Zagazig J. Pharm Sci., June 2001
Vol. 10 No. 1, pp 29-33

ISSN 1110 -5089

IBUPROFEN-QUERCETIN COMBINATION AS A NOVEL PHARMACEUTICAL COMPATIBLE SYSTEM

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ABSTRACT

The effect of oral administration of quercetin (QRT) (25 mg/kg) on an experimentally Ibuprofen-induced ulcer was studied in rats. The results of gross appearance and scanning electromicrographs of the mucous membrane and superficial cells of the stomach revealed that the quercetin reduced the damage of submucosal superficial cells. Differential scanning calorimetry, X-ray diffractometer, IR spectroscopy, solubility and partitioning studies elucidated the absence of any interaction between the two compounds and also the physical compatibility of ibuprofen (IP)-quercetin combination. It is concluded that ibuprofen and quercetin can be pharmaceutically co-formulated or co-administrated with the aim of reducing the gastrointestinal adverse effects of ibuprofen.

INTRODUCTION

In view of the drug development and therapeutic safety, it is necessary to study not only the effect of pharmaceutical formulation on the drug activity but also on the expected drug side effects, for example the main important criterion for the ideal oral NSAIDs is the lack of gastrointestinal disturbances especially after long-term treatment. Several investigations have been utilized to prevent or reduce gastrointestinal irritation of NSAIDs, including prodrug formation and microencapsulation [(1)]. One of these approaches applied to overcome the adverse reactions of NSAIDs is the incorporation of pharmaceutical agents, which can prevent or reduce the gastric disturbances, and at the same time they are compatible with the drug. The more potent antisecretory H_2-receptor antagonists do not appear to be effective in preventing NSAIDs-induced ulceration [(2)].

Quercetin, one of natural bioflavonoid, is widely distributed in edible plants and possesses vitamin P properties. It protects against vascular disorders by decreasing the permeability and fragility of capillaries. The mechanism of QRT action could be related to its antioxidant function, which can effectively protect cells and tissues against the deleterious effects of reactive oxygen species [(3-5)]. Suzuki Y et al [(6)] investigated that QRT has gastric cytoprotective and gastric healing-promoting actions. Quercetin is now increasingly used in some countries for self-care medication, because of its tolerance and safe low dose [(7)]. At the same time, QRT has a benefit as an adjunctive agent with NSAIDs because of its moderate antiinflammatory, analgesic, antipruritic and antipyretic actions [(8, 9)].

Until recently, no attempt was made to study the physical and chemical compatibilities of QRT and IP in one combination product and to ensure the cytoprotective activity of QRT against the ibuprofen-induced gastric mucosal injury. In response to this interest, the aim of this study is to test and evaluate the use of QRT for preventing, reducing or healing ibuprofen-induced gastric ulcers. Also, this study was performed to investigate the physical and chemical compatibilities of QRT with IP.

MATERIALS AND METHODS

MATERIALS

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

ANIMALS

Thirty albino rats weighing 180-200 g were fasted over night every day for 4 successive days, in grid bottomed cages, and were allowed free access to water prior to the experiment.

METHODS

Preparation of Coprecipitate

IP/QRT combination containing 1:1 molar ratio of IP and QRT was prepared by dissolving the two compounds in methanol and co-precipitated by slowly evaporation the solvent at 40 °C.

Gastric ulcerogenicity studies

A JEOL, JSM-5400 LV scanning electron microscope was used for observing mucosal injury from scanning micrographs of rat stomach specimens at the Electron Microscope Unit, Assiut University. Thirty male albino rats were allocated to five groups of rats (each group of sex rats) and were fasted over night every day for 4 successive days, prior to the oral administration of tested samples, but free access to water. The experiment was designed to optimize the procedure that would be applied to study the gastroprotective effect of QRT, as a treatment to overcome the gastrointestinal damage of repeated-use IP. Daily doses of IP (50

mg/kg) and QRT (25 mg/kg) were given orally separately or in one combination to rats as 0.5 % carboxymethylcellulose aqueous solutions or suspensions. The rats of the first group received only a daily oral dose of IP, as 1 ml suspension. The rats of the second group were prophylactically given QRT at 30 min before administration of IP. The rats of third group received the IP/QRT combination to estimate the co-administration treatment. The rats of the fourth group were treated by QRT after 30 and 60 min of IP administration. The rats of fifth group received equivalent amounts of the placebo and were considered as a control.

At the end of the four days of dosings, the rats were scarified and the stomachs were removed and opened along the greater curvature, cleaned gently by dipping in 0.9 % sodium chloride solution, examined under a binocular magnifier for possible development of histological signs of gastric ulcerations. The stomach specimens were prepared for scanning in an electron microscope according to the reported procedure by Fadl T.A. et al [10]. The results of QRT therapeutic antiulcerogenic activity were categorized as successful or failed for preventing, reducing or healing the gastrointestinal damage because of the pre-administration, co-administration or post-administration treatment.

Physicochemical Compatibility Studies

DSC-407 differential scanning calorimeter (Shimadzu Co., Japan), equipped with a computerized data station was used to obtain the thermograms of the powdered samples of IP, QRT and 1:1 molar ratio IP-QRT coprecipitate, prepared by the evaporation solvent method. The thermograms of 5 mg samples were recorded by using a heating rate of 10°C/min over the range of 30-350 °C, in a covered sample aluminum pan and under nitrogen gas flow rate of 40 ml/min.

X-ray diffraction patterns of the powdered samples were obtained with PW 1700/1710 X-ray diffractometer (Philips Co., The Netherlands). The X-ray tube was operated at voltage 40 kV, current 30 mA, wavelength 1.5418, Ni filtered Cu-Kα radiation and scanning speed 0.06°/min over a range of 4-60°.

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

Solubility and Partitioning Studies

Aqueous solubility of IP in presence and absence of QRT was experimentally calculated by shaking excess amounts of IP, QRT and IP-QRT combination in equal volumes of water (20 ml) for 72 hours. The filterates of the tested samples were assayed spectrophotometrically at 264 nm for IP and QRT after UV-spectrophotometric scanning of each drug.

Partition coefficient for IP in presence and absence of QRT was calculated in 1:20 v/v n-octanol/phosphate buffer system (pH 7.4). Diluted concentrations of IP and QRT in the phosphate buffer layer of the tested samples equal to 500 and 25 mcg/ml respectively, were prepared to neglect the activity coefficients. one ml methanol was used to dissolve IP or QRT in the aqueous layers. The partitioning samples were shacked in rotary shaker (50 rpm) at 32 °C (room temperature) for one hour. After separation, the two layers of partitioning systems, IP and QRT concentrations were calculated in phosphate buffer layers against phosphate buffer previously shacked with n-octanol at the same conditions, as a blank. The following distribution law was applied to calculate the partition coefficient (K):

$$K = [w_o/V_o]/ [(w - w_o)/V_{ph}]$$

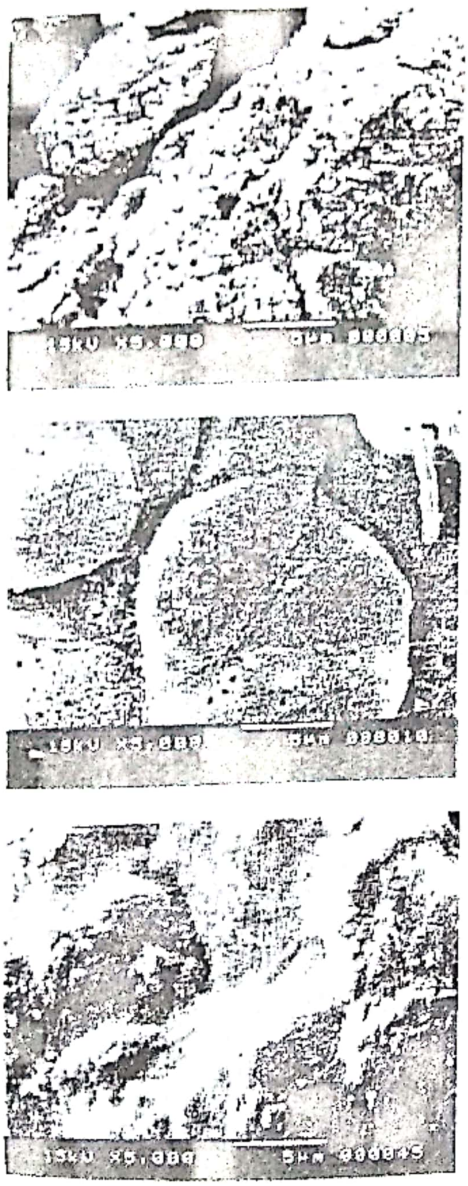
Where w_o is the amount of IP or QRT in a volume of n-octanol layer equal to V_o, w is the initial amount of IP or QRT used in preparing the tested samples and V_{ph} is the volume of phosphate buffer layer.

RESULTS AND DISCUSSION

Effect of QRT on IP-induced Gastric Ulcerations

Initial preclinical studies were conducted using rats to evaluate gastroprotective effect of QRT on IP-induced mucosal gastric injury. The results showed that 4 rats from the first group received IP alone exhibited ulcerations on gastric mucosa. The gross appearance showed stomachs with discrete ulcers. In comparison, the gastric ulcers, in the rats of the second group pre-administrated, and the rats of the third group co-administrated QRT with IP were significantly reduced by about 45 and 35 % respectively. Whereas, all rats of fourth group treated with QRT, after inducing the gastric ulcer, showed no significant effect because of the effective treatment is dose-dependent and more time is necessary. The scanning electron microscope affords a highly precise method for examination of stomach specimens. Figure 1, represents scanning electromicrographs, at a constant magnification power for stomach specimens of rats received IP, IP/QRT combination or placebo. As shown, the IP-received group (1A), was characterized by damage of some submucosal cells. A significant protection of the submucosal cells of group received IP/QRT combination is shown in 1B. These results indicate that the gastroprotective effect of QRT against IP-induced gastric mucosal

injury may result, in part, from its antioxidation and free radical-scavenging property [3,4], which inhibit lipid peroxide level in gastric mucosa. Alarcon de la Lastra C. et al. [11] investigated the gastroprotective protective action of QRT against mucosal injury produced by subcutaneous administration of indomethacin and investigated also that QRT exerts cytoprotective activity through a complex mechanism involving stimulation of prostaglandin and inhibition of leukotriene production, via mucus secretion.



(A)

(B)

(C)

Fig.1: Scanning electromicrographs of rat stomach following daily dose administration, for four successive days of , (A) IP; (B) IP-QRT combination and (C) control.

Physicochemical compatibility studies

To formulate IP and QRT in a pharmaceutical preparation, the need for preformulation information about the physical and chemical compatibilities become necessary. Thermograms of IP, QRT and 1:1 IP/QRT coprecipitate are illustrated in figure 2. The DSC curve of QRT exhibited two endothermic peaks, the first peak at 121.4°C corresponding to its water of hydration (dihydrate) and the other peak at 325°C corresponding to its melting and decomposition point. The thermogram of IP showed a sharp melting-point endothermic peak at 74°C, and another broad peak appeared at higher temperature (223.8°C), which is corresponding to non significant decomposed material. The DSC curve of IP/QRT coprecipitate did not show any peak corresponding to water of dihydrated QRT. The disappearing of peak corresponding to the water of hydration, in the thermogram of IP/QRT sample is due to the loss of the water of hydration during process of coprecepitation. Also, the results revealed a negligible lowering in the melting point of IP (73.8°C) in its coprecipitate. These results confirm undoubtedly the compatibility between IP and QRT at temperature lower than 200 °C in the coprecipitate and only the possibility of some interactions between the decomposed products of IP and QRT occurs at temperature higher than 200 °C.

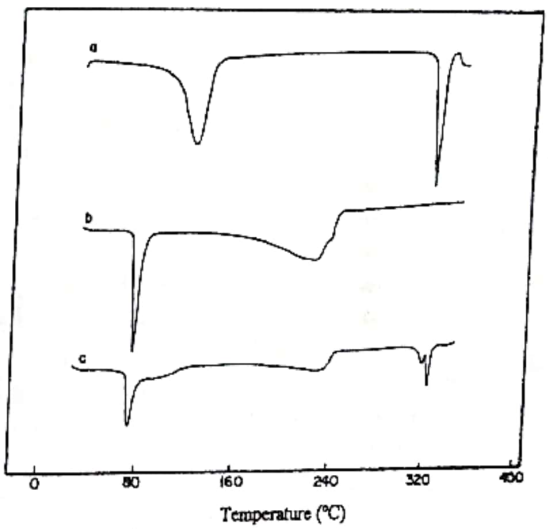


Fig. 2: DSC thermograms of (a) QRT; (b) IP; (c) IP-QRT combination.

The X-ray diffraction patterns of IP, QRT and their coprecipitate are shown in figure 3. The main diffraction peaks of QRT, 3a, appear nearly at the same 2θ of IP/QRT coprecipitate, 3c (10.71, 12.36 and 27.31). The relative intensities of characteristic peaks of IP, 3b, at 2θ = 6.04 and 22.25° decreased in IP/QRT coprecipitate at the same 2θ values. This decreasing in the relative intensities of IP in the IP/QRT coprecipitate can be explained by preventing the self-association of IP in the coprecipitate by the molecules of QRT. Also, the evaporation solvent method used for preparing the coprecipitate may affect the crystalline pattern of IP. The non-significant change of position (2θ) and distance (D-space) values of X-ray diffraction patterns of IP, QRT coprecipitate and IP/QRT coprecipitate reveal the physicochemical compatibility of IP and QRT.

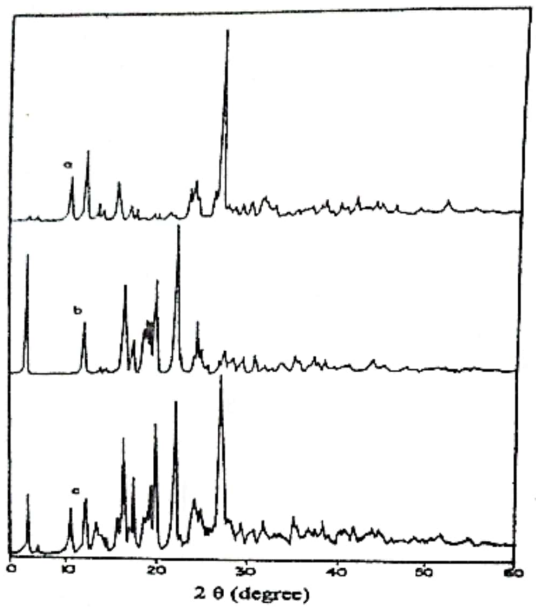


Fig. 3: Powder X-ray diffraction patterns of (a) QRT; (b) IP; (c) IP-QRT combination.

The IR spectra are presented in figure 4. The principal peaks, of IP, at wavenumbers 1720, 1232 and 779 cm^{-1} (fig. 4b) appear at the same positions in the coprecipitate (1719, 1233 and 778) without significant difference. Also, the principal peaks, of QRT (fig. 4a), at wave-numbers 1608, 1519, 1318, 1262 1168, and 1014 cm^{-1} appear at the same positions in the coprecipitate (1609, 1521, 1320, 1265, 1168 and 1014 cm^{-1}) without significant difference. The IR spectra of coprecipitate, 4c, were the summation spectra of individual constituents (IP and QRT). The relative decrease in the intensities of the IR peaks of IP/QRT coprecipitate is due to the mixing of IP and QRT in 1:1 molar ratio. The results of IR spectra agree with the results of DSC and X-ray, and confirm the compatibility physically and chemically between IP and QRT and suggest the possibility of introducing them in an oral solid dosage form.

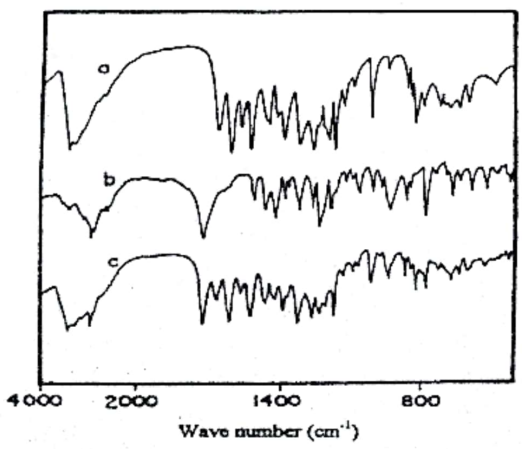


Fig. 4: IR spectra of (a) QRT; (b) IP; (c) IP-QRT combination.

Solubility and Partitioning Studies

The experimentally determined solubilities of untreated IP and QRT each alone in water, at 32°C (room temperature), equal to 0.0735 ± 0.0032 and 0.0052 ± 0.0009 mg/ml respectively. While the solubilities of IP and QRT mixed in one combination equal to 0.0980 ± 0.0024 and 0.00025 ± 0.00004 mg/ml respectively. The results of solubility revealed an increase in the solubility of IP in presence of QRT.

The calculated partition coefficients in n-octanol/phosphate buffer of IP (K_{IP}) and QRT (K_{QRT}) at 32°C each alone, were 1.5 ± 0.11 and 21.36 ± 1.68 respectively.

The results of solubility and partitioning of IP/QRT combination confirm the absence of any interaction between IP and QRT and agree the results of compatibility studies.

CONCLUSION

In general, QRT has been shown to be effective in preventing or decreasing the gastric injury, the main co-existing side effect of NSAIDs. The IP-induced gastric mucosal injury mainly was reduced in case of pre-administration and co-administration of QRT. Also, It was concluded, from DSC, IR spectra, X-ray diffraction, solubility and partitioning studies that IP and QRT could be mixed together in one combination. These observations prompt us to formulate pharmaceutical oral dosage forms from IP and QRT in suitable and effective ratio to overcome NSAIDs-associated gastric local-side effects.

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Received : March 05, 2001
Accepted : May 21, 2001

الملخص العربي

توافق الأيبيوبروفين والكورستين صيدليا

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استهدفت الدراسة اختبار تأثير الكورستين كعامـــل واقى ومضاد للتقرحات المعدية الناتجة عن تعـــاطى الأيبيوبروفين عن طريق الفم فى أزمنة مختلفة : قبل وأثناء وبعد تناول الأيبيوبروفين وأظهر فحص عينات الغشـــاء المخاطي المبطن للمعدة والتي تم فحصها بواسطة الميكروسكوب الإلكتروني مقدرة الكورستين علي حماية الغشـــاء المخاطي والجدار المعدي من تأثير الأيبيوبروفين عند تناوله قبل أو أثناء تناول الكورستين.

وأوضحت نتائج التحلل الحراري وجيود الأشعة السينية والأشعة تحت الحمراء توافق الكورستين مـــع الأيبيوبروفين فيزيائياً وكيميائياً ولم يتأثر ذوبان الأيبيوبروفين في الماء ولامعامل التوزيع الخاص به في نظام الإكتانول و محلول الفوسفات المنظم (ذات الأس الأيدروجيني ٧،٤) بوجود الكورستين مما يشير إلي إمكانية صياغة العقارين معاً في مستحضر صيدلي واحد للتغلب علي الآثار الموضعية التي يسببها عقار الايبيوبروفين عند تناوله عن طريـــق الفم.