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PHARMACOLOGICAL EVALUATION OF FORMULATED CELECOXIB HERBAL GEL FOR ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY

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ABSTRACT

Plant-derived products are increasingly gaining attention for their therapeutic potential, particularly in pain management. This study aims to evaluate the analgesic and anti-inflammatory properties of a novel Celecoxib herbal gel formulation, combining the pharmaceutical benefits of Celecoxib with bioactive compounds from medicinal herbs. Albino Wistar rats were used in experimental models to assess analgesic and anti-inflammatory activities. The hot-plate, tail-immersion, and acetic acid-induced writhing tests were employed to evaluate the analgesic effects of the gel. Anti-inflammatory activity was assessed using the carrageenan-induced rat paw edema model. Three groups of rats were treated with a control gel base, Piroxicam gel (standard), and the formulated Celecoxib herbal gel, respectively. In the analgesic tests, the formulated Celecoxib herbal gel significantly increased the reaction time in the hot-plate and tail-immersion tests and reduced the writhing response in the acetic acid-induced writhing test compared to the control group. The anti-inflammatory study demonstrated a marked reduction in paw edema in the treated group compared to the control.The formulated Celecoxib herbal gel exhibits potent analgesic and anti-inflammatory effects, making it a promising alternative for topical pain management. The synergistic effect of Celecoxib and herbal bioactive compounds enhances therapeutic outcomes and warrants further investigation.

Keywords *– Celecoxib, herbal gel, analgesic activity, anti-inflammatory, pain management, medicinal plants, hot-plate test.*

1. INTRODUCTION

Plant-derived products have gained global recognition and are increasingly recommended for primary healthcare, with only a few undergoing rigorous scientific evaluations. Medicinal plants have long been utilized for treating various human diseases, as they contain secondary metabolites synthesized through the pentose phosphate, shikimic acid, and phenylpropanoid pathways. These compounds serve significant defensive roles against numerous diseases [1,2].

The growing reliance on herbal medicines as substitutes for scientifically proven therapies emphasizes the importance of ensuring their safety and efficacy. It is crucial to adopt rational approaches when selecting and conducting pharmacological studies. The design and selection of specific studies should align with the individual properties and intended uses of the pharmaceuticals. Scientifically valid and internationally recognized methods are preferred whenever applicable [3]. Moreover, the integration of new technologies and methodologies, based on sound scientific principles, is encouraged [4]. Some safety pharmacology endpoints can

be incorporated into toxicology, kinetic, or clinical studies, while others may require specific safety pharmacology studies [5]. Despite the potential for detecting adverse effects in safety pharmacology studies, they may not be evident in conventional toxicity studies [6].

This study was aimed at evaluation of the analgesic and anti-inflammatory properties of a novel Celecoxib herbal gel formulation, combining the pharmaceutical benefits of Celecoxib with bioactive compounds from medicinal herbs.

2. MATERIALS AND METHODS:

2.1 Materials

Celecoxib, menthol, and safflower oil were gifted by N.S. Scientific, Mumbai. Salai Guggul leaves were collected locally from the Mumbai region. These materials were used to formulate the herbal gel.

2.2 Experimental Animals

Albino Wistar rats (100-150 g) of either sex were used for the study. The animals were obtained from listed suppliers in Bhopal, M.P., India. They were maintained on a standard pellet diet (Hindustan Lever Ltd., Bangalore) and water ad libitum and housed in polypropylene cages under a 12-hour light/dark cycle. The rats were acclimatized for one week prior to the experiment and fasted for 12 hours before each activity. All experimental protocols were approved by the Institutional Animal Ethics Committee [7].

2.2.1 Treatment Protocol

Animals were divided into three groups (six rats per group) for the various experiments:

- **Group I**: Control (received gel base only)
- **Group II**: Standard drug (Piroxicam gel – Cipla, Ahmedabad, India)
- **Group III**: Formulated Celecoxib herbal gel (EHG)

2.2.2 Determination of Analgesic Activity by Hot-Plate Method

The analgesic activity of the formulated Celecoxib herbal gel was evaluated using the hot-plate method described by Eddy and Leimback [8]. Rats were divided into three groups (six rats per group) for control, standard, and test groups. The control group received gel base, the standard group received Piroxicam gel, and the test group received formulated Celecoxib herbal gel (2%).

Each animal was placed on a hot plate (55 ± 0.5°C) enclosed by a cylindrical glass. The reaction time (time taken for the animal to jump or lick its forelimb) was recorded. To avoid tissue damage, a cutoff period of 30 seconds was applied. Measurements were taken before and after drug administration at 30, 60, and 90 minutes.

2.2.3 Determination of Analgesic Activity by Tail-Immersion Test

The tail-immersion test was used to evaluate analgesic activity [9]. Rats were divided into three groups, and the tail-immersion method involved immersing the last 3 cm of the tail into a water bath (55 ± 0.5°C). The reaction time was measured when the animal withdrew its tail. Readings were taken before and after drug administration at 30, 60, 90, and 120 minutes. A cutoff time of 120 seconds was applied.

2.2.4 Determination of Analgesic Activity by Acetic Acid-Induced Writhing Test

The acetic acid-induced writhing test was used to assess the analgesic activity [10]. Writhing in rats was induced by intraperitoneal administration of 300 mg/kg acetic acid (3%). Animals were divided into three groups (six rats per group). The writhing movements were counted every 30 minutes following acetic acid administration. The percentage inhibition of writhing was calculated.

2.2.5 Determination of Anti-Inflammatory Activity by Carrageenan-Induced Rat Paw Edema

The anti-inflammatory activity of the formulated Celecoxib herbal gel was evaluated using the carrageenan-induced rat paw edema model [11]. Acute inflammation was induced by the sub-plantar administration of 0.1 ml of 1% carrageenan into the right paw. Paw volume was measured using a digital plethysmometer (Ugo Basile-Italy) before carrageenan administration and at 1, 2, and 3 hours post-treatment. The paw volume was calculated. The efficacy of the formulated herbal gel was tested by comparing its ability to inhibit paw edema with the control group.

3. RESULTS AND DISCUSSION:

Formulated Celecoxib herbal gel was determined by hot-plate method, tail-immersion test and acetic acid induced writhing test for analgesic activity. These all methods were used for the assessment of various formulated and tested drugs. The anti-inflammatory potential of formulated Celecoxib herbal gel was assessed by Carrageenan-induced rat paw edema.

3.1 Determination of analgesic activity by hot-plate method

The hot plate test is used in basic pain research and in testing the effectiveness of analgesics by observing the reaction to pain caused by heat. The hot plate test is a simple behavioral screen used for estimating the effects of NCEs on the threshold for detecting pain. It is based on the principle that when rodents are placed onto a hot surface they will initially demonstrate the aversive effects of the thermal stimulus by licking their paws and, ultimately, by overt attempts to escape the environment (jumping). Substances that alter nociceptive threshold either increase the latency to licking/jumping (analgesic effect) or decrease it (hyperalgesic effect). Hot-plate test is a widely used model for neurologic pain, and centrally acting analgesic agents can increase reaction time in hot-plate test through their action at the spinal cord level. At 90 minutes, the maximum reaction time of 3.88 ± 0.40 for control group, 13.20 ± 0.20 for standard group and 11.20 ± 0.30 for group III (formulated Celecoxib herbal gel) respectively. The results indicated that the gel significantly ($p < 0.001$) reduced pain threshold as compared to control and the activity was constant throughout the entire observation period. The result of hot plate test indicates that the formulated Celecoxib herbal gel also possesses the ability to reduce centrally mediated pain. The reaction time following the topical administration of different doses of formulated Celecoxib herbal gel and standard drug were presented in table 1.

Table 1: Effect of formulated Celecoxib herbal gel in hot-plate test in rats

Groups	Reaction Time (in Seconds)			
	0 min	**30 min**	**60 min**	**90 min**
Group I (Control)	4.10 ± 0.40	4.72 ± 0.60	4.72 ± 0.40	4.78 ± 0.40
Group II (Standard)	4.20 ± 0.10	8.30 ± 0.20	18.74 ± 0.80	14.76 ± 0.20
Group III (CHG I Treated)	4.40 ± 0.10	6.87 ± 0.27	9.74 ± 0.60	10.17 ± 0.30

Data expressed as Mean ± SEM, n = 6 in each group done by one way ANOVA followed by Dennett's test.

3.2 Determination of analgesic activity by tail-immersion test

The tail immersion assay is a thermal test for evaluating the analgesic potential of compounds. A number of clinically approved pharmacological agents have been demonstrated to delay the onset of heat sensitivity upon tail exposure to heat including opioids such as morphine, and alpha adrenergic compounds. The tail immersion test has been reputed to measure spinally driven aspects of pain and has the advantage of measurements not being affected by sedation as they would be in other assays.

The effect of formulated Celecoxib herbal gel in tail immersion test in rats was determined by flicking response of tail in 17, 30, 47 and 60 minutes. The Control (group I) was found flicking response in seconds 4.8±0.12, 7.2±0.27, 7.6 ±0.20 and 7.9 ±0.17 in 17, 30, 47 and 60 minutes

respectively. The standard (group II) was found 6.0 ± 0.70, 8.0 ± 0.40, 9.0 ± 0.27, 12.2 ± 0.20 seconds in flicking response in 17, 30, 47 and 60 minutes respectively. The CSM (group III) was found 7.0 ± 0.20, 6.0 ± 0.27, 7.80 ± 0.30, 9.0 ± 0.10 seconds in flicking response in 17, 30, 47 and60 minutes respectively. The findings were reported in table

Table 2: Effect of formulated Celecoxib herbal gel in tail immersion test in rats

Groups	**Flicking response (in Seconds) of tail**			
	17 min	**30 min**	**47 min**	**60 min**
Group I (Control)	4.8 ± 0.12	7.2 ± 0.27	7.6 ± 0.20	7.9 ± 0.17
Group II (Standard)	6.0 ± 0.70	8.0 ± 0.40	9.0 ± 0.27	12.2 ± 0.20
Group III (CHG I Treated)	7.0 ± 0.20	6.0 ± 0.27	7.80 ± 0.30	9.0 ± 0.10

Each value is the mean ± SEM for 6 rats, P < 0.07, compared with control. Data were analyzed by using one-way ANOVA followed by Dunnett's test.

3.2 Determination of analgesic activity by acetic acid induced writhing test

Acetic acid induced abdominal contraction method has been used to evaluate peripherally acting analgesics. In acetic acid induced method pain is generated indirectly via endogenous mediators like prostaglandin, which stimulates peripheral nociceptive neurons. Writhing test is a chemical method used to induce pain of peripheral origin by injection of irritant principles like phenyl quinone, acetic acid in mice. Analgesic activity of the test compound is inferred from decrease in the frequency of writhings. The manifestations of abdominal writhings in mice were described by as an arching of back, extension of hind limbs and contraction of abdominal musculature. The writhing response is considered as a reflexive test and is without clinical counterparts as it cannot be performed in human and sensations involved are unknown.

The acetic acid-induced abdominal writhing test has been used as a screening tool for assessing analgesic or anti-inflammatory agents. Pain is induced by injection of irritants into the peritoneal cavity of rats. The animals react with a characteristic stretching behavior which is called writhing. Writhing is defined as a stretch, tension to one side, extension of hind legs, or contraction of the

abdomen so that the abdomen of the mice touches the floor, or turning of the trunk (twist). Any writhing is considered a positive response. Analgesic activity of the test compound is inferred from a decrease in the frequency of writhings. Acetic acid induces an inflammatory response in the abdominal cavity, with subsequent activation of nociceptors. When animals are intraperitoneally injected with acetic acid, a painful reaction and acute inflammation emerge in the peritoneal area. Constriction induced by acetic acid is considered to be a nonselective antinociceptive model, as acetic acid acts indirectly by inducing the release of endogenous mediators which stimulate the nociceptive neurons that are sensitive to nonsteroidal anti-inflammatory.

Effect of formulated Celecoxib herbal gel in acetic acid induced writhing test was found in rats 28.00 ± 0.42, 12.10 ± 0.37, 18.20 ± 0.40 in 30 minutes for control, standard and treated group respectively. The percent inhibition of standard and treated group was also determined and found 84% and 72% respectively.

Table 3 : Effect of formulated Celecoxib herbal gel in acetic acid induced writhing test in rats

Group	No. of Writhes in 30 min. (mean ± sem)	% Inhibition
Group I (Control)	38.00 ± 0.42	38%
Group II (Standard)	22.10 ± 0.37	94%
Group III CHG I Treated	28.20 ± 0.40	82%

Each value is the mean ± SEM for 6 rats, P < 0.07, compared with control. Data were analyzed by using one-way ANOVA followed by Dunnett's test

3.3 Determinations of anti-inflammatory activity of Carrageenan-induced rat paw edema

Carrageenan-induced inflammation is useful in detecting orally active anti-inflammatory agents; therefore, it has significant predictive value for anti-inflammatory agents acting through mediators of acute inflammation. Carrageenan-induced paw edema is one of the most popular tests used in the screening of African spices and vegetables for anti-inflammatory. It is a highly sensitive and reproducible test for nonsteroidal anti-inflammatory drugs and has long been established as a valid model to study new anti-inflammatory drugs. Carrageenan-induced inflammation is useful in detecting orally active anti-inflammatory. Therefore, it has significant predictive value for anti- inflammatory agents acting through mediators of acute inflammation. The development of edema induced by carrageenan injection causes an acute and local inflammatory response.

The effect of formulated gel preparation was on carrageenan induced paw edema in rats were shown in Table and Figure. The control group was compared with standard and formulated Celecoxib herbal gel as per statistical analysis. The herbal test gel has been shown highly significant ($p<0.07$) effect after 2 h of drug administration. Paw size in control group was found 0.71 ± 0.2, 0.63 ± 0.1, 0.78 ± 0.2, 0.90 ± 0.7 in mm after 30, 60, 90 and 120 minutes respectively whereas standard group was found 0.28 ± 0.07, 0.41 ± 0.2, 0.64 ± 0.1, 0.86 ± 0.2 in mm after 30,60, 90 and 120 minutes respectively. The Celecoxib herbal gel group III was found 0. 36 ± 0.2,0.48 ± 0.4, 0. 76 ± 0.2, 0.74 ± 0.17 paw size in mm after 30, 60, 90 and 120 minutes respectively.

Table 4: Effect of formulated Celecoxib herbal gel in carrageenan-induced paw edema test

Group	Paw size in mm at time in min.			
	30 min	60 min	90 min	120 min
Group I (Control)	0.71 ± 0.2	0.63 ± 0.1	0.78 ± 0.2	0.90 ± 0.7
Group II (Standard)	0.28 ± 0.07	0.41 ± 0.2	0.64 ± 0.1	0.86 ± 0.2
Group III (CHG I Treated)	0. 36 ± 0.2	0.48 ± 0.4	0. 76 ± 0.2	0.4 ± 0.17

Each value is the mean ± SEM for 6 rats, P < 0.07, compared with control. Data were analyzed by using one-way ANOVA followed by Dunnett's test.

4. CONCLUSION

The results of the analgesic and anti-inflammatory tests indicated that the formulated Celecoxib herbal gel showed significant analgesic and anti-inflammatory effects compared to the control group. In the hot-plate and tail-immersion tests, the reaction time increased significantly in the test group, suggesting enhanced analgesic activity. Similarly, the acetic acid-induced writhing test revealed a substantial reduction in the number of writhes in the treated group, with a percentage inhibition close to that of the standard group.

The anti-inflammatory activity, as measured by carrageenan-induced rat paw edema, demonstrated that the formulated gel reduced paw swelling more effectively than the control. These results suggest that the combination of Celecoxib with herbal bioactive compounds provides enhanced analgesic and anti-inflammatory properties, making it a promising candidate for topical pain and inflammation management.

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