Asian Journal of Pharmaceutical Research and Development
(An International Peer-Reviewed Journal of Pharmaceutical Research and Development)

AJPRD

Open Access Available online at http://ajprd.com/index.php Research Article

BIOLOGICAL EVALUATION OF *SAUSSUREA LAPPA* ROOT EXTRACT FOR ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY

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ABSTRACT

The present study was designed to evaluate the Phytochemical Screening for Analgesic and Anti- inflammatory activities of Ethonolic extract of *Saussurea lappa*. The collected plant root material were washed, shade dried, and size reduced into small pieces. Dried materials were coarsely powdered and macerated with petroleum ether for 72 hrs. The plant material was extracted by using ethanol by the cold maceration method. The study concluded that the root extract have potential bioactive substances that may be used to formulate new drugs. The Flavo-Glycosides content in Ethonolic extract of *Saussurea lappa* root was extracted and results were compared with the standard for Analgesic and Anti- inflammatory activities. The results were compared with the standard using statistical method. However further investigation was required for structural elucidation and pharmacological properties.

Keywords: *Saussurea lappa*, Ethonolic, Glycosides, Analgesic, Anti-inflammatory.

Article Info: Received 11 July, 2018; Review Completed 13 Aug 2018; Accepted 19 Aug 2018

Cite this article as:

Juluri Krishna Dutta Tejaswi, Biological Evaluation Of *Saussurea Lappa* Root Extract For Analgesic And Anti- Inflammatory Activity,Asian Journal of Pharmaceutical research and Development.**2018;6 (4): -35-38**

DOI: http://dx.doi.org/10.22270/ajprd.v6.i4.378

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INTRODUCTION

Pain is an unpleasant feeling often caused by intense or damaging stimuli. Most pain resolves promptly once the painful stimulus is removed and the body has healed, but sometimes pain persists despite removal of the stimulus and apparent healing of the body; and sometimes pain arises in the absence of any detectable stimulus, damage or disease. Psychological factors such as social support, hypnotic suggestion, excitement, or distraction can significantly modulate pain's intensity or unpleasantness.Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. Inflammation is a protective response involving host cells, blood vessels, and proteins and other mediators that is intended to eliminate the initial cause of cell injury, as well as the necrotic cells and tissues resulting from the original insult, and to initiate the process of repair. The classical signs of acute inflammation are pain, heat, redness, swelling, and loss of function. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues.To solve these problems, new approaches had been urgently needed on the use of plant based natural products. *Saussurea lappa* [1-4] is found at an altitude of 2,500 to 3,000m in Kashmir and neighboring Himalayan regions. From the literature review [5-7] plant was reported to be rich in Costunolide, Sitosterol, α and β-Cyclocostunolide, Guiainolides, Cynaropicrin, Reynosin, Saussurealdehyde, Isodehydrocostus-Lactone-15-Aldehyde, 11,13-Epoxy-IsozazulaninC, Lignin Glycoside, Sesquiterpenes, Guiainolides, Lappalone and 1, 6-Dihydroxycostic acid ethyl ester were isolated. In

view of this, the present study was aimed to carryout biological evaluation [8-9] of the obtained Flavo-Glycosides content in Ethonolic extract of *Saussurea lappa* [10-12] root extract for Analgesic and Anti-inflammatory activities was determined.

MATERIAL AND METHOD [13]

Preparation of Plant Extract

The aerial plants of *Saussurea lappa* (Asteracae) a branched herb were collected from the Tirupathi region, A.P. India. The collected plant root material were washed with tap water to removing adhering dust followed by distilled water, shade dried, and final product was filtered, dried and stored in polythene bags for use. The extractive values were also found to be within the limits. The % yield was calculated 1.5 and 1.7% respectively.

Animals

Albino rats (Wistar strain) of either sex weighing between 250-300 g and Albino mice of either sex weighting 20-30g and animals were acclimatized for seven days under laboratory conditions. The animals were fed with commercially available rat pellet diet.

RESULTS AND DISCCUSSION

Tail Immersion Method

The tail immersion method was used to evaluate the central mechanism of analgesic activity. On the test day, albino swiss mice were divided into 4 groups of 6 mice each. Here Aspirin (20 mg/kg) is used as standard drug as well. Ethanolic extract of *Saussurea lappa* was subjected to evaluate for analgesic activity by tail immersion method using mice as animal model. Aspirin (100 mg/kg) was taken as standard drug. Ethanolic extract of root of the *Saussurea lappa* shows moderate activity. After administration of standard and test drugs, the basal reaction time was measured by immersing the tail tips of mice (last 1-2 cm) in hot water of water bath, where temperature was previously adjusted at 51ºC. When compared with control was shown significant activity (*P<0.001)*, and compared with standard was shown to equal activity at concentration of SLEE 200 mg/kg body weight. Above results are seen in the **Table 1, Table 2** and **Fig.1**.

Table 1: Group and Dose given in Tail Immersion Method.

Group	Dose
A	Control Normal Animals Treated with Vehicle (Distilled Water).
B	Standard Aspirin (20 mg/kg body weight).
C	Dose of SLEE (100mg/Kg body weight).
D	Dose of SLEE (200mg/Kg body weight).

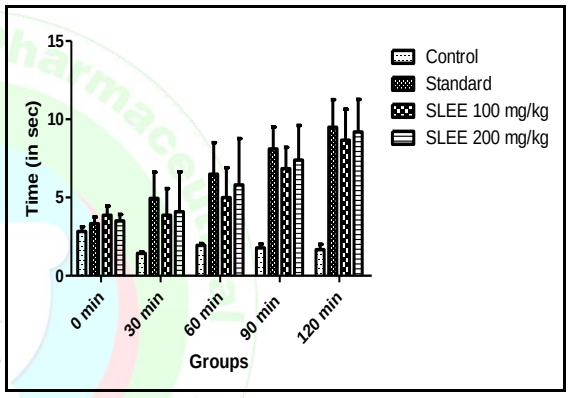

Fig.1: Analgesic Effect on Tail Immersion Method.

Table 2: Analgesic Effect of the Ethanolic Extract by Tail Immersion Test.

Group	Reaction Time (second) ± SEM				
	0 min	30 min	60 min	90 min	120 min
A	2.83 ± 0.30	1.43 ± 0.09	1.94 ± 0.12	1.78 ± 0.25	1.67 ± 0.34
B	3.33 ± 0.42	4.95 ± 1.67**	6.49 ± 2.01***	8.11 ± 1.40***	9.49 ± 1.77***
C	3.87 ± 0.59	3.87 ± 1.70*	4.99 ± 1.92**	6.84 ±1.37***	8.67± 1.98***
D	3.50 ± 0.42	4.10 ± 2.54**	5.80 ± 2.97***	7.39± 2.22***	9.20± 2.07***

-p< 0.05, **-p< 0.01, *- P<0.001 - Individual readings were compared with readings of normal control using One way ANOVA followed by tukey test. (n = 6).*

Eddy's Hot Plate Method

In this experiment, the analgesic activity of Plant extract was assessed in male albino mice, as per the method described by Eddy and Leimbach. Overnight fasted male albino mice were placed individually on a thermostatically controlled heated metal plate (Ugo Basile, Italy) within a restraining Perspex cylinder and the reaction time of each mouse was recorded. The temperature of the hot plate was maintained at 55 ± 0.5°C. The reaction time was considered as the time elapsed between placing of the mouse on the hot plate and appearance of signs of acute discomfort, characterized by flicking or licking of the hind paw, forepaw or jumping in an attempt to escape from the pain. The mice showing initial reaction time of 10 sec or less were selected for this study and were divided into 4 groups (6 in each group). After regrouping, animals in Groups II to IV were orally administered with the test substance, Plant extract at the dose rate of 100 and 200 mg/kg body weight, respectively. Group II received the standard drug Aspirin at the rate of

mg/kg i.p. and the control group (Group I) received a comparable volume of vehicle. When compared with control was shown significant activity (*P<0.001)*, and compared with standard was shown to equal activity at concentration of SLEE 200 mg/kg body weight. Results were shown in the **Table 3, Table 4** and **Fig.2**.

Table 3: Group and Dose given in Eddy's Hot Plate Method.

Group	Dose
A	Control Normal Animals Treated with Vehicle (distilled water).
B	Standard Aspirin (20 mg/kg body weight).
C	Dose of SLEE (100mg/Kg body weight).
D	Dose of SLEE (200mg/Kg body weight).

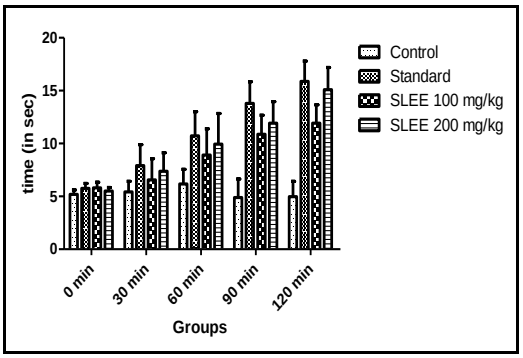

Fig.2: Analgesic Effect on Hot Plate Method.

Table 4: Analgesic Effect of the Ethanolic Extract by Eddy's Hot Plate Method

Group	Reaction Time (second) ± SEM				
	0 min	30 min	60 min	90 min	120 min
A	5.19 ± 0.42	5.42 ± 1.01	6.17 ± 1.39	4.89 ± 1.75	4.97 ± 1.45
B	5.76 ± 0.47	7.91 ± 1.98**	10.73 ±2.27***	13.79 ± 1.91***	15.88 ± 1.91***
C	5.81 ± 0.54	6.56 ± 2.00**	8.91 ± 2.49 **	10.87 ±1.80***	13.03 ± 1.72***
D	5.50± 0.34	7.37 ± 1.76**	9.94 ± 2.90**	11.93 ± 2.03***	15.09 ± 2.10***

***- P<0.01, ***- P<0.001 - Individual readings were compared with readings of normal control using One way ANOVA followed by tukey test. (n = 6).*

Paw Edema Modal

Paw Edema was induced by an intradermal injection of 0.1 ml of carrageenin (1% in normal saline solution) into the plantar surface of the right hind paw of rats (method of winter, Risley and Nuss). The acute phase of inflammatory reaction, i.e. edema volume of right hind paw was determined using Aplethysmometer modified by Hardayal Singh and Ghosh prior to and 30, 60 and 120 minutes after carrageenin injection. During in vivo anti-inflammatory activity, the paw edema was not reduced significantly in Formalin induced albino rats through introduction of Ethanolic extracts at a dosage 200 mg/Kg. The Ethanolic extract of Aerial part of *Saussurea lappa* has failed to show the significant Anti-inflammatory activity when compared with Standard drug i.e. Indomethacin. All the drugs were administered one hour prior to carrageenin. When compared with control was shown significant activity (*P<0.001)*, and compared with standard was shown to equal activity at concentration of SLEE 200 mg/kg body weight. Anti inflammatory activity results are shown in the **Table 5, Table 6** and **Fig.3**. Percentage inhibition of paw edema was calculated using the following formula.

% inhibition at given time interval =

(Paw volume in control group - paw volume in test group) X 100

Paw volume in control group

Table 5: Group and Dose given in Paw Edema Modal.

Group	Dose
A	Control (Normal saline 10ml/kg; p.o).
B	Disease Control (1.3ml of formalin solution).
C	Standard Drug - Indomethacin (10 mg/kg, p.o.).
D	Ethanolic Extract of *Saussurea lappa* (200mg/kg).
E	Ethanolic Extract of *Saussurea lappa* (400 mg/kg).

Table 6: Anti-Inflammatory Effect of Ethanolic Extract by Paw Edema Modal.

Group	Paw Edema Volume in cm ±				
	0 min	60 min	120 min	180 min	240 min
A	3.17±0.07	3.17±0.07	3.17±0.07	3.17±0.07	3.17±0.07
B	3.120±0.01	3.370±0.14	3.540±0.07	3.660±0.07	3.730±0.06
C	3.150±0.04	3.450±0.09	3.390±0.07	3.250±0.05	3.120±0.01
D	3.200±0.03	3.490±0.04	3.450±0.07	3.390±0.02	3.350±0.02
E	3.190±0.09	3.460±0.09	3.420±0.11	3.310±0.13	3.230±0.07

$ -P<0.05 – Disease control compared with normal control group,-P<0.05, **-P<0.01, ***- P<0.001 - Individual readings were compared with readings of disease control using One way ANOVA followed by Tukey test. (n = 6)*

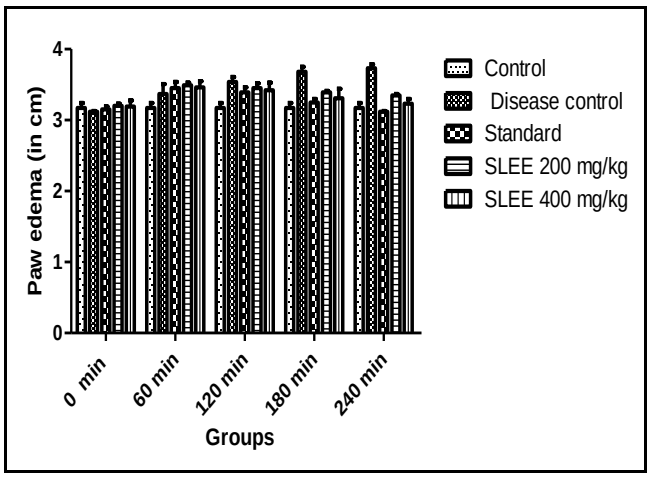

Fig.3: Anti-Inflammatory Activity on Paw Edema Modal.

CONCLUSION

The *Saussurea lappa* root was extracted, separated and isolated. The Ethonolic extract was first prepared and was screened for various phytochemical constituents. From this Ethonolic extract Ethyl acetate fraction was obtained using Solvent- Solvent separation technique. The fraction was screened for spectral studies of Phyto-constituents. The Ethyl acetate fraction of the methanol extract was found to contain Flavonoids and Glycosides. Furthermore evaluation was done for the Analgesic and Anti- inflammatory activities of the *Saussurea lappa* root extract which showed the better activity.

ACKNOWLEDGEMENT

Authors are thankful to Hindu College of Pharmacy, Amaravathi Road, Guntur, Andhra Pradesh, India to provide the infrastructures to pursue the work.

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