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Anti-inflammatory Effect of Curcumin and Licorice Against LPS-induced Inflammation

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Abstract

Many studies indicated that (Lipopolysaccharide) LPS-induced inflammation in male rats. Eighty rats were assigned into 4 groups, the first group contained 20 rats and kept as control group. The second group includes 20 rats injected with LPS. The third group administrated curcumin after LPS injection. The fourth group administra0ted licorice after LPS injection. Blood samples were collected after 3, 24, 48 hours and 1 week. The obtained results revealed significant increase in CRP, Haptoglobin, (Interleukin-6) IL-6, (Tumor necrosis factor) TNF-α, α1-globulin, α2- globulin, γ-globulin and (Nuclear factor- kappa beta) NF-KB immunostaining but showed significant decrease in total protein and albumin in LPS-group when compared with control group. In contrast, administration of curcumin and licorice improve these parameters. From the obtained results, it could be concluded that curcumin and licorice have anti-inflammatory effect against LPS-induced inflammation.

Key words: Anti-inflammatory**,** Curcumin, Licorice, LPS, Rats.

1. Introduction

Interleukin-6 and tumor necrosis factor are two-functional pro-inflammatory cytokines that are involved in the inflammation hence, the inhibition of such cytokines has currently become a major target of drug development. Curcumin is an orange-yellow compound from a spice turmeric, has been reported to have potent anti-inflammatory and anti-oxidant properties against LPS-induced inflammation [18]. Curcumin has recently been shown to be a potent immunomodulatory agent through modulates the activation of immunocytes and inflammatory factors via inactivation of NF-KB [9,6]. Licorice is an esteemed crude drug that originates from the dried roots of glycyrrhizin [10]. Licorice has been employed as a flavoring and sweetening agent as well as a demulcent and expectorant in western countries [3]. The roasted form of licorice has been reported to possess anti-oxidative and anti-inflammatory activities [16]. The aim of this study to investigate the anti-inflammatory effect of curcumin and licorice against LPS-induced inflammation in rats through measurement of pro-inflammatory cytokines, protein fractions and immunostaining for NF-KB.

2. Materials and methods

The present study was carried out on eighty male wister rats from the animal's house, Benha university for determination of the anti-inflammatory of curcumin and licorice against LPS-induced inflammation.

2.1 Experimental design

Rats were divided into four groups (each 20):

Group (1): rats are healthy and served as control.

Group (2): rats injected subcutaneously with 20 µg/kg.b.wt LPS once.

Group (3): rats treated orally with 300 mg/kg.b.wt curcumin for 7 days after and before LPS injection.

Group (4): rats treated orally with 100 mg/kg.b.wt licorice extract for 7 days after and before LPS injection.

2.2 Blood samples and tissue samples

Blood samples were collected from all study rats after 3, 24, 48 hours and 1 week by capillary tube from reto-orbital venous plexus at the medial canthus of the eye in clean dry tubes, serum was separated for analysis of CRP, haptoglobin, IL-6, TNF-α, total protein and protein fractions, were assayed according to the methods of[2,11,8,1,5,14], respectively. Also, liver tissue samples were collected for immunostaining of NF-KB and histopathological examination as previonsly described [25].

2.3 Statistical analysis

Statistical analysis was carried out with one way ANOVA test [22].

3. Results

The present data in Table (1) showed significant increase in CRP, Haptoglobin, IL-6 and TNF-α in LPS group when compared with control group. While, these parameters were decreased in curcumin and licorice group when compared with LPS group.

Table (2) LPS group showed significant decrease in total protein and albumin and increase in α1, α2 and γ-globulin. In contrast, there were improvement in these parameters in curcumin and licorice groups when compared with LPS-group.

The result of Immunohistochemical of NF-KB showed that there was a significant increase in NF-KB in the liver tissue in the hepatocytes in LPS group compared to control group: Fig (2) but in curcumin group there was a significant decreased in NF-KB expression. Fig (3) compared with LPS group: Also, Licorice group compared to LPS

group, showed significant decreased in NF-KB expression. Fig (4).

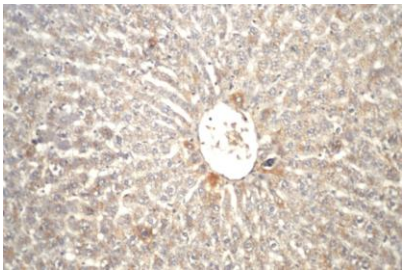

Fig (1) control group

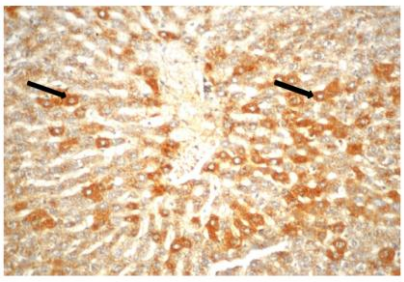

Fig (2) Liver of LPS group rats showed severe expression of NF-KB (x 40)

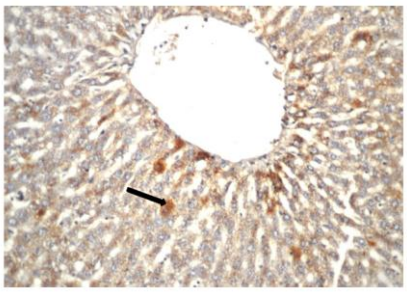

Fig (3) Liver of curcumin group rats showed Mild expression of NF-KB (x 40)

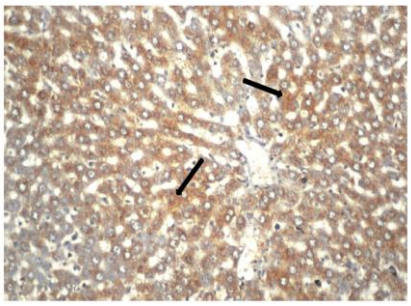

Fig (4) Liver of licorice group showed moderate expression of NF-KB (x 40)

Table (1) Changes CRP at 3, 24, 48 hours and 1 week and Haptoglobin, IL-6 and TNF-α at 24 hours in different groups of male rats

Time (hr)	Groups			
	C	LPS	LPS+Cu	LPS+Li
	CRP mg/dl			
3	6.00±0.55[a]	11.00±1.05[c]	7.40±0.51[b]	7.40±0.68[b]
24	5.60±0.51[a]	20.60±1.03[c]	11.00±1.00[b]	11.20±0.80[b]
48	5.40±0.51[a]	13.80±1.07[c]	9.20±0.58[b]	9.00±0.71[b]
1 week	6.40±0.51[a]	8.40±0.51[b]	6.40±0.51[a]	5.80±0.49[a]
Hpt (at 24 hrs g/dl)	14.03±1.76[a]	53.30±5.15[d]	21.90±1.63[b]	30.58±3.90[c]
IL-6 (at 24 hrs) ng/ml.tit	48.56±10.19[a]	487.27±23.10[c]	141.10±22.78[b]	146.30±15.31[b]
TNF-α (at 24hrs) pg/ml.tit	12.24±1.62[a]	63.69±5.93[d]	18.01±2.36[b]	25.80±3.08[c]

Results are expressed as mean ± SEM (N=5). a, b & c: Superscripts to be compared statistically within the same row. Values with different superscripts are significantly different (P<0.05).

Table (2) Changes in Total Protein at 3, 24, 48 hours and 1 week and Protien electrophoresis at 24 hours in different groups of male rats

Time (hr)	Groups			
	C	LPS	LPS+Cu	LPS+Li
Total protein g/dl				
3	5.34±0.04[c]	4.14±0.04[a]	4.83±0.10[b]	4.62±0.07[b]
24	5.29±0.11[c]	3.75±0.46[a]	4.56±0.33[b]	4.89±0.10[b]
48	5.33±0.04[c]	4.51±0.13[a]	4.90±0.08[a]	4.69±0.13[a]
1 week	5.28±0.09[a]	5.19±0.05[a]	5.23±0.04[a]	5.27±0.05[a]
Protein fractions after 24 hr				
Albumin g/dl	3.37±0.09[c]	0.90±0.06[a]	2.30±0.06[b]	2.17±0.09[b]
Alpha1 globulin g/dl	0.67±0.07[a]	0.97±0.03[b]	1.00±0.06[b]	1.03±0.03[b]
Alpha2 globulin g/dl	0.47±0.03[a]	0.83±0.03[b]	0.57±0.03[a]	0.60±0.06[a]
Beta globulin g/dl	0.47±0.03[a]	0.50±0.06[a]	0.40±0.06[a]	0.47±0.03[a]
Gamma globulin g/dl	0.33±0.03[a]	0.53±0.03[b]	0.43±0.03[a]	0.43±0.07[a]

4. Discussion

Cytokines are soluble, low molecular weight protein mediators released by one cell to bind to a specific cytokine receptor expressed by the same cell or another cell nearby. IL-6 is produced not only by activated macrophages but also by T and B cells, mast cells, vascular endothelial cells, fibroblasts, keratinocytes, and mesangial cells. It

is also produced by muscle cells during exercise. IL-6 acts on T cells, B cells, hepatocytes, and bone marrow stromal cells. It is a major stimulator of the acute-phase response. IL-6 promotes IL-2 and IL-2R production and T cell differentiation. It promotes Th2 cell differentiation (synergizes with IL-4) and the final maturation of B cells into plasma cells. IL-6 acts as a cofactor with IL-1 in IgM synthesis and with IL-5 in IgA synthesis.

The tumor necrosis Factor superfamily regulates cellular activation, viability, and proliferation through the transcription factor, NF-kB. The most important member is TNF-α, produced by macrophages, mast cells, T cells, endothelial cells, B cells, and fibroblasts. TNF-α mediates many immune and inflammatory functions and regulates the growth of many cell types. It is a potent pro-inflammatory molecule and many of its activities are shared with IL-1. TNF-α enhances the expression of adhesive molecules and triggers procoagulant activity from vascular epithelium at sites of microbial invasion. It promotes fibroblast proliferation and collagen production, a feature of importance in chronic inflammation. TNF-α activates macrophages to increase its own synthesis together with that of IL-1, IL-6, (Granulocyte-colony stimulating factor) G-CSF, and (Granulocyte macrophage- colony stimulating factor) GM-CSF. As its name implies, TNF-α can trigger killing of some tumor cells and virus-infected cells. It does so by activating caspases, the proteases that are the major mediators of apoptosis.

The term acute phase response (APR) refers to the inflammatory response of the host occurring shortly after the tissue injury. It comprises a wide variety of reactions started by different causes, like infection, tissue injury, burn, trauma, surgery, cancer or immunological disorders. These reactions aim to prevent ongoing tissue damage, isolate and eliminate the cause of the inflammation, and begin the repair process necessary to restore the normal function. Usually, the local response is accompanied by a systemic reaction characterized by the fast alteration of the concentrations of several plasmatic proteins, the APPs (Acute Phase Proteins) produced by the liver. APP whose concentrations may increase (positive APP) such as CRP and haptoglobin or decrease (negative APP) such as albumin [7].

Changes in CRP, haptoglobin, TNF-α and IL-6in this study showed significant increase in LPS group compared with control group. The results of IL-6 and TNF-α agree with [23,20].also who observed that LPS-induce endotoxemia, which activates tissue macrophages to produce IL-6 and TNF-α that stimulate a variety of cell types including endothelial cells and the production of chemotactic cytokines. After LPS injection and release inflammation, these proteins released in large amount into the circulation in response to an acute inflammation. Our results also agree with [21] who confirmed that during the acute phase of inflammation process induced by LPS, there was a characteristic increase in some plasma proteins called acute-phase reactant indicated by high levels of CRP and haptoglobin.

In contrast, curcumin group showed significant decrease in IL-6 and TNF-α when compared with LPS group. These results agree with [15] who mentioned that administration of curcumin decreased pro-inflammatory cytokine production suggesting the anti-inflammatory effect of curcumin. The results of CRP and haptoglobin indicated significant decrease, which is in agreement with [4] who stated that anti-inflammatory effect of curcumin decrease CRP and haptoglobin which considered acute-phase proteins in response for inflammation. In licorice group, there were significantly decreased IL-6, TNF-α, CRP and haptoglobin. These results agree with [17] as they found that licorice inhibited the production of pro-inflammatory cytokines. Concerning to the Immunohistochemical staining results, NF-KB showed significant increase in LPS group compared with control group. These results agree with [12]. who stated that NF-KB activation upregulated during LPS-induced inflammation as NF-KB has been shown to play an important role in regulation of the gene expression and inflammatory process. Curcumin group showed significant decrease of NF-KB when compared with LPS group. This result is in agreement with [19] who recorded a significant inhibition of NF-KB activity by curcumin, which has been shown also it preserved liver tissue integrity in early stages of hepatic damage in rats. Also, licorice group showed significant decrease of NF-KB compared with LPS group. These results agree with [24,13] as they observed that licorice directly inhibit NF-KB and reduce level of oxidative stress.

In conclusion, present results suggest anti – inflammatory effect of curcumin and licorice against LPS-induced inflammation. Meanwhile, curcumin is significantly anti-inflammatory agent than licorice .

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