Evaluation of Analgesic Activity of the Methanol Extract from the tubers of *Eulophia* species in Rats

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Abstract

The present study aims to investigate the analgesic activity of the methanol extract of the tubers of *Eulophia herbacea* (MEEH) and *E. ochreata* (MEEO) in rats using hot plate method. The hot plate method is useful in elucidating centrally mediated antinociceptive responses. The extract was administered orally at a dose of 200 and 400mg/kg to Wistar strain Albino rats while ibuprofen (100mg/kg) served as standard. The methanolic extracts exhibited significant ($p<0.001$) elongation of the latency time in thermal response. In the hot plate method both MEEH and MEEO at their higher dose (400mg/kg) significantly increased the pain reaction time (PRT) at 90 min. as maximum activity. Their range of analgesia at higher dose is 32.22-65.65% and 35.58-78.53% respectively. These studies conclude that *E. herbacea* and *E. ochreata* tubers possesses analgesic activity in a dose dependent manner.

Key words: *Eulophia herbacea*, *Eulophia ochreata*, methanolic extract, pain, hot plate method.

INTRODUCTION

In Ayurvedic medicine, *Eulophia* is generally prescribed as aphrodisiac, sperm count enhancer, cardiac and general tonic, blood purifier, remedy on impotency and gynaecological problems, expectorant, anabolic, diuretic, astringent, digestive, and soft purgative. As highlighted in some ancient texts, these species are also useful for the treatment of ear discharge, blood clotting, joint edema, and debility (Vaidya and Nighantu, 2004, Patil and Mahajan, 2013). *Eulophia* species are also used in stomatitis, purulent cough; and in the heart problems, dyscrasia, and scrofulous diseases of the neck; bronchitis, blood diseases, and as a vermifuge (Dhiman, 2006). Some species of *Eulophia* are medicinal and are widely used in other continents also. Research analysis during the last decade estimated that analgesics are one of the highest therapeutic categories on which research efforts are focussed (Farouk et al., 2008). Analgesic

compounds available in the market, still present a wide range of undesired effects (Katzung, 2004) leaving the door open for new and better compounds. Hence to prove the efficacy of *E. herbacea* and *E. ochreata* for the analgesic potential, this work was carried out.

MATERIALS AND METHODS

2.1 Plant material

The tubers of *E. herbacea* and *E. ochreata* were collected from Nasik and Jalgaon district respectively in the month of October-November. The tubers were identified, authenticated and Voucher specimens (PCA/ BOT H.S.1641 and PCA/ BOT H.S. 1642) were deposited at Pratap College, Amalner. Medicinally useful parts of the plants were studied in both fresh and dried conditions.

2.2 Extracts Preparation

The identified and certified tubers of *E. herbacea* and *E. ochreata* were washed, chopped into small pieces and shade dried. The shade dried plant material was powdered, weighed and subjected for Soxhlet extraction procedure using methanol (65^{o}C) for 48 hrs. The solvent was recovered using rotatory vacuum evaporator under reduced pressure and the extracts MEEH and MEEO were stored at 4 ^{o}C until use.

2.3 Animals

Adult wistar albino mice of either sex, weighing 200-250 g were housed in polypropylene cages in standard environmental conditions of temperature (21±2 ^{o}C), humidity (55±10%) and a 12-hour light-dark cycle. The mice were given a standard laboratory diet (Commercial pelleted food from Hindustan Lever Ltd., Bangalore) and water ad libitum. Food was withdrawn 12h before and during the experimental hours. All experimental protocols were approved by the institutional animal ethics committee having registration no. AEC/22/CPCSEA/ MJ/2014-15.

2.4 Acute Toxicity studies

Acute Toxicity study was conducted by Staircase method. The LD50 for each of the extract was determined and one tenth of the extract dose (LD50) was selected as maximum dose for the evaluation of Analgesic Activity.

Analgesic activity of methanolic extract of *E. herbacea* and *E. ochreata*

The analgesic activity of methanolic extracts of *E. herbacea* (MEEH) and *E. ochreata* (MEEO) examined using Hot plate method, described by Eddy and Leimbach in 1953. Animals were divided into six groups, each group containing four animals each. Group I served as the positive control with no protection. Group II animals received the standard drug of Ibuprofen 100 mg/kg body weight/10 ml in normal saline orally, whereas group III to VI animals were orally administered with low (200mg/kg b.w.) and high dose (400mg/kg b.w.) of MEEH and MEEO each dissolved in sterile DW. In this method heat is used as a source of pain. The paws of mice and rats are very sensitive to heat at temperatures which are not damaging the skin. The responses are jumping, withdrawal of the paws and licking of the paws. The hot plate, which is commercially available, consists of electrically heated surface (copper plate or glass surface) maintained at constant temperature (55^0C). The animals are placed on the hot plate and the reaction of animal is taken as the end point, recorded by a stop-watch. Analgesic increases the reaction time.

The animals were marked individually. Food was withdrawn 12 h prior to drug administration till completion of experiment. The animals were weighed and numbered appropriately. The reaction time was taken as the interval from the instant animal reached the hot plate until the moment animal licked its feet or jumped out. A cut off time of +10 s was followed to avoid any thermal injury to the paws. The reaction time was recorded before and after +30, +60, +90, +120- and +180-min following administration of test or standard drug.

2.6 Evaluation

The mean reaction time for each treated group was determined and compared with that obtained for each group before treatment. Percentage increase in reaction time (I %), was derived, using the formula-

$$I\% = \{(It - Io)/Io\} \times 100$$

Where It = reaction time at time t and Io = reaction time at time zero (0 h) (Prempeh and Mensah-Attipoe, 2008). The animals were subjected to the same test procedure at +30, +60, +120, and +180 min after the administration of test/standard/control drug.

2.7 Statistical Analysis

The data was expressed as mean ± SEM and the difference in the central tendencies of treatment groups was tested for statistical significance using ANOVA followed by Bonferroni's multiple comparison test for parametric data. For non-parametric data Kruskal-Wallis test followed by Dunnette's multiple comparison tests was applied; $p < 0.05$ was considered statistically significant for both these tests.

RESULT

The Eddy's hot plate method showed analgesic activity in MEEO followed by MEEH (**Table 1**). All test and standard drugs significantly reduce the pain as compared to control group. Oral administration of MEEH and MEEO resulted significant ($p<0.001$) elongation of the latency time in thermal response.

Table-1. Analgesic activity of orally treated MEEH and MEEO tuber extracts in rats.

Type	Oral dose (mg/kg)	Reaction time in min (% activity)				
		Basal	60	90	120	180
Control	Saline-2 ml	3.24±0.04	3.52±0.12 (8.64)	3.87±0.09 (19.44)	4.09±0.11 (26.23)	4.26±0.13 (31.48)
Ibuprofen	100	3.33±0.07	6.13±0.06 (84.08)	5.78±0.08 (73.57)	5.47±0.12 (64.26)	5.35±0.22 (60.66)
MEEH	200	3.31±0.11	3.68±0.13 (11.18)	3.96±0.09 (19.64)	4.48±0.05 (35.35)	4.09±0.15 (23.56)
	400	3.29±0.08	4.35±0.01 (32.22)	5.45±0.12*** (65.65)	5.17±0.16 (57.14)	4.94±0.08*** (50.15)
MEEO	200	3.22±0.02	3.73±0.05 (15.84)	4.08±0.14 (26.71)	4.62±0.21 (43.48)	4.27±0.17 (32.61)
	400	3.26±0.13	4.42±0.19 (35.58)	5.82±0.21*** (78.53)	5.49±0.11*** (68.40)	5.06±0.09*** (55.21)

( ) indicate % elongation. Each value expressed as mean ±SE, n=6, *P<0.05, **p<0.01, ***P<0.001 Vs Control

At all the reaction time intervals the analgesic activity of test plant was more than control groups but less than standard at both (200 and 400 mg/kg b. w.) the doses except at 120 min in higher dose of MEEO. There is significant effect of methanolic extract of *E. herbacea* and *E. ochreata* at their higher doses

(400mg/kg b.w.) at 90 min as maximum activity i. e. 65.65% and 78.53% respectively.

Their range of analgesia at higher dose is 32.22% -65.65% and 35.58% - 78.53% respectively. At higher doses they exhibited significant ability to prolong the latency of response of thermal induced nociception throughout the whole experiment. The low doses of *E. herbacea* and *E. ochreata* are comparatively less effective showing their range of analgesia 11.18%-35.35%and 15.84%-43.48% respectively.

2. DISCUSSION

We have used the hot plate method to screen the central nervous system for analgesic activity of a drug. The opioid agents exert their analgesic effects via supra spinal and spinal receptors (Nemirovsky et al., 2001). At higher doses they exhibited significant ability to prolong the latency of response of thermal induced nociception throughout the whole experiment. The nonsteroidal anti-inflammatory drugs (NSAIDs) have analgesic action due to their inhibition of the prostaglandin synthesizing enzymes not only through peripheral inhibition but also through a variety of other peripheral and central mechanisms. The increasing evidence that NSAIDs have a central mechanism of action that augments the peripheral mechanism. This effect may be the result of interference with the formation of prostaglandins within the CNS. At higher doses MEEH and MEEO exhibited significant ability to prolong the latency of response of thermal induced nociception throughout the whole experiment. The low doses of *E. herbacea* and *E. ochreata* are comparatively less effective.

3. REFERENCES

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