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COMPATIBILITY AND SYNERGISTIC EFFECT OF QUERCETIN AND INDOMETHACIN COMBINATION

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شملت الدراسة إستكمالاً لدراسة سابقة بيان التوافق الكيميائى والفيزيائى لعقارى الإندوميثاسين والكورستين عند نسب مختلفة من عقار الكورستين بواسطة التحلل الحرارى والتحليل الضوئى بإستخدام موجات الأشعة البنفسجية لمعرفة توافق العقارين وإمكانية التقدير الكمى للإندوميثاسين فى وجود الكورستين. تمت دراسة تاثير الكورستين علي درجة ذوبانية الإندوميثاسين بإستخدام محلول الفوسفات المنظم ذات الأس الأيدروجينى ٧,٤. اثبتت نتائج التحلل الحرارى فى وجود المعدلات المتزايدة والمتناقصة للكورستين التوافق الكيميائى والفيزيائى للعقارين في خليطهما الفيزيائى والمتراسب. وأثبتت نتائج التحليل الضوئي التوافق وإمكانية التحليل الكمى للإندوميثاسين في وجود الكورستين .

كما يهدف هذا البحث أيضا الإستفادة من عقار الكورستين ، الذي يمتلك كثيرا من التأثيرات العلاجية المختلفة ، لصياغة الإندوميثاسين والكورستين في صورة مستحضر صيدلى للتغلب على التقرحات المعدية والتي تم دراستها في بحث سابق. كما تهدف الدراسة الحالية استكشاف التأثير المعضد للكورستين (حيث ثبت أنه يمتلك مفعول مسكن ومضاد للإلتهابات) على فاعلية الإندوميثاسين كمسكن ومضاد للإلتهابات. وقد تم ذلك بعدة طرق فارماكولوجية مختلفة منها إستخدام السطح الساخن وإستخدام الحقن البريتونى لبارا بنزوكينون لدراسة المفعول المسكن وحقن محلول الخميرة لدراسة المفعول المضاد للإلتهابات. أثبتت النتائج تعضيد الكورستين لمفعول الإندوميثاسين كمسكن ومضاد للإلتهابات في حالة تحضيرهما في صيغة صيدلية واحدة.

The previous study of Indomethacin-Quercetin (IM-QRT) compatibility by thermal analysis was continued in presence of different ratios of QRT to investigate the effect of QRT on the polymorphic structure of IM. Thermal analysis results revealed that the compatibility of physical mixtures and coprecipitates were at all ratios and slightly formation of polymorph II of IM in all ratios of coprecipitates. Also, UV-spectra investigated the compatibility and possibility of quantitative analysis of IM in presence of QRT.

The synergistic effects of Quercetin on Indomethacin were studied using hot plate and p-benzoquinone methods for analgesic activity in mice and yeast-induced inflammation for anti-inflammatory effect in rats. Quercetin was found to have the ability to increase the duration of analgesic activity of Indomethacin. Also, the reaction time of Indomethacin-Quercetin combination increased significantly to reach the maximum after 4 hrs.

INTRODUCTION

The use of nonsteroidal anti-inflammatory drugs (NSAIDs), as conventional therapy, particularly for the treatment of rheumatoid and osteoarthritis forces us to reduce the NSAIDs-induced adverse affects. One of the main pharmacological and pharmaceutical techniques used to achieve available effective treatment with the minimum side effects is by pharmaceutically preparing compatible and complementary drug-drug combinations to increase the action and to reduce the dose. The concomitant use of more than one NSAID should be avoided because of the increased risk of adverse effects.[1] Madhok *et al.*[2] discussed an overview of recent

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developments including the role of complementary medicine, NSAIDs, and gastrointestinal drugs to prevent NSAID related ulcers for osteoarthritis patients. Carpentier *et al.*[3] concluded that the concomitant use of a low dose of Methotrexate with an NSAID is possible in the treatment of rheumatoid arthritis. The antioxidant, radical-trapping agents can inhibit all types of fatty acid oxygenase (cyclooxygenase or lipoxygenase) and they might seem, on that basis, to be well suited to general anti-inflammatory use.[4] The anti-inflammatory and analgesic activities[5-7] of Quercetin (QRT), naturally-occurring as powerful antioxidant suggest its use in one combination with NSAIDs to synergy their actions and overcome the side effects by reducing the amount and frequency of dose. Lyb *et al.*[5] investigated that QRT inhibits the activation of transcription factor NF-kappaB, which is responsible for the transcription of genes encoding various inflammatory mediators, and acts on different steps of the NF-kappaB pathway.

This study was designed, to continue our previous studies,[8] to estimate whether or not we can prepare a pharmaceutical compatible drug-drug combination from Indomethacin (IM) and Quercetin (QRT) to synergy the anti-inflammatory and analgesic activities of IM.

EXPERIMENTAL

Materials

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

Methods

Preparation of coprecipitate

Samples containing different ratios (1:1, 1:2, 1:3, 1:4, 1:5, 2:1, 3:1, 4:1 and 5:1 w/w) of IM and QRT were prepared by dissolving them in 1:1 v/v methanol:acetone and were coprecipitated by slowly evaporating the cosolvent at 40°. Physical mixtures of IM and QRT were prepared by simple mixing using the same ratios.

Thermal analysis studies

For continuing the compatibility study reported in previous work,[8] the thermal analysis of IM in presence of different ratios of QRT was investigated. Differential scanning calorimeter (DSC-407, Shimadzu Co., Japan), equipped with a computerized data station was used to obtain the thermograms of the powdered samples of pure IM, pure QRT and IM-QRT physical mixtures and coprecipitates. The thermograms of about 5-mg samples were recorded by using a heating rate of 10°/min over the range of 30-350°, in a covered sample aluminum pan and under nitrogen gas flow rate of 40 ml/min.

UV-spectroscopy

A comparison study between the UV-spectra of pure IM, pure QRT and IM-QRT combination in methanol and in aqueous alkali (0.01 N NaOH) is used to test and select the medium and wavelength suitable for quantitative analysis of the tested drugs presented in one combination. Methanolic solutions equal to 500 mcg/ml of each IM and QRT were prepared. The tested samples of IM, QRT and IM-QRT combination were prepared by dilution of previous methanolic solutions using phosphate buffer (pH 7.4). UV-spectra were constructed using Perkin-Elmer UV-Vis spectrophotometer, model Lambda 3B (USA), equipped with Perkin-Elmer data leader software and Star multi-font LC24-15 printer.

Solubility studies

One of the main factors governing the aqueous solubility of a drug is the sum of the drug-drug cohesive interactions.[9] Solubility of IM in water and phosphate buffer (pH 7.4) in presence and absence of QRT was experimentally calculated by shaking excess amounts of IM, QRT and IM-QRT combination in equal volumes of phosphate buffer (20 ml) for 72 hr. The filterates of the tested samples were assayed spectrophotometrically at 318 nm for IM and at 265 nm for QRT.

Anti-inflammatory activity studies

Tewenty four mature albino rats of either sex, weighing 190-200 g, obtained from the animals house of Assiut University were used in these experiments. The animals were divided into 4 groups (6 rats each). Inflammation was induced in the hind paw of the rat by subcutaneous injection of 0.1 ml of yeast suspension (20%) according to the method described by Winter *et al.*[10] After 4 hrs, the thickness of the rat paw oedema was measured using skin caliber to detect the extent of inflammatory oedema.

Suspension of the tested drugs in 5% aqueous gum acacia solution (tested samples) were orally administrated to different groups of rats in doses equal to 25 mg/kg of IM or QRT separately or in combination. Sugiyama *et al.*[11] investigated that acacia significantly increased the absorption of QRT after oral administration in Wister rats. The anti-inflammatory activity was estimated at 2, 4, 6 and 8 hrs after oral administration.

Analgesic action studies

The analgesic activities of IM and QRT and their combination were studied in mice using two different methods.

1. Hot-plate method

According to the method described by Eddy and Leimbach,[12] albino mice (25-30 g) were placed within a plexiglass cylinder placed on a hot plate surface maintained at 55°. The time taken by the mouse to lick its feet or to jump was determined. This reaction time was taken as the end point and the increase in hot plate latency was taken as a measure of the analgesic activity of the tested drugs. The control latency in each mouse was determined before the oral administration of tested samples. Then the tested samples were orally administrated according to the previously calculated doses (25 mg/kg), and the latency to reaction was again evaluated at 2, 4, 6 and 8 hrs after oral administration.

2. Writhing method

The analgesic activities of the investigated drugs and their combination were also studied using p-benzoquinone writhing method in mice.[13] The ability of the tested samples to protect mice from chemical pain induced by p-benzoquinone (pBQ) was calculated as the percentage inhibition of writhing. Before carrying out the experiment, a sensitivity test was done to determine the sensitivity of mice to pBQ. In this test, the animals were intraperitoneal injected with 0.25 ml of 0.02% pBQ aqueous solution and were observed for writhing during a period of one hour. Only animals, which responded to pBQ by writhing, were used in the main experiment, not less than 48 hr later.

Tested samples prepared in 5% gum acacia were orally administrated to different groups of mice (25-30 g), in doses equal to 25 mg/kg of IM or QRT separately or in combination, and allowed to act for different time intervals: 2, 4, 6 and 8 hrs. One group of 10 mice was used for each time interval. Following the specified time, the animals were intraperitoneal injected with 0.25 ml of 0.02% pBQ aqueous solution. The animals were observed for writhing during a period of one hour. The percentage protection against pBQ-induced writhing was determined.

Animals treated similarly after oral administration of 5% suspension of gum acacia (placebo) were used as control for analgesic and anti-inflammatory studies. The degree of variability in results was expressed as the mean $\pm$ standard error ($X \pm SE$). The significance of the differences between the tested samples was determined using student t-test.

RESULTS AND DISCUSSION

Thermal analysis studies

To formulate IM and QRT in a pharmaceutical preparation, the need for preformulation information about the physical and chemical compatibilities became necessary. Accordingly, in these DSC analysis studies we estimated the different thermal peaks of IM in presence of different ratios of QRT. Table 1 and Figures 1-2 show the effect of different ratio of QRT on the thermal behavior of IM. The results showed that increasing or decreasing QRT ratio in the physical mixture did not affect the melting point (158.2°) related to the polymorph I of IM.[14] Thermograms of coprecipitates at all ratios show a new endothermic peak at about 152°

Table 1: The effect of descending and ascending ratio of QRT on the thermogram of IM.

Thermal parameter	Pure IM	Drug : Drug Ratio (w/w)									Pure QRT
		IM:QRT ◄				1: 1	QRT:IM ►				
		5:1	4:1	3:1	2:1		2:1	3:1	4:1	5:1	
1. Physical mixture											
Temperature of water release	---*---	111.3	111.8	112.7	112.2 ◄	114.5	► 116.6	115.6	116.2	118.1	121.4
m.p of IM	158.9	158.2	158.0	158.5	158.2 ◄	158.7	► 158.7	158.0	158.2	158.6	---*---
m.p of QRT	---*---	290.1	283.0	290.1	297.0 ◄	303.2	► --*---	--*---	---*--	---*--	---*---
	---*---	295.9	289.7	295.2	331.0 ◄	323.1	► 312.1	317.5	319.4	319.2	325.0
2. Coprecipitate mixture											
Temperature of water release		--*--	87.0	88.5	103.2 ◄	128.7	► --*--	---*--	---*--	---*--	
		--*--	--*--	--*--	91.1 ◄						
m.p of IM		158.5	159.5	---*--	157.6 ◄	157.8	► 158.0	157.5	157.7	158.5	
		151.6	151.6	152.9	152.3 ◄		► 151.5	150.7	151.0	152.5	
m.p of QRT		268.3	277.1	279.1	294.7 ◄	298.1	► 308.5	311.8	314.0	312.4	
		---*--	292.9	317.8	--*--- ◄	312.1	► 318.7	---*---	---*---	--*---	

---*--- means disappearing of peaks

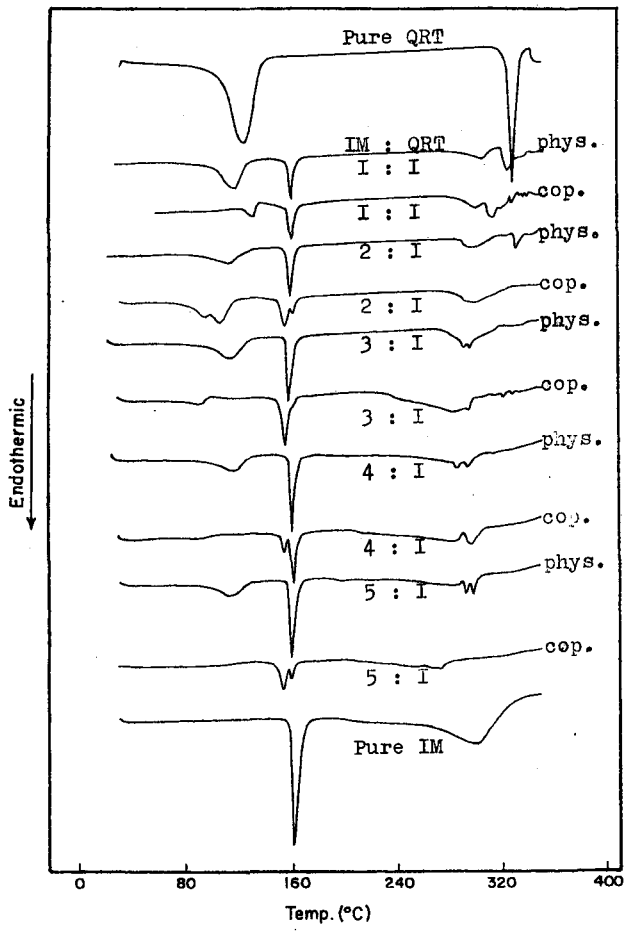

Fig. 1: The effect of descending ratio of QRT on the thermogram of IM in the physical (phys.) and coprecipitate (cop.) mixtures.

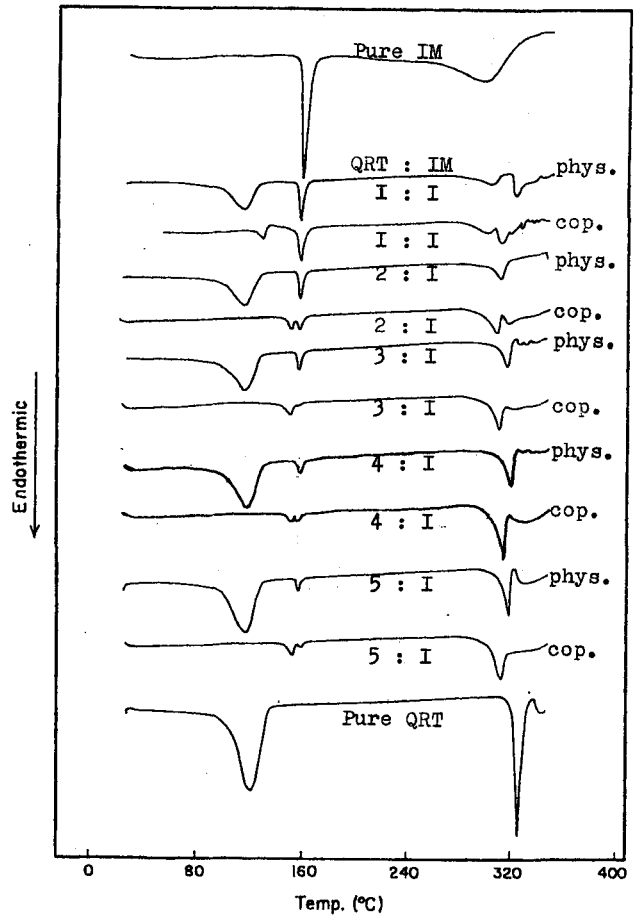

Fig. 2: The effect of ascending ratio of QRT on the thermogram of IM in the phyiscal (phys.) and coprecipitate (cop.) mixtures.

which corresponding to polymorph II of IM.[14] The hydration endothermic peak of QRT (dihydrate) slightly shifted from 114.4° at 1:1 IM:QRT physical mixture to lower values by decreasing the QRT ratio (Fig. 1) to reach the maximum lowering at 5:1 IM:QRT ratio (111.3°) and slightly increasing in the endothermic hydration peak to reach the highest value (118.1°) at 5:1 QRT:IM (or the highest ratio of QRT), as shown in Figure 2. These results revealed that the amount of heat and consequently the value of endothermic hydration peak are QRT-ratio dependant. The partial or complete disappearing of endothermic hydration peaks of coprecipitates at some ratios and shifting at other ratios revealed that the possibility of partial or complete loss of water of hydration during the process of coprecipitation.

Also, any experimental variables in the coprecipitation method, e.g. nature, amount and ratio of solvents used, temperatures and rates of evaporation and drying of the coprecipitate could be effectively affect the degree of dehydration and the net result of coprecipitation could give complete dehydration as in 2:1, 3:1, 4:1 and 5:1 QRT:IM ratios or give incomplete dehydration as in 2:1, 3:1 and 4:1 IM:QRT ratios, which show weak endothermic hydration peaks at 103.2, 88.5 and 87.0° respectively.

The possibility of some interactions between the decomposed products of IM and QRT in the physical and coprecipitate mixtures occurs at higher decomposition temperatures (more than 260°), which are negligible concerning our compatibility studies.

In previously reported study,[8] the thermograms of IM, QRT and 1:1 w/w IM-QRT combination prepared by coprecipitation from pure methanol revealed that the DSC curves exhibited a peak corresponding to water of hydration at 121.4°, which is shifted to 118.8° in the IM-QRT coprecipitate. Also, the two peaks at 158.9 and 325° corresponding to IM and QRT respectively were shifted to 152.3 and 322.2° in IM-QRT coprecipitate. In the present study, the previous peaks of water of hydrated QRT, pure IM and pure QRT were shifted to 128.7, 157.8 and 318.1° respectively in 1:1 w/w IM-QRT combination prepared by coprecipitation from 50:50 v/v mixture of acetone and methanol. The comparison between the previous and present studies suggested that the nature and ratio of solvents used in preparing the coprecipitate may play an important role in the thermal behavior and polymorphic structure of the tested samples.

These results obviously revealed the compatibility between IM and QRT in all physical and coprecipitate mixtures and the possibility of change in the polymorphic structure of IM according to the nature of coprecipitation method.

UV-spectroscopy

To evaluate the compatibility and possibility of quantitative analysis of IM in presence of QRT we studied the UV-spectra of IM-QRT combination in methanolic and aqueous alkali solutions. The λ_{max} of IM (318 nm) in methanolic solution did not change in the presence of QRT, as shown in Figure 3. Also, the UV spectra show that the percentage absorptivities of IM and QRT, at the λ_{max} of IM, equal to 76.47 and 23.53 respectively compared with the summation of pure IM and pure QRT absorptivities. At the same time, the percentage absorptivities of IM and QRT equal to 78 and 24 respectively compared with the actual total absorptivity of IM and QRT in one combination. The expected percentage of error in UV-spectrophotometric absorptivities of IM and QRT presented in one combination compared to the actual absorptivities of pure IM and pure QRT about ±1.5% and ±0.5% respectively. UV spectra revealed obviously the suitability of UV-spectrophotometric analysis of IM and QRT presented together in one combination in methanolic solution at λ_{max} of IM (318 nm).

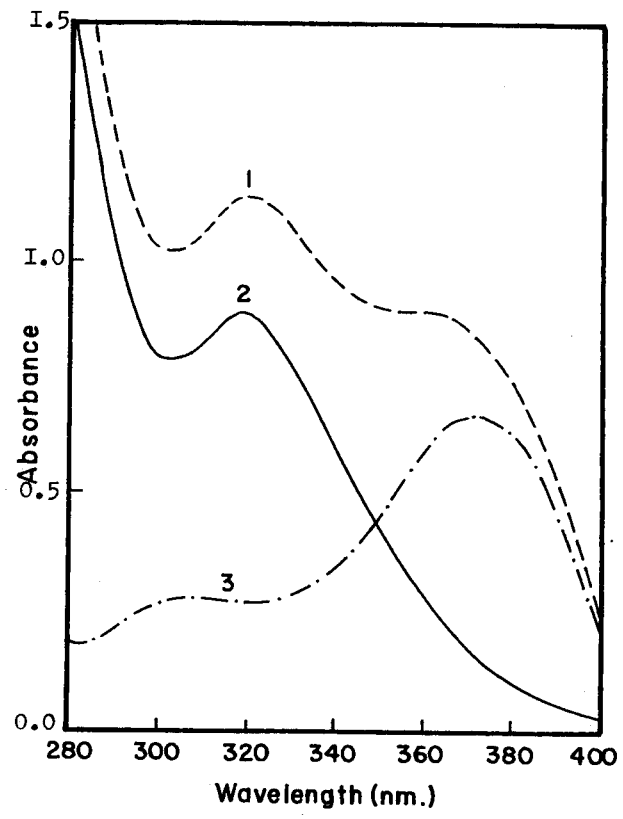

Fig. 3: UV spectra of (1) IM-QRT combination; (2) pure IM; (3) pure QRT in methanolic solution.

Figure 4 shows the λ_{max} of IM (279 nm) in aqueous alkali solution and λ_{max} of QRT at 316 nm, which shifted to 306 nm in IM-QRT combination. The results of UV-spectra in aqueous alkali solution revealed the impossibility of quantitative analysis of IM in presence of QRT because of the instability of IM[1] and QRT[15] in alkaline medium.

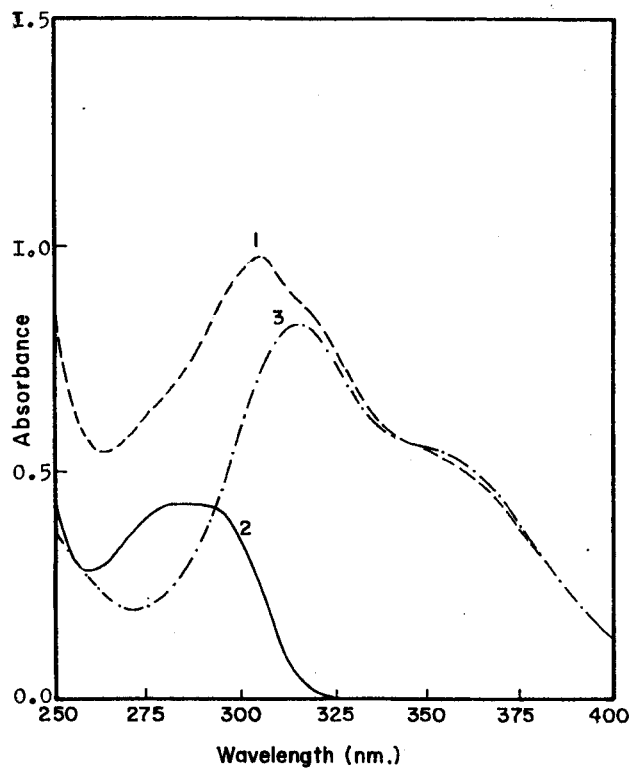

Fig. 4: UV spectra of (1) IM-QRT combination; (2) pure IM; (3) pure QRT in aqueous alkali.

Solubility

To evaluate the effect of QRT on the pharmaceutical formulation properties of IM, we studied the solubilities of IM in presence of QRT. The calculated solubilities of IM and QRT are 0.0379 ± 0.0054 and 0.0068 ± 0.0013 mg/ml respectively. Solubility of IM in phosphate buffer (pH 7.4) equals ten fold its solubility in water (0.3304 ± 0.0099 mg/ml). Whereas, the solubility of QRT slightly increased in buffer (0.0076 ± 0.0017 mg/ml). Nonsignificant change in the solubility of IM when it is mixed in one combination with QRT.

Anti-inflammatory activity

Table 2 presents the anti-inflammatory activity of IM, QRT and IM-QRT combination at different time intervals against inflammation induced by yeast in rats. It is evident from Table 2 that, the mean of paw oedema thickness of control animals ranged from 0.78 to 0.90 mm. QRT was found to decrease significantly the paw oedema thickness at 2, 4 and 6 hrs after oral administration. The maximal anti-inflammatory effect of QRT appeared after 2 hrs. IM-QRT combination was able to decrease significantly the paw oedema thickness at 2, 4, 6 and 8 hrs after administration compared to pure IM or pure QRT. The maximal anti-inflammatory effect of IM-QRT combination appeared after 4 hrs. These results indicate the synergistic effect, which appeared when IM and QRT were mixed together in one combination. NSAIDs are believed to exert their anti-inflammatory action primarily by the inhibition of the biosynthesis of prostaglandins and related cyclooxygenase-derived products. The synthesis of prostaglandines involve many free radicals and oxygen-derived intermediates. NSAIDs can also interfere with free radical generation and by trapping reactive free radical species. IM and other NSAIDs have been evaluated as inhibitors of the generation, or the activity, of oxygen free radicals in various assays.[16,17] Therefor, QRT (antioxidant and free radical scavenger) is suggested to synergy the anti-inflammatory effect of IM.

Analgesic activity

The analgesic activity results of IM, QRT and their combination revealed that all the tested samples have the ability to protect the mice from thermal pain, as shown in Table 3. The hot plate latency was found to increase when IM was given concomitant with QRT. Control mice were found to have a reaction time ranging from 4.61 to 4.92 second at different time intervals. The maximum reaction times of IM and QRT revealed after 2 hrs and were 7.25 ± 0.67 and 9.26 ± 0.61 second respectively. While, the oral administration of IM-QRT combination increased significantly the reaction time to reach the maximum after 4 hrs (18.13 ± 0.55 second) and effectively continued for 8 hrs (5.67 ± 0.20 second) compared with IM or QRT alone.

Data in Table 4 represent the percentage protection against pBQ-induced writhing. The results revealed that all the tested samples containing IM or QRT have the ability to partially protect the mice from chemical pain induced by pBQ for 6 hrs. Mice treated orally with IM-QRT combination displayed protection against writhing for 8 hrs while the control

Table 2: Anti-inflammatory activity of IM, QRT and their combination in rats.

Group treatment	Oral dose (mg/kg)	Normal paw Thickness (mm)	Inflammed paw thickness 4 hours after yeast injection (mm)	Mean of paw thickness after different intervals (hr)			
				2	4	6	8
Control	1 ml of 5% gum acacai	0.28 ± 0.02	0.98 ± 0.05	0.90 ± 0.02	0.83 ± 0.03	0.82 ± 0.03	0.78 ± 0.04
IM	25	0.28 ± 0.03	0.96 ± 0.05	0.62** ± 0.03	0.70** ± 0.04	0.71* ± 0.04	0.75 ± 0.04
QRT	25	0.30 ± 0.02	0.99 ± 0.04	0.68** ± 0.03	0.72** ± 0.02	0.69* ± 0.03	0.73 ± 0.04
IM-QRT combination	25 + 25	0.30 ± 0.03	0.98 ± 0.11	0.65** ± 0.09	0.58* ± 0.05	o.61** ± 0.04	0.62** ± 0.03

- Values are mean of 6 experiments ± SD
* Significant difference from control values at $P < 0.05$
** Significant difference from control values at $P < 0.01$

Table 3: Analgesic activity of IM, QRT ant their combination in mice by hot plate method.

Time after oral administration (hours)	Reaction time (seconds)			
	Control	IM	QRT	IM-QRT combination
2	4.61 ± 0.16	7.25** ± 0.67	9.26** ± 0.61	12.47** ± 1.10
4	4.92 ± 0.31	5.91* ± 0.27	6.61** ± 0.67	18.13** ± 0.55
6	4.70 ± 0.20	5.63* ± 0.42	5.26** ± 0.32	7.32** ± 0.19
8	4.63 ± 0.16	4.40 ± 0.10	4.99 ± 0.37	5.67* ± 0.20

- Values are mean of 6 experiments ± SE
* Significant difference from control values at $P < 0.05$
** Significant difference from control values at $P < 0.01$

Table 4: The percentage protection against pBQ-induced writhing of IM, QRT and their combination.

Drug	The percentage protection (%) against pBQ- induced writhing after different intervals (hr)			
	2 hr	4 hr	6 hr	8 hr
Control	0	0	0	0
IM	50	40	20	0
QRT	60	50	40	0
IM-QRT Combination	60	80	50	20

- Groups of 10 mice were used for each tested time interval.

animals did not show any protection against the writhing. Mice given IM, QRT and IM-QRT combination showed maximal inhibitions of writhing by percentages of 60, 80 and 50 after 2, 4 and 6 hr respectively. This increasing in the analgesic activity is an indication of the synergistic effect when IM and QRT are mixed together in one combination.

Statistical analysis of the results

The significance of the difference between samples was determined using the students t-test. The difference was regarded as significant when $P < 0.05$.

Conclusion

It can be concluded that QRT could be compatibly co-formulated or co-administrated with IM because of its synergistic effects on the anti-inflammatory and analgesic activities of IM and because of its cytoprotective action against IM-induced ulcers, as reported in previous work.[8] Therefore, we suggest using QRT as a suitable complementary drug with NSAIDs, after investigating the most suitable formulation factors, to increase the activity and to overcome the adverse affects.

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