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ANTI-INFLAMMATORY AND ANTIPYRETIC ACTIVITY OF SELECTIVE COX-2 INHIBITOR WITH CONVENTIONAL NSAIDs

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ABSTRACT

Objective: The selective COX-2 inhibitor has high cardiovascular side effects, with low GI side effects, as compare to the conventional NSAID and it is proved that low dose of any drugs is always good for the health due to chances of less adverse effects. So the main objective of this research work is to lower the dose of selective COX -2 inhibitor, combine with a conventional NSAID and find out the pharmacological activity of combination drug, selective cox-2 inhibitor alone and other standard NSAIDs and compare with control.

Methods: Carrageenan induced hind paw edema in Rats and Brewer's yeast induced pyrexia in rat models were used for the evaluation of anti-inflammatory and antipyretic activity.

Results: The % age of inhibition of edema at peak hrs i.e. at 3 and 4 hrs were (51.8%) and (49.7%) for standard drug (62.1%) and (59.3%) for test-1 drug (71.4%) and (70.1%). The percentage of reductions in rectal temperature of tests and standard group was shown in Graph-1 b. The present results show that the test-2 possesses a significant antipyretic effect in yeast-provoked elevation of body temperature in rats, and its effect is less than that of paracetamol.

INTRODUCTION: Non steroidal anti-inflammatory drugs (NSAIDs), including selective cyclooxygenase (COX-2 inhibitors), have come to play an important role in the pharmacologic management of arthritis and pain. Clinical trials have established the efficacy of etoricoxib in osteoarthritis, rheumatoid arthritis, acute gouty arthritis, ankylosing spondylitis, low back pain, acute postoperative pain, and primary dysmenorrhoea. Comparative studies indicate at least similar efficacy with etoricoxib versus traditional NSAIDs.

NSAIDs are 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 cancer [1]. 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 cyclooxygenase enzymes involved in the first step of the arachidonic acid cascade [2-3]. 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, naproxen, aspirin etc.[4]

Although basic NSAIDs such as glafenine and floctafenine 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]. The selective COX- 2 inhibitor has high cardiovascular side effects, with low GI side effects, as compare to the conventional NSAID and it is proved that low dose of any drugs is always good for the health due to chances of less adverse effects. So the main objective of this research work is to lower the dose of selective COX -2 inhibitor, combine with a conventional NSAID and find out the pharmacological activity of combination drug, selective Cox-2 inhibitor alone and other standard NSAIDs and compare with control. So the present study was intended to find out that whether the low dose combination of a selective Cox-2 inhibitor is effective to the single dose and to the other conventional NSAIDs.

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), Carrageenan (Inflammation inducer), Brewer's yeast (Pyrexia inducer) and paracetamol (standard drug)

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/kg body weight) was dissolved together in sufficient quantity of solvent (normal saline) & use in the treatment. Ibuprofen (100mg/kg body weight) was dissolved in sufficient quantity of solvent in normal saline and Carrageenan was prepared by using normal saline of strength of 1%w/v. Paracetamol (200mg/kg body weight) for Antipyretic activity was dissolved in sufficient quantity of solvent in normal saline and use in the treatment. Brewer's yeast (20% w/v) suspension was prepared by using normal saline.

Selection of Experimental Animals: Healthy Wistar 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, S 'O' A 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 *ad libitum*. All the experimental procedures and protocols used in this study were reviewed and approved via the Approval No. 17/09/IAEC/SOAU by the Institutional Animal Ethical Committee (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 Anti-inflammatory Activity [7, 8, 9]**:** The anti-inflammatory activity of the test and standard compounds was evaluated by carrageenan induced rat paw edema method. Thereby measuring the carrageenan induced inflammation by slide calipers. Inflammation is response of the tissue to an infection, irritation or due to any foreign substance e.g. Carrageenan. The Carrageenan induced rat paw edema is standard method for testing anti-inflammatory drugs. One of the cardinal signs of inflammation is the presence of edema.

Wistar albino rats 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, 0.1ml of 1% w/v carrageenan was injected in the sub plantar region of the left paw of control, test and standard drug treated groups. The paw length of all the groups of rats were noted at 0,1, 2, 3, 4, 5, 6 hrs after carrageenan challenge. The percentage inhibition of inflammation in the standard or test drug treated animals was recorded and calculated using the formula as below

% of inhibition= 100 [1-(a-x) / (b-y)]

Where, a=Mean paw volume of treated animal after Carrageenan inj.

b= Mean paw volume of control animal after Carrageenan inj.

X= Mean paw volume of treated animal before Carrageenan inj.

Y= Mean paw volume of control animal before Carrageenan inj.

The results of carrageenan induced hind paw edema in rats were tabulated in **Table 1**.

Evaluation of antipyretic activity [10]: The subcutaneous injection of Brewer's yeast suspensionis known to produce fever in rats. Pyrexia was induced by subcutaneously injecting 20% w/v brewer's yeast suspension (10ml/kg) into the animal's dorsum region. 17h after the injection, the rectal temperature of each rat was measured using a clinical thermometer. Only rats that showed an increase in temperature of at least 0.7 ^{0}C were used for experiments.

A group of Wistar albino rats were taken and 20% w/v brewer's yeast suspension (10ml/kg) was administered via subcutaneous route. 17hr after the injection, the rectal temperature of each rat was measured using a clinical thermometer. Only rats that showed an increase in temperature of at least 0.7 ^{0}C were used for experiments. Then they are divided into four different groups each containing six animals, the animals were marked individually. The

animals were weighed and numbered appropriately. The test and standard drugs were given orally. The rectal temperature was measured after 60 minutes and at a time intervals of 0, 1, 2, 3, 4, 5, 6 hrs. The percentage of reduction in rectal temperature was calculated using the formula as below;

$$\% \text{ of reduction} = (B\text{-}C_n / B\text{-}A) \times 100$$

Where, A= Initial rectal temperature (before yeast injection); B= Rectal temperature at 0 hr; C_n= Rectal temperature at time interval after administration of drug

The results of brewer's yeast induced pyrexia in rats were tabulated in **Table 2**.

TABLE 1: ANTIINFLAMMATORY ACTIVITY BY CARRAGEENIN INDUCED HIND PAW EDEMA IN RAT

Group	Treatment	Dose (mg/kg)	Edema length (cm) at time intervals (hrs.						
			0	1	2	3	4	5	6
Control	Normal Saline	-------	0.32±0.002	0.761±0.019	0.801±0.023	0.905±0.024	0.971±0.050	0.916±0.026	0.876±0.027
Standard	Ibuprofen	100	0.33±0.002	0.511±0.006 [c]	0.55±0.006 [c]	0.61±0.016 [c]	0.658±0.021 [c]	0.655±0.022 [c]	0.636±0.023 [c]
Test – 1	Etoricoxib	10	0.325±0.003	0.461±0.014 [c]	0.486±0.011 [c]	0.54±0.005 [c]	0.59±0.006 [c]	0.576±0.017 [c]	0.53±0.018 [c]
Test - 2	Etoricoxib + Diclofenac Potassium	5+10	0.325±0.003	0.418±0.010 [c]	0.45±0.011 [c]	0.491±0.010 [c]	0.52±0.006 [c]	0.501±0.007 [c]	0.485±0.006 [c]

Each value is the mean ± SEM for 6 rats, [a] P < 0.05; [b] P < 0.01; [c] P < 0.001 compared with control. Data were analyzed by using One-way ANOVA followed by Dunnett's test

TABLE 2: ANTIPYRETIC ACTIVITY BY BREWER'S YEAST INDUCED PYREXIA IN RATS

Group	Treatment	Dose (mg/kg)	Rectal Temperature at time intervals (hrs.) (mean ± sem)						
			0	1	2	3	4	5	6
Control	Water	-------	36.9±0.068	36.93±0.091	36.95±0.076	36.93±0.061	36.93±0.061	36.91±0.047	36.91±0.060
Standard	Paracetamol	200	37.1±0.044	35.81±0.030 [c]	35.76±0.042 [c]	35.75±0.022 [c]	35.63±0.042 [c]	35.75±0.076 [c]	35.71±0.087 [c]
Test – 1	Etoricoxib	10	37.01±0.060	36.78±0.040 [a]	36.61±0.047 [a]	36.35±0.042 [b]	36.21±0.047 [b]	36.2±0.036 [b]	36.21±0.030 [b]
Test - 2	Etoricoxib + Diclofenac Potassium	5+10	36.96±0.066	36.41±0.079 [b]	36.11±0.065 [b]	36.03±0.120 [c]	35.96±0. 033 [c]	35.96±0.042 [c]	35.98±0.047 [c]

Each value is the mean ± SEM for 6 rats, [a] P < 0.05; [b] P < 0.01; [c] P < 0.001 compared with control. Data were analyzed by using One-way ANOVA followed by Dunnett's test

RESULTS AND DISCUSSION: The most widely used primary test to screen new anti-inflammatory agent measure the ability of a compound to reduced local edema induced in the rat paw by injection of an irritant agent. Carrageenan induced edema has been commonly used as an experimental animal model for acute inflammation & is believed to be biphasic. The early phase (1-2hrs) of the carrageenan model is mainly mediated by histamine, serotonin & increased synthesis of prostaglandin in the damage tissue surrounding. The late phase is sustained by prostaglandin release & mediated by bradykinin, leukotriene, polymorphonuclear cell & prostaglandin produced by tissue macrophages [11, 12].

Here in this study, all the test and standard drugs significantly ($p<0.001$) inhibit the inflammation as compare to the control group (Table 1). By applying Student Newman-Keuls test, there is significant ($p<0.001$) difference between test-2 and standard at 3, 4, 5 and 6 hrs and at ($P<0.01$) there is significant difference between test-1 and test-2 at 3, 4 and 5 hrs. The % of inhibition of edema at peak hrs i.e. at 3 and 4 hrs were (51.8%) and (49.7%) for standard drug, (62.1%) and (59.3%) for test-1 drug, (71.4%) and (70.1%) (**fig. 1 a & b**).

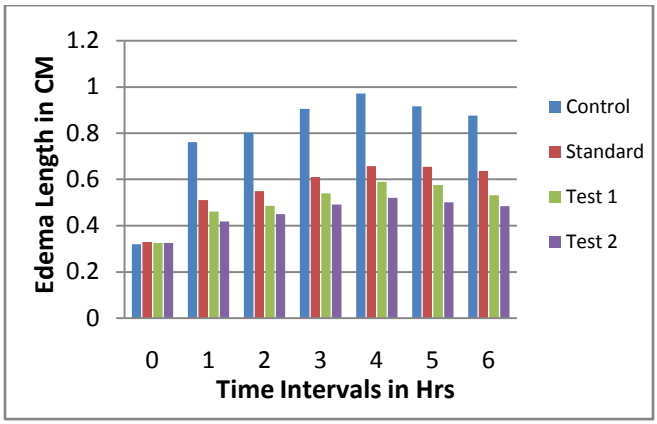


FIG. 1A: ANTI-INFLAMMATORY ACTIVITY BY CARRAGEENAN INDUCED IN PAW EDEMA IN RATS

Standard- Ibuprofen (100mg/kg), Test 1- Etoricoxib (10mg/kg), Test 2- Etoricoxib (5mg/kg) + Diclofenac potassium (10mg/kg)

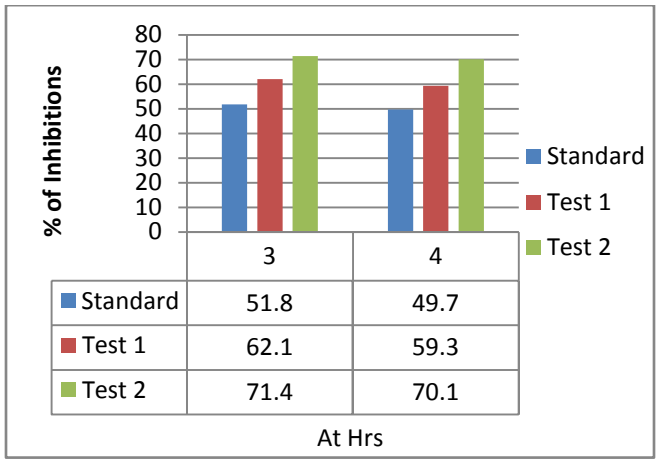


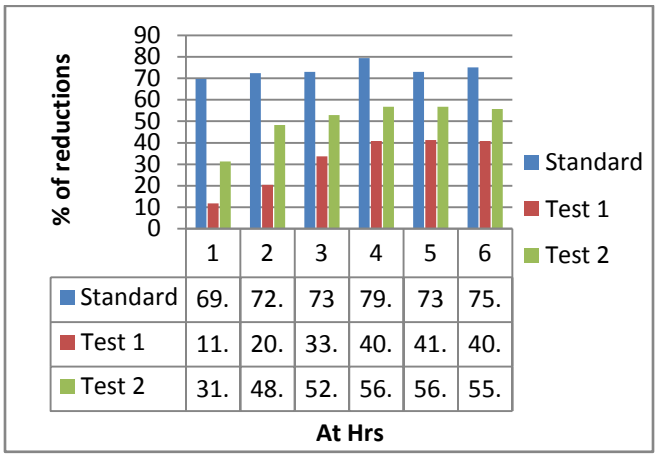
FIG. 1B: % OF INHIBITIONS OF PAW EDEMA

Standard – Ibuprofen (100mg/kg), Test 1 – Etoricoxib (10mg/kg), Test 2 – Etoricoxib (5mg/kg) + Diclofenac potassium (10mg/kg)

Fever may be due to infection or one of the sequels of tissue damage, inflammation, graft rejection, or other disease states. 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. In fever this set point elevates and a drug like paracetamol does not influence body temperature when it is elevated by the factors such as exercise or increase in ambient temperature.

Yeast induced fever is called pathogenic fever. Its etiology includes production of prostaglandins which set the thermoregulatory center at a lower temperature. The standard group produced significant ($p<0.001$) as compare to control group. At ($p<0.01$) the test-1 was significant to the control at 3, 4, 5 and 6 hrs and at ($p<0.05$) significant at 1 and 2 hrs. The test-2 was significant at 3, 4, 5 and 6 hrs ($p<0.001$) and at 1 and 2 hrs ($p<0.01$) as compare to the control

group. The % of reductions in rectal temperature of tests and standard group was shown in Fig. 2.



	1	2	3	4	5	6
Standard	69.	72.	73	79.	73	75.
Test 1	11.	20.	33.	40.	41.	40.
Test 2	31.	48.	52.	56.	56.	55.

FIG. 2: % OF REDUCTION IN RECTAL TEMPERATURE BY BREWER'S YEAST INDUCED PYREXIA IN RATS

Standard- Ibuprofen (100mg/kg), Test 1- Etoricoxib (10mg/kg), Test 2- Etoricoxib (5mg/kg) + Diclofenac potassium (10mg/kg)

The present results show that the test-2 possesses a significant antipyretic effect in yeast-provoked elevation of body temperature in rats, and its effect is less than that of paracetamol (standard drug). There are several mediators or multiprocesses underlining the pathogenesis of fever. Inhibition of any of these mediators may bring about antipyretics.

CONCLUSION: The selective cox-2 inhibitor has high effective than the conventional NSAIDs and has low gastrointestinal 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 anti-inflammatory activity as compared to the Etoricoxib and conventional NSAIDs alone and for antipyretic activity it was found that Etoricoxib in combination with diclofenac shows more effective than the Etoricoxib alone but lees as compare to the paracetamol.. 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 chance of side effects of combination products is more as compare to the single drug. More study on combination drug therapy may overcome this problem.

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