Article

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Anti-inflammatory potential of curcumin and quercetin in rats: Role of oxidative stress, heme oxygenase-1 and TNF-α

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Abstract

Flavonoids are group of compounds that have been shown to possess potent anti-inflammatory effects in both cellular and animal models of inflammation. In the current study, the single and combined effects of the two flavonoids, curcumin and quercetin, against carrageenan-induced acute inflammation in rats were evaluated with emphasis on the role of oxidative stress, anti-inflammatory enzyme, heme oxygenase-1 (HO-1) and proinflammatory cytokine, tumor necrosis factor-alpha (TNF-α). Curcumin (50 mg/kg), quercetin (50 mg/kg) and a combination of both were orally administered for 14 days before carrageenan injection in rats and compared with the reference nonsteroidal anti-inflammatory drug, indomethacin (10 mg/kg). The percentage increase in paw thickness was calculated. Frozen hind paws were used for the estimation of lipid peroxides (malondialdehyde, MDA), nitric oxide (NO), reduced glutathione (GSH), TNF-α level and HO-1 messenger RNA (mRNA) expression. Formalin-fixed hind paws were used for histopathological examination. Results showed that both curcumin and quercetin caused reduction in carrageenin-induced edema and lymphocytes infiltration along with the decrease is being even higher in case of their combination. Additionally, both flavonoids reduced MDA and NO formation, and restored GSH contents in the paw. Furthermore, both flavonoids increased HO-1 mRNA expression and decreased the elevated TNF-α level. Results showed that both flavonoids moderately lowered inflammation, while their combination was more effective. Accordingly, this study suggests that the reduction in oxidative stress and modulation of HO-1 mRNA expression and TNF-α release by curcumin and quercetin may contribute to the synergistic anti-inflammatory effects of these two flavonoids upon combination.

Keywords

Curcumin, quercetin, inflammation, oxidative stress, heme oxygenase-1, TNF-α

Introduction

Inflammation is an essential protective process preserving the integrity of organisms against physical, chemical and infective insults. However, it is frequent that the inflammatory response to several insults erroneously leads to the damaging of normal tissues (Kemp et al., 2010). In addition, the systemic inflammatory response is associated with the production of reactive oxygen species (ROS), nitric oxide (NO) and cytokines such as tumor necroses factor-alpha (TNF-α). Furthermore, there is substantial evidence that much of the cytotoxicity is due to a deleterious

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action of oxygen- and nitrogen-derived free radicals (Gad and Khattab, 2000).

Heme oxygenase-1 (HO-1) is an inducible enzyme that catalyzes the first and rate-limiting step in the oxidative degradation of free heme into ferrous iron, carbon monoxide (CO) and biliverdin, the latter being subsequently converted into bilirubin. HO-1, which is expressed ubiquitously and highly induced by numerous stress stimuli, plays an important role in host defense against oxidative injury (Abraham and Kappas, 2008). Several lines of evidence suggest an involvement of HO-1 in the anti-inflammatory activity of cells and tissues (Ndisang, 2010; Pae and Chung, 2009). Bilirubin and CO have been reported to effectively inhibit histamine release from mast cells and inhibit nitric oxide synthase (iNOS) messenger RNA (mRNA) induction in cytokine-stimulated intestinal epithelial cells (Chung et al., 2008). Moreover, it has been demonstrated that HO-1-deficient mice are susceptible to increased inflammatory reactions (Kapturczak et al., 2004), whereas HO-1 transgenic mice are protected from pulmonary inflammation induced by hypoxia (Minamino et al., 2001). Therefore, HO-1 is an important therapeutic target in various disease models, and compounds that induce HO-1 possess great therapeutic potential against diseases associated with oxidative stress and inflammation (Abraham and Kappas, 2008).

The use of conventional therapies for inflammation, nonsteroidal anti-inflammatory drugs (NSAIDs), have discouraging profile of side effects, despite their important role in managing pain and inflammatory conditions (James and Hawkey, 2003). This demonstrates the need for new and safe anti-inflammatory drugs. Flavonoids are a group of naturally occurring polyphenolic compounds that are found in most plants and are found to exert a significant impact on the inflammatory process. Curcumin and quercetin are among the most widely distributed flavonoids, which have demonstrated to have potent anti-oxidant, anti-inflammatory and anti-carcinogenic activity (Gonzalez et al., 2011). Curcumin and quercetin inhibit the formation of proinflammatory mediators such as prostaglandins and leukotrienes (Katsori et al., 2011; Nair et al., 2006). Moreover, both flavonoids have been shown to lower the generation of ROS and NO release by macrophages (Gonzalez et al., 2011). Curcumin have been shown to reduce edema in rats and to moderately reduce the clinical symptoms in rheumatoid arthritis patients (Jurenka, 2009). Quercetin inhibits mast cell degranulation, release of histamine and also inhibits phospholipase A2, cyclooxygenase and lipoxygenase activity (Morales et al., 2006). Although previous studies suggest that both curcumin and quercetin exhibit significant anti-inflammatory and anti-allergic properties, the molecular mechanisms of their biological responses and the combined effects of both flavonoids remain to be delineated. Accordingly, the current study was designed to investigate the beneficial anti-inflammatory activities of both flavonoids and their combination against carrageenan-induced acute inflammation in rats with emphasis on the changes in the anti-inflammatory enzyme, HO-1 mRNA and the proinflammatory cytokine TNF-α.

Materials and methods

Chemicals

Curcumin, quercetin, indomethacin and carrageenan sodium were purchased from Sigma (St Louis, MO, USA). Assay kit for reduced glutathione (GSH) was purchased from Biodiagnostic (Cairo, Egypt). Enzyme-linked immunosorbent assay (ELISA) kit for TNF-α was purchased from Genzyme Diagnostics (Cambridge, Massachusetts, USA). All other chemicals were of the highest available commercial grade. Curcumin, quercetin and indomethacin were suspended in 0.5% aqueous solution of carboxymethyl cellulose.

Animals and experimental design

A total of 48 adult male albino rats weighing 180–200 g were obtained from the animal house of Faculty of Agriculture, Minia University, El-Minia, Egypt. Rats were acclimatized for at least 1 week prior to any experiment. The experiments were conducted according to the Institutional Animal Ethics Committee guidelines for the care and use of laboratory animals. Rats were divided into six groups of eight animals each. Groups I (normal control) and II (Carg group) were given 0.5% aqueous solution of carboxymethyl cellulose by intragastric tube, while groups III (Carg + Curm), IV (Carg + Qurs) and V (Carg + Curm + Qurs) received curcumin (50 mg/kg, orally), quercetin (50 mg/kg, orally) and a combination of them, respectively, for 14 consecutive days before the day of the experiment (injection of carrageenan). Animals in group VI (Carg + Ind) were treated with indomethacin (10 mg/kg, orally) as standard anti-inflammatory drug for 14 consecutive days before the day of the experiment.

Dosing volume was kept constant (10 ml/kg) and was completed with 0.5% aqueous solution of carboxymethyl cellulose when required. The choice of the used doses and time of measurement was based on pilot studies in our laboratory.

Induction and assessment of carrageenan-induced paw edema

Animals were fasted 16 h before the experiment, with free access to water alone. Following 30 min of oral administration of the last doses of the treated drugs, carrageenan-induced paw edema was persuaded according to the method described by Winter et al. (1962). Briefly, group I received 0.1 ml saline and served as control group, while groups II, III, IV, V and VI received 0.1 ml carrageenan sodium (1.5% solution in saline) subcutaneously on the plantar surface of the right hind paw. The thicknesses of injected paws were measured before (time 0) and at 1, 2, 3 and 4 h after injection of carrageenan, using a calibrated micrometer (scale 0.01 mm). The paw edema was quantified by measuring the difference between the paw thickness before carrageenan injection (zero time) and at 1, 2, 3 and 4 h after carrageenan injection. For each time point, measurement was repeated three times and the average is then calculated. All the assessments were performed by the same investigator in order to reduce any potential interoperator differences. The percentage increase in paw thickness was calculated by dividing the difference in paw thickness at each time point by its zero time thickness and multiplied by 100.

Sample collection

Following 4 h of carrageenan injection, animals of each group were decapitated. The hind paw specimens were collected. Frozen hind paws were used for the estimation of lipid peroxides, NO, GSH, TNF-α level and HO-1 mRNA expression. Formalin-fixed hind paw was used for the histopathological examination.

Determination of the paw tissues oxidative stress parameters

Soft tissues were dissected from the entire rat paw, immediately placed in cold normal saline four times their volume and homogenized at −4°C using ice around the homogenization tube. Then the homogenate was centrifuged at 12,000 rpm/min for 10 min. The supernatant was obtained and stored at −20°C for the assessment of different oxidative stress parameters. The lipid peroxidation product, malondialdehyde (MDA), was estimated by the determination of thiobarbituric acid-reactive substance levels (Uchiyama and Mihara, 1979). The product formed following the reaction of tissue homogenate with thiobarbituric acid in boiling water bath was extracted with *n*-butanol. The difference in optical density developed at two distinct wavelengths; 535 nm and 525 nm was a measure of the tissue MDA content.

GSH was determined according to the method described earlier by Beutler et al. (1963). The procedure is based on the reduction in 2-nitrobenzoic acid by glutathione to produce a yellow compound that was measured spectrophotometrically at 405 nm. NO level was measured as total nitrite/nitrate, the stable degradation products of NO, by reduction of nitrate into nitrite using copperized cadmium, followed by color development with Griess reagent in acidic medium (Sastry et al., 2002).

Determination of TNF-α level

TNF-α was assayed using ELISA kit. Briefly, soft tissues were dissected from the entire rat paws, rinsed in ice-cold normal saline and were homogenized in phosphate-buffered saline (pH 7.4) for 30s. They were then centrifuged at 12,000 rpm/min for 20 min. Briefly, 100 µl of the supernatant was added to the 96-well microtiter plate pre-coated with monoclonal antimouse TNF-α antibody (coupled to horse radish peroxidase) and incubated for 2 h at 37°C. After thorough washing, the substrate solution was added. Color development was allowed for 10 min and the reaction was stopped by the application of stop solution. Color absorbance was read in a microplate reader (Dynex Technologies, Chantilly, Virginia, USA) at 450 nm. Protein content was determined using the Lowry method (Lowry et al., 1951).

Detection of HO-1 mRNA expression with semi-quantitative reverse-transcriptase polymerase chain reaction (RT-PCR)

The soft tissues were dissected from rats' paws and total RNA was extracted using RNA extraction kit (Bio Basic Inc., Markham, Ontario, Canada) according to the manufacturers' instructions. RNA was then transcribed using revert aidTM first-strand

cDNA synthesis kit (Ferments life science, Fort Collins, Colorado, USA). The cDNA products were amplified by PCR in a total volume of 50 µl containing 2.5 U Taq DNA polymerase (Promega) and 10 pmol each of the upstream and downstream primers. After predenaturation at 94°C for 2 min, 35 cycles were allowed to run for 45s at 94°C, followed by 45s at 56°C and 45s at 72°C, and a final extension at 72°C for 5 min. The primers for HO-1 were: sense 5′-CTGGAAGAGGAGATAGAGCGAA-3′ and antisense 3′-TCTTAGCCTCTTCTGTCACCCT-5′.

As an internal control, we also estimated the expression of β-actin mRNA using the following primers sequences: sense 5′-GAGACCTTCAACACCCAGC C-3′ and antisense 5′-GCGGGGCATCGGAACCG CTCA-3′ (Shanghai Bioengineering Ltd., Shanghai, China). The predicted sizes of the amplified HO-1 and β-actin DNA products were 433 bps and 374 bps, respectively. Amplified products (5 µl) were loaded onto 1.5% agarose gels previously stained with 0.5 µg/ml ethidium bromide, electrophoresed at 100 V for 30 min and then examined under a UVP gel imaging system (UVP Co., USA). Images were analyzed with the Gel-Pro Analyzer Version 3.0, and the semi-quantitative measure of mRNA expression was expressed as the ratio of the optical density (OD) of HO-1 to that of β-actin.

Histopathological examination of hind paws

Formalin-fixed hind paws (taken 4 h after injection of carrageenan) were embedded in paraffin wax, serially sectioned (3–5 mm) and stained with hematoxylin and eosin, for the assessment of histopathological changes.

Statistical analysis

Results are expressed as the mean ± SEM. Comparison between different groups was carried out by one-way analysis of variance followed by the Tukey–Kramer post-analysis test for multiple comparisons to detect significant differences among individual mean values of all groups. The level of significance was set at $p < 0.05$

Results

Effect of curcumin, quercetin and their combination on carrageenan-induced paw inflammation in rats

Intraplantar injection of carrageenan to rats resulted in severe discernible inflammation with significant and time-dependent increase in paw thickness compared with the baseline (before carrageenan injection) over 4-h period (Figure 1). In accordance to our data, the percentage increase in paw thickness in groups received different treatment started to decrease. Curcumin, quercetin and their combination showed anti-inflammatory effects at all hours after carrageenan injection. Nevertheless, the most significant anti-inflammatory response occurred at the fourth hour. The percentage increase in paw thickness, at the 4th hour, was 69, 72, 51 and 73% for curcumin, quercetin, their combination and indomethacin, respectively. However, combination of curcumin and quercetin showed the largest effect in reducing paw thickness compared with the two individual flavonoids.

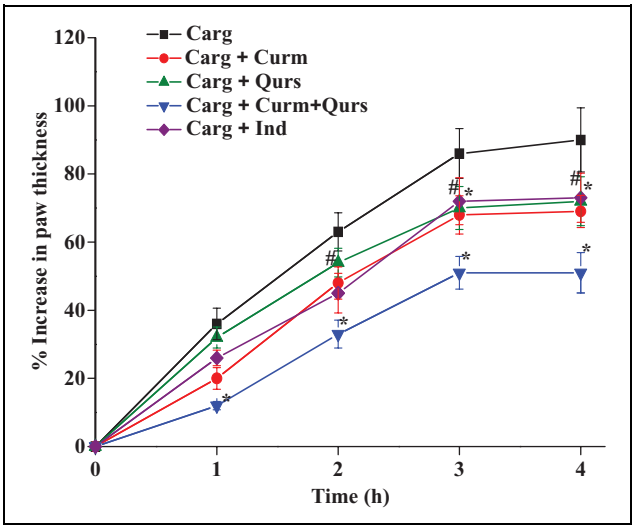

Figure 1. Effect of curcumin, quercetin, their combination and indomethacin on the carrageenan-induced paw edema formation. Rats were evaluated for paw edema at 1, 2, 3 and 4 h post-carrageenan injection. Results were expressed as percentage increase in paw thickness. Each point represents the mean ± SEM of eight rats. *Statistically significant compared with carrageenan group and #statistically significant compared with curcumin + quercetin-treated group at $p < 0.05$.

Effect of curcumin, quercetin and their combination on oxidative stress parameters

At 4 h following the intraplantar injection of carrageenan, paw tissues were analyzed for the oxidative stress parameter such as MDA, NO and GSH levels. As shown in Table 1, injection of carrageenan resulted in more than threefold increase in MDA compared with control group. Curcumin, quercetin and their combination significantly prevented carrageenan-induced elevation in MDA

Table 1. Effect of Curm, Qurs, their combination and Ind on paw lipid peroxides (MDA), NO and GSH levels in carrageenan-induced rat hind paw edema (4th hour).[a]

	MDA (nmol/g tissue)	NO (nmol/g tissue)	GSH (nmol/g tissue)
Control	27 ± 2.8	5 ± 0.42	4.5 ± 0.21
Carg	88 ± 6.5[b]	38 ± 2.21[b]	1.6 ± 0.18[b]
Carg + Curm	42 ± 3.2[c,d]	21 ± 0.71[c,d]	3.1 ± 0.41[c,d]
Carg + Qurs	56 ± 6.1[c,d]	29 ± 1.9[c,d]	2.6 ± 0.19[c,d]
Carg + Curm + Qurs	31 ± 2.8[c]	12 ± 1.23[c]	4.2 ± 0.33[c]
Carg + Ind	59 ± 11.6[c,d]	27 ± 3.1[c,d]	2.1 ± 0.17[d]

Carg: carrageenan; Curm: curcumin; GSH: reduced glutathione; Ind: indomethacin; MDA: malondialdehyde; NO: nitric oxide; Qurs: quercetin.
[a]Data represent the mean ± SEM of observations from eight rats.
[b]Statistically significant compared with control group at $p < 0.05$.
[c]Statistically significant compared with Carg group at $p < 0.05$.
[d]Statistically significant compared with Carg + Curm + Qurs-treated group at $p < 0.05$.

level by 52, 36 and 65%, respectively, compared with carrageenan-injected rats. The percentage decrease in MDA level afforded by indomethacin treatment (32%) from carrageenan group was much lower than that afforded by the treatment with combination of curcumin and quercetin (65%).

Carrageenan challenge caused about eightfold increase in paw NO production compared with control group (Table 1). Pretreatment of rats with curcumin, quercetin and their combination guarded against carrageenan-induced NO elevation by 44, 23 and 68%, respectively, compared with carrageenan-injected group. Similarly, indomethacin treatment significantly lowered NO level by 28%.

As shown in Table 1, intraplantar injection of carrageenan produced significant decrease in paw GSH content (64%) compared with control group. Pretreatment of rats with curcumin, quercetin and their combination protected against carrageenan-induced reduction in GSH level by 93, 62 and 162%, respectively, compared with carrageenan-injected group. Indomethacin did not improve (31%) the loss in GSH level compared with other treatments. Nevertheless, the protection conferred by the combination of curcumin and quercetin against carrageenan-induced biochemical changes was more effective than that conferred by either individual flavonoids or indomethacin treatments.

Effect of curcumin, quercetin and their combination on HO-1 mRNA expression

Intraplantar injection of carrageenan significantly evoked HO-1 mRNA expression compared with normal control group (Figure 2). Curcumin, quercetin and their combination significantly increased HO-1 mRNA expression compared with carrageenan-injected rats. As shown in Figure 2, combination of curcumin and quercetin showed the largest effect in upregulation of HO-1 mRNA expression (more than sixfold increase from carrageenan group) compared with the two individual flavonoids (four- and twofold increase from carrageenan group for curcumin and quercetin, respectively). On the other hand, indomethacin did not change carrageenan-evoked HO-1 mRNA expression compared with carrageenan-injected rats.

Effect of curcumin, quercetin and their combination on TNF-α level

Injection of carrageenan into the rat hind paw caused about fourfold increase in TNF-α level compared with control animals (Figure 3). Pretreatment with curcumin, quercetin and their combination reduced TNF-α release by 32, 28 and 63%, respectively, compared with carrageenan-injected rats. Indomethacin, serving as positive control caused a reduction mounting to 30% of TNF-α level in rats of group VI.

Effect of curcumin, quercetin and their combination on carrageenan-induced histopathological alterations

Microscopic examination of paw sections of rats from normal control group revealed normal histopathology (Figure 4). Meanwhile, examined sections from carrageenan injected rats showed

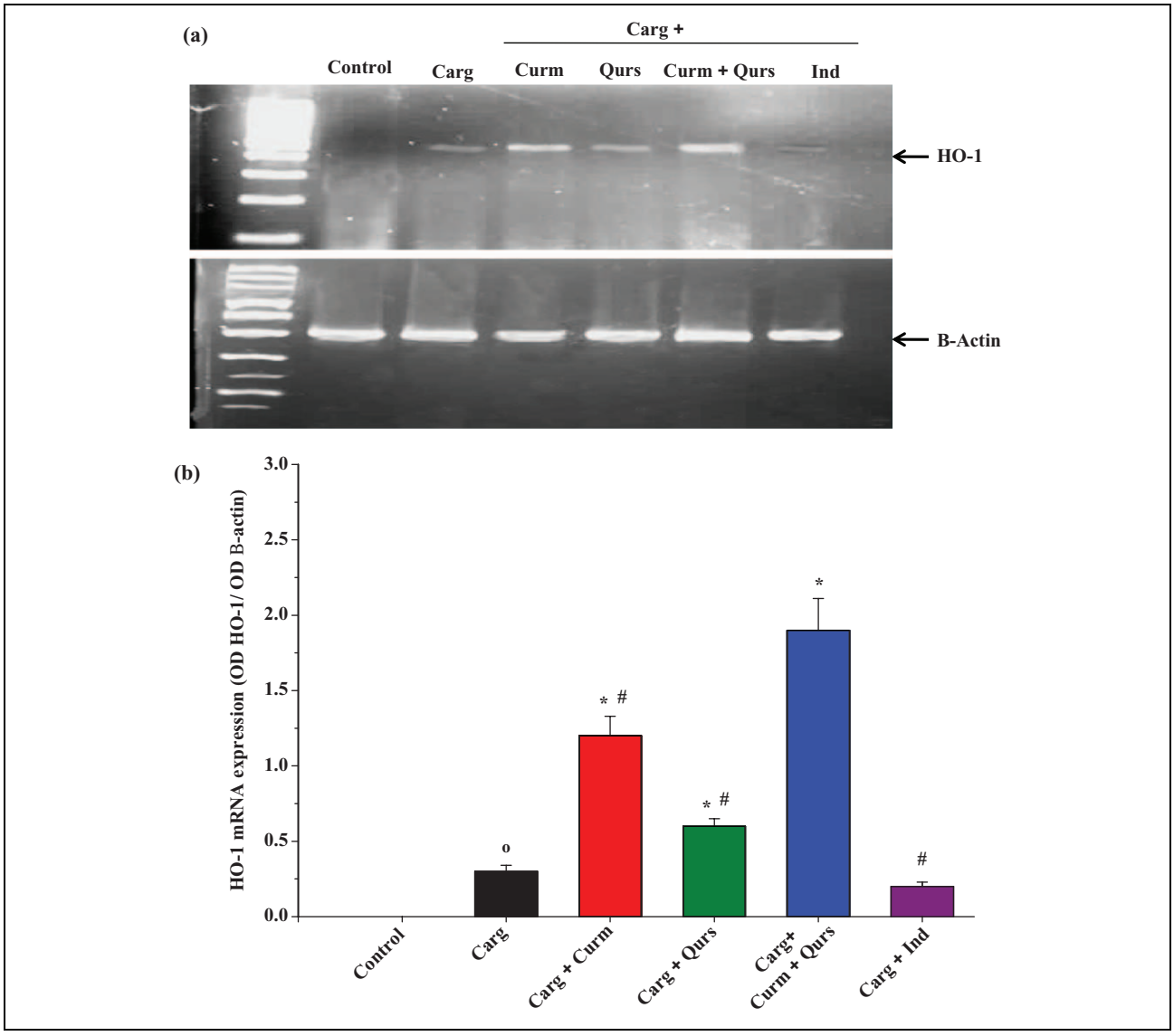

Figure 2. Effect of curcumin, quercetin, their combination and indomethacin on paw HO-1 mRNA expression (RT-PCR) in carrageenan-induced rat hind paw edema (4th hour). Data are reported as: (a) representative electrophoretic analysis of RT-PCR products; (b) a graph presents the ratio of densitometric measurements (OD) of samples to the corresponding reporter gene (B-actin). °Statistically significant compared with control group, *statistically significant compared with carrageenan group and #statistically significant compared with curcumin + quercetin-treated group at $p < 0.05$. HO-1: heme oxygenase-1; mRNA: messenger RNA; OD: optical density; RT-PCR: reverse-transcriptase polymerase chain reaction.

marked intermuscular edema with dispersed muscle bundles far away from each other and associated with massive inflammatory cells infiltration (Figure 4, Carg-1 and Carg-2). On the other hand, paw sections of curcumin, quercetin and indomethacin-treated rats showed mild to moderate inflammatory reaction with intermuscular infiltration of neutrophils. However, curcumin + quercetin-treated group showed very weak inflammatory reaction with slight intermuscular edema in some examined sections.

Discussion

Inflammation is the most common aspect of tissue pathology and is implicated in the pathogenesis of many diseases, including cancer, diabetes, cardiovascular, neurodegenerative and other life-threatening and debilitating diseases (Lawrence et al., 2002). The effects of flavonoids on a variety of inflammatory processes and immune functions have been extensively reviewed, and it has been demonstrated that they may inhibit several enzymes

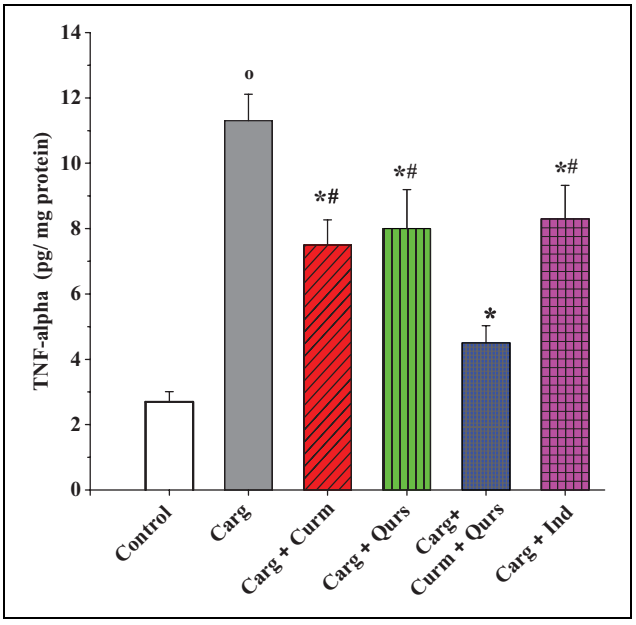

Figure 3. Effect of curcumin, quercetin, their combination and indomethacin on paw TNF-α in carrageenan-induced rat hind paw edema (4th hour). Each point represents the mean ± SEM of eight rats. °Statistically significant compared with control group, *statistically significant compared with carrageenan group and #statistically significant compared with curcumin + quercetin-treated group at $p < 0.05$. TNF-α: tumor necrosis factor-alpha.

that are activated in certain inflammatory conditions (Gonzalez et al., 2011; Katsori et al., 2011; Nair et al., 2006). In the present study, coadministration of the two flavonoids, curcumin and quercetin, have an anti-inflammatory effect on carrageenan-induced rat paw edema and this anti-inflammatory potency was compared with that of the NSAID, indomethacin. Experimental results indicate that the anti-inflammatory effect of curcumin combined with quercetin is more potent than that of indomethacin and this anti-inflammatory effect is due to suppression of oxidative stress, induction of the anti-inflammatory enzyme HO-1 mRNA expression and reduction in the proinflammatory cytokine TNF- α release.

The production of ROS at the site of inflammation is proposed to be a major cause of the cell and tissue damage associated with many inflammatory diseases. Lipid peroxidation may produce injury by compromising the integrity of membranes and by covalent binding of reactive intermediates to important biological molecules such as GSH. Moreover, the resulting free radicals may provoke inflammatory responses resulting in the release of proinflammatory cytokines, which may lead to cellular death (Gad and Khattab, 2000). In the present study, MDA and NO were elevated, while GSH level was decreased during carrageenan-induced acute inflammation in rats. The results of this study demonstrated that both curcumin and quercetin reduced paw MDA and NO formation and restored the depleted GSH contents in the paw. It has been reported that the antioxidant mechanisms of curcumin and quercetin include scavenging of free radicals, interacting with oxidative cascade, inhibiting oxidative enzymes and chelating oxidative metal ions (Motterlini et al., 2000; Zhang et al., 2011). Both Myhrstad et al. (2002) and Moskaug et al. (2005) showed that flavonoids such as quercetin increase the expression of the rate-limiting enzyme in the synthesis of GSH; c-glutamylcysteine synthetase. Increased level of paw GSH contents may be the possible reason for the reduction in MDA level observed in the curcumin- and quercetin-treated groups. Previous reports had showed that both curcumin and quercetin inhibit NO production and iNOS expression and inhibition of iNOS by both flavonoids may be one of the mechanisms responsible for their anti-inflammatory effects (Morales et al., 2006; Motterlini et al., 2000).

The aim of this work was to explore the mechanism of action, at the molecular level, of curcumin and quercetin related to their anti-inflammatory properties. In this sense, although the antioxidant properties of curcumin and quercetin could exert a beneficial effect against inflammation as previously described, it is probable that additional mechanisms are also involved. The data of the present study revealed that both flavonoids ameliorate inflammation by upregulation of HO-1 mRNA expression with simultaneous reduction in the cytokine, TNF-α, release.

HO-1 has attracted significant attention due to its apparent involvement in the modulation of the inflammatory response (Pae and Chung, 2009; Ndisang, 2010). *In vitro* studies revealed that CO modulates intracellular signal transduction systems and its biochemical effects result in the reduced production of proinflammatory cytokines such as TNF-α (Minamino et al., 2001). Previous study also revealed that bilirubin inhibits iNOS expression and NO production (Wang et al., 2004). In the current study, the induction of HO-1 mRNA expression by curcumin and quercetin was observed, which is consistent with previous studies (Bao et al., 2010; Motterlini et al., 2000). A notable finding was that synergistic induction of HO-1 expression by

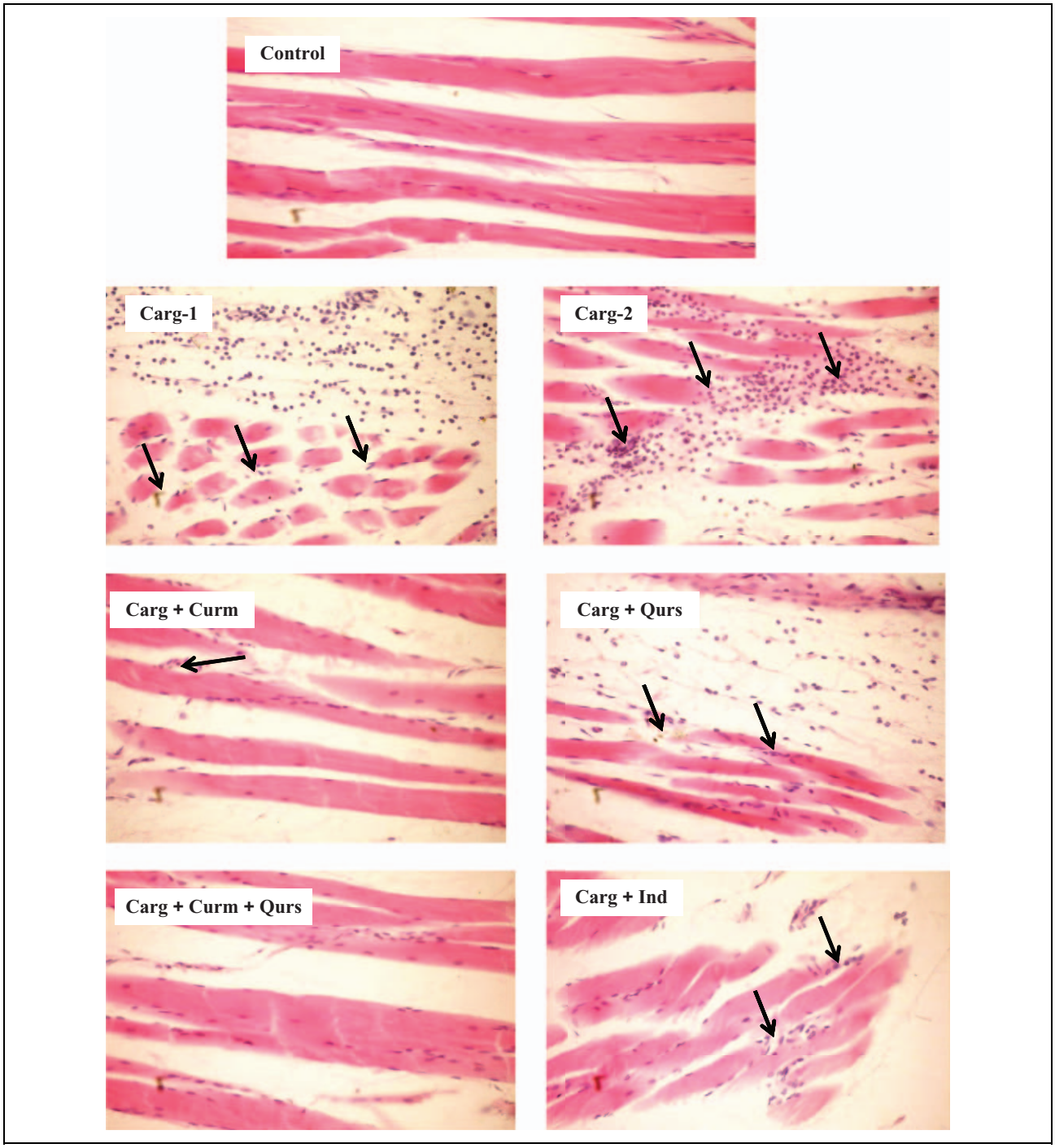

Figure 4. Light micrograph of rat paw in all studied groups. Control group showing no histopathological changes. Carrageenan-treated group showing marked (++++) inflammatory reaction associated with intermuscular infiltration with massive number of neutrophils (Carg-1) and marked intermuscular edema (Carg-2). Carg + Curm is curcumin-treated group showing mild (+) inflammatory reaction. Carg + Qurs is quercetin-treated group showing moderates (++) inflammatory reaction. Carg + Curm + Qurs is curcumin + quercetin-treated group showing very weak (±) inflammatory reaction. Carg + Ind is indomethacin-treated group showing moderate (++) inflammatory reaction (H&E, ×200). H&E: hematoxylin and eosin.

coadministration of curcumin and quercetin was observed, while administration of indomethacin produced insignificant increase in HO-1 expression compared with carrageenan group. Emerging evidence indicates that HO-1 inducers have the ability to suppress immune/inflammatory response through

several mechanisms including the suppression of macrophage infiltration and abrogation of oxidative/inflammatory transcription factors (Ndisang, 2010). In agreement with our results, it was reported that plant-derived flavonoids or polyphenols may act as nonstressful and noncytotoxic HO-1 inducers and accordingly maximize cellular intrinsic antioxidant/anti-inflammatory potential (Matsushima et al., 2009).

TNF-α is a cytokine that plays a critical role in both acute and chronic inflammation. It has been shown that TNF-α facilitates inflammatory cell infiltration by promoting the adhesion of neutrophils and lymphocytes to endothelial cells (Holtmann and Neurath, 2004). Curcumin and quercetin; in particular their combination, strongly inhibited TNF-α release. The reduction in TNF-α release by curcumin and quercetin is in agreement with the previous studies (Rushworth and Micheau, 2009; Sergent et al., 2010). Several studies have reported that the ability of some flavonoids to inhibit both cyclooxygenase and 5-lipoxygenase pathways of the arachidonate metabolism, as well as cytokine production, may contribute to their anti-infammatory properties (Nair et al., 2006; Rushworth and Micheau, 2009). Therefore, the prophylactic effect of coadministration of curcumin and quercetin is likely considered to originate from the enhancement of HO-1 mRNA expression, which in turn reduced TNF-α release. The obtained results are in agreement with that detected by Minamino et al. (2001) and El-Bassossy et al. (2009) who demonstrated that the induction of HO-1 protected against TNF-α impairment by a mechanism involving a reduction in iNOS-derived NO production.

In conclusion, we have demonstrated that pretreatment with curcumin, quercetin and their combination ameliorated carrageenan-induced acute inflammation in rats. However, combination treatment of both flavonoids exhibited a more pronounced anti-inflammatory effect compared with each flavonoid alone. The protective anti-inflammatory effect of curcumin combined with quercetin seems to result from reduction in oxidative stress, increased HO-1 mRNA expression and inhibition of TNF-α release. The current study suggests that the anti-inflammatory and the antioxidant activities of curcumin can be enhanced in a synergistic fashion by combining with quercetin. Accordingly, curcumin, quercetin and, in particular, their combination may be considered promising therapeutic strategy that could be useful in the management of various inflammatory diseases.

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Conflicts of interest

The authors declared no conflicts of interest.

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