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In Vivo Studies Using Herbal Ointment Made Of Methanolic Extract Of Elaeocarpus Ganitrus Leaves And Cymbopogon Citratus Oil For Treating Pain In Albino Wistar Rats.

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Abstract:
***Background**: **Elaeocarpus ganitrus** is an evergreen tree with a hard and extremely attractive stone endocarp known as bead or nut and is well known in India as rudrakasha. Traditionally, the herb has been used to cure mental illnesses, hypertension, and epilepsy among other ointments. **Cymbopogon citratus** is an aromatic perennial tall grass commonly known as Lemon grass with rhizomes and densely tufted fibrous roots. It is a commonly grown plant in India and is the major used in kitchens but has many medical properties and lemon grass tea is also used to relieve pain. In this ointment was formulated to check its analgesic activity for that methanolic extract of **Elaeocarpus ganitrus** leaf and **Cymbopogon citrate** oil is used. The phytochemicals present in the leaf extract are responsible for analgesic activity.*
***Materials and Methods**: Extract was prepared using the process of maceration, the solvent used is methanol. Ointment was prepared using the procedure in table no 1, and the extract was added as an API. Evaluation parameters were carried such as organoleptic characteristics, pH, spread ability, dissolution test, and in vivo studies such as: skin irritation test, hot plate test, formalin induced paw edema test, tail immersion test on rats. In this prospective randomised study, 12 healthy rats of Albino Wistar rat strain were used. The rats were divided into 4 groups containing 3 rats in each group. Group 1: Control group received no treatment, Group 2: standard group received the treatment of diclofenac ointment, Group 3: test group 1 received the treatment of ointment of **Elaeocarpus ganitus** extract, Group 4: test group 2 received the treatment of ointment prepared using extract + **Cymbopogon citratus** oil. The results of in vivo studies were compared by the rats of group 2 receiving the treatment of diclofenac ointment.*
***Results**: The ointment prepared has good strength, spreadability with no sign of skin irritation and the pH (6.80-7.02) was measured and found to be skin friendly. In vivo tests confirmed that ointment prepared did shows positive result in skin irritation test with no sign of edema or irritation, in hot plate test the paw licking time was noted, in tail immersion test the tail flick response was noted, in formalin induced paw edema test the flinching response was noted. Results showed that formulation 2 prepared using the extract and oil showed positive result then that of formulation 1 prepared using the extract.*
***Conclusion**: Two Ointments were prepared using the **Elaeocarpus ganitrus** extract and **Cymbopogon citratus** essential oil. In vivo results showed that formulation 2 showed much resistant in pain then that of formulation 1, and can be compared to the standard one diclofenac ointment.*
***Key Word**: Methanol, Pain, In vivo, Ointment, Adverse drug reaction, Maceration.*

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I. Introduction

Pain helps the body mend injured tissues and defends against further harm. But chronic pain continues to be a substantial clinical concern that, if left unaddressed, severely lowers the quality of life for those who experience it[1].

In 1996 the International Association for the Study of Pain (IASP) defined pain as 'an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage'.

Nociception as nociception serves as the body's defence system against harm or any injury, but acute pain results from avoidant behaviour and is controlled by a mesolimbic threshold process that opens the door from nociceptive activity to conscious pain[2,3].

Analgesics

Analgesics are commonly known as Painkillers; they are the medications that are used to treat acute or chronic pain. It is also known as nociperception.

There are many medications as well as ointments and sprays that are used to treat pain, there are also combination medications that include both analgesics and other types of drugs, like acetaminophen and codeine, to enhance pain relief[15].

Ointment

An oil-based topical formulation with a semi-solid texture and a greasy appearance that can be applied to the skin is called an ointment. As per the ointment meaning, the therapeutic substances are dispersed in the medium. The medium generally has 80% oil and 20% water[4].

Herbal Drugs

Since ancient times, herbal remedies have been used extensively around the world. Both doctors and patients have acknowledged the superior therapeutic benefits of herbal remedies, noting that they have less side effects than contemporary medications. Because medicinal plants have the potential to greatly help society or maybe all of humanity especially in the field of medicine, they are currently receiving more attention than ever before. By lowering the toxicity and adverse effects of medications while simultaneously increasing their bioavailability, the herbal treatment contributes to their increased therapeutic value[14].

Elaeocarpus ganitrus

Elaeocarpus ganitrus (Rudraksha) is a member of the *Elaeocarpaceae* family that grows primarily in India's imalayan area. They are referred to as the king of herbal medicine, with numerous spiritual and medicinal properties both preventative and curative. Elaeocarpus is a Greek term formed by combining the words Elaei and Carpus. Elaei means "wild olive" tree in Greek, and Carpus means "fruit," hence Elaeocarpus refers to the seed of wild olive-like trees[3,5].

Figure 1. *Elaeocarpus ganitrus* plant

Cymbopogon citratus

It is an aromatic grass belonging to the genus Cymbopogon that contains finely flavoured essential oils. Lemongrass is widely used as a vital component health in Asia. It is used as central nervous system sedatives in India[6,7].

Figure 2. *Cymbopogon citratus* leaves

II. Material And Methods

Ingredients:

Elaeocarpus ganitrus leaves, *Cymbopogon citratus* oil, Methanol, Bees wax, Hard paraffin, White soft Paraffin, Cetosteryl alcohol.

Preparation of extract:

Extract was prepared using the process of maceration, 50gm of leaves powder was soaked in 200ml methanol and was allowed to macerate for 24 hours, after 24 hours the solvent was filtered out and kept in China dish at water bath(60-700C) for evaporation, and the extract was obtained.

Two ointments were prepared using the extract: formulation 1: 40%w/w Ointment was prepared using the extract. Formulation 2: 40%w/w Ointment using the extract + *Cymbopogon citratus* essential oil.

Table no 1: Formulation Table

Chemicals	Formulation A	Formulation B	Uses
Wool Fat	1.25gm	1.25gm	Emollients
Hard Paraffin	1.25gm	1.25gm	Solid hydrocarbon obtained from petroleum
White soft paraffin	21.25gm	21.25gm	Hydrophobic and an oil soluble component
Cetostearyl Alcohol	1.25gm	1.25gm	Emollients and moisturizing agent
Elaeocarpus ganitrus extract	10gm	10gm	Active Pharmaceutical Ingredient
Cymbopogon citrates oil	NA	2 to 3 drops	Active Pharmaceutical ingredient

Study Design: Prospective open label observational study.

Animal Approval: The experiment was performed as per rules and guidelines of Institutional Animal Ethics Committee, School of Pharmacy & Emerging Sciences, Baddi University of Emerging Sciences & Technology, Baddi, Solan, Himachal Pradesh- 173205, Regd. No: BUEST/SPES/2024/002 Dated: 02.03.2024. IAEC approval certificate (Approval No: BUEST/SPES/2024/040) was issued for this experimentation.

Study Duration: June 2024 to June 2024.

Sample size: 12 Animals.

Sample size calculation: The sample size was estimated on the basis of a single proportion design. The species of rats chosen was Albino Wistar rats. We planned to include 12 animals (Group I- Control, Group II- standard group, Group III- test group 1, Gorup IV- test group 2 for each group).

Inclusion criteria:

- Species and strain: Albino Wistar rats
- Either sex
- Aged ≥ 7-8 weeks
- Weight: 150-180gm

Procedure methodology:

After the extraction of active pharmaceutical ingredient, ointment base was prepared, in formulation 1 only *Elaeocarpus ganitrus* extract was added whereas in formulation 2 *Elaeocarpus ganitrus* extract + *Cymbopogon citratus* oil was added. Some evaluation parameters of the ointment were carried out such as pH, organoleptic characteristics, Spread ability.

In vivo tests:

- **Skin Irritation test:** The ointment was undergone for skin irritation test to check of any sign of erythema or edema

  0 = No erythema/edema, 1 = Very slight erythema/edema, 2 = Well-defined erythema/edema, 3 = Moderate to severe erythema/edema, 4 = Severe erythema/eschar formation/edema.
- **Hot plate Method:** Each group of 3 rats of either sex with an initial weight of 150-180gm were used for each dose. The hot Plate, which was commercially available, consisted of electrically heated surface. The surface of the hot plate was heated to a constant temperature of 50-52° C. The latency was recorded before and after 30min, 1 hr, 2 hr and 3 hr following test compound (all polyherbal formulation).

- **Tail immersion Method:** The animals were divided into 4 different groups containing 3 animals in each group, the control group did not receive any treatment, the standard group's animal was treated with Diclofenac (30min) prior the test, test group 1 was treated with Test ointment 1 (30min) prior and test group 2 was test ointment 2 (30min) prior the test. response time was noted at 30min, 1hr, 2hr, and 3hr after the drug application.
- **Formalin induced paw edema test:** On the plantar surface of the right hind paw, 0.3gm of ointment was applied and gently rubbed 50 times with the index finger. This process was repeated with standard (Diclofenac), test group A and test group B. 30min later, 50µL of 2.5% formalin was administrated by an intraplanar injection into the dorsal right hind paw of rats. The number of flinching events of the paw was monitored between 0–5min (first phase) and 15–60min (second phase) after the injection of formalin.

Table no 2: Animal Grouping

Sr. no.	Group	No. of animal used
1.	Control group	3
2.	Standard group (Diclofenac ointment)	3
3.	Test Group 1 (Methanolic extract ointment) (40%w/w)	3
4.	Test Group 2 (Methanolic extract ointment + lemongrass oil) (40%w/w)	3

All the treatments will be topically administered and the animals will be caged 30 minutes before the experiment, the screening methods chosen do not cause any harm to the animals after 30 minutes the animals have undergone for the experiment. At the end of the experiment the tested groups are compared with the control and standard groups and other biochemical parameters will be evaluated for the formulation, the experiment will be carried out for 7 consecutive days.

III. Result

Phytochemical screening:

Table no 3. Phytochemical screening

Observation	Result
1. Alkaloids	+ve
2. Flavonoids	+ve
3. Terpenoids	+ve
4. Phenolic compounds	+ve
5. Glycosides	+ve
6. Tannins	+ve
7. Steroids	+ve
8. Saponins	+ve

The experiment was carried out for 7 days consecutive days in rats.

Skin irritation test:

Table no 4: Skin irritation evaluation

Group of animals	Formulation A		Formulation B	
Time observed	After 4 hours	After 24 hours	After 4 hours	After 24 hours
Group A	0	0	0	0
Group B	0	0	0	0

Table 4 shows, both the ointment showed no sign of skin irritation, redness or swelling or any discomfort to the rat hence no erythema/ edema and was safe to use for the further testing.

Hot Plate Method (Eddy's Hot Plate):

Table no 5: Hot Plate Test

Treatment	Reaction time in seconds			
	30 min	60 min	120 min	180 min
Control(n=2)	1	2.5	1.5	2.0
Standard(n=2)	15	15	12.4	11.5
Test group 1(n=2)	11	10.4	9.5	8
Test group 2(n=2)	14	13.5	12	11

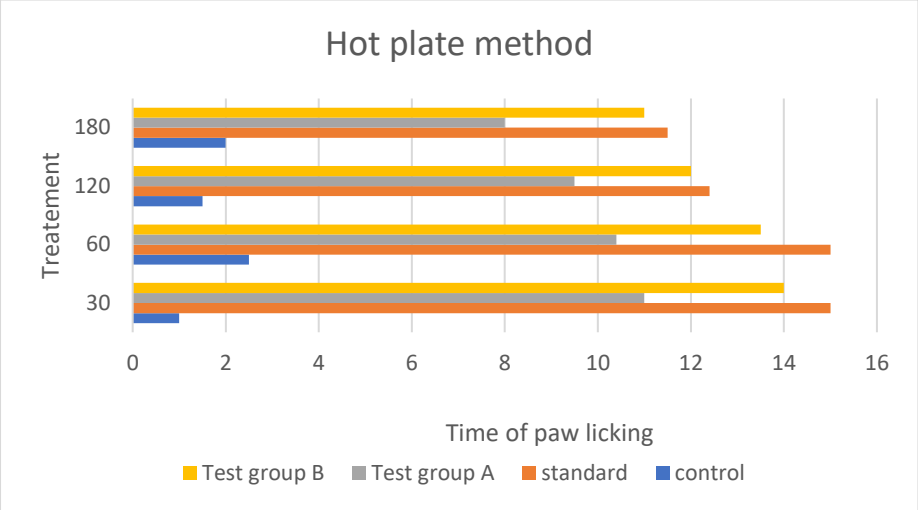

Table 5 shows, hot plate test in rats, we got to know the difference between the treatment received by the rats of different groups and after comparing the paw licking time with standard and control group, that test ointment 2 was much more affective in pain then that of test ointment 1, and is comparable with the standard.

Tail immersion Method:

Table no 6: Tail immersion Test

Treatment	Reaction time in seconds			
	30 min	60 min	120 min	180 min
Control (n=2)	2.0	2.45	2.5	2.0
Standard (n=2)	15	13	11	8
Test group 1(n=2)	12	11.2	10	8.5
Test group 2(n=2)	14	12	11	9

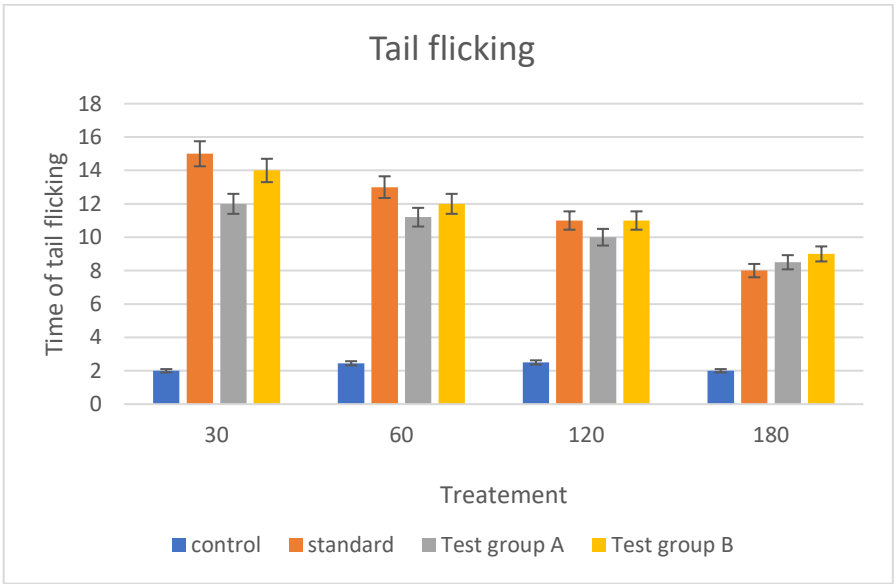

Table 6 shows, tail immersion test in rats, we got to know the difference between the treatment received by the rats of different groups and after comparing time of tail flicking with standard and control group we came to a conclusion that test ointment 2 was much more affective in pain then that of test ointment 1, and is comparable with the standard.

Formalin induced paw edema test:

Table no 7: The Effect of Different Concentrations of Formalin on Behaviours

Group	Phase 1 response		Phase 2 response	
	Flinching (Freq.)	Licking (Sec.)	Flinching (Freq.)	Licking (Sec.)

Control(n=2)	2±2	0	0	0
Standard(n=2)	12±18	28	(118-223) 170	230
Test group A(n=2)	23±18	24	(174-240) 185	125
Test group B(n=2)	19±12	26	(89-178) 130	90

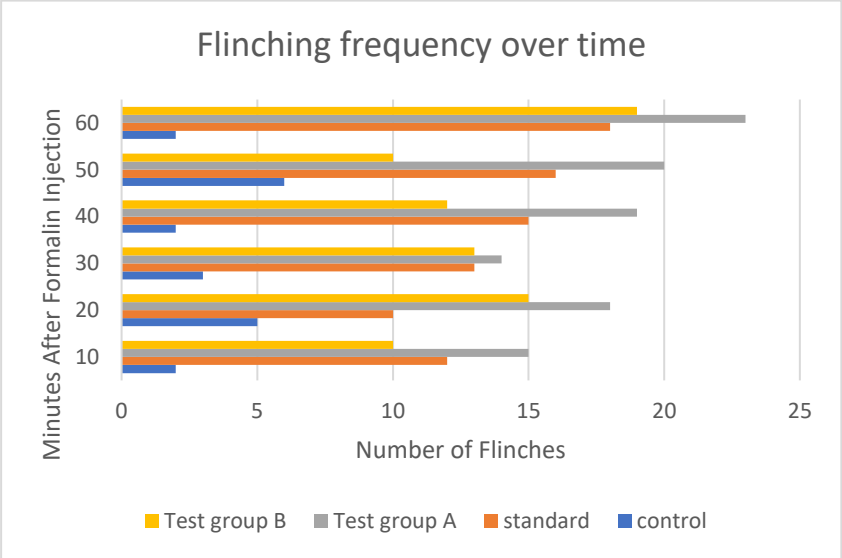

Table 7 shows, Formalin-induced paw edema test in rats and got to know the difference between the treatment received by the rats of different groups and after the result was compared with standard and control group. Test ointment 2 was much more affective in pain then that of test ointment 1, and is comparable with the standard.

IV. Discussion

Pain is a complex sensory and emotional experience that serves as a vital protective mechanism. Traditional treatments include analgesics, physical therapy, and cognitive behavioural therapy. However, new approaches are emerging to address pain more effectively[7,8]. These include targeted drug delivery systems, neuromodulation techniques like transcranial magnetic stimulation, and personalized pain management based on genetic profiles. Additionally, researchers are exploring innovative methods such as virtual reality therapy, mindfulness-based interventions, and bioelectric medicine. As our understanding of pain mechanisms deepens, the focus is shifting towards multimodal, patient-centred approaches that combine pharmacological and non-pharmacological strategies to improve pain management and quality of life[9,10,11].

Numerous conventional herbs and medications used in Indian medicine have analgesic activity claims. Claims are meaningless because these herbs haven't been the subject of any study. Scientists have yet to investigate systematically several traditional plants[13]. There are numerous other treatments having side effects. To overcome this, it is crucially necessary to discover alternative medications that are easily assessable and easy to use and the treatment that can be withdrawn easily in case of discomfort[11,14].

The goal of the study was to determine whether *Elaeocarpus ganitrus* leaf extract *and Cymbopogon citrate* oil had any analgesic effects on pain. Wistar albino rats were used as the study's animal model. Rats were chosen as an animal model because of their multi-purpose characteristics, including as physiological resemblance to humans, ease of care, and greater survival rates. According to animal protection guidelines, rats were kept in standard diurnal conditions with easy access to food. Rats were acclimatized for two weeks before experimentation. Following plant authentication, *Elaeocarpus ganitrus* leaves were collected, dried, and extracted accordingly. Extract was prepared using methanolic solvent to obtain the Active pharmaceutical ingredient and oil of *Cymbopogon citrate* was used.

The ointment was prepared using the Active pharmaceutical ingredient and oil and named them test group A (leaf extract), test group B (leaf extract+ oil). And diclofenac was used as standard drug.

For the study, rats were divided into 4 groups namely, control, standard, Test group A, and Test group B and each group contain 3 animal each, and the experiment was carried out for 7 days, during which animals of different groups were administered with different drug treatments and different types of tests were carried out.

Animals receiving standard treatment (Diclofenac ointment) experienced less pain in comparison to others, and formulation B that contains *Elaeocarpus ganitrus* methanolic extract and *Cymbopogon citrate* oil matches is much more effective in pain then formulation B that only contained *Elaeocarpus ganitrus* methanolic

extract. Group 4 receiving test ointment 2 (40%w/w) had the greater analgesic as it was comparable to the group receiving standard treatment.

V. Conclusion

The main purpose of this research was to determine whether an ointment prepared from the extract made from the leaves of the plant *Elaeocarpus ganitrus* and *Cymbopogon citrates* oil has analgesic properties. Previous research on the same plant has shown that it has a variety of pharmacological properties but not in combination with each other. With this research we came to a conclusion that ointment prepared using *Elaeocarpus ganitrus* leaf extract and *Cymbopogon citrate* oil in combination can be positively used for the treatment of pain, and formulation 2 is better than formulation 1 and can be easily compared with the standard (diclofenac ointment), however require further studies.