Journal of Scientific and Innovative Research 2015; 4(1): 13-16
Available online at: www.jsirjournal.com

Research Article

ISSN 2230-4818
JSIR 2015; 4(1): 13-16

Received: 12-11-2014
Accepted: 13-12-2014

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Sub-acute anti-inflammatory activity of ethanolic extract of leaves of *Leucas indica* in Wistar albino rats

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Abstract

Newer anti-inflammatory drugs are still under intense investigation, especially from herbs. Ethanolic extract of leaves of Leucas indica could be used to treat sub-acute inflammatory conditions. The objective of the study was to evaluate sub-acute anti-inflammatory activity of ethanolic extract of the leaves of *Leucas indica* in Wistar albino rats. Ten Wistar albino rats were randomly assigned to five groups as Controls, standard and three doses of the test drug viz 75, 150 & 300 mg/kg, orally respectively. The adsorbent sterilized cotton pellet (20±1 mg) was implanted subcutaneously. Drugs were administered once daily for 7 days and on 8th day rats were sacrificed. The mean weight of the cotton pellets (dry, wet and transudative) were weighed and compared between the groups. Ethanolic extract of leaves of *Leucas indica* showed significant sub acute anti-inflammatory activity at the dose of 150 mg/kg and 300 mg/kg by cotton pellet induced granuloma pouch in Wistar albino rats compared to controls. Our study reveals that ethanolic extract of the leaves of *Leucas indica* has shown significant sub-acute anti-inflammatory activity in Wistar albino rats.

Keywords: Ethanolic extract, *Leucas indica*, Sub-acute inflammation.

Introduction

Inflammation is a complex biological response of vascular tissue to harmful stimuli caused by injury, infection, environmental agents, malignancy and cellular changes.[1]

Inflammation may be acute or chronic depending on the disease course. Acute inflammation is characterized by heat, erythema, pain, swelling and loss of function. Chronic inflammation on the other hand, results in a progressive shift in inflammatory cells characterized by simultaneous destruction and healing of the injured tissue.[2] The use of herbs and minerals processed with herbs which were used to treat ailments is as old as antiquity.[3]

The use of traditional medicine and medicinal plants in most of the developing countries in order to maintain good health, has been widely practised.[4]

Plants of genus *Leucas* have been widely employed by the traditional healers to cure many diseased conditions which indicate that this genus have immense potential for the discovery of new drugs or lead molecules. Our previous study has shown acute anti-inflammatory activity of ethanolic extract of the leaves of *Leucas indica* by carrageenin induced paw oedema at the dose of 150 mg/kg and 300 mg/kg respectively in Wistar albino rats.[5] Till date the effect of this drug on sub-acute inflammation has not been done.

As our previous study has shown acute anti-inflammatory activity of ethanolic extract of the leaves of *Leucas indica*, The present study intends to focus on the role of ethanolic extract of the leaves of *Leucas indica* on sub-acute inflammation. Hence the present study to know its effect on sub-acute inflammation was undertaken.

Materials and Methods

Institutional Animal Ethical Committee (Licence No. CPCSEA /347) approval was obtained from Yenepoya University, Deraalakatte, Mangalore, India, before conducting the experiments Ref No. PhD 1/ 2010 dated 6th May 2010. All the animals were handled and taken care, according to the guidelines of "Principles of Laboratory Animal Care" (NIH Publication No.85- 23, Revised 1985) and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Plant Material

The whole plant was collected from rural regions of Manjanady, in Mangalore region, Karnataka, India, between June – August 2010. It was authenticated by Dr. Krishnakumar G., Head, Department of Applied Botany, Mangalore University, Mangalore, India.

Extraction Procedure

Leaves of *Leucas indica* were carefully separated, cleaned, shade dried, mechanically grinded and coarsely powdered. About 1000 gm of air dried leaf powder was extracted with 90% ethanol in a Soxhlet extractor for 36 hr. It was concentrated to dryness under reduced pressure and controlled temperature (40-50ºC) using a rotary evaporator. The collected leaf extract was stored in a desiccator. A suspension of the extract prepared in 1% gum acacia was used in experimental studies.

Procedure for Sub acute anti-inflammatory activity

Wistar Albino rats (male and female in equal no.) were divided into five groups of 10 rats each. Adsorbent cotton wool was weighed (20±1 mg) and made into pellet. Each pellet was then sterilized in a hot air oven at 120º C for 2 hr. The rats were anaesthetized with pentobarbitone sodium (30 mg/kg, orally). The nape of the back of the neck was shaved cleanly, swabbed with 70% ethanol and sterilized cotton pellet was implanted subcutaneously. The animals which received the drug indomethacin 10 mg/kg orally served as reference the standard and the control group received 1% gum acacia 3 ml/kg orally. Three doses of the test drug viz ethanolic extract of the leaves of *Leucas indica* (75, 150 & 300 mg/kg, orally) respectively, were administered once daily throughout the experimental period for 7 days and on the 8th day one hour after drug administration, rats were sacrificed with high doses of pentobarbital sodium. The pellets were dissected out and dried at 60º C for 18 hr, weighed after cooling. The mean weight of the cotton pellets of the control group as well as of the test / standard groups was calculated. The transudative weight, granuloma formation and percent granuloma inhibition of the test compound were calculated and compared between the groups (Table & Fig 1).[6,7]

Statistical Analysis

ANOVA followed by Dunnett's multiple comparison test. The observations are mean ± S.E.M. $p<0.05$ as compared to control was considered as statistically significant.

Results

It was observed, there was significant sub acute anti-inflammatory activity with respect to wet weight ($p<0.05$), dry weight ($p<0.05$) and transudative weight ($p<0.05$) at the dose of 150mg/kg and with respect to wet weight ($p<0.05$), dry weight ($p<0.05$) and transudative weight ($p<0.05$) at the dose of 300mg/kg by cotton pellet induced granuloma pouch in Wistar albino rat**s** compared to control with a significant mean percentage of inhibition of sub acute inflammation.

Table 1: Sub-acute anti-inflammatory activity of EELLI by cotton pellet induced granuloma pouch in Wistar albino rats

Groups / Drugs / Dose	WW (mg)	DW (mg)	TW (mg)
Control (1% Gum acacia) 3 ml/kg, p.o	204.5±3.31	57.03±0.88	151.2±2.24
Standard (Indomethacin) 10 mg/kg, p.o	114.7±2.89 (p<0.01)	44.82±1.10 (p<0.01)	77.43±1.87 (p<0.01)
EELLI 75 mg/kg, p.o	198.2±3.15 (p>0.05)	56.85±3.23 (p>0.05)	149.6±4.62 (p>0.05)
EELLI 150 mg/kg, p.o	192.6±3.11 (p<0.05)	48.56±2.10 (p<0.05)	138.7±3.35 (p<0.05)
EELLI 300 mg/kg, p.o	192±2.08 (p<0.05)	48.45±2.32 (p<0.05)	137.6±3.52 (p<0.05)

The observation are mean ± S.E.M. p> 0.05- Not Significant, p<0.05-Significant, p< 0.01- Highly Significant as compared to control

(ANOVA followed by Dunnett's multiple comparison test), WW-Wet weight, DW-Dry weight, TW- Transudative weight, EELLI- Ethanolic Extract of the leaves of Leucas indica, p.o- per oral

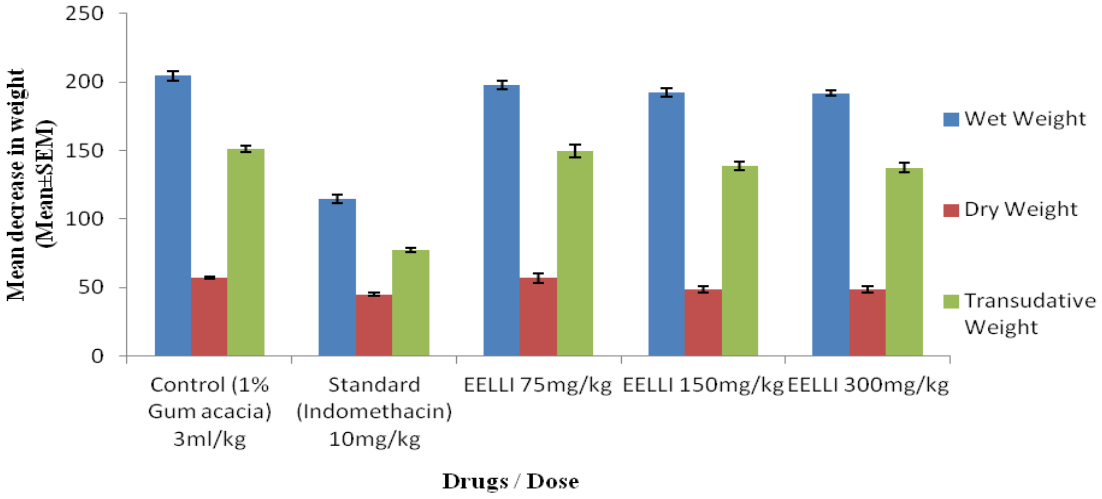

Figure 1: Sub-acute anti-inflammatory activity of EELLI by cotton pellet induced granuloma pouch in Wistar albino rats. EELLI- Ethanolic extract of the leaves of *Leucas indica*

Discussion

Preliminary phytochemical screening of EELLI has shown the presence of phytosterols, triterpenoids, flavinoids, lactones, fats and fatty acid, phenolic compound and tannins, glycoside.[8] Many researches has shown that the plants containing these phytochemicals possesses anti-inflammatory activity which is evident from our study.[9-19]

Immediately after an injury, inflammation occurs which is characterized by pain, swelling, and redness that happen at the injury site. This natural response by the body is its way of protecting the injured part of the body and releasing chemicals that will help to resolve the damage, but with the cost of pain and discomfort. Scar tissue also starts to form at this stage of healing. In this stage the body starts to grow more tissues, and starts repairing what was damaged in the first phase. Sub-acute inflammation includes the proliferation of fibroblasts and the infiltration of neutrophils and exudation. Sub acute inflammation occurs by means of the development of proliferative cells. These cells can be spread from granuloma form. Efficacy of anti-inflammatory agents in sub acute inflammatory states are indicated by their ability to inhibit the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation. [20]

It was observed in our study that EELLI exhibited significant sub acute anti-inflammatory activity with respect to wet weight ($p<0.05$), dry weight ($p<0.05$) and transudative weight ($p<0.05$) at the dose of 150mg/kg and with respect to wet weight ($p<0.05$), dry weight ($p<0.05$) and transudative weight ($p<0.05$) at the dose of 300mg/kg by cotton pellet induced granuloma pouch in Wistar albino rats compared to control with significant mean percentage of inhibition of sub acute inflammation.

Conclusion

Based on our previous preliminary phytochemical screening and results of the present study, EELLI has shown sub-acute anti-inflammatory activity in animal model and this could become a potential drug in conditions with chronic inflammations. As the cost and side / adverse effects of modern drug's influences greatly on health as well as economy state of an individual, we opine that this drug should go for the benefit of the mankind in future.

Acknowledgements

The authors are thankful to Dr.S.N Rao, Senior Prof. & HOD for providing required facilities to carry out this research work in the Dept of Pharmacology, Yenepoya Medical College, Yenepoya University, Derlakatte, Mangalore 575018, India.

References

1. Denko CW: A role of neuropeptides in inflammation. In: Whicher JT, Evans SW Biochemistry of inflammation. Kluwer Pub. London 1992; pp. 177-181.

2. Cotran RS, Kumar V, Collins T, 2001: Robbins pathological basis of disease. 6th ed. WB Saunder's company, 2001; p.51.

3. Ogunyemi A. O. The origin of herbal cure and its spread; proceedings of a conference on African medicinal plants. Sofowora A. (Ed.) University Press, Ile-Ife. 1979; Pg. 20-22

4. Sofowora A. Medicinal Plants and Traditional medicine in Africa. John Wiley and Sons Ltd. 1st edition. 1982; 131: 168 –171

5. Chandrashekar R, S. N. Rao. Acute anti-inflammatory activity of ethanolic extract of leaves of *Leucas indica* by carrageenan induced paw oedema in Wistar albino rats. International Journal of Basic & Clinical Pharmacology. 2013; 2(3):302-305.

6. Swingle KF, Shideman FE. Phases of the inflammatory response to subcutaneous implantation of a cotton pellet and their modification by

certain anti-inflammatory agents. J Pharmacol Exp Ther. 1972; 183(1):226-34.

7. Panthong A, Kanjanapathi D, Taesotikul T, Wongcome T, Reutrakul V. Anti-inflammatory and antipyretic properties of *Clerodendrum petastites* S. Moore J Ethnopharmacol. 2003; 85:151–6.

8. Chandrashekar.R, Rao. S.N."Phytochemical analysis of ethanolic extract of leaves of *Leucas indica* (EELLI)". International Journal of Pharma and Bio Science. 2012; 3(4): (P) 673 – 677.

9. Awad AB, Toczek J, Fink CS. Phytosterols decrease prostaglandin release in cultured macrophages. Prostaglandins Leukot Essent Fatty Acids. 2004; 70(6):511-520.

10. Navarro A, De las Heras B, Villar A. Anti-inflammatory and Immunomodulating Properties of a Sterol fraction from *Sideritis foetens* Clem. Biol PhaBull. 2001; 24(5):470-473

11. J.L. Ríos, M.C. Recio, S. Máñez, R.M. Giner. Natural triterpenoids as anti-inflammatory agents. Studies in Natural Products Chemistry.2000; 2: 93–143.

12. Lindenmeyer MT, Tubaro A, Sosa S, Merfort I. New sesquiterpene lactones from *Arnica tincture* prepared from fresh flower heads of Arnica Montana. Planta Med. 2005; 71:1044-52.

13. Ferrante A, Seow WK, Rowan-Kelly B, Thong YH. Tetrandrine, a plant alkaloid, inhibits the production of tumour necrosis factor-alpha (cachectin) by human monocytes. Clin Exp Immunol. 1990; 80:232-5.

14. Teh BS, Seow WK, Li SY, Thong YH. Inhibition of prostaglandin and leukotriene generation by the plant alkaloids tetrandrine and berbamine. J Immunopharmacol. 1990; 12:321-6.

15. Serafini M, Peluso I, Raguzzini A. Flavonoids as anti-inflammatory agents. Proc Nutr Soc. 2010; 69(3):273-278.

16. Azuma Y, Ozasa N, Ueda Y, Takagi N. Pharmacological studies on the anti-inflammatory action of phenolic compounds. J Dent Res. 1986; 65(1):53-56.

17. Mota ML, Thomas G, Barbosa Filho JM. Anti-inflammatory actions of tannins isolated from the bark of *Anacardium occidentale* L. J Ethnopharmacol. 1985; 13(3):289-300.

18. Gomes A, Sharma RM, Ghatak BJ. Pharmacological investigation of a glycosidal fraction isolated from *Maesa chisia* D. Don var. angustifolia Hook f and Th. Indian J Exp Biol. 1987; 25:826-31.

19. Sun HX, Xie Y, Ye YP. Advances in saponin-based adjuvants. Vaccine. 2009; 27(12):1787-96.

20. ME Arrigoni. Inflammation and anti-inflammatory, Spectrum publication Inc., New York, 1988; 119-120