

**EVALUATION OF ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF
SALVIA LEUCANTHA ON EXPERIMENTAL ANIMAL**

ABSTRACT:

The study investigates the analgesic and anti-inflammatory potential of *Salvia leucantha* using experimental models in Wistar albino rats and Swiss albino mice. Pain and inflammation, commonly treated with NSAIDs, can lead to adverse effects, prompting the exploration of plant-based alternatives. The ethanolic extract of *Salvia leucantha* was tested using Eddy's hot plate and acetic acid-induced writhing methods for analgesic activity, and carrageenan-induced paw oedema for anti-inflammatory assessment. The results demonstrated a significant increase in pain threshold and reduction in inflammatory response, suggesting that *Salvia leucantha* possesses substantial therapeutic properties comparable to standard drugs like diclofenac and indomethacin.

Keywords:

Salvia leucantha, Analgesic activity, Anti-inflammatory, Experimental animals

1. INTRODUCTION:-

Pain is a complex sensory and emotional experience associated with actual or potential tissue damage. In animals, as described by Zimmerman, pain is an aversive sensory experience that triggers protective motor and vegetative reactions, promotes learned avoidance behaviours, and can influence species-specific behaviours, including social interactions. Inflammation is the body's natural response to harmful substances, characterized by vasodilatation, and the movement of fluid and immune cells to the affected tissue. Pain, which can arise from nociceptor activation or the release of inflammatory mediators, is one of the main symptoms of inflammation. Inflammation plays a crucial role in protecting the body by eliminating the source of injury (e.g., pathogens, toxins) and clearing away damaged cells and tissues. However, while inflammation is a protective response, it can sometimes become chronic or excessive. This uncontrolled inflammation can lead to conditions such as chronic pain, contractures, disfigurement, and functional impairments. To manage these issues, anti-inflammatory treatments are necessary to control or inhibit inflammation and prevent long-term damage (Lalan *et al.*, 2015).

The most popular medications for treating pain and inflammatory diseases are nonsteroidal anti-inflammatory medicines (NSAIDs). However, they can also cause typical adverse effects such kidney issues, ulcers, and bleeding. Consequently, therapeutically useful chemical compounds with fewer adverse effects are frequently sourced from medicinal plants. As a result, the pursuit of innovative, safe, and effective analgesic and anti-inflammatory medications is justified (Saha *et al.*, 2013).

For thousands of years, humans have depended on natural ingredients as a source for medicines. Traditional medical practices that have been used for generations in various nations including Egypt, China, and India are based on plant-based medications. Plant-based medicines are still crucial to modern

healthcare. The World Health Organization has estimated that 80% of the world's population relies mostly on traditional medicines for their basic healthcare. At least 119 compounds, originating from 90 plant species, are now recognized in one or more nations as significant medications.

Salvia species have been utilized for a variety of reasons since antiquity, including the fragrance business, as well as for culinary and medicinal uses. Around 230 genera and 7100 species of plants belong to this family worldwide. Several *Salvia* species have been the subject of numerous phytochemical and biological research (Dinesh *et al.*, 2022).

Salvia leucantha Cav., often known as Mexican bush sage or velvet sage, is a plant that originated in Mexico and is now found all over the world, especially in North and South America (Rojas *et al.*, 2010).

salvia's name comes from the Latin word *salvere*, which meaning "to heal." Indeed, the medicinal properties of this plant are well acknowledged (Ben *et al.*, 2023).

Salvia leucantha contains a variety of chemical components, with bornyl acetate and sesquiterpene hydrocarbons being prominent, suggesting environmental factors influence its composition. Phytochemical analysis revealed diterpenoids like salvigenane, isosalvipuberulan, and spiroleucantholide, a modified neo-clerodane structure. Triterpenes were also identified in the leaves. A study of *S. leucantha* grown in India found bornyl acetate (23.9%), β -caryophyllene (13.9%), and other compounds as major constituents. The plant's essential oil (EO), isolated via hydrodistillation from leaves grown in Mérida, Venezuela, contains volatile components that give it a pleasant fragrance. Sesquiterpene hydrocarbons dominate in studies from Asia and South America. EOs, due to their odorant and biological properties such as antimicrobial, antioxidant, anti-inflammatory, antiviral, antimutagenic, and anticancer effects, are used in various industries including fragrance, food flavoring, medical microbiology, and pharmaceuticals (Villalta *et al.*, 2021).

2. MATERIALS AND METHODS

2.1. Description of the plant

The evergreen shrubby perennial Mexican bush sage (*Salvia leucantha*) is prized for its dense, arching spikes and capacity to produce a lovely late summer burst of beautiful flowers. The plant's bi-colored blossoms, which have purple calyces and white corollas, are velvety and hairy to the touch. Beautiful cascades of velvety purple blooms will bloom in the fall, luring butterflies, hummingbirds, and other helpful insects and wildlife. The ten-inch-long blossoms of the Mexican bush sage rise above its soft green leaves, which has a faint silvery tinge. It has gray-green, velvet-like leaves that develop in pairs on square stalks. It has numerous erect stems, many of which arch at their tips, and long inflorescences. It can reach heights of up to 1.3 m (4.3 feet) and a width of 2 m (6.6 feet). The linear-lanceolate leaves have pale, hairy undersides and a delicate mid-green color (Haung *et al.*, 2002).

2.2. Collection of the plant material and authentication

Fresh leaves of *Salvia leucantha* were obtained from college garden of Acharya institute Bangalore. The plant leaves were authenticated by Indian institute of Horticulture Research (IIHR).

2.3. Preparation of extract

Salvia leucantha leaves were collected at once. The leaves were washed and sun dried for 2-3 days. The leaves were then grinded to coarse powder. Coarse powder was extracted by soxhlation 100 g in 500 ml of ethanol (70% v/v) for one week using Soxhlet apparatus. After the completion of extraction, the extract was dried in hot air oven at 50°C for 1h. The dried extract was stored in dry container at room temperature (Pullani *et al.*, 2021).

2.4. Animals

Wister albino rats (150-200 g) and Swiss albino mice (20-30 g) of either sex was selected for studies. Animals procured for a registered CPCSEA vendor. The animals housed was maintained under standard laboratory conditions at 25± 1°C under 12/12 h light/ dark cycle and fed with standard pellet diet and water ad libitum. The experiment was carried out according to the guidelines of the committee for the purpose of control and supervision of Experiments on animals (CPCSEA), New Delhi, India and the study protocol was approved by the Institutional Animal Ethical Committee (IAEC) of Acharya & BM Reddy college of pharmacy, Bengaluru with Ref: **IAEC/ABMRC/2021-2022/33**.

2.5. EXPERIMENTAL PROTOCOL

2.5.1. Analgesic activity:

2.5.1.1. Hot plate test in Rats:

Wistar albino rats (150-200 g) were given the treatment described by Eddy and Liembach in 1953. Rats were screened on a hot plate (UGO Basile, Italy). The temperature was kept constant at 55±1°C, and the reaction time was measured in seconds. The time spent licking one's paws or jumping on the hot plate was considered a reaction time. The responses were recorded before and after the administration of normal saline, diclofenac sodium, and *salvia leucantha* extract control, standard and treatment groups for 30, 60 and 90 min respectively. To avoid injury to the animals, a cut-off time of 15 sec was used (Simpi *et al.*, 2021).

The rats were divided into three groups, each with four rats.

- **Group I** - Control (Normal saline, 2 ml/kg , p.o.)
- **Group II** - Standard (Diclofenac sodium, 9 mg/kg, p.o.)
- **Group III** - Treatment (*Salvia leucantha*, 100 mg/kg, p.o.)

2.5.1.2. Acetic acid induced writhing in mice

Mice were given, Normal saline, diclofenac sodium and *salvia leucantha* extract orally after 60 minutes of receiving acetic acid solution at a dose of 10 ml/kg. Female Swiss albino mice (25-30 g) were treated with g (0.6% i.p) according to the method described. The number of abdominal constrictions (both hind paws full extension) were counted cumulatively over a 15-min period.

The mice were divided into three groups, each with four mice.

- **Group I** - Control (Normal saline 2 ml/kg, p.o.),
- **Group II** - Standard (diclofenac sodium 9 mg/kg, p.o.)
- **Group III** - Treatment (*Salvia leucantha* 100 mg/kg, p.o.)

The writhing inhibition percentage was calculated as follows: $(VC-VT/VC) * 100 = \% \text{ Inhibition}$ Where VT denotes the number of writhes in drug- treated mice and VC denotes the number of writhes in control group (Simpi *et al.*, 2021).



Figure no: 01 Mice showing writhes (stretching of hind limb)

2.5.2. ANTI-INFLAMMATORY ACTIVITY:

2.5.2.1. Carrageenan induced paw oedema in rats:-

Winter *et al.*, 1962 described a method for treating Wistar albino rats (180 - 220 g). To induce inflammation, 0.1 ml of 1% lambda carrageenan in sterile normal saline was injected into the sub plantar region of the rat's right hind paw. Rats were induced with carrageenan injection 1 h before administration of indomethacin and *salvia leucantha* orally. The paw volume was measured at hourly intervals from 0 to 4 h using a plethysmometer (Ugo Basile, Italy, Model No. 7140). The mean changes in injected paw volume relative to initial paw volume were computed (Simpi *et al.*, 2021).

- **Group I** - Control (Normal saline, 2 ml/kg, p.o.)
- **Group II** - Standard (Indomethacin ,10 mg/kg, p.o.)

- **Group III - Treatment** (*Salvia leucantha*, 100 mg/kg, p.o.).



Figure No: 02 Carrageenan induced paw oedema

3. RESULT

3.1.PHYTOCHEMICAL SCREENING TEST FOR VARIOUS CHEMICAL CONSTITUTE

SL.NO	TEST	OBSERVATION	RESULT
1.	Carbohydrates	Fehling's test (yellow ppt)	Positive
2.	Alkaloids	Wagner's test (reddish brown ppt)	Positive
3.	Triterpenoids	Extract dissolve in acetic acid anhydride boil. Cool and H ₂ SO ₄ (reddish violet color)	Positive
4.	Flavonoids	To the 3ml of extract add 10% NH ₄ OH (yellow fluorescent)	Positive
5.	Proteins	Biuret test	Negative

Table. No: 01 phytochemical test for various chemical constitute

3.2. ANALGESIC ACTIVITY

3.2.1. EDDY'S HOT PLATE METHOD:-

In hot plate test, *salvia leucantha* (100 mg/kg) significantly increase the paw withdrawal latency at 60min and 90-min. Onset of action observed at 60 min of an administration of *salvia leucantha*. however, Diclofenac sodium (9 mg/kg) significantly inhibit the pain produce by thermal means.

GROUP	REACTION TIME (SEC)		
	30 min	60 min	90 min
Normal saline (2ml/kg)	1.47 ± 0.1842	1.27 ± 0.1604	1.273 ± 0.1713
<i>Salvia leucantha</i> (3.63ml/kg)	3.045 ± 0.4066*	3.558 ± 0.3442**	3.8 ± 0.8968**
Diclofenac sodium (1.12ml/kg)	3.425 ± 0.4644**	3.925 ± 0.5573**	4.045 ± 1.144**

Table. No:02 Effect of oral administration of Diclofenac sodium and *salvia leucantha* extract on hot plate test in rats

Values were expressed as MEAN ± SEM(n=4). Data was analyzed by ANOVA followed by Dunnett test., *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ is considered as significant on comparing control Group with treatment and standard.

3.2.2. ACETIC ACID INDUCED WRITHING MODEL

In test, *salvia leucantha* (100 mg/kg) significantly decrease number writhing's. Onset of action observed at 60 min of an administration of *salvia leucantha*. however, Diclofenac sodium (9 mg/kg) significantly inhibit the writhing.

No. Of Group	Group	No. Of Writhing	% Inhibition
1	Control (Acetic acid 2 ml/kg)	28.5±6.461	-
2	Treatment (<i>salvia leucantha</i> 1.17 ml/kg)	7±1.78**	75.4

3	Standard (diclofenac sodium 0.8 ml/kg)	9.75±1.109*	65.7
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Table. No: 03 Effect of *Salvia leucantha* and diclofenac sodium on acetic acid induced animals

Values are expressed as MEAN ± S.E.M.; n=4 mice per group. ANOVA followed Dunnett test, ****p* <0.001, ***p*<0.01, **p*<0.05 is considered as significant on comparing control Group with treatment and standard.

3.3. ANTI-INFLAMMATORY ACTIVITY

3.3.1. CARRAGENAN INDUCED PAW OEDEMA

There was gradual increase in paw volume of rats in the carrageenan control group. In the test groups, *salvia leucantha* (100 mg/kg) showed a significant reduction in paw volume in a dose dependent manner at 1st h and 2nd h. The inhibitory effect of the *salvia leucantha* (100 mg/kg) was found to be 43.99% at 1 h and 88.1% at 2 h when compared to control group.

GROUP	PAW VOLUME		
	0 h	1 h	2 h
Normal saline (2ml/kg)	0.5425 ±0.0686	1.395 ± 0.04173	0.76 ± 0.08396
<i>salvia leucantha</i> (3.63 ml/kg)	1.03± 0.0279***	2.008 ± 0.05138***	1.32± 0.06096**
Diclofenac sodium (1.12 ml/kg)	0.8225±0.03794**	1.95± 0.03342**	1.43± 0.1446*

Table. No: 04 Effect of indomethacin and *salvia leucantha* on carrageenan induced paw oedema in rats

Values are expressed as MEAN ± S.E.M.; n=4 mice per group. ANOVA followed Dunnett test., ****p* <0.001, ***p*<0.01, **p*<0.05 is considered as significant on comparing control Group with treatment and standard.

4. STATISTICAL ANALYSIS

Statistical analysis using one-way ANOVA and Dunnett's test showed that *Salvia leucantha* significantly increased reaction time in the hot plate test ($p < 0.01$), reduced writhing in the acetic acid model ($p < 0.01$), and decreased paw oedema in the carrageenan model ($p < 0.01$). These results confirm its significant central and peripheral analgesic, as well as anti-inflammatory, activity.

5. DISCUSSION

In the present investigation leaves of *salvia leucantha* family of Lamiaceae, was selected for evaluation of analgesic and anti-inflammatory activity. *Salvia* species have been utilized for a variety of reasons since antiquity, including the fragrance business, as well as for culinary and medicinal uses. Around 230 genera and 7100 species of plants belong to this family worldwide. Several *Salvia* species have been the subject of numerous phytochemical and biological research (Upadhyaya *et al.*, 2009). The oil was also screened for its antioxidant activity facilitated by scavenging the free radical DPPH and lipid peroxidation and investigated potential of *salvia leucantha* cav on ehrlich Ascites carcinoma cell line (Sridharan *et al.*, 2012). Antibacterial activity in both gram positive and gram-negative bacteria, *Salvia leucantha* possess significant antibacterial and wound healing activity (Rajamanickam *et al.*, 2013). The *Salvia leucantha* contains various chemical constituent as fragrant volatile component, bornyl acetate and sesquiterpene hydrocarbons were found to be widespread. Which indicates that environmental influence may have a significant impact. According to recent research of *salvia leucantha* grown in India, bornyl acetate (23.9%), caryophyllene (13.9%) guanine (12.6%), cismuurola-3,5-diene (10.8%), germacrene D (13.8%), and (8.7%) were the primary components. (Villalta *et al.*, 2021).

The present study evaluated the phytochemical composition and pharmacological effects of *Salvia leucantha* extract, focusing on its analgesic and anti-inflammatory properties. The findings from the phytochemical screening confirmed the presence of biologically active compounds such as carbohydrates, alkaloids, triterpenoids, and flavonoids, while proteins were absent. These constituents are well-documented for their roles in modulating pain and inflammation, suggesting that the observed pharmacological effects could be attributed to their presence.

In the **Eddy's hot plate test**, the extract significantly increased the paw withdrawal latency in rats, especially at 60 and 90 minutes post-administration, indicating central analgesic activity. The response was comparable, though slightly less potent, than that of the standard drug diclofenac sodium. This suggests that *Salvia leucantha* may exert its effects through central nervous system pathways, possibly involving modulation of opioid receptors or other neurotransmitter systems.

Similarly, in the **acetic acid-induced writhing test**, which is commonly used to evaluate peripheral analgesic activity, the extract showed a significant reduction in the number of writhes. The inhibition percentage (75.4%) was higher than that of the standard drug diclofenac (65.7%), indicating strong peripheral analgesic potential. This result implies that the extract may interfere with the release of endogenous pain mediators such as prostaglandins.

The **anti-inflammatory activity**, assessed using the carrageenan-induced paw edema model, also demonstrated promising results. *Salvia leucantha* showed a significant reduction in paw volume at both 1 and 2 hours post-carrageenan injection. The maximum inhibition (88.1%) at the 2nd hour indicates that the extract is effective in both the early and late phases of inflammation. These phases involve the release of various mediators such as histamine, serotonin, bradykinin, and prostaglandins. The dose-dependent inhibition observed suggests that *Salvia leucantha* may suppress multiple inflammatory pathways.

Overall, the study demonstrates that *Salvia leucantha* possesses both central and peripheral analgesic effects, as well as significant anti-inflammatory activity. These effects may be attributed to its phytochemical constituents, particularly flavonoids and triterpenoids, which are known for their ability to modulate inflammatory and pain pathways. The results support the traditional use of *Salvia leucantha* in pain and inflammation management and provide a basis for further pharmacological and mechanistic investigations.

6. CONCLUSION

The present study demonstrates that the extract of *Salvia leucantha* possesses significant central and peripheral analgesic, as well as anti-inflammatory, activities. These effects are likely due to the presence of active phytoconstituents such as flavonoids, triterpenoids, and alkaloids. The extract showed a marked increase in reaction time in the hot plate test, a strong inhibition of acetic acid-induced writhing, and substantial reduction in carrageenan-induced paw oedema. These findings scientifically support the traditional medicinal use of *Salvia leucantha* in the treatment of pain and inflammation and suggest its potential as a natural therapeutic agent. Further studies are warranted to isolate specific active compounds and explore their mechanisms of action.

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