level of prostaglandins involving in part peritoneal receptor [16] and inflammatory pain by capillary action[17]. It is widely used for analgesic screening and predominately involves induction of prostaglandins. In our present study ethanolic extract of *Cleodendrum serratum* linn leaves (EECS) and standard drug diclofenec significantly reduced the no of writhing compared to the control groups. This suggests mechanism of analgesic effect of *Cleodendrum serratum* linn leaves is probably due to a blockade of capillary permeability or release of endogenous substances like prostaglandins.

CONCLUSION

The results of the present study support the folklore use of this plant in pain. Thus, the results of this study confirmed the traditional uses, claiming that the ethanolic extract of plant *Clerodendrum serratum* linn showed significant analgesic activity which is comparable to the standard drug Diclofenac sodium. Presently available synthetic analgesic agents are rapidly losing their therapeutic value. So conjoint the use of medicinal plants (naturally occurring analgesic agents) and standard drugs may prove useful in future medical practice. Further studies involving the purification of the chemical constituents of the plant and the investigations in the biochemical pathways may result in the development of a potent analgesic agent with a low toxicity and better therapeutic index.

REFERENCES

1. Mukesh Kr. Singh, Gaurav Khare, Shiv Kr. Iyer, Gotmi Sharwan and D.K. Tripathi; *Clerodendrum serratum*: A clinical approach; *Journal of Applied Pharmaceutical Science*. **2012**; 2 (0):11-15.
2. N Narayanan, P Thirugnanasambantham ; Anti-inflammatory, Antinociceptive and antipyretic effects of ethanol extract of *Clerodendron serratum* roots in experimental animals; *Journal of Ethnopharmacology*,**1999**;65(3):237-241.
3. Vidya, S. M; Antibacterial activity of *Clerodendrum serratum* L.; *Electronic Journal of Environmental, Agricultural and Food Chemistry*. **2010**; 9(6):1059-1063.
4. Vidya SM, Krishna V, Manjunatha BK. Evaluation of hepatoprotactive activity of *Clerodendrum serratum* L, *Indian J Exp Biol*. **2007;** 45(6) :538-542.
5. Ali Jimale Mohamed; Antioxidant, antiangiogenic and vasorelaxant activities of methanolic extract of Clerodendrum serratum (Spreng.) leaves; *Journal of Medicinal Plants Research*. **2012**; 6(3):348-360.
6. Ashish S. Zalke1, Ashish V. Kulkarni, Devendra S. Shirode1, B. Duraiswamy ;

In vivo anticancer activity of Clerodendrum serratum (L) moon.; *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. **2010**; 1(3):89-98.
7. Ravikumar R, Lakshmanan AJ, Ravi S. Chemical constituents from *Clerodendron serratum. J Asian Nat Prod Res.* **2008**;10(7-8):659-662.
8. Kokate CK, Practical Pharmacognosy, 5th Edn, Vallabh Prakasham, **1991**, 107-121.
9. Gad SC, Chengalis CP, Acute Toxicity Testing, 2nd edition, Academic press, New York., **1998**, 25-45.
10. Wani TA, Kumar D, Prasad R, Verma PK, Sardar KK, Tandan SK, Kumar D, Analgesic activity of the ethanolic extract of Shorea robusta resin in experimental animals. *Indian J Pharmacol*. **2012**;44(4):493-499.
11. Tjolsen A, Lund A, Berge OG, Hole K. An improved method for tail-flick testing with adjustment for tail-skin temperature. *J Neurosci Methods.* **1989** ;26(3):259-265.
12. Holanda FR, Coelho-de-Sousa AN, Assreuy AM, Leal-Cardoso JH, Pires AF, do Nascimento KS, Teixeira CS, Cavada BS, Santos CF. Antinociceptive activity of lectins from Diocleinae seeds on acetic acid-induced writhing test in mice. *Protein Pept Lett.* **2009**;16(9):1088-1092.
13. Saini N, Singhal M. Evaluation of anti-nociceptive activity of methanolic extract of Tecomaria capensis Thunb. (Bignoniaceae) leaves in rats. *Asian Pac J Trop Med.* **2012**;5(10):781-784.
14. Bjorkman R. Central antinociceptive effects of non-steroidal anti-inflammatory drugs and paracetamol. Experimental studies in the rat. *Acta Anaesthesiol Scand Suppl*. **1995**;103:1-44.
15. Almasi R, Petho G, Bolcskei K, Szolcsanyi J. Effect of resiniferous toxin on the noxious heat threshold temperature in the rat: a novel heat allodynia model sensitive to analgesics. *Br. J. Pharmacol* **2003**; 139: 49-58.
16. Riedel W, Neeck G. Nociception, pain, and antinociception: current concepts. *Z Rheumatol*. **2001***;* 60(6):404-415.
17. Adachi KI. A device for automatic measurement of writhing and its application to the assessment of analgesic agents. *J Pharmacol Toxicol Meth.* **1994**; 32:79–84.