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NON-OPIODERGIC LIKE MECHANISM FOR ANTINOCICEPTIVE ANALGESIC AND ANTIPYRETIC ACTIVITY OF ETHANOLIC ROOT EXTRACT OF *OPERCULINA TURPETHUM* IN SWISS ALBINO MICE

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ABSTRACT

Non-opioid analgesics including both selective and non-selective cyclooxygenase (COX) inhibitors and acetaminophen are the most widely used treatments for pain. Inhibition of COX is thought to be largely responsible for both the therapeutic and adverse effects of this class of drugs. So it is necessary to find some of the alternate effective measures. The root extract of *Operculina turpethum* has been used as an anti-inflammatory,purgative,hepato-protective agent and antipyretic.The present study was done to evaluate the analgesic activity of the root extract of this plant by acetic acid writhing method, formalin test, hot plate method and tail flick method along with the antipyretic potential.Oral administration of *O. turpethum* extract at the doses of 250,300 and 350 mg/kg b.wt. significantly decreased the number of contortions, stretchings and licking activity in pain induced mice in comparison to standard drug.The present findings indicated that *Operculina turpethum* possesses genuine antiinflammatory and antinociceptive properties, lending pharmacological support to folkloric or anecdotal use of the plant in the treatment and management of painful inflammatory conditions.

KEYWORDS: **Analgesic, Anti-inflammatory, Antipyretic, Formalin, Acetic acid**

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INTRODUCTION

Inflammation is considered to be a pathophysiological response of mammalian tissues to a variety of agents including infectious organisms, toxic chemical substances, physical injury , tumor growth or other noxious stimuli leading to local accumulation of plasmic fluid and blood cell[1]. Pain has been described by the International Association for the Study of Pain as an "unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. Currently drugs like opioid analgesics, corticosteriods and non-steroidal or non-opioid drugs like NSAID's and immunosuppressive agents are used to control the symptoms of inflammation and pain[2] .Non-opioid analgesics are among the most widely used medications due to their efficacy for a wide range of pain and inflammatory conditions[3].The use of these drugs might cause side effects like respiratory depression, sedation, constipation, tolerance, spasm, gastrointestinal disturbances, renal and hepatic damage, bone marrow depression, suppression of response to infection or injury, osteoporosis, development of Cushing's syndrome etc [4].Therefore the search for new analgesic compounds devoid of these side effects of opioid agonists as well as of nonsteroidal anti-inflammatory drugs has attracted considerable attention in recent years.

Mechanism of nociception

Nociception is the mechanism whereby noxious peripheral stimuli are transmitted to the central nervous system[5].Pain is a subjective experience, not always associated with nociception. Polymodal nociceptors (PMN) are the main type of peripheral sensory neuron that responds to noxious stimuli. The majority are non-myelinated C-fibres whose endings respond to thermal, mechanical and chemical stimuli[6]. PMN are sensitised by prostaglandins, which explains the analgesic effect of aspirin, diclofenac sodium, ibuprofen like drugs, particularly in the presence of inflammation. NSAIDs have their predominant action via inhibition of the cyclo-oxygenase (COX) enzyme which regulates synthesis of prostaglandins and autacoids such as thromboxanes[7] (Figure 1). Diclofenac is a non-steroidal anti-inflammatory drug (NSAID), widely used in therapeutics, that exhibits potent analgesic and anti-inflammatory properties [8]. It is known that diclofenac, as other nonselective NSAIDs, is able to impair prostaglandin synthesis by the inhibition of the cyclooxygenase isozymes COX-1 and COX-2 in the injured tissues and the central nervous system[9]. However, there is evidence that additional prostaglandin-independent mechanisms are involved in the antinociceptive action of diclofenac at both the peripheral and central levels[10]. Similar mechanism is thought to be responsible for the analgesia of various plant derived drugs.

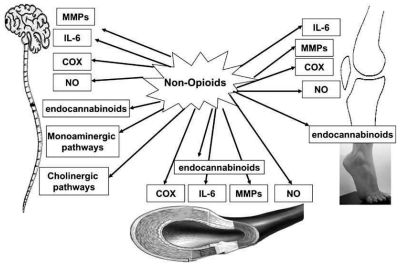


Figure 1

Targets contributing the analgesic effects, anti-inflammatory action or adverse effects of non-opioids in the central nervous system, the peripheral sites of inflammation or the blood vessels.[7]

Phytomedicines offer an alternative source of therapy for inflammation treatment and also provide some information about the pathogenesis of inflammation. The investigations of the efficacy of plant-based drugs used in the traditional medicine have been paid great attention because they are cheap and have little side effect[11].*O. turpethum* is the source of the drug known as Turpeth or Indian Jalap and it is widely grown throughout India and it is occasionally cultivated in gardens as an ornament. The root bark of Trivrit is rich in turpethin resin consisting of 10% 'turpethin' which is a glycoside analogue of Jalapine and Convolvulin and also contains Turpethinic acids-A, B, C, D, & E, , volatile oil, albumin, starch, lignin salts, ferric oxide, Scopoleptin, Betulin, lupiol & beta- sitosterol[12]. The roots are bitter, acrid, sweet, thermogenic, analgesic, purgative, carminative, antihelmintic, expectorant, antipyretic, hepatic, stimulant and hydragogue. They are useful in colic constipation, dropsy, vitiated conditions of vata, paralysis, myalgia, hyperlipidemia[13], arthralgia, pectoralgia, bronchitis, obesity, helminthiasis, gastropathy, ascites, inflammations, intermittent fever, leucoderma, puritus, ulcers, erysepelas, haemorrhoids, tumors, jaundice, ophthalmia, employed in drug formulations, dropsical effusions and rheumatism.

MATERIALS AND METHODS

Experimental animals

Healthy male Swiss albino mice (*Mus musculus*) (4-6 months old, weighing 20-30 g) were procured from C.C.S. Haryana Agricultural University (Hisar, India). They were housed under standard laboratory conditions of light (12:12 h L: D cycle), temperature (23 ± 2°C) and relative humidity (55 ± 5%). Animals lead free access to standard food pellet diet (Hindustan Lever Limited: metal contents in parts per million dry weight: Cu 10.0, Zn 45.0, Mn 55.0, Co 5.0, Fe 75.0) and drinking water *ad libitum* throughout the study.

Plant Material

Operculina turpethum was collected from Pharmacological garden of CCSHAU Hisar , Haryana, India in the month of august 2012. The plant was identified with the help of available literature and authenticated by Botanist of Krishi Vigyan Kendra Rohtak, Haryana, India.

Preparation of Ethanolic Extract

The *Operculina turpethum* roots were dried in shade and coarse powder was extracted. Dried powdered material was placed in the Soxhlet thimble with 80% ethanol in 500 ml flat bottom flask. Further refluxed for 18 h. at 80^{o}C for two days. Collected solvent was cooled and poured in a glass plate. The marc was dried in hot air oven below 50^{o}C for 48 h and kept in dissector for 2 days[14]. The yield of the extract was 14 % w/w of powdered plant material for further exploration. Collected dried extract was stored at 5^{o}C in air tight containers.

ETHICAL CLEARANCE

The animal experiments were carried out according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The Institutional Animal Ethics Committee approved experimental design performed in this study for the use of Swiss Albino mice as an animal model for the study.

ANTINOCICEPTIVE ACTIVITY

Formalin test

The procedure was similar to that described by Hanskaar and Hole[15] and Gorski *et al.,*[16] .The formalin test possesses two distinctive phases, which possibly reflecting different types of pain. Mice were treated orally with OTE (250, 300 or 350 mg/kg b.wt.) and diclofenac (10 mg/kg b.wt, i.p.) . After 1 h, 20 µl of 1 % formalin was injected subcutaneously under the dorsal surface of hind paw. Mice were observed in glass chamber. The number of licks in the injected paw was counted till 5 minutes (early phase) and from 15 to 20 min (later phase) after formalin injection. The early phase represents neurogenic pain while later phase is of inflammatory pain. The percentage of inhibition was calculated as follows:

$$\% \text{ Inhibition} = \frac{(\text{Control group mean}) - (\text{Test group mean})}{(\text{Control group mean})} \times 100$$

Acetic acid induced writhing method

The acetic acid induced abdominal writhing test in mice was performed according to the method described by Ahmed *et al* .,[17]. The test was performed to assess the ability of OTE (*Operculina turpethum* extract) to affect the nociceptive response induced by noxious chemical stimulus. Swiss albino mice were divided into 5 groups each group containing 6 mice. Acetic acid is administered intraperitoneally to the experimental animals to create pain sensation. Diclofenac (10 mg/kg b.wt, i.p.) was used as positive control. The plant extract was administered orally in three different doses (250, 300 and 350 mg/kg body weight) to the mice after an overnight fast. Test samples were administered orally 1 h prior to intraperitoneal administration of 0.7% v/v acetic acid solution (0.1ml/10g b.wt.) but diclofenac was administered 15 minutes prior to acetic acid injection. Each mice was observed individually for counting the number of writhing they made in 15 minutes commencing just 5 minutes after the intraperitoneal administration of acetic acid solution. Antinociceptive activity was indicated by the reduction in the mean of the number of writhings in the test groups compared with the control group. The percentage of antinociception was calculated based on the formula described as:

$$\% \text{ of antinociception} = \frac{(\text{Control group mean}) - (\text{Test group mean})}{(\text{Control group mean})} \times 100$$

ANALGESIC ACTIVITY

Tail flick method

Before the study, Swiss albino mice were screened for sensitivity test by placing the tip of the tail on the radiant heat source. Any animals that held to withdraw its tail in 5 second was rejected from the study. The selected animals were divided into five groups of six mice each. And were given doses as OTE (250, 300, 350mg/kg b.wt.) orally, diclofenac (10 mg/kg b.wt., i.p.). Analgesia was assessed with a tail flick apparatus (Analgesiometer). The basal reaction time was measured initially and another set of four measures were taken as 15, 30, 45 and 60 minutes interval and the reaction of the animals considered as the post drug reaction time[18].

Eddy's Hot plate method

The hot plate test was used to measure the response latencies. In this experiment the hot plate was maintained at 55-56^{o}C to observe the pain responses of mice. The response consists of paw licking and jumping of Swiss albino mice. The latent time before the occurrence of the pain response was recorded as an analgesic parameter. The untreated mice exhibiting latency time greater than 30 s or less than 5 s were excluded. The latency time was determined before and 30, 60 min after administration of ethanolic root extract (250, 300 or 350 mg/kg b.wt.) and diclofenac solution (10 mg/kg b.wt., i.p.) as a standard drug[19].

ANTIPYRETIC ACTIVITY

Yeast induced pyrexia method

A suspension of Brewer's yeast (15%) in saline (0.9%) was prepared. Five groups each containing 6 mice of either sex were taken. The animals were fevered by injection of brewer's yeast suspension (10 mg/kg b.wt.) subcutaneously in the back below the nape of the neck. The sight of injection was massaged in order to spread the suspension beneath the skin. Immediately after yeast administration, food was withdrawn, and then the rise in temperature was recorded after 30 minutes. The dose of the test compound and standard drug was given orally. The rectal temperature was recorded again after 60, 120 and 180 minutes. Diclofenac (10 mg/kg b.wt.) was selected as a standard drug[20].

STATISTICAL ANALYSIS

Results are expressed as Mean ± S.D. The difference between experimental groups was compared by One-way Analysis of Variance (ANOVA) followed by tukey's test. The results were considered statistically significant when $P<0.01$, $P<0.001$, $P<0.05$.

RESULTS AND DISCUSSION

Formalin Test

The antinociceptive activity of OTE assessed using the formalin test is shown (Table 1).

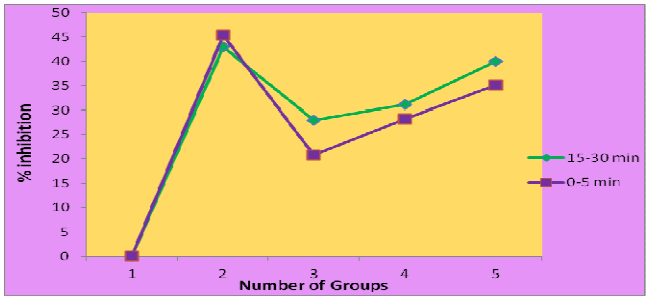
Table 1
Formalin induced stress response in various groups of mice at various doses

Treatment	Dose mg/kg	Total time spent in licking (s)			
		0 –5 min	% Inhibition	15–30 min	% Inhibition
Group 1	Control (0.9% saline)	76.83±1.60	0	68.67±4.46	0
Group 2	Diclofenac (10 mg/kg)	42.00±2.97*	45.334	39.17±3.43*	42.96
Group 3	OTE 250mg/kg	60.83±4.12*	20.825	49.5±2.17*	27.92
Group 4	OTE 300 mg/kg	55.17±3.13*	28.192	47.33±3.72*	31.08
Group 5	OTE 350mg/kg	49.83±3.19*	35.143	41.17±2.71*	40.05

Values expressed in table are mean ± standard deviation (n=6) in each group,
****p<0.01; significantly different from the control.***

The 250, 300 and 350 mg/kg OTE was found to produce significant (P<0.01) antinociceptive activity in a dose-dependent manner in both phases of the test (Figure 2). The extract at 350 mg/kg was found to produce almost similar strength of activity when compared to diclofenac(10 mg/kg b.wt.). The results presented in Table 1 show that pretreatment of the animals with OTE at 350 mg/kg b.wt. Produced significant and complete antinociceptive effect as compared with the animals pretreated with standard drug.

Figure 2
Percentage inhibition of formalin induced stress



Acetic acid-induced writhing test

The antinociceptive activity of *Operculina turpethum* extract (OTE) assessed using the acetic acid induced writhing test is shown in (Table 2).

Table 2
Percentage inhibition of antinociception in acetic acid induced writhing method

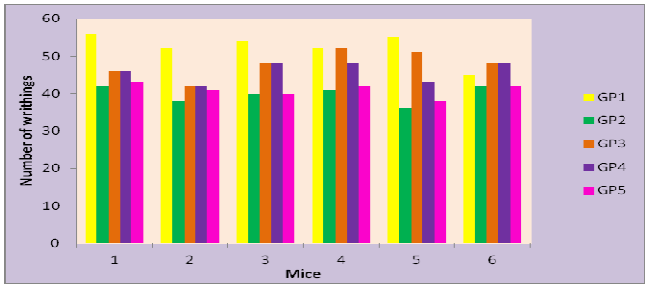
Groups	Treatments	Doses (mg/kg)	No. of Writhings Mean± SD	% Inhibition
Group I	Control (0.9% saline)	-	53.5±1.76	0.00
Group II	Diclofenac (10 mg/kg)	10	39.8 ±2.40*	25.61%
Group III	OTE 250mg/kg	250	47.8±3.60*	10.65%
Group IV	OTE300 mg/kg	300	45.83±2.71*	14.34%
Group V	OTE 350 mg/kg	350	41.0±1.79*	23.36%

Values expressed in table are mean ± standard deviation (n=6) in each group,
****p<0.01; when compared with control group***

All the doses of OTE showed significant reduction ($p<0.01$) of writhing induced by the acetic acid after oral administration in a dose dependant manner. After oral administration of three different doses (250, 300 and 350 mg/kg body weight), the percent inhibition was 10.65%, 14.34 % and 23.36% whereas diclofenac showed inhibition of 25.61% respectively. The reference drug diclofenac was found more potent than the plant extract at all of the dose levels (Figure 3).

Figure 3

Acetic acid induced writhing response in various groups of mice

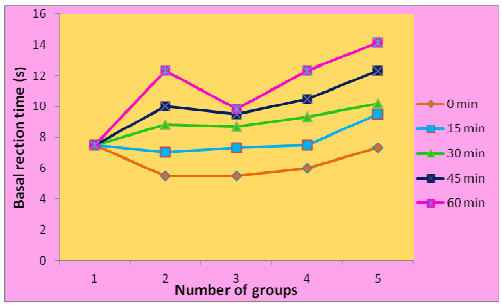


Tail flick method

In tail flick method, the ethanolic extract of *Operculina turpethum* root showed analgesic activity ($P < 0.001$), (14.17± 1.72) at a dose of 350mg/kg after 60 minutes whereas at a dose of 300mg/kg it showed ($P< 0.01$), (12.33±1.37) analgesic activity after 60 minutes (Figure 4). The extract significantly and dose-dependently protected the mice against thermally induced pain stimulus in mice.

Figure 4

Basal reaction time at various doses after various time intervals in tail flick method



Hot plate method

The interpretation of hotplate test is presented in (Table 3). The OTE was found to exhibit a dose-dependent increase in latency time when compared with control.

Table 3

Hot plate method latency time of different groups at different time intervals

		Latency time (sec)		
Groups	**Treatment**	**At 0 min**	**At 30 min**	**At 60 min**
Group I	Control(0.9%saline)	7.17±1.72	7.17±1.72	7.17±1.72
Group II	Diclofenac(10mg/kg)	8.83±0.75	14.5±2.81*	21.17±1.72*
Group III	OTE 250mg/kg	9±1.41	13.67±2.42*	15±2.37*
Group IV	OTE300 mg/kg	9.5±1.22*	13.5±2.35*	17.17±0.98*
Group V	OTE 350 mg/kg	10.33±1.03*	12.33±2.58*	19.5±1.05*

Values expressed in table are mean ± standard deviation (n=6) in each group, *p<0.01; when compared with control group

Treatment of animals with the OTE (250, 300 and 350 mg/kg) caused a significant (P<0.01). increase in the latency response in the hot-plate test (from 7.17±1.72 in control group to 19.5±1.05 in the OTE 350 mg/kg) treated groups, respectively.

Antipyretic activity

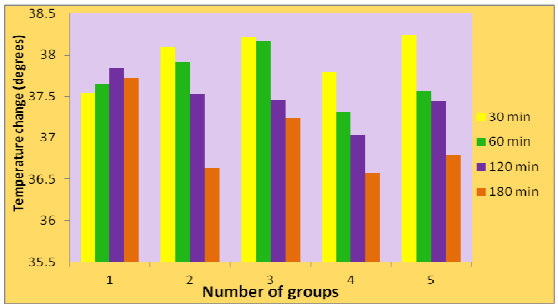
In yeast-induced pyrexia OTE significantly reversed hyperthermia at both doses (300mg/kg and 350 mg/kg) whereas the dose of 300mg/kg was found to be most effective. Time of peak effect obtained was 2 to 3h after oral administration of OTE. The standard drug, diclofenac (10mg/kg) also suppressed hyperthermia induced by yeast significantly (p<0.01) in all the observations when compared with control values (Table 4).

Table 4
Antipyretic effect of OTE in Swiss albino mice

Groups	Dose	Rectal temperature (°C) at different time			
		30 min	60 min	120 min	180 min
Group I	Control(0.9%saline)	37.54±0.51	37.64±0.64	37.84±0.72	37.72±0.71
Group II	Diclofenac (10 mg/kg)	38.10±0.52*	37.91±0.61*	37.53±0.57*	36.63±0.66*
Group III	OTE 250mg/kg	38.22±0.22*	38.17±0.06*	37.45±0.34*	37.24±0.14*
Group IV	OTE 300 mg/kg	37.80±0.44*	37.31±0.43*	37.03±0.54*	36.58±0.72*
Group V	OTE 350 mg/kg	38.24±0.40*	37.56±0.66*	37.44±0.20*	36.79±0.44*

Values expressed in table are mean ± standard deviation (n=6) in each group,
****p<0.01; significantly different from the control***

Figure 5
Graphical representation of the effect of OTE on hyperthermia induced by yeast in different groups



In the present study, these initial observations were confirmed and extended by demonstrating that the OTE (*Operculina turpethum* extract) administered orally, produced dose-related and marked antinociception in several models of chemical nociception, namely the formalin-induced licking response and acetic acid-induced abdominal constriction and it has also shown significant analgesia in Eddy's hot plate method and tail flick test.

DISCUSSION

Indigenous drug systems can be source of variety of new drugs which can provide relief in inflammation The antinociceptive effect of ethanolic root extract of *Operculina turpethum* was investigated in this study. Inflammation responses occur in three distinct phases and apparently mediated by different mechanisms: acute phase by local vasodilatation and increase capillary permeability, subacute phase by infiltration of leukocytes and phagocytic cells and chronic proliferative phases, in which tissue degeneration and fibrosis occurs[21]. Acetic acid induced writhing in mice attributed visceral pain finds much attention of screening analgesic drugs [22] .The abdominal constriction elicited by acetic acid has also been used to assess the potential analgesic activity of drugs. It is postulated that acetic acid acts indirectly by releasing endogenous mediators, which stimulate the

nociceptive neurons, is sensitive to nonsteroidal anti-inflammatory drugs, narcotics and other centrally acting drugs[23]. In writhing response, acetic acid causes pain by inducing release of endogenous mediators, such as PGE2 (prostaglandin E2) and PGF2α in peritoneal fluids as well as lipooxygenase products, which stimulate the nociceptive neurons sensitive to NSAIDs [24].Therefore, the results of the acetic acid-induced writhing strongly suggest that the mechanism of test extract. The OTE produced anti-nociceptive effect against thermal induced pain stimuli in mice in tail flick method at various points. In the present study, diclofenac also inhibited the pain produced by tail flick method. The hot plate and tail immersion methods elucidated the centrally acting antinociceptive effects of the ethanolic extract. Formalin induced stress also shows a biphasic response and originate mainly from neurogenic inflammation followed by participation of kinins and leukocytes with their pro-inflammatory factors including prostaglandins[25].Antipyretic are the agents, which reduce the elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus regulates the set point at which body temperature is maintained. Yeast induced fever is called pathogenic fever[26]. Its etiology includes production of prostaglandins, which set the thermoregulatory center at a lower temperature. Therefore, the antipyretic activity of ethanolic extract of *Operculina turpethum* is probably by inhibition of prostaglandin synthesis in hypothalamus.The results of this study exhibited that ethanolic (*Operculina turpethum* extract) OTE possess analgesic, anti-inflammatory and antipyretic activities which may be mediatedby central and peripheral mechanisms. This offers a new perspective in the treatment of pain. Further studies are in progress and are aimed to identify all active constituents responsible for the anti-inflammatory and anti-nociceptive properties.

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