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Protective Effects of Quercetin on Lipopolysaccharide -Induced Inflammation and Lipid Peroxidation in BALB/c Male Mice

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Abstract

In the resent years, there is a robust scientific interest in discovery of new anti-septic and anti-oxidant naturally products with no/or limited side effects.

The current study aimed to investigate the protective role of the quercetin on inflammations induced by lipopolysaccharide (LPS) in male mice

A number of criteria included i.e. liver and spleen index and IL-6 and IL1-β cytokines level in spleen homogenate were considered. Sixty male mice (8-9 week age) was divided into six groups and treated for 5 days as the following: the first group represented control, the second and third group were injected with 5, 10 mg/kg b.w doses of quercetin respectively. While the fourth and fifth groups were co-treatment with (5, 10 mg/kg b.w.) intraperitoneally (i.p) quercetin followed by LPS for 90 min, and the sixth group was injected LPS only (represented the positive control).

The results shown highly significant increase in IL-6 and IL1-β cytokine levels in LPS challenge mice along with significant MDA level increased compared to control group. Administration of quercetin showed a significant improvement by decreasing the cytokines of interest level and MDA in both quercetin only and co-treatment groups compared with LPS mice.

Thus, it appears that short-term pretreatment with quercetin was effective in preventing acute injury and suppression of inflammation induced by LPS, with suggestion of potentially therapeutic role. Further study of the effects of other herbal constituents are critical to evaluating efficacy and elucidating their mechanisms of action.

Keywords: Quercetin, Inflammation, Hepatic MDA, Lipopolysaccharide, spleen pro-inflammatory cytokines, organs index.

INTRODUCTION

Quercetin, one of the flavonoids family member, can be found in many vegetables, fruits, and beverages with a noticeable nutritional pharmacological properties, including antioxidants, anti-inflammatory effects, as well as anti-tumorigenic action,[1,2]. Literature demonstrates that quercetin can provoke protective effects in different organs injury models in tissue and molecular level, [3,4].

lipopolysaccharide (LPS), a component of the outer cell wall of most Gram-negative bacteria, attributed possibly in many critical organ failure and collapse via stimulating the expression of cytokines and adhesion molecules that contribute to the inflammatory response that accompany endotoxemia; endotoxin in many cell types, [5,6]. Lipopolysaccharide, binding to its receptor and provokes the activation of pro-inflammatory transcription factor, nuclear factor kB, [7]. Therefore, we hypothesized that quercetin could potentially protect against short-term LPS-induced oxidative stress and inflammation in vivo where BALB/ c mice used.

MATERIALS AND METHODS

Chemicals and Reagents

Quercetin (3,3',4',5,7-pentahydroxyflavone, ≥ 95%) was obtained from Sigma Aldrich Co. (St. Louis, MO, USA) and prepared in dimethyl sulfoxide (DMSO). Kits for estimation of IL-6 and IL1-β was obtained from Diaclone, and all other tissue culture chemicals and RPMI-1640 media were of analytical grade and purchased from GIBCO (Rockville, MD, USA). LPS (serotype E. Coli 0111:B4) was obtained from Fluka and prepared in pyrogen- saline.

Animals and experimental design

Swiss albino male mice (20–25 g) were obtained and housed in the animal house of biological department in college of education for pure sciences/Ibn Al-Haitham under controlled environmental conditions (12L:12D light cycles; 25C°±2 temperature). Water and food were given *ad libitum*. Animals were divided into six groups each consisting of ten animals and i.p. injected with Q for 5 days as the following: Group I (negative control) received (100 µl DMSO) Group II, received (100 µl LPS) Group III injected with of 5 mg/kg Q, Group IV injected with 10 mg/kg Q. Group V and VI received 5 and 10 mg/kg Q respectively followed by i.p injected with LPS for 90 min.

Determination of Organ Indexes and tissue preparation

At the end of experiment the mice were sacrificed by cervical dislocation, the spleens and livers were carefully dissected, washed and weighed to calculate organ indexes according to Liu *et al.*, [8]. Their then immediately minced to prepare the homogenate in ice-cold sterile phosphate buffered saline (50 mM PBS, pH 7.4), the homogenate was centrifuged at 13000 *g* for 10 min at 4 °C and the Supernatant kept at -20 for further experiments.

Organ indexes (organ weights relative to the final body weight) were calculated as the following:

Organ indexes % = weight of organ (mg)/weight of animal body (g) × 100

Determination of cytokines by ELISA

Interleukin-6 (IL-6) and IL-1β from spleen homogenate were measured by Enzyme-linked immunosorbent assay (ELISA) using available commercial kits. The assay were carry out according to the manufacturer's instructions.

Determination of lipid peroxidation

Lipid peroxidation was determined according to Guidet and Shah, [9]. Briefly; 1 ml supernatant of liver homogenise tissue mixed with 1 ml of trichloroacetic acid 17.5 % and 1ml of 0.6% thiobarbituric acid. The mixture was incubated in water bath at 100° for 15 min, after cooling 1ml of 70% TCA was add and left to stand at room temperature for 20 min. samples mixture then centrifuged at 2000 rpm for 15 min, and the absorbance was measured at 532 nm.

Statistical analysis

Statistical analysis was performed using SPSS software version 16.0. All results are presented as means ± standard error. Significance was calculated using one-way analysis of variance (ANOVA) followed by Tukey's test. $P < 0.05$ was considered statistical significant.

RESULTS

Organs weight index

The protective effect of quercetin on relative liver and spleen weight are described in Table 1. Treatment mice with quercetin only without LPS did not shown any adverse effect compared with control group in liver and spleen index. However, there was a significant elevation ($p < 0.05$) in the spleen relative weight with the LPS challenge, as well as in croups co-treated with quercetin when compare to control group. Additionally, injection with quercetin significant reduced the increased in spleen index in co-treated groups in contrast to LPS only.

Lipid peroxidation in liver

The impact effect of quercetin on malondialdehyde (MDA) level of liver tissue was shown in Figure 1. Treatment with Q significantly improved the induction of MDA level in the groups treated with Q only ($p < 0.001$) and groups treated with Q plus LPS compared to LPS challenge group. In addition, Q suppressed MDA level in the co-treated groups relative to the control group. However, there was no significant difference between Q group only and control mice.

Cytokine levels

Quercetin impact on IL-6 and IL1-β levels of spleen tissue in normal and LPS-inducing inflammation in mice are describe in Table 2, 3. The results shows that inflammation increased cytokine secretion as the level of the both interleukins were significantly elevated ($p < 0.05$) in LPS challenge group compared with control mice. In the other hand, Q treatment mice show an ameliorated anti-inflammation reaction by which a significant decreased in the levels of IL-6 and IL-1 β were observed in mice co-treated with Q plus LPS. However, no significant effect was observed in the IL-6 and IL-1 β levels in Q only injected mice compared to control as well as compared to co-treated mice.

Table 1: Organs weight index in mice injected quercetin for 5 days and treated with or without LPS. Data (mean ± SE).

Treatment Groups (No. 8)	Dose Concentration	Liver index (%)	Spleen (%)
1 Control (DMSO + PBS)	5 μg ml^{-1}	5.99 ± 0.107	0.50 ± 0.039$^{\#}$
2 LPS only	5 μg ml^{-1}	6.35 ± 0.101	0.62 ± 0.038*
3 Quercetin only	5 μg kg^{-1}	6.09 ± 0.123	0.48 ± 0.041$^{\# NS}$
4 Quercetin only	10 μg kg^{-1}	6.17 ± 0.104	0.52 ± 0.039$^{\# NS}$
5 Quercetin + LPS	5 μg kg^{-1} + 5 μg ml^{-1}	6.19 ± 0.139	0.46 ± 0.043$^{*\#NS}$
6 Quercetin + LPS	10 μg kg^{-1} + 5 μg ml^{-1}	5.57 ± 0.053	0.42 ± 0.017$^{*a\#NS}$

(*) indicate significant differences at $P < 0.05$ versus control

(#) indicate significant differences at $P < 0.05$ versus LPS

(a) significant ($P < 0.05$) between quercetin versus quercetin with LPS

(NS) not significant ($P > 0.05$) between same quercetin treatment groups with different concentration

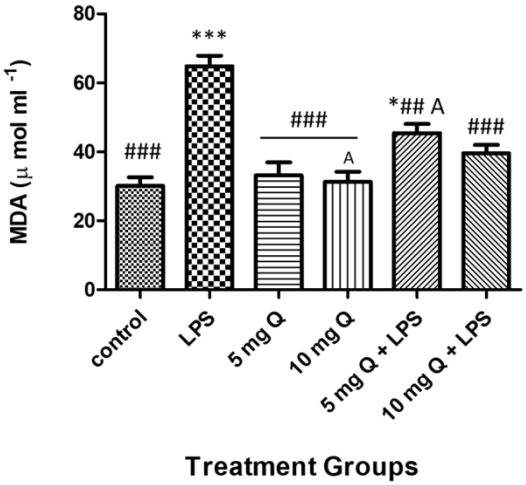

Figure 1: Treatment effect on the level of lipid peroxidation (malodialdihyed) in mice liver homogenate. Data represents the mean of (10) animals ± standard error (SE), * Significantly different from control group at ($p \leq 0.05$), ** at ($p \leq 0.001$). $^{\#\#}$ Significantly different from LPS group at ($p \leq 0.01$), $^{\#\#\#}$at ($p \leq 0.001$). ^{A} Significantly different between (10 mg) quercetin versus (5 mg quercetin) with LPS.

Table 2: IL-6 level in spleen homogenate of mice injected quercetin for 5 days and treated with or without LPS. Experiment was performed in duplicate.

Treatment Groups(No. 8)	Dose Concentration	IL-6 Level (Mean ± SE) (pg ml^{-1})
1 Control (DMSO + PBS)	5 μg ml^{-1}	71.98 ± 8.64$^{\#}$
2 LPS only	5 μg ml^{-1}	583.44 ± 10.91*
3 Quercetin only	5 μg kg^{-1}	146.15 ± 17.04*$^{\# NS}$
4 Quercetin only	10 μg kg^{-1}	182.11 ± 12.04*$^{\# NS}$
5 Quercetin + LPS	5 μg kg^{-1} + 5 μg ml^{-1}	305.06 ± 15.42*$^{\# a NS}$
6 Quercetin + LPS	10 μg kg^{-1} + 5 μg ml^{-1}	257.19 ± 13.62*$^{\#a NS}$

(*) indicate significant differences at $P < 0.05$ versus control
(#) indicate significant differences at $P < 0.05$ versus LPS
(a) significant ($P < 0.05$) between quercetin versus quercetin with LPS
(NS) not significant ($P > 0.05$) between same quercetin treatment groups with different concentration

Table 3: IL-1β level in spleen homogenate of mice injected quercetin for 5 days and treated with or without LPS. Experiment was performed in duplicate.

Treatment Groups(No. 8)	Dose Concentration	IL-1β Level (Mean ± SE) (pg ml^{-1})
1 Control (DMSO + PBS)	5 μg ml^{-1}	172.83 ± 7.58$^{\#}$
2 LPS only	5 μg ml^{-1}	1545.14 ± 11.80*
3 Quercetin only	5 μg kg^{-1}	181. 23 ± 15.93 $^{\#NS}$
4 Quercetin only	10 μg kg^{-1}	187. 46 ± 14.88 $^{\#NS}$
5 Quercetin + LPS	5 μg kg^{-1} + 5 μg ml^{-1}	1095.48 ± 16.38*$^{\# a NS}$
6 Quercetin + LPS	10 μg kg^{-1} + 5 μg ml^{-1}	1104.42 ± 8.52*$^{\#a NS}$

(*) indicate significant differences at $P < 0.05$ versus control
(#) indicate significant differences at $P < 0.05$ versus LPS
(a) significant ($P < 0.05$) between quercetin versus quercetin with LPS
(NS) not significant ($P > 0.05$) between same quercetin treatment groups with different concentration

DISCUSSION

Quercetin an active flavonoid component has been reported to its ability as free radical scavenging and in the suppression and progression of carcinogenesis along with its ability as anti-inflammatory, [10,11], antioxidant of ROS and RNS like NO, [12,13]. This anti-oxidative ability of quercetin is recognize to the presence of two antioxidant pharmacophores inside the molecule that have the best pattern for free radical scavenging, [1].

LPS has been shown to cause inflammatory reactions and injured in animal's organ. The toxic role of LPS has been referred to release mediator such as pro-inflammatory cytokines and superoxide. The other mechanism involved in LPS induced damage in organs is the oxidative stress; where its ability as anti-inflammatory explained by reducing macrophages inflammatory response that might be involved in pro-tumral developments of cell *in vitro*. However, studies to understand there mechanisms effects *in vivo* still limited, [14]. Therefore, it has been used to establish an inflammation model in the present study where BALB/c mice used. In toxicological studies organs weight are a vital indicator to compounds induced toxicological damage; therefore, organs weight changes between treated and untreated animals have been used to assessed the noxious consequence of materials. Liver as one of important organ in metabolism system and spleen as an important immune organ in which a number of lymphocytes i.e. T, B-lymphocytes, and macrophages are accrued consider as a good indicator of strength immunity. The present study results demonstrated that i.p injection of LPS caused a severe injury changes, including significant increase in organ indexes. Meanwhile, Q supplement could notably alleviate these alteration effects. Previous studies have shown that oral administration of LPS causes liver damage and elevated serum ALT, AST, and LDH enzymes levels; where the body weight and absolute and relative liver weight in rats have not negatively affected, [15,16]. The effect of quercetin on spleen weight was also determined as an indirect measure of inflammation; spleen weight has been reported to be increased following infection and inflammation. It has been reported that spleen weight decrease was associated with IL-6 decreased in male Apc Min /+ mice exposure to oxidative stress, whereas quercetin supplement can significantly decrease spleen weight in Apc Min /+ mice, [17].

Literature showed that during LPS stimulation the secretion and up-regulation of cytokines levels increased and elevation of pro-inflammatory cytokines from neutrophils in the liver were associated with liver tissue damage, [18, 19]. In the present study, LPS significantly increased the level of spleen IL-1β and IL-6. Injected mice with Q former to the LPS challenge was capable to decrease IL-1β and IL-6 levels observed in the homogenate tissue compare to control animals. Pretreatment with a series of flavonoids protected mice from two types of endotoxin lethality. Quercetin and other flavonoids pretreatment can reduced the serum cytokines levels in mice injected with LPS, [20]. The suggestions mechanisms that flavonoids improved the cytokines due to the excess of antioxidant inhibit the synthesis of TNF-α synthesis and subsequently synthesis of IL-6, [21]. Although the level of nuclear factor- κB (NF-κB) for its important role in the inflammation, it is also proposed that inhibiting the activation of NF-κB by flavonoids can be another mechanism in the reduction of

TNF-α and IL-6 hepatic levels, [22]. Moreover, LPS stimulates cascades of phosphorylation of mitogen-activated protein kinase family members, thus blocking LPS-stimulated phosphorylation has been proposed as a good target for the development of novel therapy for patients with sepsis, [23]. Quercetin has been reported as competitive inhibitors with respect to the ATP binding site on kinases, [24], thus, flavonoids are able to block the activity of both lipid and protein kinases in addition to block the activity of a variety of enzymes involved in inflammation, [25, 26]. Yang *et al*., [27] demonstrated that quercetin decreased IL-1 β-induced expression of ICAM-1 mRNA, the mechanism suggested that quercetin actively inhibited inhibitory protein of NF-kappa B activity, and the inhibitory effect of Q on ICAM-1 expression was mediated by the progressive reduction of the c-fos and c-jun mRNA expressions.

LPS-induced lipid peroxidation is an indicator of oxidative stress; literatures have reported the increased of lipid peroxidation in many tissues in rodents, [28, 29]. In oxidative stress, free radicals attack the polyunsaturated fatty acids (PUFAs) of cell membranes causing disintegration and alteration in membrane permeability, causing protein degradation and leads to cell lysis, [30]. In the present study, hepatic MDA was measured, as marker of lipid peroxidation. Elevated levels of MDA was observed in LPS treatment. Co-treatment in the LPS-challenged mice inhibited the formation of MDA in the liver. The ability of quercetin to reduce lipid peroxidation and modulate oxidative stress has been demonstrated in animals as well as in human, [31, 32]. The protective effect of Q observed in this study may be due to its ability in maintain the integrity cell membrane as well as in cytokines production.

CONCLUSIONS

In conclusion, we have demonstrated that the flavonoid Quercetin can protect mice from LPS-induced inflammation *in vivo*. Additionally, Q inhibits LPS-induced IL-6 and IL-1 β production in tissues. More studies of this bioactive compound and may be preparation of synthetic analogues might yield molecules with increased effectiveness and efficacy that could be promising therapy.

Declaration of interest

There are no conflicts of interest.

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