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# MODULATION OF ANTI-INFLAMMATORY DRUGS' ULCEROGENICITY VIA SOLID DISPERSION WİTH SKIMMED MILK ON THE EXAMPLE INDOMETHACIN

# ANTIENFLAMATUAR İLAÇLARIN ÜLSEROJENİTESİNİN AZ YAĞLI SÜT İLE HAZIRLANAN KATI DISPERSİYON YOLUYLA MODÜLASYONUNA ÖRNEK OLARAK INDOMETASİN

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*Indomethacin (IDN) which is used for analgesic, anti-inflammatory and antipyretic effects in therapy is not soluble in water and causes disturbance in gastrointestinal system. In this study, in order to improve solubility and modulate IND's ulcerogenicity, physical mixture (PM) and solid dispersion (SD) of IND with skimmed milk (SM) (maximal 1% fat content) were prepared and investigated by using rat stomachs. Due to test results, particularly SD containing IND was found to create less ulcerogenicity on stomach in a significant manner.*

*Analjezik, antienflamatuvar ve antipiretik etkileri nedeniyle tedavide kullanılan indometasinin (IND) suda çözünmeme ve gastrointestinal sistemde tahriş gibi istenmeyen etkileri vardır. Bu çalışmada, IND'in çözünürlüğünü arttırmak ve ülserojenik etkisini azaltmak üzere az yağlı süt (SM) (en fazla %1 yağ içeren) ile fiziksel karışımı (PM) ve katı dispersiyonu (SD) hazırlanmış ve bu amaçla sıçan mideleri kullanılmıştır. Testler sonucunda özellikle IND içeren SD'nin mide üzerinde ülser yapıcı etkisinin anlamlı şekilde azaldığı test edilmiştir.*

**Keywords**: *Anti-inflammatory drugs; Solid dispersion; Skimmed milk; Ulcerogenicity; Biopharmacy*

**Anahtar kelimeler:** *Antienflamatuvar ilaçlar; Katı dispersiyon; Az yağlı süt; Ülserojenisite; Biyofarmasi*

## Introduction

Many nonsteroidal anti-inflammatory drugs (NSAIDs) cause gastrointestinal disturbance and have poor solubility (1). Biopharmaceutical studies have been performed in order to find solutions to these problems (2-7).

In order to improve the solubility of NSAIDs, addition of surface active agents and formation of water-soluble salts (2) and to enhance dissolution and absorption rate, reduction of particle size have been reported (3). For reducing particle size, SD (4,5) has been used. Formation of SD of the drug with a water soluble carrier is one of the several techniques that can be used to improve the dissolution properties of low water-soluble drugs. It has been known that against gastro-intestinal disorders of NSAIDs, amino acids have been proposed (6,7).

Indomethacin (IND) is one of them that used for its analgesic, anti-inflammatory and antipyretic properties in therapy. It is practically insoluble in water and shows either systemic or local ulcer in gastro-intestinal tract (1).

In this study, SM has been used as the carrier for SD and investigated for its modulation on ulcerogenicity of IND. To determine the modulation of ulcerogenicity of IND with the use of SM in PM or SD, a modified test was performed (7,8).

### Materials

Indomethacin gift of Ratiopharm GmbH&Co., Germany, skimmed milk used as purchased, Pontamin sky blue 6BX (Ferak, Germany), all other reagents and chemical substances were of analytical grade.

### Apparatus

Microscope with stereoscopic loop (triocular-side lighting) Olympus, SZH 10-11 Japan. Pentax Macro camera 100 with F/2-8 object lense. Lyovac GT 2 (Leybold Heraeus) used for lyophilization.

### Methods

In this study, IND, PM and SD of IND with SM were investigated for their ulcerogenic effects on stomachs of rats.

*1. Preparation of skimmed milk powder*

After freeze-drying (SM was lyophilized until the sample's humidity reduced to maximum 3%. According to preliminary studies, lyophilization time was chosen as 72 h to reduce humidity), 25 ml SM yielded ≅ 2.615 g powder (SMP). The powder was sieved through 250 µm mesh.

*2. Preparation of the physical mixtures*

500 mg micronized drug was uniformly mixed with 2.615 g SMP using an agate mortar and pestle. The prepared PM were kept in a dessicator over calcium chloride (0% relative humidity) at room temperature.

*3. Preparation of the solid dispersions*

500 mg IND was suspended in 25 ml SM. Suspension was mixed in a water bath having 50°C temperature for 30 minutes by using a magnetic stirrer. It was freezed by keeping in fluid nitrogen bath and lyophilized, the yield (SD) was sieved though 250 µm mesh.

*4. Experiments on ulcerogenic ativity of IND and its different formulations*

Male rats (Wistar albino) of 100-150 g weight were deprived of food but allowed free access to water for 24 h before experiments. IND, its PM and SD with SM were suspended in a 1% carboxymethylcellulose (CMC) solution containing a trace amount of Tween 80 were given s.c. at 40 mg/kg for IND in a volume of 0.2 ml/100 g. 10 minutes prior to sacrifice of rats, 1 ml 5% Pontamin sky blue 6BX in physiological saline were injected to vene in tail of rats. The animals were sacrificed 24 h after the administration by a blow on the head. The stomach of each was removed, 10 ml 1% formalin solution was injected and the somach was immersed in 1% formalin solution. The stomach was then incised along the greater curvature and the length (mm) of each mucosal ulcer as dark spots upon blue base developed in the glandular portion was measured under a dissecting microscope (5x). The sum of the length of each mucosal ulser per rat was used as the ulcer index. Student's t-test was employed to determine the statistical significance of the data.

Rats were divided into four groups: Blank (these animals were given 1% CMC solution containing a trace amount of Tween 80); Control (IND 40 mg/kg); Test A (PM of IND with SMP 249.2 mg/kg equivalent to 40 mg/kg IND); Test B (SD of IND with SM 249.2 mg/kg equivalent to 40 mg/kg IND).

As ulcer index, the ratio of total ulceration areas in stamachs of rats to rat number was used. Inhibition in ulcerogenicity was calculated according to the formula below.

$$\text{Inhibition}\% = \frac{\text{Control group index-Test group index x100}}{\text{Control group index}}$$

Control group index = Amount of mucosal lesions in rats that were injected IND over rat number
Test group A index = Amount of mucosal lesions in rats that were injected PM of IND with SM over rat number
Test group B index = Amount of mucosal lesions in rats that were injected SD of IND with SM over rat number

The pictures of rat stomachs were taken by Pentax Macro camera 100 with F/2-8 object lense.

## Results and Discussion

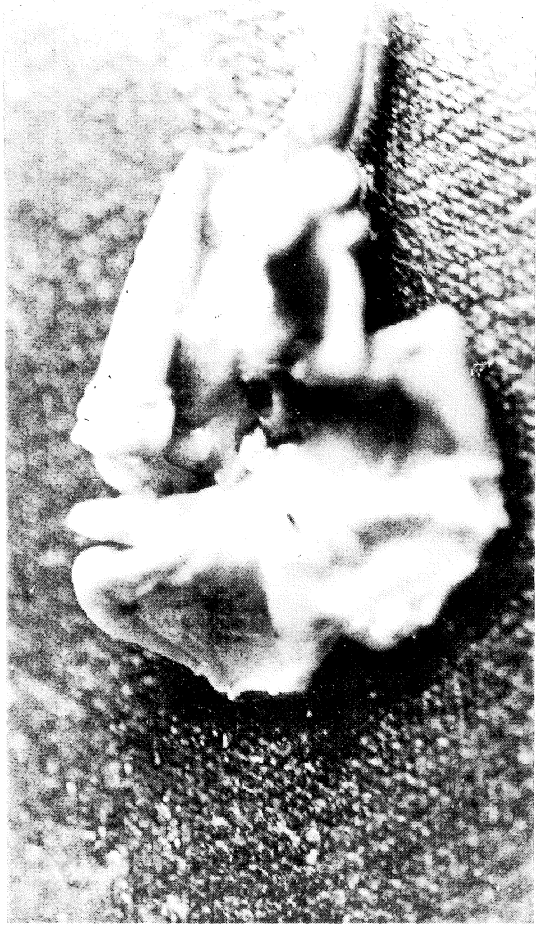
As it is shown in Table 1, the SD of IND with SM (in a dose of 249.2 mg/kg containing 40 mg/kg IND; ulcer index 0.248) was remarkably less ulcerogenic than IND (40 mg/kg) alone (ulcer index 0.847). However, PM of IND with SMP (in a dose of 249.2 mg/kg containing 40 mg/kg IND; ulcer index 0.378) was also shown to be less ulcerogenic than IND alone. It could be concluded that SM inhibited the ulcerogenicity of IND in animals when prepared as SD of IND.

In pictures 1-4, the stomachs of tested rats are shown. It has been observed that there was not any dark blue areas due to ulcerogenic lesions in stomachs of rats used blank in Picture 1. However in other pictures respectively, stomach of rat which was injected IND as plain drug found to have the largest ulcerotion

Table 1. Effects of various forms of IND on IND-induced gastric ulcers in rats

| Treatment | Number of rats | Ulcer index (mm) s.d. | Inhibition (%) |
|---|---|---|---|
| Blank (1% CMC) | 5 | - | - |
| Control (IND) | 5 | 0.847 0.14 | - |
| Test A | 5 | 0.378 0.12 | 55.37 |
| Test B | 5 | 0.248 0.09 | 70.72 |

Test A: PM of IND with SMP
Test B: SD of IND with SM
$p<0.05$, for student t test



Picture 1. Stomach of a rat among ones which were used as blank

Picture 2. Stamach of a rat among ones which were injected IND alone

area in Picture 2, stomach of rat which had taken PM of IND with SMP by injection seemed to have less ulcer areas than the one in previous Picture 3 and finally the stomach of rat which was injected IND as SD with SM has shown the least ulcer areas in Picture 4.

According to the same pictures, the modulation percentage of ulcerogenicity of IND by preparation of PM and SD are as follows, stomachs of the animals which were injected PM of IND with SPM and SD of IND with SM have less ulcer areas (55.37 and 70.72 % less, respectively) than the one which was injected IND alone.

In case of peroral administration, the lesser ulcerogenic activity with drug-SM SD might be expected to be formed due to drug-carrier water-soluble complex. could be more absorbable, therefore, less resident in the stomach and consequently causes less local ulcerogenicity than plain drug.

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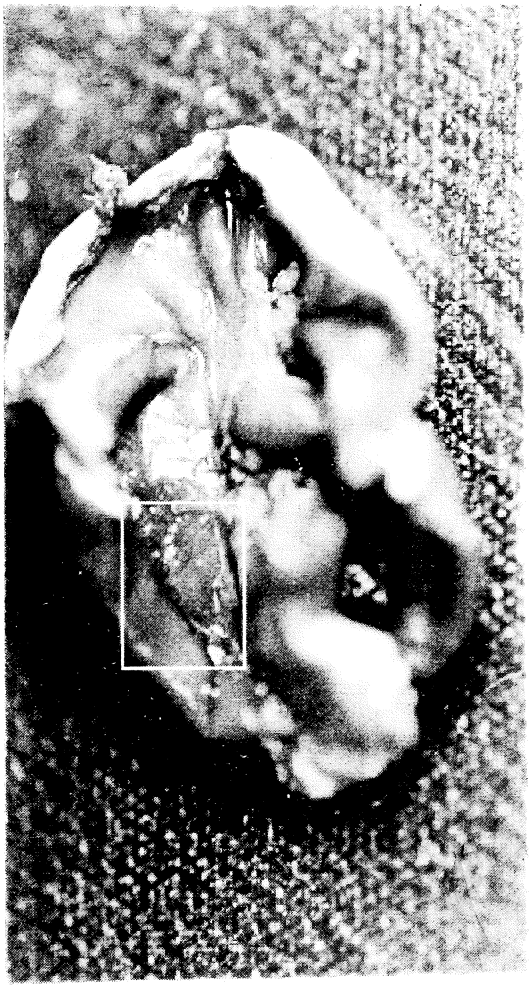
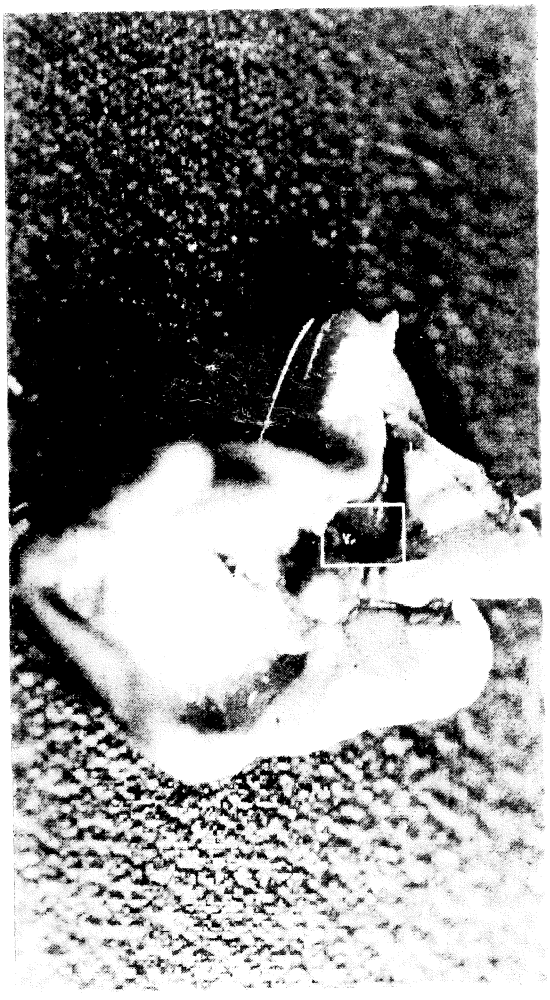


Picture 3. Stomach of a rat among ones which were injected PM of IND with SMP



Picture 4. Stomach of a rat among ones which were injected SD of IND with SM8. Kasuya,

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