AN EXPERIMENTAL STUDY OF ANALGESIC ACTIVITY OF SELECTIVE COX-2 INHIBITOR WITH CONVENTIONAL NSAIDs

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ABSTRACT
The present study was carried out to investigate the analgesic activity of Etoricoxib (10 mg) for individual drug therapy and etoricoxib (5 mg) for combination therapy with diclofenac potassium (10 mg) using Acetic acid induce writhing, Hot plate and Tail immersion methods. The test and standard drugs significantly (p<0.001) reduced the number of abdominal constriction and stretching of hind limb induce by the injection of acetic acid in a dose dependent manner. The Hot plate and Tail immersion test useful in the elucidating centrally mediated antinoceptive responses, which focused mainly on changes above the spinal cord level. All the test and standard drugs significantly (p<0.001) reduced the pain as compare to the control group. The results of pharmacological tests performed in the present studies suggest the combination of Etoricoxib and diclofenac potassium possess potent analgesic activity.

Key words: Etoricoxib, cox-2 inhibitor, diclofenac potassium, analgesic.

INTRODUCTION
Nonsteroidal anti-inflammatory drugs (NSAIDs) reduce pain and edema by suppressing the formation of prostaglandins, by inhibiting the activity of the enzyme Cyclooxgenase (COX-1 and COX-2). However, prostaglandins are key mediators of several components of GI mucosal defense, so suppression of synthesis of prostaglandins (PGs) by NSAIDs greatly reduces the resistance of the mucosa to injury as well as interfering with repair processes. Selective COX-2 inhibitors were thought to be the solution to this conundrum as it is required that NSAIDs suppress prostaglandin synthesis at sites of inflammation, and not in the GI tract.

However, it is now clear that both COX-1 and COX-2 isoforms contribute to mucosal defense. Selective COX-2 inhibitors elicit less GI damage and bleeding than conventional NSAIDs, although the magnitude of this reduction continues to be contested in the literature. As widely reported in the lay-press, the selective COX-2 inhibitors also cause significant adverse effects in the renal and cardiovascular systems, possibly more serious than those caused by conventional NSAIDs. The market for NSAIDs is expanding rapidly because of an aging population in developed countries and the associated increase in the prevalence of diseases like arthritis. Use of Aspirin is also increasing because of its utility in reducing the incidence of a number of common disorders including stroke, myocardial infarction, Alzheimer's disease and cancer1.

However, their use is limited by their significant side effects upon the stomach and the kidney. Their side effects as well as their therapeutic actions are related to their ability to inhibit cyclooxgenase enzymes involved in the first step of the arachidonic acid cascade.2-4 In addition, the damaging effect of some NSAIDs upon the stomach and intestine is in part due to their acidic nature, as with indomethacin, ibuprofen, diclofenac, naproxene, aspirin, etc.4 Although basic NSAIDs such as glafenine and flufenacet sodium are expected to be devoid of the primary insult effect, their damaging effect upon the stomach and kidney is still prominent as they inhibit prostaglandin biosynthesis as strongly as indomethacin.5,6

In the recent years, several novel approaches for reducing the GI toxicity of NSAIDs with promising results have been reported. These mainly involve structural modification of existing NSAIDs such that inhibition of COX is maintained, but other attributes are added that diminish GI (and other) toxicity, and in some cases boost efficacy and/or potency1. The mortality rate for NSAID induced GI bleeding is 5-10% in the world population.

MATERIALS AND METHODS
Selection of Drugs and Chemicals
For the purpose of this work we selected Etoricoxib (Selective COX – 2 inhibitor), Diclofenac potassium (Conventional NSAID), Ibuprofen (Standard drug ) and Glacial acetic acid.

Preparation of drugs and Chemical solutions
Etoricoxib (10mg/kg body weight) was dissolved in sufficient quantity of solvent in normal saline and use in the treatment. Etoricoxib (5mg/kg) and Diclofenac potassium (10mg/kgbody weight) was dissolved together in sufficient quantity of solvent(normal saline)& Glacial acetic acid was prepared by using normal saline of strength of 1%v/v.

Selection of Experimental Animals
Healthy Swiss albino rats of either sex weighing 220-250g were used in this study. All the animals were obtained from Animal house of the School of Pharmaceutical Sciences, SOA University, Bhubaneswar, Orissa. The animals were housed comfortably in a group of six in a single clean plastic cage with a metal frame lid on its top. They were housed under standard environmental conditions of temperature (24±1°C) and relative humidity of 30-70 %. A 12:12 h light dark cycle was followed. All animals had free access to water and standard pelletized laboratory animal diet diet ad libitum. All the experimental procedures and protocols used in this study were reviewed and approved via the Approval No. 17/09/IAEC/SAU by the Institutional Animal EthicalCommittee (IAEC) of School of Pharmaceutical sciences, S’ O’ A University, Bhubaneswar, Orissa (Regd. No. 1171 /C/08/CPCSEA) constituted in accordance with the guidelines of the CPCSEA, Government of India.

Evaluation of Analgesic activity7-10
Pain is not easily or satisfactorily defined and therefore is often interpreted as a suffering that results from the perception of painful stimuli. It’s a common symptom and it indicates that something is wrong in the body and may give a clue to the nature of disease. Hence, “pain is a specific sensation with its own peripheral and central mechanisms independent of other five senses.” Pain itself is not a disease; it is by far the most common medical complaint. It is usually perceived as an indication of ill health and most diseases have a component of pain. The control of pain is one of the most important uses to which drugs are put. Pain can be defined as the effect produced in consciousness by the arrival of nerve impulses generated by noxious stimuli in the brain. Drugs, which alter the pain sensitivity or remove pain, are called as painkiller or analgesics.

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Acetic acid induced writhing in mice

Acetic acid induced writhing method was adopted for evaluation of analgesic activity. Writhing is defined as a stretch, tension to one side, extension of hind legs, contraction of the abdomen so that the abdomen of mice touches the floor, turning of trunk (twist). Any writhing considered as a positive response. Swiss albino mice weighing between 15-35g were used for evaluation of analgesic activity; in each group six albino mice were kept.

A solution of acetic acid (1% v/v) in distilled water was prepared. A solution of Ibuprofen (dose-100mg/kg/10ml) was prepared in normal saline water. Test - 1 : A solution of Etoricoxib (10mg/kg/10ml). Test - 2 : A solution Etoricoxib (5mg/kg) in combination with Diclofenac potassium (10mg/kg) was prepared in 10ml of normal saline water.

Wistar albino mice of either sex were divided into four different groups each containing six animals, the animals were marked individually. Food was withdrawn 12 hours prior to drug administration till completion of experiment. The animals were weighed and numbered appropriately. The test and standard drugs were given orally. After 60 minutes writhing was induced by intraperitoneal injection of 1% acetic acid in volume of 0.1 ml/kg body weight. The writhing episodes were recorded for 30 minutes; stretching movements consisting of arching of the back, elongation of body and extension of hind limbs were counted.

Percentage of inhibition was evaluated using following formula: The results of Acetic acid induced writhing method in mice was tabulated in Table-1.

**Hot plate method in rats**

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 an electrically heated surface. The temperature is controlled for 55° to 56 °C. This can be a copper plate or a heated glass surface. The animals are placed on the hot plate and the time until either licking or jumping occurs is recorded by a stop-watch.

Swiss albino rats weighing between 100-150g were used for evaluation of analgesic activity; in each group six albino rats were kept. A solution of Ibuprofen (dose-100mg/kg/10ml) was prepared in normal saline water. Test - 1 : A solution of Etoricoxib (10mg/kg/10ml). Test - 2 : A solution Etoricoxib (5mg/kg) in combination with Diclofenac potassium (10mg/kg) was prepared.

Wistar albino mice of either sex were divided into four different groups each containing six animals, the animals were marked individually. Food was withdrawn 12 hours prior to drug administration till completion of experiment. The animals were weighed and numbered appropriately. The test and standard drugs were given orally. After 60 minutes, the observations were recorded and at the time interval of 90, 120 and 180 minutes. The results of tail immersion test in mice was tabulated in Table-3.

**Tail immersion test in mice**

The procedure is based on the observation that morphine-like drugs are selective incapable of prolonging the reaction time of the tail withdrawal reflex in rats induced by immersing the end of the tail in warm water of 55 °C.

The lower 5 cm portion of the tail is marked. This part of the tail is immersed in the water bath of exactly 55 °C. Within a few seconds the rat reacts by withdrawing the tail. The reaction time is recorded in 0.5 s units by stopwatch. After each determination the tail is carefully dried. The reaction time is determined before and periodically after oral administration of the test and standard substance.

**RESULTS**

All the test and standard drugs significantly (p<0.001) reduce the number of abdominal constrictions and stretching of hind limb induced by the injection of acetic acid in a dose dependent manner. (Table-1)

As all the drugs are standard analgesics, by applying Student Newman-Keuls test, it was shown that no significant difference between the tests and standard. The standard drug exhibited a
Each value is the mean ± SEM for 6 rats, *P < 0.05; **P < 0.01; ***P < 0.001 compared with control. Data were analyzed by using One-way ANOVA followed by Dunnett’s test.

**DISCUSSION**

The abdominal constriction response induced by acetic acid is a sensitive procedure to evaluate peripherally acting analgesics.14 In general, acetic acid causes pain by liberating endogenous substances such as serotonin, histamine, prostaglandins (PGs), bradykinins and substance P, endings. Local peritoneal receptors are postulated to be involved in the abdominal constriction response.15

The Hot plate and Tail immersion test useful in elucidating centrally mediated antinociceptive responses, which focuses mainly on changes above the spinal cord level. All the test and standard drugs significantly (p<0.001) reduce the pain as compared to the control (Table 2). By applying Student Newman-Keuls test, it was shown that there is significant (p<0.01) effect of test-2 as compared to the standard at 60 and 90 minutes. But there is no significant difference between test-1 and standard.

In Tail immersion method all the test and standard drugs significantly (p<0.001) reduce the pain as compared to the control group (Table 3). By applying Student Newman-Keuls test, it was shown that there is significant (p<0.01) effect of test-1 &test-2 as compared to the standard at 90 minutes and there is significant (p<0.05) effect of test-2 and standard group at 90 and 120 minutes.
levels of PGE2 and PGF2α in peritoneal fluids as well as lipoxygenase products.16

The significant increase in pain threshold produced by tests and standard in these models suggests involvement of central pain pathways. Pain is centrally modulated via a number of complex processes including opiate, dopaminergic descending noradrenergic and serotonergic systems.17-19. The analgesic effect produced by the tests and standards may be via central mechanisms involving these receptor systems or via peripheral mechanisms involved in the inhibition of prostaglandins, leukotrienes, and other endogenous substances that are key players in pain.

The selective cox-2 inhibitor has high effectiveness than the conventional NSAIDs and has low GI and high cardiovascular side effects than to the conventional NSAIDs. Etoricoxib is a cox-2 inhibitor with a high degree of selectivity of its target. It provides an alternative to other selective and traditional NSAIDs in treating patients with arthritis and other painful conditions.

Here in this research work we found that Etoricoxib is more effective than the conventional NSAIDs. The low dose combination of etoricoxib with conventional NSAIDs has more effective for analgesic activity as compare to the etoricoxib and conventional NSAIDs. Here we conclude that the combination product was more effective than the single drug, it may be due to different mechanism of actions of different drugs in combined products. But the chances of side effects of combination products are more as compare to the single drug. More study on combination drug therapy may overcome these problems.

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REFERENCES


