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# Beneficial Effects of Vegetable Oils (Rice Bran and Mustard Oils) on Anti-inflammatory and Gastro Intestinal Profiles of Indomethacin in Rats

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**ABSTRACT**

Non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin are used in the treatment of inflammation, fever and pain. They are prescribed for a variety of acute and chronic inflammatory conditions and diseases. However, NSAIDs cause gastric damage as a major adverse reaction. In this study, the anti-inflammatory activity of vegetable oils (rice bran and mustard oils) alone and in combination with indomethacin was studied using carrageenan-induced rat paw edema. The gastro-protective effect of vegetable oils was also studied using model of indomethacin induced gastric damage and compared with omeprazole. The results showed that rice bran oil significantly ($p<0.001$)reduced the paw edema (97.08%) and improved the anti-inflammatory effect of indomethacin in carrageenan-induced paw edema. However, mustard oil did not show any significant anti-inflammatory activity. Besides, the administration of indomethacin together with the vegetable oils did not cause a statistically significant gastric lesions. However, administration of indomethacin alone caused statistically significant gastric lesions. These results suggest that rice bran oil improved the anti-inflammatory effect of indomethacin in acute model of inflammation and rice bran and mustard oils reduced the ulcerogenic effect of indomethacin on the gastrointestinal tract when they are used together with indomethacin.

**Key words:** Carrageenan inflammation, Indomethacin, gastric damage and vegetable oils

## INTRODUCTION

Non-steroidal Anti-inflammatory Drugs (NSADs) are among the commonly prescribed drugs for a variety of acute and chronic inflammatory conditions and diseases. The rate of conversion of arachidonic acid into prostanoids depends on the availability of two cyclooxygenase (COX) enzymes, COX-1 and COX-2[1]. The COX enzymes constitute the main targets of action of NSAIDs, most of which inhibit both isomers, whereas some of them selectively inhibit COX-2[2]. However, commercial NSAIDs cause gastric lesions as an important adverse effect by inhibiting prostaglandin biosynthesis and suppression of the formation of prostaglandins. Indomethacin, which is a NSAID, has been used to reduce inflammation, pain and fever in humans. However, it causes injury, as other NSAIDs do, on the gastric mucosa, mainly due to inhibition of the COX enzymes and suppression of prostaglandins biosynthesis[3].

The search for newer and novel compounds which possess anti-inflammatory activity without the unwanted side effects has been an important frontier in the research of anti-inflammatory agents. The vegetable oils such as rice bran, mustard, sesame, sunflower and olive oils have been shown to possess anti-inflammatory, anti-ulcerogenic, hypocholesterolemic and anti-cancer activities. This study has therefore been designed to evaluate the anti-inflammatory and anti-ulcerogenic potential

of rice bran and mustard oils and also to evaluate their beneficial effects on the anti-inflammatory and gastrointestinal profiles of indomethacin in rats.

## MATERIAL AND METHODS

### Animals

A total of 60 rats of either sex weighing between 150 to 250 g were used in the present study. The animals were kept under standard conditions with standard pellet food. The experimental protocol was approved by Institutional Animal Ethics Committee.

### Carrageenan-induced paw edema in rat[4]

The paw edema in rat was induced by injection of 0.1 ml of 1% carrageenan solution into the right hind paw plantar surface and paw volume was measured by plethysmometer. The animals were divided into six groups of six animals in each group and administered orally with vehicle, 2 ml/kg, rice bran oil, 2 ml/kg and mustard oil, 2 ml/kg and indomethacin, 25 mg/kg alone ; indomethacin 25 mg/kg together with rice bran oil, 2 ml/kg and mustard oil, 2 ml/kg. One hour later, 0.1 ml of 1% carrageenan solution was injected into the right hind paw plantar surface and then changes in paw volumes of rat were measured at I h interval for 4 h by plethysmometer . The results were presented as the paw volume variations in relation to the control volumes.

### Indomethacin-induced gastric damage[5,6]

The gastroprotective effect of the vegetable oils on indomethacin-induced gastric damage was determined and compared with reference drug, omeprazole. Animals were divided into four groups each consisting of six rats and were starved for 24 h before the experimentation. Vehicle, 2 ml/kg, Vegetable oils, rice bran oil, 2 ml/kg, mustard oil, 2 ml/kg and omeprazole 20 mg/kg were administered orally to the assigned groups of rats. Five minutes later, indomethacin, 25 mg/kg was administered to all animals orally to induce gastric damage. Six hours after the administration, animals were sacrificed with ether anaesthesia. The stomach of rat was removed and opened along the greater curvature and then washed with water. The wideness of ulcer area was determined by a millimetric paper and magnifier. Protective effects of the vegetable oils and omeprazole were determined by comparing with indomethacin group.

### Statistical analysis

The experimental data were expressed as mean ± SE. The significance of difference among the various treated groups and control group were analyzed by means of one way ANOVA followed by Dunnet's t-test. P values <0.05 were considered as statistically significant and p<0.01 as highly significant.

## RESULTS

### Carrageenan-induced paw edema in rat

Injection of 1% carrageenan solution caused a time dependent increase in rat paw edema. In the present study, the mean paw volume of control group (carrageenan group) reached its peak at about 4 h. The administration of vegetable oils, rice bran oil and mustard oil, alone reduced carrageenan-induced paw edema by 97.08% and 2.38% respectively at about 4 h, where as indomethacin alone reduced paw volume by 94.4%. The administration of indomethacin with rice bran oil and mustard oil decreased paw volume by 99.47% and 89.41% respectively. These results indicate a similarity in the anti-inflammatory profile of rice bran oil and indomethacin. It also indicates that indomethacin , rice bran oil and the combination of rice bran oil with indomethacin showed a highly significant anti-inflammatory activity (p<0.001), which could not be demonstrated with mustard oil (Table 1).

### Indomethacin (NSAID)-induced gastric ulcer

The anti-ulcer activity was examined by noting the number of ulcers, calculating the mean surface area of ulceration, calculating the ulcer index and the percent inhibition of gastric ulcer formation in the model of indomethacin-induced gastric ulceration in rat. The results obtained are shown in Table 2. The administration of indomethacin caused gastric damage with a mean ulcer area of 32 ± 0.45 mm in gastric tissue. The administration of rice bran oil or mustard oil along with indomethacin limited the gastric damage to 1.67 ± 0.33 mm and 2.67 ± 0.21 mm respectively, while the standard anti-ulcer drug omeprazole decreased the mean area of ulceration to 1 ± 0.00 mm . The percent inhibition of gastric-ulcer was 94.78%, 91.66% and 96.88% by rice bran oil, mustard oil and omeprazole respectively. These results show that rice bran oil and mustard oil significantly inhibited the gastric damage induced by indomethacin and their efficacy as anti-ulcer

**Table 1: Effect of vegetable oils (rice bran and mustard oils) and indomethacin on carragenaan-induced paw edema in rats**

| Treatment n=6 | Dose | Paw volume before inflammation | Paw volume after inflammation (ml) | Percentage inhibition (ml) at 4 h |
|---|---|---|---|---|
| Control (Distilled water) | 2 ml/kg | 1.08 ± 0.02 | 1.70 ± 0.04 | - |
| Rice bran oil | 2 ml/kg | 1.08±0.04 | 1.06 ±0.04* | 97.08 |
| Mustard oil | 2 ml/kg | 0.95±0.04 | 1.57± 0.10 | 02.38 |
| Indomethacin | 25 mg/kg | 1.15 ±0.02 | 1.11±0.03* | 94.44 |
| Rice bran oil | 2 ml/kg | | | |
| Indomethacin | 25 mg/kg | 1.15±0.03 | 1.15±0.03* | 99.47 |
| Mustard oil | 2 ml/kg | | | |
| Indomethacin | 25 mg/kg | 1.12±0.01 | 1.19±0.02 | 89.41 |

n-number of rats (6). The values represent the mean±SEM of the variation in the paw volume (ml) of six animals for each group. Vegetable oils and indomethacin treated groups were statistically compared with control group. P<0.01 as compared to control (one way ANOVA)

**Table 2: Effect of vegetable oils (rice bran and mustard oils) and omeprazole on indomethacin-induced gastric damage in rats**

| Treatment N = 6 | Dose | Mean number of ulcers | Mean ulcer area, mean ± SEM | Percentage inhibition | Ulcer index |
|---|---|---|---|---|---|
| Control (Distilled water) | 2 ml/kg | 00 | 00 | 00 | 00 |
| Indomethacin | 25 mg/kg | 16 | 32+0.45 | 00 | 28 |
| Indomethacin + | 25 mg/kg | | | | |
| Rice bran oil | 2 ml/kg | 1.66 | 1.67+0.33 | 94.7 | 13 |
| Indomethacin + | 25 mg/kg | | | | |
| Mustard oil | 2 ml/kg | 2.66 | 2.67+0.21 | 91.66 | 14 |
| Indomethacin + | 25 mg/kg | | | | |
| Omeprazole | 20 mg/kg | 1.00 | 1.0+0.00 | 96.88 | 12 |

Data expressed as mean +SEM. Statistical analysis by one-way ANOVA followed by Dunnett's Multiple Comparison. P< 0.01 compared to control. n = number of animals.

agents was comparable to that of the proton pump inhibitor, omeprazole.

## DISCUSSION

The objective of the present study was to evaluate the gastro-protective effect of vegetable oils (rice bran & mustard oils) and omeprazole on indomethacin-induced gastric damage in rats. Indomethacin caused a significant gastric damage in rats compared with untreated rats, as reflected by an increase in ulcerated areas. However, indomethacin administered with vegetable oils did not cause a significant gastric damage. These results indicated that the vegetable oils had a potential to prevent the gastric damage resulting from indomethacin administration. A similar protective effect of the vegetable oils in the NSAID-induced gastric damage has been reported earlier[7,8]. The study also revealed the gastro-protective effect of omeprazole.

Non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin induce an injury to gastrointestinal mucosa in experimental animals and humans and their use is associated with a significant risk of hemorrhage, erosions and perforation of

both gastric and intestinal ulcers[8]. The molecular basis for the gastrointestinal toxicity of NSAIDs is widely believed to be their inhibitory activity against cyclooxygenase, which causes them to block the production of prostaglandins. This is associated with reduction of gastric mucosal blood flow, disturbance of microcirculation, decrease in mucus secretion, lipid peroxidation, and neutrophil activation, which are involved in the pathogenesis of gastrointestinal mucosal disorders[9]. The findings of the present study show that vegetable oils were able to attenuate the development of gastric ulcer.

Gastrointestinal wall integrity is known to be controlled by two opposing forces: aggressive and defensive[10]. The aggressive force encompasses the increase in acid output and subsequent lipid peroxidation, which is a result of the reaction between oxy-radicals and the polyunsaturated fatty acids. The defensive actions are gastro-protective in nature and involve the anti-oxidative enzymes; superoxide dismutase which catalyses the dismutation of superoxide radical anion in to less noxious hydrogen peroxide and catalase or glutathione peroxidase that inactivate hydrogen peroxide to water[11]. Indomethacin has been known to cause lipid peroxidation with depletion of endogenous anti-oxidants[12].

Vegetable oils administered along with indomethacin have been reported to increase the level of anti-oxidants leading to decrease in reactive oxygen species and hydrogen peroxide level in gastric tissues, and also increase in glutathione levels. Hence vegetable oils possess a reducing effect against gastric damage in rat stomach tissues induced by indomethacin and their gastro-protective effects can be attributed to their enhancing effects on anti-oxidant defense systems[13]. Vegetable oils have also been reported to decrease the activity of myeloperoxidase resulting in reduction of neutrophil infiltration, lipid peroxidation and onset of inflammatory response and also attenuation of the mucosal damage by activated neutrophils.

Another objective of this study was to investigate if the vegetable oils (rice bran & mustard oils) alter the anti-inflammatory effect of indomethacin. In the current study, the effects of vegetable oils on anti-inflammatory action of indomethacin were also studied in rats. Our results showed that the vegetable oils enhanced efficacy of indomethacin for reducing paw edema induced by carrageenan. It has also been found that the vegetable oils possess anti-inflammatory effect against paw edema when given alone. Previous reports also showed that vegetable oils have anti-inflammatory effects[14, 15, 16, and 17]. Inflammation induced by carrageenan involves three distinct phases of the release of mediators, including serotonin and histamine in the first phase (0-2 h), kinins in the second phase (3 h) and prostaglandins in the third phase (>4 h). Vegetable oils showed significant inhibition of the edema in all the three phases. Indomethacin, a known cyclooxygenase inhibitor also significantly reduced the paw edema in rats.

In conclusion, the vegetable oils (rice bran & mustard oils) possess a reducing effect against gastric damage in rat stomach tissues induced by indomethacin and their gastroprotective effect can be attributed to their enhancing effects on anti-oxidant defense systems. The vegetable oils possess beneficial effects on preventing the oxidative damage induced by indomethacin and neutrophil infiltration into gastric mucosa. Moreover, the vegetable oils (rice bran oil) enhanced the anti-inflammatory effect of indomethacin. Enhanced anti-inflammatory effect and gastro-protective effect of vegetable oils administered along with indomethacin may reduce the limitation of use of indomethacin like drugs.

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