Research Article **www.ijcps.com**

International Journal of
Chemical and Pharmaceutical Sciences
2016, Mar., Vol. 7 (1)

ISSN: 0976-9390
IJCPS

Analgesic activity of methanolic leaf extract of *Leucaena leucocephala*

Siddhanadham Arun Satyadev*, Naidu Venkata Narayana, Ashok krishnan and Ch.Nandini.

A.U. College of pharmaceutical sciences, Andhra University, Visakhapatnam, Andhra Pradesh, India.

* **Corresponding Author:** E-Mail: arun.satyadev673@gmail.com

Received: 06th Jan 2016, Revised and Accepted: 10th Jan 2016

ABSTRACT

The aim of the present research deals with the analgesic efficacy of methanolic leaf extracts of *Leucaena leucocephala* (Leguminosae). Most of the species belonging to this family are renowned for their wide range of biological activities. The analgesic activity was tested in albino mice by giving oral doses of 100, 200 and 300 mg/kg body weight using tail immersion method, acetic acid induced writhing and formalin induced analgesic effect. The outcome of the analgesic activity showed significant and dose dependent analgesic effect ($P<0.001$). The brink was found to be 60 min after treatment with 100, 200, and 300 mg/kg of the methanolic leaf extract. The activity produced by the extract was significantly lower than the standard drug (Diclofenac sodium). The dose of the standard drug used is 100 mg/kg. The results clearly states that methanolic extracts showed delayed response towards thermally induced pain, decreased acetic-acid induced writhing and also showed significant inhibition in both the phases of formalin induced pain test. The analgesic activity of the extracts can be attributed to their central and peripherally mediated secondary messengers in the management of pain. The phytochemical constituents extracted by the respective solvents may contribute for the analgesic activity. The results finally support the use of folkloric use over the conventional dosages in the management of pain and these natural sources are to be explored as an alternate remedy as a potential analgesic agents.

Keywords: *Leucaena leucocephala*, methanolic extract, tail immersion, acetic acid induced writhing test, Formalin test, analgesic activity.

1. INTRODUCTION

Pain/ Analgesia are one of the major alarming problems around the world [1]. The available conventional analgesic drugs in the market possess many side effects like depletion of the mucosal layer in the stomach and thereby causing ulcers which lead to discomfort and change in the biological processes. Most of the marketed analgesic drugs which are used in the tolerance of pain namely NSAIDS (Non Steroidal anti inflammatory drugs) [2], opiod analgesics, corticosteroids, Sustain release analgesics drugs etc are known for their common side effects like tolerance, dependence, increase in the lipid profile, obesity etc. So it is necessary to explore the folklore and find out an alternate remedy for the management of pain which is biologically cause less risk and has fewer side effects [3].

Leucaena leucocephala (Leguminosae) is a small, leguminous and native to tropical America; it is mostly distributed in southern Asia and neighboring islands. It is widely distributed in Africa, Oman, Mauritius [4,5]. In India it is mostly distributed in Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Daman and Diu, Delhi, Goa, Gujarat, Haryana, Himachal Pradesh and other parts [6]. Other names of this plant are Oriya: nagarjuna Hindi: Subabul [7]. Common names include White lead tree, jumbay and white popionic. It has been used as livestock feed due to their high contents of alkaloid, saponin, flavonoid, mimosin, leukanin, protein, fat, calcium, phosphor, iron, vitamin A and vitamin B [8-10]. Literature review studies have shown that the extract of *L.leucocephala* was found to exhibit various pharmacological effects [11,12]. The seeds of *L. leucocephala* are emollient and had beneficial effect in ascariasis; the bark and roots can also be used as an emmenagogue. It was promoted as a "miracle tree" [13] for its multipurpose use. It is also called as "Conflict tree" as it spreads like a weed in some of the tropics. Other uses or *L. leucocephala* are: for fuel, ornament, timber,

erosion control, shade or shelter, reclamation for forest covers, soil improver, its fibre is used for paper production, and has a high nitrogen-fixing potential. serine proteinase inhibitor isolated from *L. leucocephala* (LITI) seeds was purified to homogeneity. LITI is a 174 amino acid residue protein which shows high homology to plant Kunits inhibitors. *L. leucocephala* have many different chemical properties which are traditionally used by people to cure certain diseases [14,15]. Analyzing on the chemical compounds, and constituents of these plants would help explain medicinal capabilities. Investigating on the activities would clear out the questions on how these plants work for health treatment. Furthermore, the knowledge could be the key to understanding the mechanisms of *Leucaena leucocephala* and could be the first step in the possible synthesis to approved medicines and treatments for health. This legume is also used as fodder for cattle, fire wood and charcoal production [16].

Based upon the existing chemical, clinical literature and folkaire claims, our objective were to gratifying the analgesic activity of leaf extracts namely methanol. Natural sources especially this genus it is necessary to find out alternate remedy for side effects caused by traditional system of medicine. The active principle which is responsible for the activity has to be found out by different chromatographic techniques.

1.2. Objective

The aim and objective of the work was to identify the analgesic activity of the methanolic extract in the management of pain response against the experimental models. The analgesic activity is determined by tail immersion method, acetic acid induced writhing model and formalin test.

3. MATERIALS AND METHODS

3.1. Collection and authentication of plant material

The leaves of *Leucaena leucocephala* are collected from the local areas (Visakhapatnam and vizianagaram) of Andhra Pradesh in the month of September and October. It was authenticated by Dr. S.B.Padal, Dept. of Botany, Andhra University and Sample specimen was kept in our laboratory for future reference. The collected leaves are garbled and freed from the unwanted dust material. The leaves are first washed with tap water followed by distill water to remove all the debris attached to the leaves. Then these are allowed to dry in shade and after drying the leaves are pulverized into a course powder and required amount of the extract is taken for preparation of the extract and the remaining powder is stored in a tightly closed container for future use.

3.2. Preparation of Plant extracts

To 1 Kg of *Leucaena leucocephala* leaf powder, 2 liters of solvent, viz. methanol was added consequently for preparing the extract (flow chart-1).Extraction with the solvent was done for 24 hours at 27^0C, after maceration the supernatant of each solvent was recovered by filtering through whatmann filter paper. This process was repeated thrice and the respective solvent from the supernatant was evaporated in a rota vapor to obtain crude extract which are to be stored at 4^0c until used for evaluation.

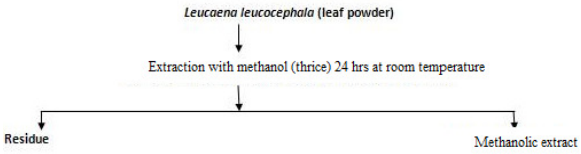

Figure - 1: Schematic representation of showing methanolic extraction procedure from leaves of *Leucaena leucocephala.*

3.3. Experimental animals

Analgesic activity was carried out on 2 weeks old swiss wister albino mice weighing 25-30 grams were procured from Mahaveer enterprises, Hyderabad, Telangana state. The animals are taken utmost care and housed in standard cages, with 5 mice in each cage, under a 12 hr day and 12 hr night catalog at an optimum room temperature (26^0c) so as to make them acclimatize to the laboratory conditions. The experimental animals were fed with water and food *ad libitum* before a week prior to the commencement of the experiment. The experimental animals were treated according to the rules of institutional and international guidelines mentioned for the use of experimental animals. The animal ethical committee registration number: 916/a/07/CPCSEA.

3.4. Screening of suitable animals for the experiment

Total number of albino mice used for this experiment was 20 weighing between 150-250grams. These total rats were grouped in three groups containing 5 albino rats in each group. The tail of the rat was immersed in the water which is maintained at 55 ± 5^0C withdraws its tail normally in 3-6 seconds when exposed to hot water., if any animal fails to withdraw its tail in that stipulated reaction time those rats were rejected for the study. The rats which are normal and suitable for the experiment were labeled accordingly for the identification purpose.

3.5. Preparation of standard drug

Diclofenac sodium is used as a standard drug in this method. 400mg diclofenac sodium tablets were procured and powdered. Then 40ml of distilled water was added to get 10mg of diclofenac in 1ml of solution. Group I is maintained as standard group which received 0.1ml/ 100 gram body weight of albino rats.

3.6. Tail immersion assay

The experimental animals are allowed to embrace to the laboratory conditions and in the cage for about half an hour prior to the commencement of the experiment. Analgesic activity is performed by the method described by Chandrashekar et al, with slight modification. The mice selected for the experiment are divided into five groups of four animals each. Group I mice received standard drug Diclofenac sodium 10mg/kg. Group II, III, IV mice were pretreated with 100-300mg/kg (i.p) of methanolic extract. Group V received physiological salt solution (PSS) as control. After half an hr of administration of the drug, each mouse was held in a apposition such that the tail is extended out of the cage. The tail was marked about 3 cm and was immersed into the water bath which was maintained at temperature 55 ± 5^0C. The mouse has given response within few seconds which was recorded as the withdrawal time. The latency was evaluated at 0, 30, 60, 90 and 180 minutes with 0 minute being the initial reading. The mean increase in the reaction time after administration of the drug indicates the potency of the extract and the standard. To prevent the damage to the tail of the mice, the immersion time was limited to 10 seconds.[17]

3.7. Analgesic activity of *Leucaena leucocephala* by Acetic acid induced writhing method

For the acetic acid induced writhing assay method total number of rats taken is 25 which were divided as 5 rats per group. The writhing were induces as described in koster et al with slight modification. The standard group received the standard drug 100mg/kg body weight of diclofenac sodium. Control group received 0.3ml physiological saline solution and the test groups received 100,200 and 300 mg/kg of methanolic *Leucaena leucocephala* extract intra peritoneal. Firstly the experimental rats are allowed to fast for 15hrs and after that the treatment is started. After 60 min of the treatment, the rats were administered with acetic acid acid (0.6%, v/v in normal saline, 10 mL/kg), which acts as a source for the contraction of the abdominal muscle (Writhing). The stretching contractions and jerking of the hind limb was noted between 5 to 30 minutes after the administration of the acetic acid. The standard group rats are compared with that of the test and control group. Inhibition of the writhing activity was taken as the mark of analgesia and the percentage of writhing inhibition is calculated [18].

$$\% \text{ Inhibition of writhing} = \frac{\text{Mean writhing by control} - \text{Mean writhing by test}}{\text{Mean writhing by control}} \times 100$$

3.9. Formalin test

Acute pain can be determined efficiently by this method. This method was done as described by shibata et al with minor modification. This method involves the injection of 20ml of 5% formalin subcutaneously to the right hind paw of mice to produce pain response. The time (in seconds) taken by the mice to lick and bite the hind paw was taken as the indicator of pain induction. The responses are measured from 0-5 minutes in the early phase and 25-40 minutes after the administration of formalin injection. The experimental mice selected suitable for the experiment were divided into four groups of five animals each. Group I, II, III received the methanolic extract 100-300 mg/kg (i.p) administered 60 minutes prior to formalin injection. Group IV received the standard drug (diclofenac sodium) 10mg/kg (i.p) was administered half an hour prior to formalin injection. Group V received Physiological Salt Solution as control.

4. RESULTS AND DISCUSSIONS

The analgesic effect produced by the methanolic extract by performing tail immersion method is well elucidated in Table I. Methanolic extract showed % inhibition of the pain at 48.1, 18.1 and 38.1 tail withdrawals by inducing 100, 200, 300 mg/kg doses respectively. While the standard drug showed % inhibition of 63.6 at 100mg/kg dose which shows that the extract produced inferior effect than that of the sample. Methanolic extract showed significant ($P<0.001$) dose dependent inhibition of analgesia. The recordings are done without causing damage to the tissue of the tail.

Acetic acid induced writing assay results were well illustrated in table II. The methanolic extract showed percentage inhibition of writhing (30 minutes) of 6.45, 12.9, 29.8 writhing responses at 100mg, 200mg, 300mg dose. The results obtained are found to be of high significance ($P<0.001$) when compared with that of the control and standard doses. The extract showed significant inhibition at high dose level of 300mg/kg, but the effect produced is inferior to the effect produced by the standard drug (Diclofenac sodium 100mg/kg).

Formalin test results were shown in table III. There was a significant dose-dependent

inhibition in both phases of the formalin-induced pain response in mice, with a more potent effect in the second phase. Diclofenac sodium also inhibited pain in both phases, but its effect on the first phase was not significantly (P > 0.001) different from that produced by 300 mg/kg of the extract.

The results are displayed in the tables below.

Table -1: Effect of methanolic extract of *L.Leucocephala* on tail immersion test

Group	Dose (mg/kg)	Drug administration		% Inhibition
		Before	After	
Control	--	2.2± 0.68	2.3±0.41	--
L. *leucocephala* extract	100	2.1±0.32	2.0±0.56	48.1
	200	2.2 ±1.68	2.6±0.77*	18.1
	300	2.1±1.80	2.8±0.62*	38.1
Diclofenac sodium	100	2.2± 0.70	3.7±0.27*	63.6

Values are means ± S.E.M. * P < 0.001, signifi cantly diff erent from control; Student's t-test (n = 5).

Table - 2: Effect of acetic acid induced writhing in albino rats when administered with Standard dose, Methanolic extract at different doses of *L. leucocephala*

Experimental group	No. Of writhing (30 minutes)	% inhibition of writhing
Diclofenac sodium (100mg/kg b.w)	68.1±1.02*	66.2
Control (0.3 ml normal saline)	72.8±0.51	---
L. leucocephala (100mg)	63.4±1.52	6.45
L. leucocephala (200mg)	51.1±0.28*	12.9
L. leucocephala (300mg)	24.6 ±1.13*	29.8

Values are means ±S.E.M. * P < 0.001, significantly different from control; Student's t-test (n = 5).

Table - 3: Effect of methanolic extract of *L. leucocephala* on the early and late phase of Formalin-induced pain in rat

Group	Dose (mg/kg)	0-5 min	% inhibition	15-30 min	% inhibition
Control	--	93±1.3		100±1.3	
L.Leucocephala	100	91±2.2	2.5	64±1.8*	36.6
	200	83±2.4*	10.1	46±1.6*	54.2
	300	71±1.8*	22.4	35±2.1*	65.4
Diclofenac sodium	100	66±2.8*	27.7	21 ±2.5*	79.5

Values are means ± S.E.M. * P < 0.001, significantly different from control; Student's t-test (n = 5).

5. CONCLUSIONS

Tail immersion method performed in albino rats was found to be significant by the administration of methanolic extract of *l.leucocephala*. The standard drug used was diclofenac sodium, test dose (300mg/kg) has produced significant effect same as that of standard drug but during course of time the reaction time for tail withdrawal has gradually decreased. Abdominal contractions induced by acetic acid are used to evaluate the peripheral analgesic activity; the pain may be induced by the secondary messengers like PG (Prostaglandins), TNF, which are responsible for nociceptive activity. The phytochemical constituents like terpenoids may contribute to the anti nociceptive activity, Literature review states that terpenoids inhibits both peripheral and centrally mediated analgesia.

Finally from the above observations it is evident that methanolic extract of *L.leucocephala* has given an promising results in the management of nociception action and strongly supports the use of herbal or natural medicines in contrast to the conventional drugs which are mostly renowned for their side effects. There are no symptoms of high toxicity found during the

process of experiment which clearly indicates high margin of safety.

Acknowledgement

Author like to thank Prof. Y. Rajendra Prasad, Head of the department, Pharmaceutical chemistry, Andhra University and K. Kishore naidu, Research scholar, Pharmacology department, Andhra university for their constant support and encouragement during the course of research.

6. REFERENCES

1. Farnsworth NR, Akerela O, Bingel AS, Soejarto DD and Guo Z Bull. **W. H. O,** 1989; 63: 965.
2. Balandrin MF, Kinghorn AD and Farmsworth NR. **In: Human Medicinal Agents from Plants**. Ed By Kinghorn, A.D. and Balandrin, M.F., ACS symposium series, Washingto, DC. 1993; 534: 2.
3. Neil MO and Lewis JA. **In: Human Medicinal Agents from Plants.** Ed By Kinghorn AD and Balandrin, M.F., ACS symposium series, Washingto, DC. 1993; 534: 48.
4. Anonim, **Standard of ASEAN Herbal Medicines,** ASEAN Countries, Jakarta, 2004; 2: 142-150.
5. Erickson AJ, Ramsewak RS, Smucker AJ and Nair MG. Nitrification Inhibitors from the roots of Leucaena leucocephala. **Journal of Agricultural and Food Chemistry,** 2000; 48: 6174-6177.
6. Xuan TD, Elzaawely AA, Deba F, Fukuta M and Tawata S. Mimosine in *Leucaena leucocephala* as a potent bio-herbicide. **Agronomy for Sustainable Development,** 2006; 26: 89-97.
7. Kaufusi PH, Forsberg LS, Tittabutr P and Borthakur D. Regulation of exo polysaccharide synthesis in rhizobium sp. strain TAL1145 involves an alternative sigma factor gene, rpoH2. **Microbiology,** 2004; 150: 3473-3482.
8. Yeung PKK, Wong FTW and Wong JTY. Mimosine, the allele chemical from the leguminous tree *Leucaena leucocephala*, selectively enhances cell prolixferation in dinoflagellates. **Applied and Environmental Micro-biology,** 2002; 68, 5160-5163.
9. Soedarjo M and Borthakur D. Mimosine, a toxin produced by the tree-legume leucaena provides competition advantage to mimosine-degrading rhizobium strains. **Soil Biology and Biochemistry,** 1998; 30: 1605-1613.
10. Kamada Y, Oshiro N, Miyagi M, Oku H, Hongo F and Chinen I. Osteopathy in broiler chicks fed toxic mimosine in *Leucaena leucocephala*. **Bioscience Biotechnology and Biochemistry,** 1998; 62: 34-38.
11. Villasenor IM, Gajo RMT and Gonda RC. Bioactivity studies on the alkaloid extracts from seeds of *Leucaena leucocephala*. **Phytotherapy Research,** 1998; 11(6): 15-617.
12. Collins LA and Franzblau SG. Microplatealamar blue assay versus BACTEC 460 system for high- throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium.* **Antimicrob Agents Chemother**, 1997; 41: 1004-1009.
13. Chibbar SS, Butt SK, Sharma RP and Sharma A. **Indian J. Chem.,** 1981**;** 20 B: 626.
14. Cragg GM, Newman DJ and Snader KM**. J. Nat. Prod,** 1997; 60: 52–60.
15. Ile CH and Wender SH. **J. American, Chemical Society**, 1953; 75: 117.
16. Swati Paul and Dibyajyoti Saha. **In Analgesic Activity of Methanol Extract of *Plumbago Indica* (L.) by Acetic Acid Induced Writhing Method.** 2002; 74: 76.
17. Satyajit dutta, Anurag yadav and Mariappan G. **In analgesic activity of *taxus baccata* linn. bark extract.** 2006; 1-3.
18. Sreedam Chandra Das, Subrata Bhadra, Sumon Roy, Sajal Kumar Saha, Md. Saiful Islam and Sitesh Chandra Bachar. **In: Analgesic and Anti-inflammatory Activities of Ethanolic Root Extract of *Swertia chirata* (Gentianaceae).** 2012; 31-32.