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INFLUENCE OF QUERCETIN ON DISSOLUTION BEHAVIOR AND ULCEROGENIC ACTIVITY OF INDOMETHACIN

M. G. Abdel-Mohsen and M. Fathy

Department of Pharmaceutics, Faculty of Pharmacy, Assiut University, Assiut, Egypt

أستهدفت هذه الدراسة التعرف على قدرة عقار الكورستين فى تقليل الآثار الجانبية (قرح المعدة) التى يحدثها عقار الاندوميثاسين. وقد تم أختيار عقار الكورستين لما له من درجة أمان عالية حيث أنه من المواد الطبيعية. كما أنه يعتبر من مضادات الأكسدة وله القدرة على إزالة الشق الطليق وأيضا له تأثير مسكن ومضاد للإلتهابات. وقد تمت التجارب الفارماكولوجية على الجرذان بإعطاء مجموعة منها عقار الإندوميثاسين فى صورة معلق عن طريق الفم ، وإعطاء مجموعات أخرى الإندوميثاسين وأعطى الكورستين فى توقيتات مختلفة (قبل أو مع أو بعد إعطاء الإندوميثاسين) فى صورة معلق عن طريق الفم أيضا. وتم التعرف على حالة المعدة من خلال التصوير بالميكروسكوب الإلكترونى الماسح وفحص الخلايا والغشاء المخاطى المبطن للمعدة. ثم تمت دراسة التوافق الفيزيائى والكيميائى للعقارين معا بواسطة الأشعة تحت الحمراء والتحلل الحرارى وحيود الأشعة السينية وأيضا تمت دراسة معدل الإنطلاق المعملى للإندوميثاسين فى وجود الكورستين بإستخدام محلول الفوسفات المنظم ذات الأس الايدروجينى ٧,٢.

وقد إتضح أن عقار الكورستين له قدرة كبيرة فى منع ظهور قرح المعدة التى أحدثها عقار الإندوميثاسين عند إستخدامه بمفرده وذلك عند إعطائهما معا كما أن العقارين متوافقين فيزيائيا وكيميائيا. وقد وجد أيضا أن عقار الكورستين لم يؤثر على معدل الانطلاق المعملى للإندوميثاسين. ولهذا يوصى بإستخدام عقار الكورستين مع عقار الإندوميثاسين فى صيغة صيدلية واحدة وذلك تجنبا للآثار الجانبية التى يسببها عقار الإندوميثاسين للمعدة.

The results of in-vivo studies, in rats, revealed that the use of Quercetin in combination with Indomethacin highly reduced the risk of adverse reaction (gastric ulcerative effect) related to the peroral administration of the drug. Gross observations and scanning electromicrographs of the mucous membrane and superficial cells of the stomach showed that the Quercetin reduced the damage of mucosal membrane and destruction of the gastric superficial cells presented under the mucous membrane. The physical and chemical compatibilities of a double blend of Indomethacin and Quercetin prepared by solvent evaporation technique were confirmed using IR spectroscopy, differential scanning calorimetry and X-ray diffractometer. Also, the results showed that the presence of Quercetin in coprecipitated mixture with Indomethacin does not affect the dissolution characteristics of the drug.

The results suggest that a compatible blend from Indomethacin and Quercetin could be pharmaceutically formulated with the aim of reducing the gastrointestinal adverse effects of Indomethacin.

INTRODUCTION

The value of most nonsteroidal antiinflammatory drugs (NSAIDs) is limited because of their unacceptable adverse effects.[1-3] A number of natural active constituents, used primarily for dietary supplement, established successful gastric antiulcerogenic activity.[4,5] One of these natural products is Quercetin (QRT), which appears to possess anticarcinogenic, antiulcer, free radical scavenging and antioxidant activities.[4-11] Now, it is commonly used in some countries for self-care medication, because of its tolerance and safe low dose.[12] Castagnino E.

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et al.[13] investigated that QRT did not show signs of toxicity or any significant effects on the autonomic system when tested at doses up to 1 g/kg. Suzuki Y. *et al.*[4] investigated that QRT possesses gastric cytoprotective and gastric healing-promoting actions. El-Deen E.Z. *et al.*[5] investigated that the number of animals that showed ulcers increased by 3 fold in the case of phenylbutazone alone as compared with a combination of phenylbutazone and QRT. At the same time, QRT has a benefit as an adjunctive agent with NSAIDs because of its moderate antiinflammatory, analgesic, antipruritic and antipyretic activities and as a powerful antioxidant.[14,15]

The purpose of current studies are to investigate the effect of QRT to overcome the mucosal damage and ulceration, as local side effects associated with long-term treatment by indomethacin (IM). This was carried out through evaluating the role of QRT in preventing, reducing or healing the gastrointestinal mucosal damage, in rats, accompanied by oral route. Also, this study was performed to investigate the interaction of QRT with IM, and influence of QRT on the *in-vitro* dissolution behavior of IM.

EXPERIMENTAL

Materials

The following compounds were used as received from the suppliers without further purification: Indomethacin (EIPICO Co., Egypt); Quercetin (E. Merck, Darmstadt, Germany). All other materials and solvents used were of analytical grade.

Methods

Gastric-ulcerogenicity studies

Scanning electron microscope JEOL, JSM-5400LV (Electron Microscope Unit, Assiut University) was used for observing mucosal injury from the scanning micrographs of stomach specimens. Thirty rats were allocated to five groups of rats (each group of six rats) and were fasted for 24 hr prior to the administration of aqueous drug suspensions. The experiment was designed to optimize the procedure that would be applied to overcome the gastrointestinal damage of repeated-use IM. Daily dose of IM (9 mg/kg) and treated dose of QRT (5 mg/kg) were given perorally in combination or separately to rats as suspensions in 0.5% carboxymethylcellulose aqueous solution. The rats of the first group received a daily peroral dose of IM as a 1-ml suspension, for 4 successive days. The rats of second group were prophylactically treated by administration of QRT at 30 min before administration of IM. The rats of third group received double blend of IM and QRT with a daily dose of IM and QRT to test the co-administration treatment. The rats of the fourth group were treated by QRT after 30 and 60 min of IM administration. The fifth group was administrated equivalent amounts of the placebo and considered the control group.

By the end of the experiment of each tested group, the rats were scarified and the stomachs were removed and opened along the curvature, cleaned gently by dipping in saline and prepared for scanning in a scanning electron microscope according to the reported procedure by Fadl T.A. *et al.*[16] The rats of each group are examined for the development of histological signs of gastric ulcerations. The results of QRT therapeutic activity (antiulcerogenic activity) were categorized as successful or failed for preventing, reducing or healing the gastrointestinal damage because of the pre-administration, co-administration or administration treatment.

Compatibility studies

The thermograms of the samples were recorded on DSC-407 differential scanning calorimeter (Shimadzu Co., Japan) equipped with a computerized data station. The thermal behavior of 1:1 molar ratio IM/QRT double blend, prepared by the solvent evaporation technique, was studied by heating about 5 mg of the sample at a scanning rate of 10°/min in a covered sample aluminum pan, under nitrogen gas flow rate of 40 ml/min, and compared by the thermal behavior of IM and QRT each alone.

Data of X-ray diffraction, from 4° to 60°, for IM alone and its coprecipitate mixture with

QRT were obtained by using PW 1700/1710 X-ray diffractometer (Philips Co., Netherlands).

The IR spectra, as KBr disks compressed under a pressure of 6 ton/cm^2, were recorded on a Shimadzu IR-476 infrared spectrometer.

Dissolution rate measurement

Dissolution studies of IM and its blend with QRT were conducted on USP XIX dissolution apparatus using simulating intestinal buffer (phosphate buffer, pH 7.2). The dissolution rate studies from tested samples of IM were done in 250 ml- solutions at 37±0.5° and 100 rpm in the absence and the presence of QRT. At the appropriate intervals, sample of 2 ml was pipetted through a cotton plug, diluted with aqueous alkaline media (0.01 N NaOH) and assayed spectrophotometrically at 318 nm for IM. Equivalent volume (2 ml) of fresh phosphate buffer, preheated at 37° was added. A correction was applied for the absorbance of QRT at the same wavelength (318 nm) by considering the samples from dissolution of QRT alone as blanks for IM dissolution from the double blend. The maximum relative absorbance of QRT in double blend did not exceed 3.17%, which was subtracted from the total abosorbance. Also, a correction was applied for the cumulative dilution caused by replacement of the sample by equal volume of the dissolution medium.

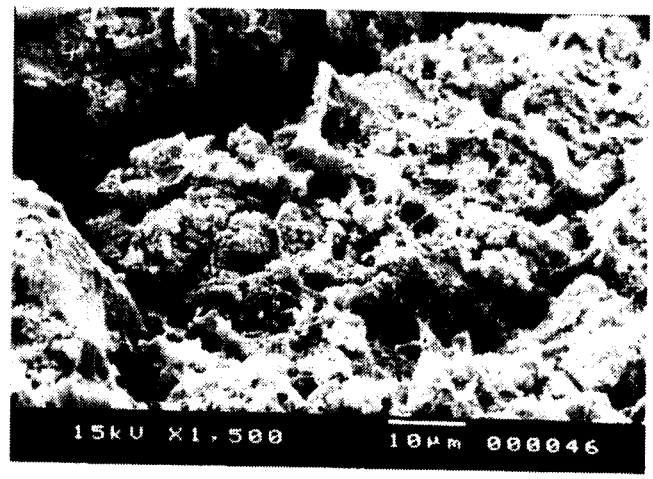
RESULTS AND DISCUSSION

The peroral administration of IM, without any treatment by QRT, exhibited ulceration on gastric mucosa of all the rats of first group, as can be seen from the gross observation, pale stomachs with discrete ulcers are characteristic for all rats. In comparison, the rats of the second and third groups showed a reducing in gastric ulcerations. The gastric ulcers induced in the rats of second group by preadministration of QRT were reduced. Also, the co-administration of QRT with IM (group 3) significantly protected the gastric mucosa by about 50% reducing of gastric ulcerations. Whereas, the treatment with QRT, after development of gastric ulcer in rats of fourth group showed no significant effect, because of the treatment may require suitable long-term treatment or may be the effective treatment is dose-dependent. Examination of stomach specimens of the treated rats under scanning electron microscope affords a highly precise method for investigation of the gastric ulceration of IM. Figure 1, represents scanning electromicrographs, at a constant magnification power for stomach specimens of rats treated with chronic doses of IM and the co-administration of IM and QRT in double blend. As shown in Figure 1, the IM-received group (1A), was characterized by damage of the mucous layer besides destruction some submucosal cells. A significant reduction in the gastric mucosal injury and valuable protection of the submucosal cells of double blend-treated group are shown in 1B. These results indicate that the peroral administration of QRT markedly reduced the IM-induced gastric mucosal ulceration because of its free radical-scavenging property.[11]

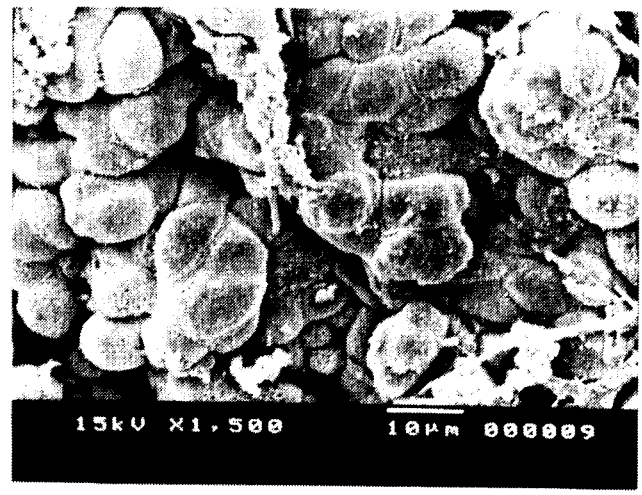
The DSC curves of IM, QRT and their double blend are shown in Figure 2. The DSC curve of QRT (2a) exhibited two endothermic peaks, the first peak at 121.4° corresponding to its water of hydration and the other peak at 325° corresponding to its melting point. The DSC curve of IM (2b) showed a sharp melting-point endothermic peak at 158.9° and another broad peak at higher temperature (298.1°) than its melting point corresponding to nonsignificant decomposed material. The DSC curve of IM/QRT (2c) double blend showed a significant shift for the peak corresponding to water of hydrous QRT (111.8°), also a lowering for the melting-point peaks of IM and QRT corresponding to 152.3 and 322.2° respectively. The results revealed the compatibility between IM and QRT in the blend mixture and the possibility of formation eutectic mixture between them.

The X-ray diffraction patterns of IM, QRT and their double blend prepared by evaporation solvent method are shown in Figure 3. The main diffraction peaks of QRT (3a) appear nearly at the same 2θ of IM/QRT system (3c) (2θ = 10.71, 12.36 and 27.31°). The relative intensities of characteristic peaks of IM, 3b (2θ = 11.59, 16.66, 21.82 and 26.62°) changed in IM/QRT system. The results revealed that QRT

(A)



(B)



(C)

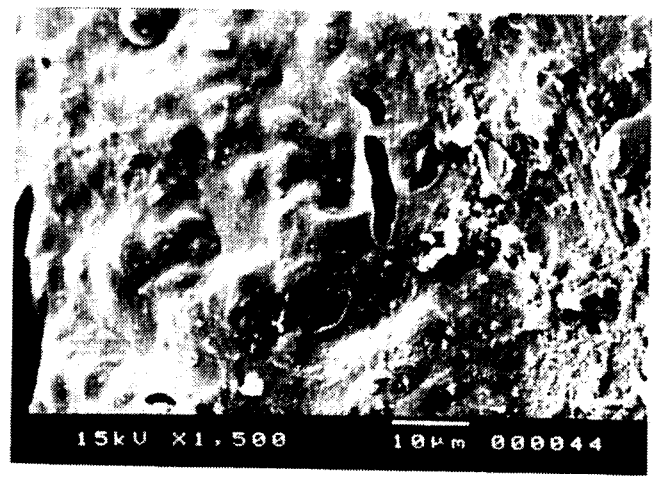


Fig. 1: Scanning electromicrographs of rat stomach following daily dose administration, for four successvie days, of (A) IM (9 mg/kg); (B) Double blend of IM (9 mg/kg) and QRT (5 mg/kg); (C) Control.

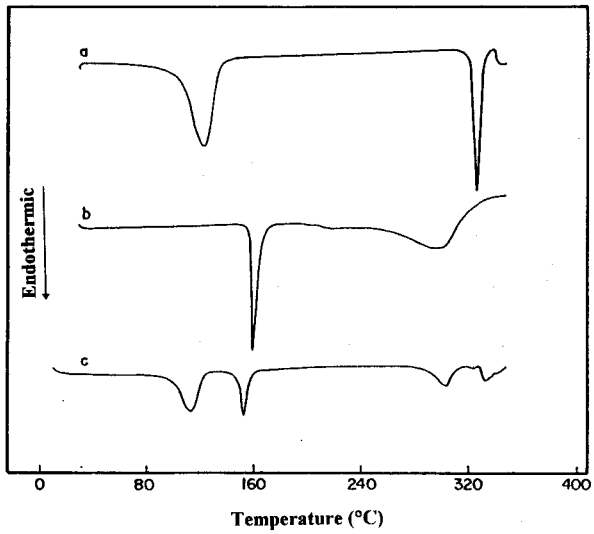


Fig. 2: DSC thermograms of IM/QRT systmes. (a) QRT; (b) IM; (c) IM/QRT double blend.

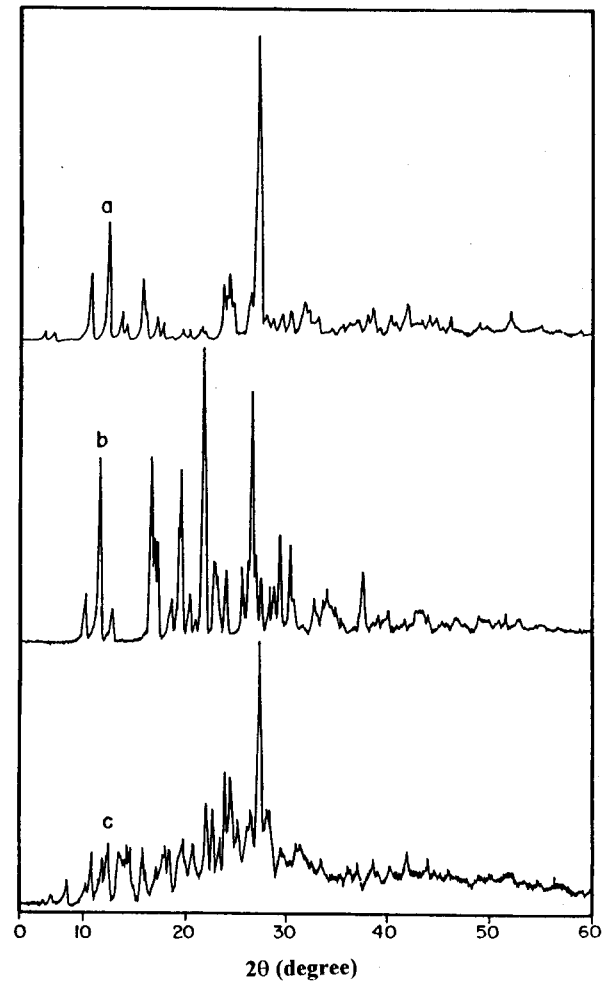


Fig. 3: Powder x-ray diffraction patterns of IM/QRT systems. (a) QRT; (b) IM; (c) IM/QRT double blend.

is compatible with IM in the blend. Quercetin may influence the crystallization kinetics by preventing the self-association of IM molecules, also the solvent method used for preparing the double blend may affect the crystalline pattern of drug.

The IR spectra are presented in Figure 4. The principal peaks of IM at wave numbers 1689, 1235, 1713 and 1066 cm^{-1} (4b) appear at the same position in the double blend without significant difference except slightly shifting from 1713 to 1724 cm^{-1}. The IR spectra of double blend (4c) were the summation spectra of individual constituents (IM and QRT). The relative decrease in the intensities of the peaks of 4c IR spectrum is due to the mixing of IM and QRT in 1:1 ratio in the blend mixture. The results of IR spectra, with the results of DSC and X-ray, confirm the compatibility between IM and QRT and successfully suggest the possibility of formulation of them in a solid dosage form.

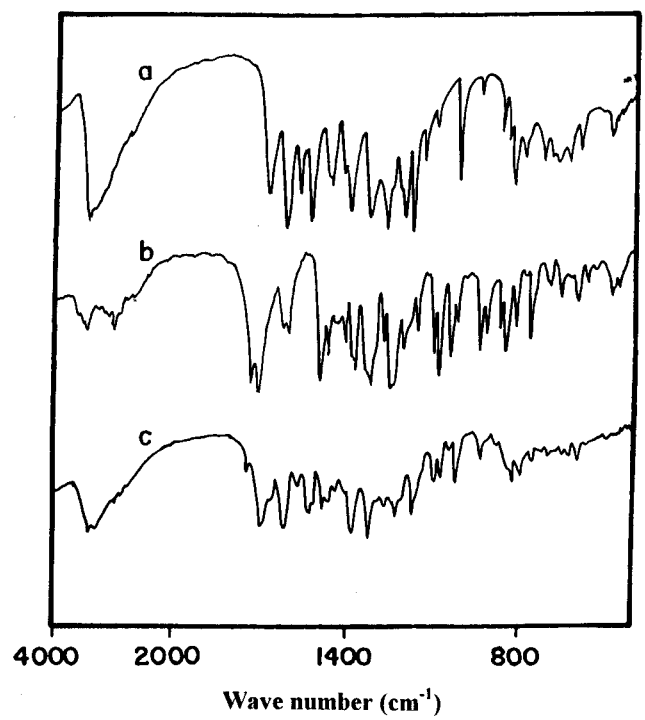


Fig. 4: IR spectra of IM/QRT system. (a) QRT; (b) IM; (c) IM/QRT double blend.

In-vitro dissolution profiles of IM with tested powder samples containing IM/QRT double blend or IM alone are illustrated in Figure 5. The dissolution rate of IM slightly increased from the double blend especially at the first 10 minutes. The results, fortunately, revealed that QRT does not confuse the dissolution rate of IM and confirm the physical and chemical compatibilities of the two substances.

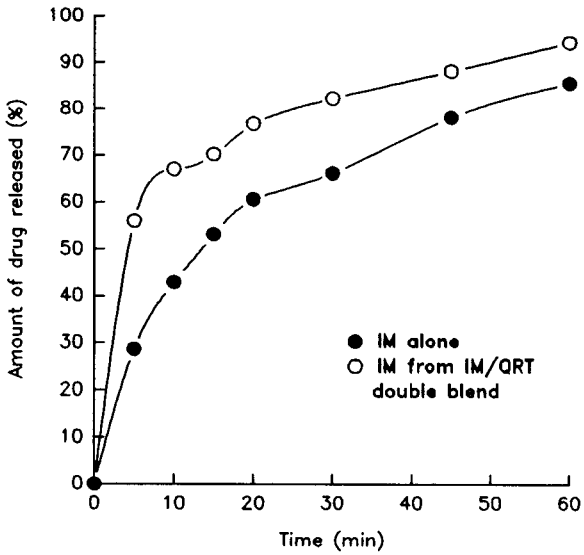


Fig. 5: Dissolution rate of IM in phosphate buffer (pH 7.2).

Conclusion

In general, the ulcerogenic activity of IM was reduced in pre-administration and co-administration tested samples including IM and QRT as compared with that containing IM alone. Specifically, the co-administration of QRT has been shown to be more effective than pre-administration in decreasing the gastric ulceration induced by IM peroral administration.

The dissolution rates of IM from coprecipitated double blend and physical mixture of IM and QRT did not change compared with the dissolution of the drug alone. It could be concluded that IM and QRT could be given together. These observations prompt us to predict the healing efficacy of QRT and to suggest pharmaceutical preparations to be prepared from IM and QRT in suitable and effective ratio to overcome NSAIDs-associated gastric local-side effects.

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