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PRELMINARY EVALUATION OF QUERCETIN EFFECT ON DICLOFENAC SODIUM ASSOCIATED GASTRIC ULCER IN RATS.

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ABSTRACT

The effect of quercetin (QRT) in preventing or reducing the diclofenac sodium-induced gastric ulcers was evaluated in rats following over night fasting by using a scanning electron microscopy. The scanning micrographs of stomach specimens showed a marked reduction in the gastric ulceration only when the quercetin is given before the administration of diclofenac sodium. The physicochemical characteristics of diclofenac sodium and quercetin combination were studied using differential scanning calorimetry, IR spectroscopy and X-ray diffractometery. The results revealed that a chemical incompatibility of diclofenac sodium and quercetin undoubtedly has been occurred when they were mixed in one combination. According to aforementioned results, we advise orally taking quercetin only before administration of diclofenac sodium as a prophylactic treatment to protect against its expected gastric disturbances.

INTRODUCTION

The extremely widespread use of nonsteroidal antiinflammatory drugs (NSAIDs) and their clinical usefulness nowadays is restricted because of their unacceptable and non-tolerated gastric adverse effects, especially for treatment of elder patients [1-3]. NSAIDs induce the damage of gastric mucosa by direct effect of its acidic group and locally inhibiting the production of protective prostaglandins [2]. Discontinuing NSAIDs use is not always feasible, particularly when NSAIDs are used for the treatment of rheumatoid and osteoarthritis. Consequently, availability of effective treatment for the prevention or reducing of NSAIDs-induced ulceration is important. Misoprostol and other prostaglandin analogs have been shown to be effective as a conventional therapy in healing peptic ulcers, but their propensity to cause diarrhea precludes their routine use for therapy at this time [3]. Also until recently, no treatment has been proven to prevent NSAIDs-induced gastric ulcers and bleeding. The addition of H_2-antagonists or sucralfate has not been shown to reduce the severity or incidence of NSAIDs-induced gastric ulcers or bleeding [2].

The antiulcerogenic effect of Quercetin (QRT), the most abundant bioflavonoid, and some other flavonoids have been frequently reported [4-6]. QRT is a powerful antioxidant, which is widely distributed in edible plants, mainly as glycosides such as rutin [7,8]. Budavari et al [9] investigated that LD_{50} of QRT taken orally in mice equals 160 mg/kg. Also, Castagnino et al [10] investigated that QRT did not show signs of toxicity or any significant behavioral effects on the autonomic system when tested at doses up to 1000 mg /kg. At the same time, QRT has a benefit as an adjunctive agent with NSAIDs because of its moderate antiinflammatory, analgesic, antipruritic and antipyretic activities [11,12].

This study has the important aim of determining whether double blend combination of Diclofenac Sodium (DS) with QRT well be effective in reducing the most frequent DS-induced gastric ulcerations. It will also provide information on the physicochemical compatibility of DS and QRT. This study is to design and evaluate a novel drug-drug combination for reducing the gastric adverse actions of NSAIDs.

MATERIALS AND METHODS

MATERIALS

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

Preparation of Coprecipitate

DS/QRT double blend containing 1:1 molar ratio of DS and QRT was prepared by dissolving the two compounds in methanol and were coprecipitated by slowly evaporating the solvent at 40 °C.

Gastric ulcerogenicity studies

Thirty male albino rats weighing (180-200 g) were allocated to five groups (each group of sex rats) and were fasted over night prior to the administration of tested compounds, in 5 % aqueous solutions or suspensions. The experiment was designed to optimize the procedure that would be applied to overcome the gastrointestinal damage of repeated-use of DS. Daily dose of DS (9 mg/kg) and treatment dose of QRT (5 mg/kg) were given perorally in combination or separately to rats. The rats of the first group received a daily peroral dose of DS as a 1-ml solution, for 4 successive days. The rats of second group were prophylactically treated by QRT at 30 min before administration of DS. The rats of third group received double blend of DS

and QRT to test the co-administration treatment. The rats of the fourth group were treated by QRT after 30 and 60 min of DS administration. The fifth group received equivalent amounts of the placebo and considered the control group.

At the end time of experiment, the rats were sacrified and the stomachs were removed, opened along the curvature, cleaned gently by dipping in saline, and prepared for scanning in electron microscope JEOL, JSM-5400LV (Electron Microscope Unit, Assiut University) for observing any mucosal injury. The scanning micrographs of stomach specimens were prepared according to the reported procedure by Fadl et al [13]. The rats of each group were examined for the development of histological signs of gastric ulcerations. The results of therapeutic antiulcerogenic activity of QRT were categorized as successful or failed for preventing or reducing the DS-induced gastric damage by its prophylactic pre-administration, protective co-administration or after administration treatment.

The Physicochemical Compatibility Studies

In this preformulation study, the thermograms, diffractograms and IR spectra of the tested samples were investigated. DSC-407 computer-mediated differential scanning calorimetry (Shimadzu Co., Japan) was used to study the drug-drug interaction between DS and QRT. The samples were run at a scanning rate of 10 °C/min in a covered sample aluminum pan, under nitrogen gas flow rate of 40 ml/min.

X-ray powder diffraction patterns of the tested samples were obtained using a PW 1700/1710 X-ray diffractometer (Philips Co., Netherlands). The X-ray tube was typical operated at voltage 40 kV, current 30 mA, wavelength 15418 Å, nickel-filtered Cu-Kα radiation and scanning speed 0.06°/min starting from 4° to 60°.

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

RESULTS AND DISCUSSION

Examination of stomach specimens of the treated rats under scanning electron microscope affords a highly precise method for investigation of the gastric ulceration of DS. Figure 1 represents scanning electromicrographs for the stomach specimens of rats of the first and second groups, at the same constant magnification power. The peroral administration of DS, without any treatment by QRT, exhibited ulcers on gastric mucosa of all the rats of first group. The ulcers formed were characterized by damage of the mucous layer beside destruction of some submucosal cells, as can be seen in the scanning electromicrograph (Fig. 1A). The reactive oxygen species derived from neutrophils may play a role in the formation of ulcers during the DS-induced acute gastritis [14]. In comparison, pretreatment with QRT (second group) significantly protected the gastric mucosa and consequently prevented the destruction of submucosal cells as shown in Figure 1B. But, the gross observation and the scanning electromicrographs of stomach specimens of the third group rats, in which QRT was co-administrated with DS, did not show any change or reduction in the gastric ulceration. Whereas, the treatment with QRT, after development of gastric ulcer in rats of fourth group showed non significant effect. Yokoo and Kitamura [15] reported that the peroral receiving of QRT before or after the administration of DS may markedly reduce the DS-induced gastric mucosal ulceration, according to the dosing regimen, and the antiulcer activity of QRT is due to its free radical-scavenging and powerful antioxidation properties. More in vivo studies are required to estimate the QRT dose suitable for complete protection against DS-induced gastric ulcer.

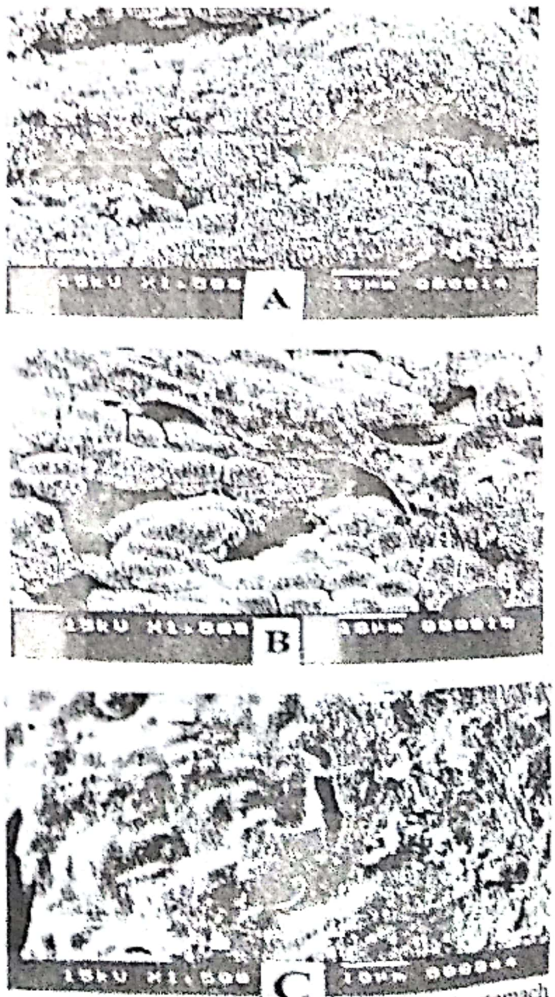

Fig.1: Scanning electromicrographs of stomach specimens treated by (A) DS; (B) DS/QRT combination and (C) placebo.

The DSC is effective thermal method for the rapid evaluation of the compatibility of drug-drug or drug-excipient combinations.

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Thermograms of DS, QRT and their double blend are presented in Figure 2. As shown, QRT has a well-defined two endothermic peaks, the first peak around 121.4°C corresponding to its water of hydration and the other peak at 325°C corresponding to its melting point. Thermogram of DS showed a sharp melting-point endothermic peak at 286.4°C, which is followed with another slightly broad exothermic peak at higher temperature (293.1°C) above its melting point corresponding to reacted or decomposed materials. The DSC curve of DS/QRT double blend showed two endothermic broad peaks in two new positions (at 60.8 and 104.7 °C), and appearing two new very weak broad endothermic peaks at 255.6 °C and 270.7°C. Also, the DS/QRT double blend thermogram showed a complete disappearing of the two melting-point endothermic peaks related to DS (286.4°C) and QRT (325°C). The results clearly revealed a chemical interaction between DS and QRT in the mixture and the possibility of formation of new chemical substances. This chemical interaction might due to the reaction between the sodium ion of DS and the reactive hydroxyl groups of QRT. This chemical incompatibility explains the loss of antiulcer activity of QRT when it is mixed with DS in one combination.

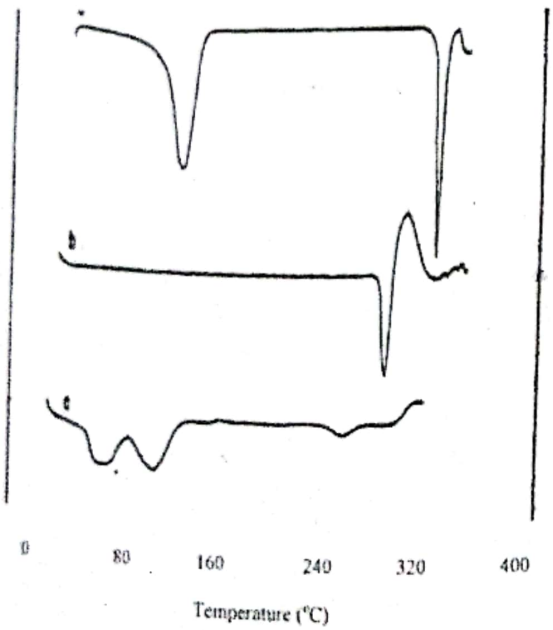

Fig. 2: DSC thermograms of (a) quercetin; (b) diclofenac sodium; (c) diclofenac sodium/. quercetin cmbination.

The X-ray diffraction patterns of DS, QRT and their double blend are shown in Figure 3. The diffractograms are significantly different with respect to both the position and intensity of the X-ray diffractions. In spite of the main diffraction peaks of QRT (Fig. 3a) appear nearly at the same 2θ of DS/QRT double blend as shown in Figure 3c (10.300, 12.440 and 27.360) the relative intensity of characteristic peak of DS, Fig. 3b, (2θ = 6.654°) completely changed in DS/QRT system. The results confirm that QRT might react with DS in the blend. In comparison with the DSC data, the results suggest that the chemical incompatibility between DS and QRT may proceed at elevated temperature or in liquid medium.

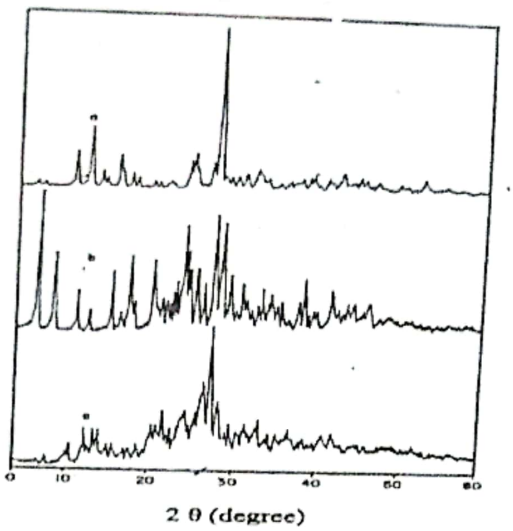

Fig. 3: X-ray spectra of (a) quercetin; (b) diclofenac sodium; (c) diclofenac sodium/. quercetin cmbination.

The IR spectra are presented in Figure 4. The principal peaks of DS appear at wavenumbers 1574, 1505, 1303 and 1281 cm^{-1} (Fig. 4b). The IR spectrum of DS/QRT double blend revealed a complete disappearance of the main peaks of DS at wavenumbers 1574 and 1281 cm^{-1}. Also, slight shifting from 1505 to 1514 cm^{-1} and from 1303 to 1314 cm^{-1} occurred for the other characteristic peaks (Fig. 4c). The results of IR spectra, with the results of DSC and X-ray, strongly confirm the chemical incompatibility between DS and QRT and impossibility of formulating them in one oral dosage form.

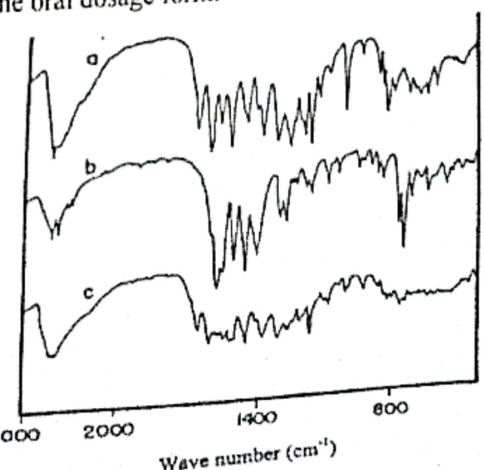

Fig. 4: IR spectra of (a) quercetin; (b) diclofenac sodium; (c) diclofenac sodium/. quercetin cmbination.

study demonstrate the superior effectiveness of QRT in preventing or reducing the Diclofenac

sodium-induced gastric ulceration, when it is used before administrating of DS. The results revealed clearly the chemical incompatibility between DS and QRT when they mixed together in one combination. Consequently, the results confirm the impossibility of preparing DS and QRT in one combination or their simultaneous oral co-administration. These results may play important role in future NSAIDs therapeutic strategies.

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الملخص العربى

تقييم أولي لدراسة تأثير الكورستين علي التقرحات المعدية الناتجة عن إستخدام ديكلوفينات الصوديوم في الفئران

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استهدفت الدراسة اختبار تأثير الكورستين كعامل واقى ومضاد للتقرحات المعدية الناتجة عن تناول ديكلوفينات الصوديوم بطرق تناول مختلفة: قبل وأثناء وبعد تناول ديكلوفينات الصوديوم وأظهرت عينات للغشاء المخاطي المبطن للمعدة والتي تم فحصها بواسطة الميكروسكوب الإلكتروني عدم تأثير الكورستين علي حماية الغشاء المخاطي والجدار المعدي عند تناوله أثناء تناول ديكلوفينات الصوديوم .

وأثبتت نتائج التحلل الحراري وحيود الأشعة السينية والأشعة تحت الحمراء تفاعل الكورستين مع ديكلوفينات الصوديوم كيميائيا نتيجة تفاعل أيون الصوديوم في ديكلوفينات الصوديوم مع مجموعات الهيدروكسيل الموجودة في جزئ الكورستين مما يشير إلي عدم إمكانية صياغة العقارين معا في مستحضر صيدلي واحد للتغلب علي الأثار الموضعية التي يسببها عقار ديكلوفينات الصوديوم وتقتصر إمكانية تناول الكورستين فقط في حالة تناوله قبل تناول ديكلوفينات الصوديوم .