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Influence of indomethacin-mastic combinations on dissolution, absorption and gastrointestinal mucosal damage in rats

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Abstract

Coprecipitates and physical mixtures of indomethacin (IM), lactose and mastic were studied to determine the effect of mastic on drug dissolution rate, bioavailability and ulcerogenic effects in rats. The drug was received as oral suspensions of IM preparations containing 20 mg/kg. The mean particle diameter of the coprecipitate was increased with increase of mastic concentration. The X-ray diffraction pattern showed a crystalline IM in the coprecipitate. The drug release rate in phosphate buffer (pH 7.2) was improved by the addition of polysorbate-80 to the release media. The presence of mastic retarded drug dissolution rate. This retardation was dependent on the percentage of mastic in the preparation as well as the method of preparation. The results of bioavailability showed a sustained effect of drug from IM-mastic physical mixture and coprecipitate. The ulcerogenic activity of IM was reduced in all the preparations containing mastic as compared with that containing IM alone. © 1997 Elsevier Science B.V.

Keywords: Indomethacin; Mastic; X-ray diffraction; Particle size analysis; Dissolution; Bioavailability; Anti-ulcerogenicity

1. Introduction

Indomethacin (IM) is a nonsteroidal anti-inflammatory drug widely used in the treatment of rheumatoid arthritis. IM is one of the powerful anti-inflammatory, but potentially more toxic agent. IM is associated with gastrointestinal (GI) side effects which include ulceration, sometimes with perforation and hemorrhage (Flower et al., 1985). The incidence of GI damage of IM was reduced by complexation with zinc (Singla et al., 1990) and calcium glycerophosphate (Foda and Said, 1991), amphoteric gel formulation (Liverside et al., 1989), microencapsulation (Hilton and Summers, 1987), solid dispersion using water soluble cellulose polymers (Chowdary and Suresh-Babu, 1994) and prodrug preparation (Tammara et al., 1993).

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Mastic is a natural oleoresin obtained from a cultivated variety of *Pistacia lentiscus* (Martindale, 1982). A monograph for it was given in the BPC (1973). Mastic consists mainly of triterpenoid acids, including masticadienonic acid, triterpene alcohols and about 2% volatile oil. It is officially used as a temporary material for carious teeth and as a protective covering for wounds. There was a scant amount of research work referred to in the literature concerning the use of mastic in pharmaceutical preparations (Georgarakis et al., 1987; Panagopoulou and Georgarakis, 1990; Abdel-Aleem, 1996). Mastic is used in folk medicine for its gastric and duodenal antiulcer activity (Hassan, 1989). It has been reported that mastic produced a significant reduction in the intensity of the experimentally induced gastric and duodenal ulcers in rats (Al-Said et al., 1986).

The aim of this work is to investigate the effect of coadministration of mastic with IM on the GI damage produced by the drug. Also, the effect of mastic on drug dissolution and bioavailability in rats was studied.

2. Experimental

2.1. Materials

Indomethacin (IM) was donated by Kahira Pharm. and Chem. Ind. Co., Cairo, Egypt (manufactured by Merck and Co., USA). Mastic, the commercial grade was supplied by Chios' Gum Mastic Growers Association, Greece, and lactose monohydrate from ElNasr Chem. Co., Egypt. Phenolsulfonphthalein (phenol red), carboxymethyl cellulose sodium salt (CMC), heptane, isoamyl alcohol, diethyl ether and other chemicals were of reagent grade.

2.2. Preparation of physical mixtures

Physical mixtures (20 g) of IM, lactose and mastic were prepared by mixing constant weight of IM (10%) with various ratios of lactose and mastic (90%) using mortar and spatula. The concentration of mastic in the final mixtures were 0, 10, 20, 30 and 40%. The uniformity of drug distribution was done by the analysis of random samples equivalent to 25 mg of drug from the powders.

2.3. Preparation of coprecipitates

The same percentages of the ingredients in the physical mixtures were used. IM and mastic were dissolved in chloroform and lactose was added to the chloroformic solution. The solvent was evaporated under reduced pressure in a rotary evaporator at 50°C. The solid was dried under vacuum at 50°C for 24 h. The dried powder was pulverized

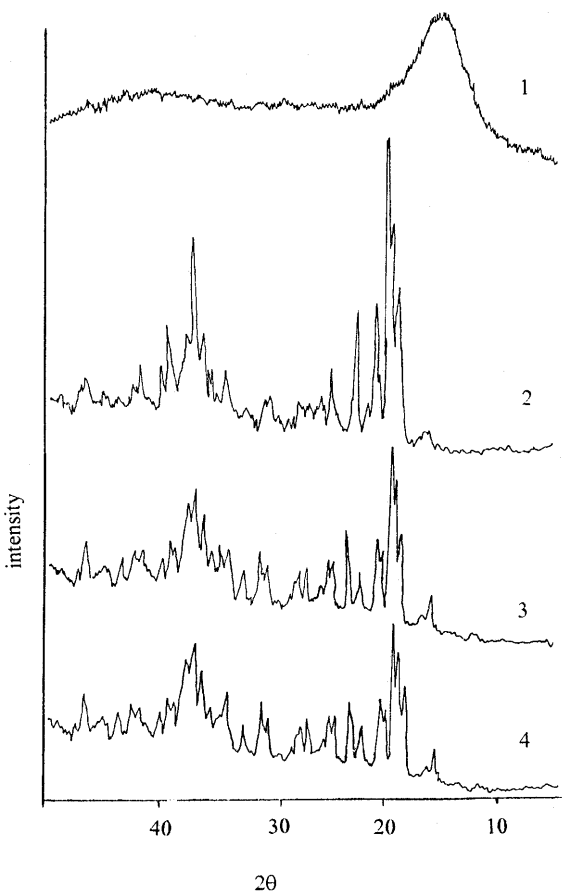

Fig. 1. (1) Powder X-ray diffractrograms of mastic, (2) IM-physical mixture containing 40% mastic, (3) coprecipitate containing 10% mastic and (4) coprecipitate containing 40% mastic.

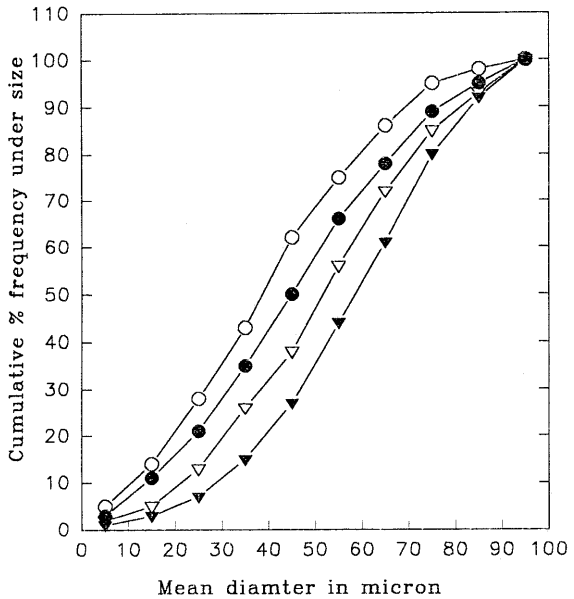

Fig. 2. Plot of cumulative percent frequency under size of IM coprecipitate powders containing different concentrations of mastic. (○), 10%; (●), 20%; (▽), 30%; (▼), 40%.

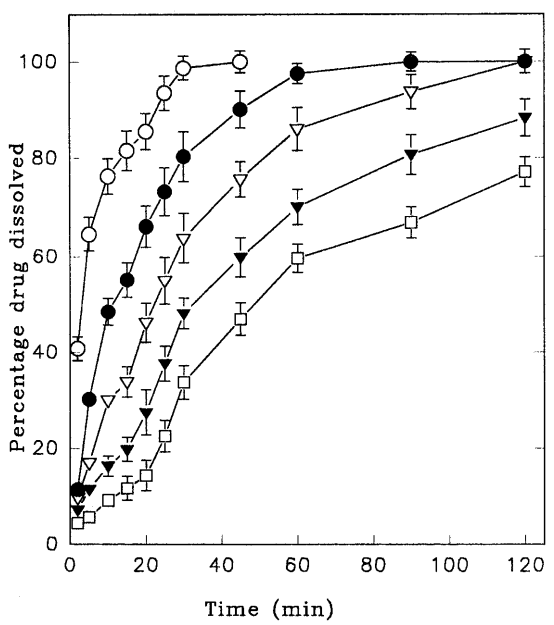

Fig. 3. Dissolution profiles of indomethacin (IM) from physical mixtures containing 25 mg drug, lactose and various concentrations of mastic in buffer of pH 7.2. (○), 0%; (●), 10%; (▽), 20%; (▼), 30%; (□), 40% mastic.

and stored in a desiccator until tested. All the powders were passed through a sieve with opening of 100 μ after preparation.

2.4. Powder X-ray diffraction

The X-ray diffraction of powders was performed using a Philips® PW 1050/70 diffractometer system, Cu-Kα radiation and a scan speed of 2θ/min.

2.5. Particle size analysis

The coprecipitate powders were suspended in distilled water and analyzed by the optical microscope using eyepiece micrometer. The diameter of 200 particles was measured and classified into different size range.

2.6. In-vitro release study

A weight of each of the prepared formulae, 250 mg (< 100 μ), equivalent to 25 mg pure IM, was used for dissolution from powders. The dissolution behavior of IM was examined using jacketed beakers containing 500 ml phosphate buffer of pH 7.2 with or without 0.02% polysorbate-80. The temperature was 37 ± 0.5°C and stirring speed was 60 rpm. The amount of IM dissolved was determined spectrophotometrically at 318 nm. The experiments were performed in triplicate and the mean percent dissolved was calculated. The presence of mastic did not interfere with the measurement

2.7. In-vivo absorption studies

Male albino rats weighing 150–200 g were divided into five groups, each of six rats. All animals were fasted 24 h before the experiments, but had free access to water. The water supply was, however, withdrawn during the experiments. The animal received the drug or the preparations in the form of suspension in 1% CMC (particle size < 100 μ). A dose level of 20 mg/kg of the drug or its equivalent was kept constant in all treatments. This was achieved by withdrawing 1 ml of the suspension by means of plastic syringe and feed-

ing it to the rat through metallic gastric tube under light ether anesthesia. Each group of rats received one of the following; pure IM, physical mixture containing 40% mastic (mastic concentration was arbitrary chosen), coprecipitates containing 40% mastic and pure mastic (80 mg/kg which is equivalent to that in the preparations) followed after 15 min by administration of pure IM. The fifth group received 1% CMC as a control. Blood samples (0.5 ml) were taken at 1, 2, 3, 5 and 7 h from retro-orbital sinus (Gaballah et al., 1991) under light ether anesthesia. Plasma (0.1 ml) was separated for determination of indomethacin level using the spectrophotofluorometric method (Hucker et al., 1966).

2.8. In-vivo evaluation of GI mucosal damage

Another five groups of rats received the same preparations used in the in-vivo absorption study, but the dosing was repeated daily for a period of 4 days. The animals were housed under similar conditions and fed the same diet of bread with free access to water. Under light ether anesthesia, 2 μmol of phenol red in 2 ml saline was administered to the rats by gastric intubation, 15 h after the last treatment. Following the intubation, the animals were placed individually in metabolic cages for urine collection. The cumulative amounts of urine excreted during the first 8 h after dosing were collected quantitatively. The volume of each collection was measured and the phenol red content was determined colorimetrically (Nakamura et al., 1983). Urine from animals which had received saline instead of phenol red was used as a blank. The urinary recovery of phenol red was expressed as percent of dose.

3. Results and discussion

3.1. Powder X-ray diffraction

The powder X-ray diffractograms of mastic alone, IM physical mixture containing 40% mastic and IM-coprecipitates containing 10% and 40% mastic are shown in Fig. 1 as illustrative examples. Mastic diffractogram exhibited broad peak,

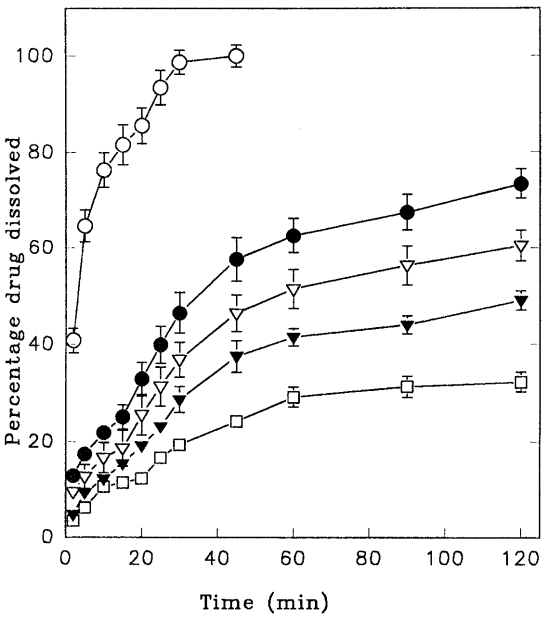

Fig. 4. Dissolution profiles of indomethacin (IM) from coprecipitates containing 25 mg drug, lactose and various concentrations of mastic in buffer of pH 7.2. (○), 0%; (●), 10%; (▽), 20%; (▼), 30%; (□), 40% mastic.

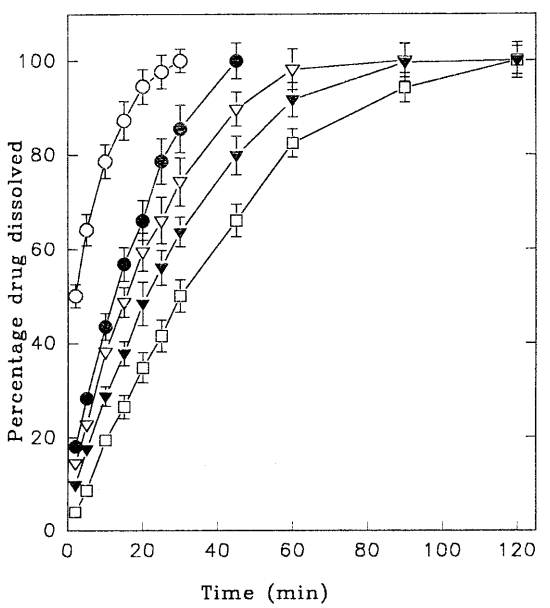

Fig. 5. Dissolution profiles of indomethacin (IM) from physical mixtures containing 25 mg drug, lactose and various concentrations of mastic in buffer of pH 7.2 containing 0.02% polysorbate-80. (○), 0%; (●), 10%; (▽), 20%; (▼), 30%; (□), 40% mastic.

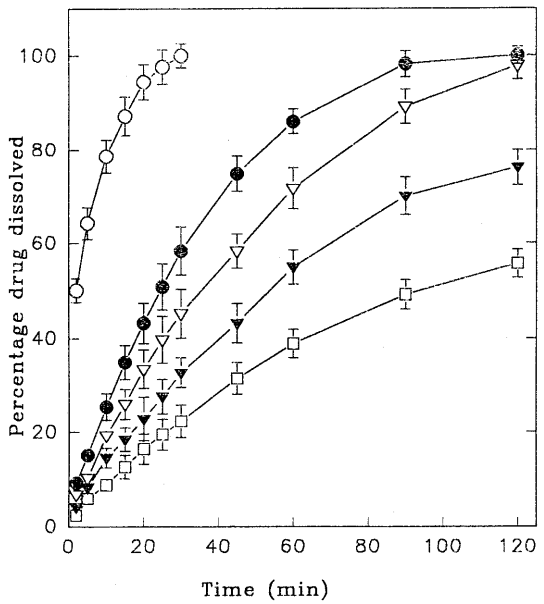

Fig. 6. Dissolution profiles of indomethacin (IM) from coprecipitates containing 25 mg drug, lactose and various concentrations of mastic in buffer of pH 7.2 containing 0.02% polysorbate-80. (○), 0%; (●), 10%; (▽), 20%; (▼), 30%; (□), 40% mastic.

while that of IM-mastic physical mixture and coprecipitates showed sharp peaks corresponding to the drug crystals. The intensity of drug peaks were decreased in the coprecipitates. This indicated that mastic reduced the drug crystallinity during copreciptation, but didn't prevent drug crystallization in the concentration range of the used mastic.

3.2. Particle size analysis

The cumulative percent frequency undersize of coprecipitate powder is shown in Fig. 2. From the figure, the percent of the coprecipitate particles smaller than 50 μ were 34, 47, 58 and 67% for 10, 20, 30 and 40% mastic respectively. Furthermore, the calculated mean diameters (volume-surface area) for coprecipitate powder were 46.6, 50.08, 56.0 and 62 μ for 10, 20, 30 and 40% mastic respectively. The previous results demonstrated that the increase of mastic concentration in the coprecipitates resulted in an increase in the particle diameter. This may be attributed to the binding properties of mastic (Abdel-Aleem, 1996).

3.3. In-vitro dissolution

The effect of mastic on the dissolution rate of IM from the different formulae is shown in Figs. 3–6. It was observed that the powders containing mastic clumped during dissolution in buffer medium and this clumping effect was increased with the increase of mastic percentage in the formulae. However, this clumping effect was disappeared in presence of 0.02% polysorbate-80 in the release medium (Figs. 5 and 6). The hydrophobic nature of mastic resulted in a lower dissolution rate of both the physical mixtures and the coprecipitates compared with that of IM alone. Similar results were reported on the release of KCl from mastic microcapsules, where the release of KCl was retarded by increasing the layer of mastic wall (Georgarakis et al., 1987). Analysis of the dissolution data according to Hixon-Crowell's cube root law (Martin et al., 1993) up to 30 min resulted in straight lines (Fig. 7). The cube root

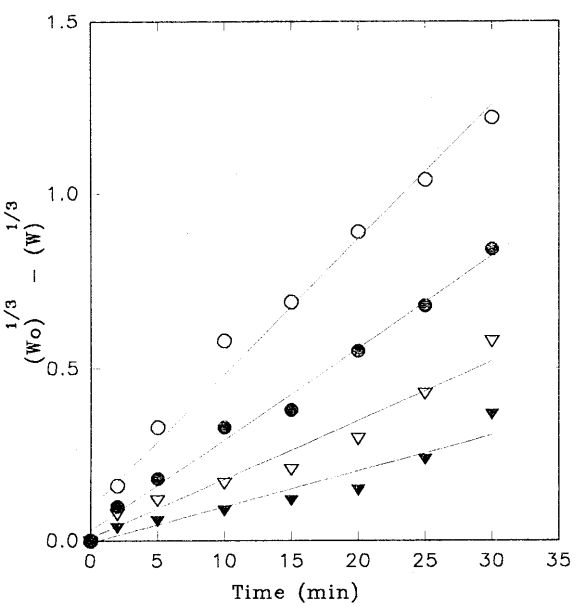

Fig. 7. Plot of $(W_o)^{1/3} - (W)^{1/3}$ as a function of time for dissolution data of IM-mastic physical mixtures in buffer of pH 7.2. (○), 10%; (●), 20%; (▽), 30%; (▼), 40%.

Table 1
Cube root dissolution rate constant ($\times 1000$) of IM from powders containing different concentrations of mastic in phosphate buffer (pH 7.2) with and without 0.02% polysorbate-80

Mastic conc.	Buffer		Buffer with 0.02% polysorbate-80	
	Physical	Coprecipitate	Physical	Coprecipitate
0	45.5 (0.975)*		55.3 (0.998)	
10	31.6 (0.978)	8.03 (0.985)	41.8 (0.997)	22.9 (0.999)
20	24.2 (0.987)	6.3 (0.978)	32.6 (0.998)	16.88 (0.999)
30	16.5 (0.995)	4.54 (0.965)	26.1 (0.998)	11.0 (0.997)
40	9.8 (0.964)	3.04 (0.978)	19.3 (0.999)	7.5 (0.998)

* correlation coefficient (r^2).

dissolution rate was calculated from the plots and represented in Table 1. The results in Table 1 showed that the presence of polysorbate-80 in the release medium improved IM release rate from the powders compared to that without polysorbate-80. In addition, the drug release rate was decreased with increase of mastic concentration in the powder. A linear relationship was obtained when the cube root dissolution rate were plotted as a function of the percentages of mastic in the different formulae (Fig. 8). Table 1 and Fig. 8 also depict that the release of IM from the different formulae was not only dependent on the percentage of mastic, but also on the method of preparation of the different formulae. At equal percentages of mastic, the coprecipitate experienced lower dissolution rate than the corresponding physical mixtures. This could be attributed to the decrease in the effective surface area of the

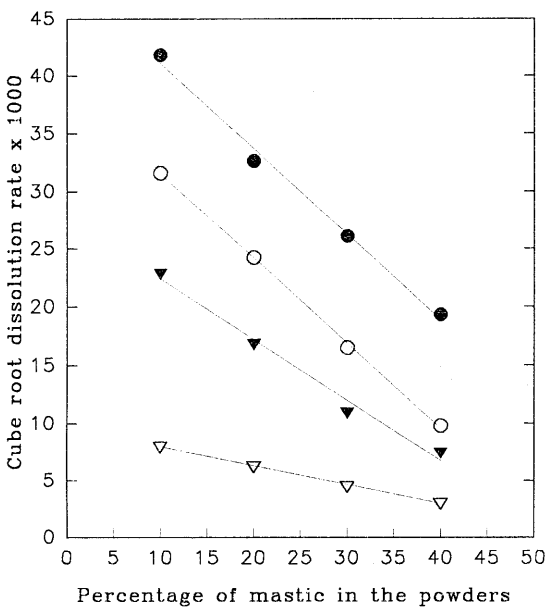

Fig. 8. Plot of cube root dissolution rate constant as a function of mastic concentrations in the powder. Physical mixture in buffer with (●) and without polysorbate-80 (○) and coprecipitate in buffer with (▼) and without polysorbate-80 (▽).

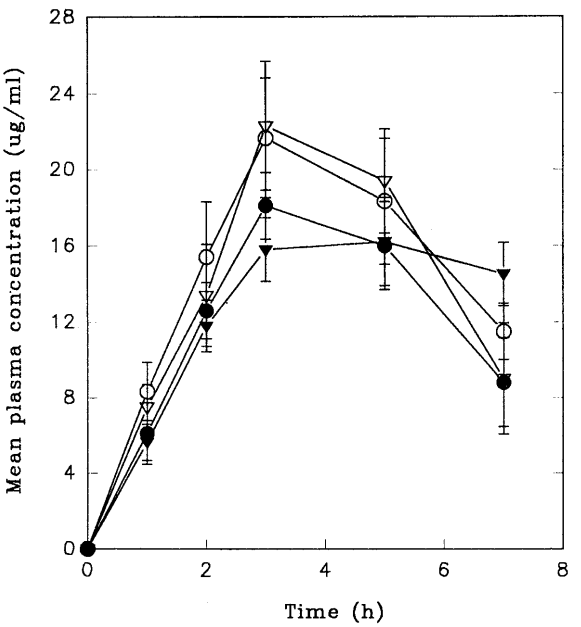

Fig. 9. Plasma levels of IM following the oral administration of different preparations of IM-mastic combinations equivalent to 20 mg/kG. (▽), IM preceded by mastic; (○), IM alone; (●), physical mixture; (▼) coprecipitate.

Table 2
Bioavailability parameters for IM following oral administration of IM preparations[a]

Preparation	t_{max} (h)	$C_{max} \pm$ S.D. (μg/ml)	$AUC_{(0-7)} \pm$ S.D. (μg/ml per h)
IM alone	3.6 ± 0.045 (1.0)[b]	20.58 ± 1.313 (1.0)	104.38 ± 13.78 (1.0)
Physical mixture	3.9 ± 0.085 (1.09 ± 0.0077)	17.99 ± 0.738 (0.087 ± 0.765)	86.65 ± 15.44 (0.86 ± 0.246)
IM preceded by mastic	3.91 ± 0.053 (1.07 ± 0.098)	21.58 ± 1.4 (1.05 ± 1.2)	91.15 ± 16.34 (0.94 ± 0.196)
Coprecipitate	4.167 ± 0.154 (1.016 ± 0.096)	16.6 ± 1.857 (0.8 ± 1.63)	87.82 ± 10.54 (0.86 ± 0.145)

IM, indomethacin.
[a] Values represent the mean $\pm$ S.D. of six rats.
[b] Number in parentheses is the ratio of bioavailability parameters of the preparations to that of drug alone.

drug through its impeding by the insoluble mastic matrix (Yuasa et al., 1991). The results of the release study showed that mastic can be used as a controlled release carrier.

3.4. In-vivo absorption studies

The plasma levels of IM following the oral administration of the drug alone, physical mixture, coprecipitate and IM alone preceded by mastic are shown in Fig. 9. The bioavailability parameters were calculated from the plasma level-time curves up to 7 h post-administration, and the results are summarized in Table 2. The AUC was calculated by the trapezoidal rule (Gibaldi and Perrier, 1982). Also, the bioavailability parameters were calculated as ratios to that of IM alone (Table 2; values in parenthesis). Statistical analysis among the different parameters of the different preparations were performed using super ANOVA software®, followed by both student-Newman-keuls and Tukey-Kramer tests. There was significant differences ($P = 0.1$) in the area under the plasma concentration-time curve (AUC_{O-7}) among drug alone and each of physical mixture and coprecipitate. Also, the t_{max} and C_{max} of the coprecipitate and the physical mixture were significantly different from that of the drug alone. These results indicated that mastic decreased the rate and extent of IM absorption. The decrease in bioavailability might be due to the slow dissolution rate of drug in GI fluid. The results of IM alone and IM preceded by mastic showed comparable bioavailability.

3.5. Evaluation of GI mucosal damage

To study the effect of IM on the GI mucosa, phenol red was used as a marker compound. Phenol red is a highly charged molecule and, as such, is poorly absorbed from GIT. It was used to measure the change in permeability and mucosal damage of GIT (Meshali and Nightingale, 1976). The permeability of phenol red was reported as a measure of mucosal damage caused by IM (Nakamura et al., 1983). In this study the rats were treated with IM preparations (the same as in the in-vivo study) for 4 days (arbitrary time) to investigate the protective effect of mastic on the ulcerative properties of IM upon prolonged use. The urinary recovery of phenol red administered orally from animals received the different preparations is represented in Fig. 10. There was a significantly higher (student-Newman-Keuls) urinary recovery of phenol red from the animals who had received the drug alone compared with the control animals and those that received drug-mastic combinations. However, the administration of mastic with IM in the different preparations reduced the recovery of phenol red to a level equal to that recovered from animals who didn't receive IM (control). There is no significant difference in the urinary recovery of phenol red between the control and any of the preparations containing mastic. These results revealed that mastic protected the GI tract from the damaging effect of IM. This protective effect of mastic might be attributed to its mild antisecretory and localized adaptive cytoprotectant action (Al-Said et al., 1986).

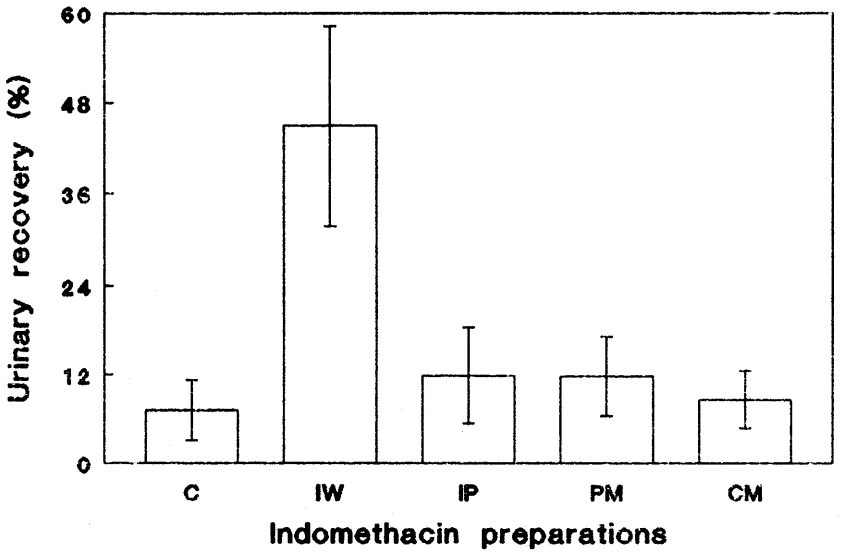

Fig. 10. Urinary recovery in 8 h of phenol red administrated orally in rats pretreated with IM preparations (orally) for 4 days. (C), control; (IW), drug without mastic; (IP), drug preceded by mastic; (PM), physical mixture; (CM), coprecipitate.

4. Conclusion

The result of this work suggested that the concurrent administration of IM with mastic, protected the GI tract from the severe side effects of IM. In addition, drug-mastic physical mixture and coprecipitate can be used to control the IM release and also protect GI membrane from its harmful effect.

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