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# EVALUATION OF ANTI - INFLAMMATORY AND ANALGESIC ACTIVITY OF POLYHERBAL FORMULATION

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**ABSTRACT**

Currently used analgesic agents are opioids & NSAID. All of these are associated with certain adverse effects, so research in new potent herbal formulations is urgently needed. Therefore, a polyherbal formulation from four well-known herbs that had been already individually tested for its analgesic & anti-inflammatory property was prepared, thinking that synergistically it would act better. ***Aims & objective:*** To evaluate the analgesic & anti-inflammatory properties of Ethanolic extract of polyherbal formulation (EEPHF) in Wister rats. ***Materials and Methods:*** Analgesic activity was evaluated by using Eddy's hot plate method. Anti-inflammatory activity was tested by using inj. carrageenan-induced hind paw oedema test. Rats were divided into 6 groups, viz. I to VI. Group I served as the Normal, B serves as control, III serves as Standard and IV, V, VI as drug group. ***Results:*** EEPHF had also shown a significant reduction in hind paw edema induced by carrageenan injection, indicating anti-inflammatory action. EEPHF had shown a significant increase in the hotplate latency period ($p<0.01$) in dose dependent manner indicating central analgesic activity. ***Conclusion:*** PHZ has potent analgesic & anti-inflammatory action, which supports its clinical use.

## INTRODUCTION

Inflammation is a local response of living mammalian tissues to injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agent. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury.[1] The mechanisms of inflammation involve a series of events in which the metabolism of arachidonic acid plays an important role. It can be metabolized by the Cyclooxygenase (COX) pathway to prostaglandins and thromboxane A2, or by the 5-lipoxygenase (5-LOX) pathway to hydroperoxy-Eicosatetraenoic acids (HPETE's) and leukotrienes (LT's), which are important biologically active mediators in a variety of inflammatory events.[2] Inflammation is a complex biological response of vascular tissue to harmful stimuli, pathogens, irritants characterized by redness, warmth, swelling and pain. Prolonged inflammation leads to the rheumatoid arthritis. Anti-inflammatory drugs like NSAIDs used to reduce the swelling and pain of inflammation. But these agents carry the risk of gastro-intestinal toxicity, cardiovascular and other side effects for prolonged use. Therefore, to overcome this problem, there is a need for prepared safe and potent therapeutic activity herbal drug.

At the tissue level, inflammation is characterized by redness, swelling, heat, pain, and loss of tissue function, which result from local immune, vascular and inflammatory cell responses to infection or injury. In response to tissue injury, the body initiates a chemical signaling cascade that stimulates responses aimed at healing affected tissues. These signals activate leukocyte chemotaxis from the general circulation to sites of damage. These activated leukocytes produce cytokines that induce inflammatory responses.[3] The international association for the study of pain (IASP) defines pain as "unpleasant sensory and emotional experience that is caused by actual or potential tissue damage". The emotional component differs from one person to the other and in the same individual from time to time and it can be classified in several ways, but in therapeutic application into; nociceptive and neuropathic.[4] In the body, Sensory nerve endings are generally found in every part of the body such as the blood vessels, internal organs, muscles, joints, and the skin. Damage caused by the chemical, mechanical, and thermal stimuli sensitizes nociceptors. When cells are damaged a number of chemical mediators are released which then activate and sensitize nociceptors to other mediators of pain.[5] Sensation of pain due to mechanical, thermal and electrical stimuli is initiated by peripheral receptors.[6] In the brain pain stimulus are processed and generated impulses are send down the spinal cord following the appropriate nerves and instructs the body to respond, for instance withdrawing your hand from fire. Fast pain nerve endings secrete a neurotransmitter called glutamate, which transmits fast pain impulses to the brain in the cortex. Therefore, localization of pain in certain part of the body becomes relatively precise.[7] Peripheral nerves transmit pain stimulus to the spinal cord which then links to the brain. Two types of nerve fibers are involved in this process; slow pain fibers and Fast pain fibers. Transmission of fast pain is through the A delta fibers (Aδfibers) to the spinal cord. The activity of fast pain fibers is terminated at luminal in the spinal cord.

A second neuron is excited which follows the neospinothalamic pathway and terminates its transmission in the brainstem. Bradykinin, histamine, serotonins and prostaglandins are the major mediators of pain. It is a sensory modality that is essential for survival of an organism from harmful stimuli. It provides a warning signal to the nervous system to initiate a response that would otherwise minimize injury to the tissues.[8] Hence attempts are made in this present study to evaluate the anti-inflammatory and analgesic activity of polyherbal formulation. Preliminary phytochemical screening also carried out for the extract to identify the nature of chemical constituents present.

## MATERIALS AND METHODS

### Animals

Experimental animals of either sex weighing 150-170 g were obtained from Raghavendra enterprises. The animals were housed in stainless steel cages at a controlled room temperature of 24C, under a 12 h light and 12 h dark cycle. After one week of acclimatization, the experimental animals were divided randomly in to 8 groups (n=6).

### Chemicals

Ibuprofen was obtained from Sigma-Aldrich, Bangalore. Carragenan was obtained from SD fine chemicals Ltd Mumbai and all other reagents used were of analytical grade.

### Instruments

Plethesmometer, hot plate and electronic balance (Shimadzu, Model no: DS-852 J).

## PARAMETERS MEASURED

### 1. Measurement of paw volume

### Principle

Carrageenan induced inflammation is the most reliable and reproducible model for. In the present study inflamation was induced by administration of carragenan 1% through subplantar route.

### Procedure

### Carrageenan induced paw edema

Acute inflammation was induced by sub plantar injection of 0.1 ml of 1% suspension of carrageenan in normal saline, in the right hind paw of the rats. After one hour the standard and test drugs were administerd orally for individuaval groups. The paw volume was measured plethysmometrically at 0, 1, 2, 3 and 4 h after the carrageenan injection. The difference between the two readings was taken as the volume of edema and the percentage anti-inflammatory activity was calculated. With Ibuprofen (15 mg/kg) as standard, Ethanolic extract of Polyherbal formulation at dose of 100, 200 and 400 mg/kg was given orally by oral feeding needle. % inhibition of paw edema is calculated by comparing the control.

**Tail immersion method:** The tail immersion method was used to evaluate the central mechanism of analgesic activity. Here the painful reactions in animals were produced by thermal stimulus that is by dipping the tip of the tail in hot water. The animals were fasted for 12 hours with water *ad libitum*. The experimental animals were previously treated (p.o.) with different doses 100mg/kg, 200mg/kg and 400mg/kg of Ethanolic extract of Polyherbal formulation. Standard group was treated with ibuprofen 15mg/kg and control group was treated with normal salaine. After 1h, the basal reaction time was measured in a regular interval of 30 minutes, by immersing the tail tips of the rats (Last 1-2 cm) in hot water heated at temperature of temperature (55 ± 1) °C. The actual flick responses of rats i.e. time taken in second to withdrawn its tail from hot water source were compared with control group.

### Hot plate method

The paws of rats were very sensitive to heat at temparature which are not damaging the skin. The animals were fasted for 12 hours with water *ad libitum*. The experimental animals were previously treated (p.o.) with different doses 100mg/kg, 200mg/kg and 400mg/kg of Ethanolic extract of Polyherbal formulation. Standard group was treated with ibuprofen 15mg/kg and control group was treated with normal salaine. After 1 h of respective treatments the animals were individually placed on the hot plate maintained at 55°C. The response time was noted as the time at which animals reacted to the pain stimulus either by paw licking or jump response, whichever appeared first. The cut off time for the reaction was 15 seconds.

**Statistical analysis:** All the data was expressed as mean ± S.E.M. Statistical significance between more than two groups was tested using one way ANOVA followed by the Tukey test using computer based fitting program (Prism graph pad 5.0). Statistical significance was set accordingly.

### Pharmacological studies

- ✓ Phytochemical screening
- ✓ Acute toxicity study
- ✓ Anti-inflammatory activity
- ✓ Analgesic activity

***A. Phytochemical screening:*** Shown in Table 1.

***B. Acute toxicity study:***

The EEPHF was found to be safe since no animal died at the maximum single dose of 4000 mg/kg when administered orally and the animals did not show any gross behavioural changes. Hence 400mg/kg were used as high dose

and 200mg/kg were used as medium dose and 100 mg/kg used as low dose in the subsequent study respectively.

***C. Anti-Inflammatory Activity***

Rats are treated with carrageenan were significantly induced paw volume when compared with normal group. Oral dose of 100, 200 & 400mg/kg of EEPHF showed significant and dose dependent decrease in paw volume when compared with control group against carrageenan induced inflammation.

***D. Analgesic activity***

**i**. *Hot plate Method:* The analgesic activity of test drug was evaluated against Eddy's hot plate method in at 55±0.5$^{o}$C at 30, 60, 120, and 180 minutes. The experimental groups were treated with 100mg/kg (T1), 200 mg/kg (T2) and 400 mg/kg (T3) of EEPHF. At 30 minutes no significant analgesic activity was observed in all the test groups when compared with control group. At 60 and 120 and 180 min all the test drug doses were shown a significant and dose dependent ($p<0.001$) analgesic activity when compared with control group.

*ii. Tail immersion method:* The analgesic activity of test drug was evaluated against tail immersion method in hot water at 55±0.5$^{o}$C at 30, 60, 120, and 180 minutes. The experimental groups were treated with 200 mg/kg (T1) and 400 mg/kg (T2) of EEPHF. At 30 minutes no significant analgesic activity was observed in all the test groups when compared with control group. At 60 and 120 and 180 min all the test drug doses were shown a significant ($p<0.001$) analgesic activity when compared with control group.

**DISCUSSION**

It is well known that Carrageenan-induced paw edema in rats is a biphasic events, and the early phase (2.5–3 h) of the inflammation is due to the release of vasoactive amines such as histamine and serotonin. The later phase (4.5–6 h) is due to the activation of kinin-like substances such as prostaglandins, proteases and lysosome.

In the present study the result indicated that oral administration of EEPHF can inhibit the exudation on the process of acute inflammation, and the early phase inflammatory responses related to the release of pro-inflammatory mediators, such as histamine, serotonin, kinins. The hot plate method is considered to be selective for the drugs acting centrally. The hot plate test measures the complex response to a non-inflammatory, acute nociceptive input and is one of the models normally used for studying central nociceptive activity.

**Table.1. Phytochemicals**

| Phytochemicals | Ethanolic extract of Polyherbal formulation |
|---|---|
| Alkaloids | + |
| Flavonoids | + |
| Steroids | + |
| Saponins | + |
| Cardiac glycosides | + |
| Phenolics | - |
| Terpenoids | + |

**Table.2. Effect of EEPHF on carrageenan induced paw edema in rats**

| S.No | Treatment groups | 0'min | 60min | 120min | 180min | 240min |
|---|---|---|---|---|---|---|
| I | Normal | 0.200±0.00 | 0.200±0.00 | 0.200±0.00 | 0.200±0.00 | 0.200±0.00 |
| II | Control | 0.2167±0.01 | 0.4900±0.0285 | 0.5083±0.019 | 0.4750±0.021 | 0.3833±0.021 |
| III | Standard | 0.2333±0.03 | 0.2700±0.02*** | 0.2671±0.013*** | 0.2583±0.015*** | 0.2589±0.015*** |
| IV | Test-1 | 0.2333±0.01 | 0.3417±0.30** | 0.3467±0.012*** | 0.3200±0.025*** | 0.3150±0.011* |
| V | Test-2 | 0.2167±0.01 | 0.3050±0.09*** | 0.2550±0.011*** | 0.2367±0.008*** | 0.2233±0.010*** |
| VI | Test-3 | 0.2500±0.00 | 0.3500±0.02** | 0.3267±0.009*** | 0.2933±0.014*** | 0.3250±0.011* |

All values are shown as mean ±SEM and n=6.

*p<0.05 when compared with control (II) group. **p<0.01 when compared with control (II) group. ***p<0.001 when compared with control (II) group.

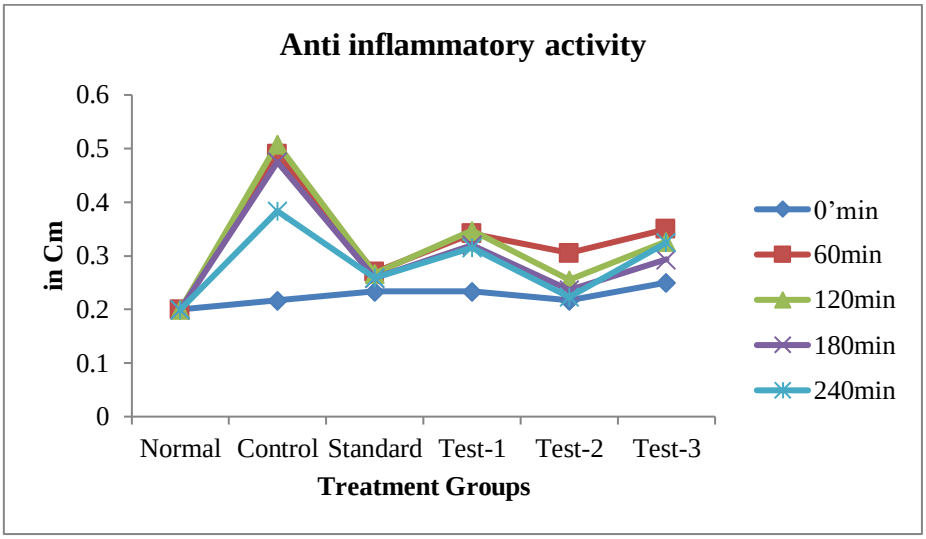


**Fig.1. Effect of EEPHF on carrageenan induced paw edema in rats**

**Table.3. Effect of EEPHF on against hot plate method in rats**

| S.No | Treatment groups | 30min | 60min | 120min | 180min |
|---|---|---|---|---|---|
| I | Normal | 1.180±0.12 | 1.180±0.12 | 1.180±0.12 | 1.180±0.12 |
| II | Control | 1.180±0.12 | 1.180±0.12 | 1.180±0.12 | 1.180±0.12 |
| III | Standard | 2.26±0.10*** | 4.173±0.41*** | 5.050±0.40*** | 3.233±0.09*** |
| IV | Test-1 | 1.548±0.08ns | 2.250±0.20* | 3.033±0.27*** | 2.198±0.12*** |
| V | Test-2 | 1.822±0.06*** | 2.900±0.15*** | 3.710±0.18*** | 2.550±0.13*** |
| VI | Test-3 | 1.985±0.05*** | 3.350±0.29*** | 4.250±0.26*** | 3.017±0.28*** |

All values are shown as mean ±SEM and n=6. *p<0.05 when compared with control (II) group.

**p<0.01 when compared with control (II) group. ***p<0.001 when compared with control (II) group.

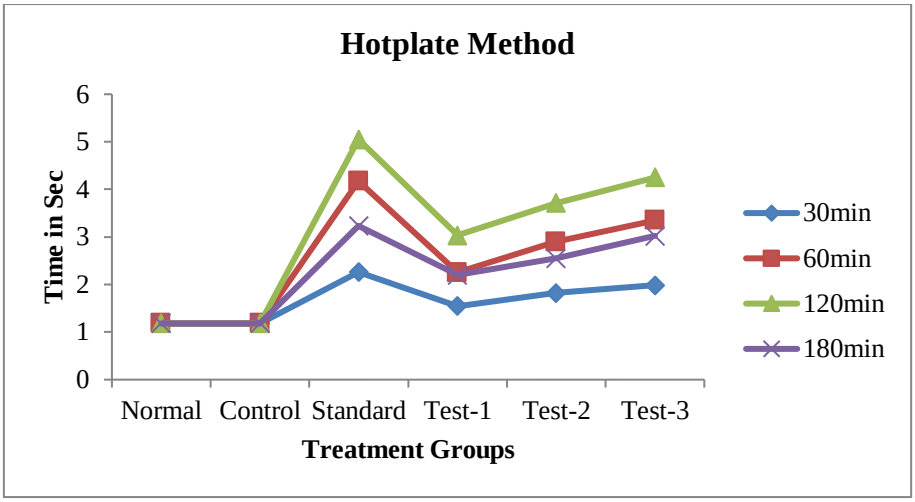


**Fig.2. Effect of EEPHF on against hot plate method in rats**

**Table.4. Effect of EEPHF on against tail immersion method in rats**

| S.No | Treatment groups | 30min | 60min | 120min | 180min |
|---|---|---|---|---|---|
| I | Normal | 1.117±0.17 | 1.117±0.17 | 1.117±0.17 | 1.117±0.17 |
| III | Standard | 2.883±0.30*** | 4.050±0.24*** | 5.118±0.21*** | 2.952±0.54*** |
| IV | Test-1 | 1.317±0.17 | 2.00±0.13* | 2.633±0.16*** | 1.730±0.10 |
| V | Test-2 | 1.467±0.18 | 2.550±0.16*** | 3.283±0.16*** | 2.500±0.17* |
| VI | Test-3 | 1.767±0.10 | 3.333±0.21*** | 4.000±0.13*** | 2.517±0.25* |

All values are shown as mean ±SEM and n=6.

*p<0.05 when compared with control (II) group.

**p<0.01 when compared with control (II) group.

***p<0.001 when compared with control (II) group.

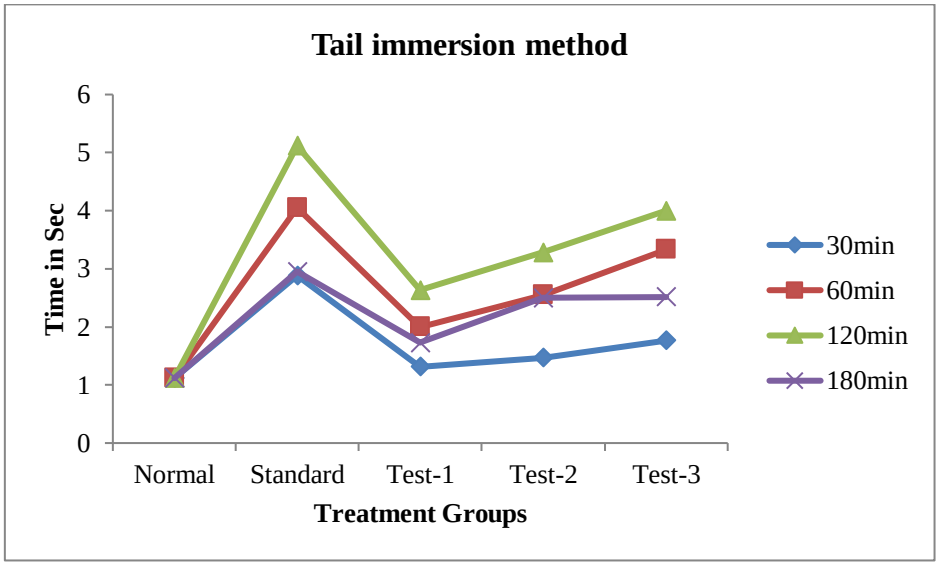


**Fig.3. Effect of FPCM on against tail immersion method in rats**

## CONCLUSION

The ethanolic extract of Polyherbal formulation has shown anti-inflammatory with a significant decrease in paw oedema. It also showed significant increase in latency period than control in Hot plate and Tail immersion method for Analgesic activity. Hence, the results obtained in this study proved the efficacy of ethanolic extract of Polyherbal formulation as anti-inflammatory and analgesic agent and the effect was observed to be dose dependent. Further studies for better understanding of the mechanism of action might be note-worthy.

## REFERENCES

1. Rajesh, S. Gopi, R. Sanjeev, Invitro Antinflammatory and antioxidant activity of leaf extracts of Datura metal, Asian journal of pharmaceutical and research. 2013; Volume (6) Issue (14): 146-149.
2. Rashmi Y, Vijender S. Invitro Anti inflammatory activity o oral polyherbral formulations. The pharma innovation, 2018; Volume (7) Issue (2): 272- 276.
3. Linlin C, Huidan D, Hengmin C. Inflammatory responses and inflammation associated diseases in organs. Oncotarget, 2018; Volume (9) Issue (6): 7204-7218.
4. Rajagopal. Pain - Basic Considerations: Indian Journal of Anaesthetic, 2006; Volume (5): 331–334.
5. Thorp, C.M. Pharmacology for the Health Care Professions. A John Wiley & Sons, Ltd. Lodon, 2008; 243.
6. Guyton, A.C., and Hall, J.E. Textbook of Medical Physiology. Elsevier Saunders, Philadelphia, 2006; Volume (11): 429-434.
7. Rang, H.P., Dale, M.M., Ritter, J.M., and Flower, M.C. Pharmacology, Churchill Livingstone, 2006; Volume (7): 202-210.
8. Arome, D., Akapabio, I.S., Onalike, E.I., and Agbafor, A.. Pain and inflammation: Management by conventional and herbal therapy. Indian Journal of Pain, 2016; Volume (28): 5-12.