

Evaluation of Antinociceptive Effect of Orlistat Using Different Methods of Screening Techniques in Mice

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ABSTRACT:

Orlistat was investigated for possible antinociceptive effect in mice. Three methods were used to study the antinociceptive effect, A. Thermal method (Eddy's hot plate, Tail flick method and Tail immersion method), B. Physical method (Tail clip method) and C. Chemical method (Acetic acid induced writhing test). Based on the researches conducted on Orlistat, it was identified that Orlistat possesses Phospholipase A2 inhibitory property. Phospholipase A2 catalysis the hydrolysis of membrane phospholipids resulting in the production of arachidonic acid and lysophospholipid which are precursors for inflammatory pain mediators such as eicosanoids. With this background of knowledge, the present study has been designed to explore the antinociceptive effect of Orlistat. Orlistat was administered in the dose range of 5.4, 10.8 and 21.6 mg/Kg orally 1 h prior to pain induction. Pentazocine 5 mg/ kg i.p was used as standard. Oral administration of Orlistat revealed antinociceptive potency at all therapeutic dose levels in all the models. It blocked the neurogenic and inflammatory pain and its activity was comparable with the reference drug. The results indicate that Orlistat possesses a significant $p < 0.001$ antinociceptive activity.

Key Words: Orlistat, Phospholipase A2, Antinociceptive effect, Arachidonic acid, Eddy's hot plate, Acetic acid induced writhing.

INTRODUCTION:

The sensation of pain is due to the stimulation of free nerve endings known as nociceptor. These receptors are found in both somatic and visceral structure distinguished between noxious and innocuous stimuli, and they are activated and sensitized by thermal, physical and chemical impulses. These underlying mechanisms of these noxious stimuli may be due to the release of bradykinins, leukotrienes (LTs), prostaglandins (PGs) that sensitize and/or activate the nociceptors. [1] Phospholipase A2 (PLA2) catalyze the hydrolysis of membrane phospholipids which results in the stoichiometric production of two potentially bioactive lipids that include non-esterified fatty acids principally arachidonic acid (AA) and lysophospholipid. Both of these phospholipid metabolites serve as precursors for inflammatory pain mediators such as eicosanoids. These mediators have been reported to cause sensitization of primary afferent neurons and to produce allodynic behaviors. [2-6]

Orlistat (tetrahydrolipstatin), a hydrogenated derivative of lipostatin, US Food and Drug Administration (FDA)-approved drug for body weight loss is an irreversible inhibitor of pancreatic and gastric lipases. Orlistat also inhibits gastric lipase, carboxyl ester lipase, and PLA2 that are all serine hydrolase. Orlistat is expected to inhibit eicosanoid synthesis at the level of PLA2. The effect being much like that of mechanism of action of glucocorticoids, potentiating antinociceptive effect. Action of PLA2 on membrane phospholipids trigger the production of AA which intern synthesis PGs and thromboxanes (TX) with the help of enzyme cyclooxygenase (COX). [7, 8] Orlistat is expected to inhibit eicosanoids synthesis at the level of PLA2. The effect being much like that of mechanism of action of glucocorticoid, potentiating antinociceptive effect. With this background, the present study has been designed to explore the anti- nociceptive effect of Orlistat using thermal, physical and chemical methods of screening techniques.

MATERIALS AND METHODS:**Dose Calculation:** [9]

$$\begin{aligned} \text{Rat dose (mg/ 200g body weight)} &= \text{Human therapeutic dose} \times \text{CF (0.018)} \\ &= 120 \text{ mg} \times \text{CF (0.018)} \\ &= 2.16 \text{ mg / 200 gms} \end{aligned}$$

Dose	Dose (mg/kg)	Mice [30 gms] (mg/ 30g)
½ Therapeutic Dose	5.4	0.16
Therapeutic Dose	10.8	0.32
2 Therapeutic Dose	21.6	0.6

Animals: Albino Mice of either sex, around 16 months old and weighing 30 ± 2 gms were selected and used. Animals were procured from disease free environment for the experiments. They were acclimatized to the laboratory conditions for 5 days. They were kept in sufficient polypropylene cages under controlled temperature and humidity conditions. The animals had free access to food and water and were housed under standard light-dark cycle, 12 hr each. All the experiments were carried out during day time from 0900 to 1600 hrs.

Drugs: The drug Orlistat used in the study was obtained from Sanmour Pharma Pvt Ltd, India. Orlistat being insoluble in water was administered orally by suspending in 5% acacia. [10] Pentazocine (5mg/kg i.p.) was used as standard.

Groups of animals: In the present study the animals were divided in to 5 groups, each consists of 5 mice. Each group of mice was administered with respective doses 1h prior to commencement of screening techniques.

- Group I served as control (5% gum acacia suspension)
- Group II treated with Pentazocine (5mg/kg i.p)
- Group III Orlistat (5.4 mg/Kg., p.o)
- Group IV Orlistat (10.8 mg/Kg., p.o)
- Group V Orlistat (21.8 mg/ Kg., p.o)

Laboratory Models for testing Antinociceptive Effect:**A. THERMAL METHOD-****1. Eddy's hot plate method:**

Mice were divided in to five groups with five mice in each group. They were placed on Eddy's hot plate maintained at 55 ± 1 °C individually. The surface can be a copper plate or a heated glass. The mouse was placed on the hot plate and the time until either licking or jumping occurs is recorded by a stop-watch. The reaction time in control and treated animals were recorded at 0 min, 30 min, 60 min, 120 min and 180 min after the treatment. The mouse was kept on the hot plate for not more than the maximal period of 30 sec to avoid tissue damage. [11, 12]

2. Tail flick method:

Albino mice were divided into five groups each containing five mice. The reaction time in control and treated animals were recorded at 0 min, 30 min, 60 min, 120 min and 180 min after the treatment. A cut off period of 10-12 sec is given to prevent damage to the tail. The tail of the mouse was placed on the hot wire (5.35 ampere) at a distance of 1 cm and the time taken by the mouse to flick its tail from the hot source was taken as the Basal Reaction Time (BRT). [12, 13]

3. Tail immersion method:

Albino mice were selected by immersing the tail in hot water at 55 ± 0.2 °C. The animals that showed a positive response within 5 sec by withdrawing of tail clearly out of water were selected and they are divided into five groups containing five mice in each group. BRT was recorded at 0 min, 30 min, 60 min and 90 min after the administration of the test compounds and standard drug. [13, 14, 15] The cut off time of the immersion is 15 sec. The withdrawal time of untreated animals is between 1 and 5.5 sec. A withdrawal time of more than 6 sec therefore is regarded as a positive response. [16]

B. PHYSICAL METHOD-**4. Tail clip method:**

Albino mice were selected by applying a metal clip to the base of tail. The animals which showed efforts to dislodge the clip within 15 seconds were used for the experiments and such animals were divided into five groups containing five animals in each. The tail clip was applied at 0, 30, 60 and 90 minutes after drug administration and the BRT was noted. [13-15].

C. CHEMICAL METHOD-**D. Acetic acid induced writhing in mice:**

A group of mice were administered 0.1 ml/10g of 0.3% (v/v) acetic acid *i.p.* The mice exhibiting the writhing episodes (stretching hind limbs and bending of trunk) were selected for the study. These mice were randomly divided into five groups each with five mice. One group was administered with diclofenac sodium 15 mg/kg orally 1 hr prior the acetic acid injection. The numbers of writhing episodes were counted for 25 min following acetic acid administration at three different time intervals. [17]

RESULT:

Antinociceptive effect of Orlistat was evaluated using various screening techniques. All the techniques show a significant activity at therapeutic dose level. The standard Pentazocine 5mg/kg *i.p.* exert a significant analgesic effect ($p < 0.001$) at 30 min. The following are the results obtained from individual screening methods.

Eddy's hot plate method results shows that Orlistat at all the three doses 5.4, 10.8 and 21.6 mg/kg., *p.o.* shows significant analgesic effect ($p < 0.001$) at 30 min and Orlistat 21.6 mg/kg., *p.o.* shows significant analgesic effect ($p < 0.01$) at 60 min (Table 1).

In tail flick method, the antinociceptive effect of Orlistat at all the three doses 5.4, 10.8 and 21.6 mg/kg., *p.o.* shows significant analgesic effect ($p < 0.001$) at 30 min (Table 2).

In tail immersion method, Orlistat 5.4 mg/kg., *p.o.* does not show any significant effect. Whereas Orlistat 10.8 and 21.6 mg/kg., *p.o.* shows significant analgesic effect ($p < 0.001$) at 30 min (Table 3).

In tail clip method, Orlistat 5.4 mg/kg., *p.o.* does not show any significant effect. Orlistat 10.8 mg/kg., *p.o.* show significant analgesic activity ($p < 0.001$) at 90 min. Additionally Orlistat 21.6 mg/kg., *p.o.* show significant analgesic activity ($p < 0.001$) at 30 min (Table 4).

Acetic acid induced writhing was produced by treating mice with 0.1ml/10g of 0.3 % (v/v) acetic acid *i.p.* The writhing was noted for about 25 min with intervals as 5-10 min, 10-20 min, 20-25 min. Orlistat 5.4 mg/kg., *p.o.* does not show any significant effect at all intervals. Orlistat 10.8 and 21.6 mg/kg., *p.o.* show significant analgesic activity ($p < 0.001$) at 5-10 min and 10-20 min whereas it produces $p < 0.01$ analgesic activity at 20-25 min (Table 5).

DISCUSSION:

The present study suggests that Orlistat 10.8 and 21.6 mg/kg., *p.o.* possesses antinociceptive activity and Orlistat 5.4 mg/kg *p.o.* possesses mild antinociceptive activity. Pain is a major problem because majority of tissues and organs are innervated by special sensory receptors (nociceptors) connected to primary afferent nerve fiber of different diameters. PLA2 hydrolyzes the sn-2 position of glycerophospholipids to release AA and lysophospholipid and subsequently generates lipid mediators such as PGs, LTs, platelet-activating factor (PAF) and lysophosphatidic acid. [18, 19] These phospholipid metabolites serve as precursors for inflammatory pain mediator. Additionally Lyso PAF formed during the process converts to PAF by acetylation is an important mediator of inflammation and allergies. [8]

Under normal conditions, pain is associated with electrical activity in small diameter primary afferent fibers of peripheral nerves. These nerves have sensory endings in peripheral tissues, and are activated by stimuli of various kinds thermal, physical and chemical. Orlistat was evaluated using various pain models like thermal (Eddy's hot plate, tail flick and tail immersion), physical (tail clip) and chemical (acetic acid induced writhing) screening techniques. In all models it possessed significant analgesic activity at dose levels 10.8 mg/kg and 21.6 mg/kg *p.o.* and mild activity was seen in lower dose of Orlistat 5.4 mg/kg *p.o.* The abdominal writhing induced by acetic acid involves the production and release of AA metabolite via COX and PG biosynthesis. [20, 21] Which is the exact mechanism by which Orlistat is expected to act and the results shows significant activity at therapeutic dose level.

CONCLUSION:

In conclusion, one can say that, Orlistat showed significant antinociceptive effect at therapeutic dose levels. Hence our findings indicate that further development of Orlistat as antinociceptive drug is warranted. Further studies could provide important therapeutic options, pharmacologic profiles and perhaps to improve the efficacy of this drug.

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