The use of curcumin in obstructive jaundice

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Abstract

Introduction: The goal of this study is to evaluate the effects of curcumin on hepatic oxidative stress.
Material and methods: Thirty rats were divided into 3 groups of ten each. Cholestasis was achieved by double ligature of the common bile duct. Group I, sham-operated, group II, our control group, ligation and division of the common bile duct (BDL), group III, curcumin-treated and BDL ligated group. In group III, rats were administered curcumin at a dosage of 20 mg/kg once a day by gastrogavage, starting from 15 days pre-BDL to 7 days post-BDL, and to the control group (group II) an equal volume of corn oil was administered instead. Hepatic oxidative stress markers were evaluated by changes in the amount of lipid peroxides, measured as malondialdehyde (MDA), superoxide dismutase (SOD), nitric oxide (NO), myeloperoxidase (MPO), catalase (CAT) and reduced glutathione (GSH) in the liver tissue. Liver samples were examined under light microscope.
Results: All parameters between the sham-operated group (group I) and the other two groups (group II and III) were statistically different. The liver-tissue levels of MDA, SOD, NO, MPO, CAT and GSH were significantly different between the curcumin and BDL groups (group II) ($p < 0.05$). In group III, significant reductions in the size of enlarged hepatocytes and the oedema were demonstrated. In light microscopic examination, histopathologically cirrhotic level of the liver tissue was statistically lower than the control group ($p < 0.05$).
Conclusions: The results of the current study revealed that curcumin improved hepatic functions and reduced hepatic injury by minimizing oxidative stress, decreasing the liver lipid peroxidation, reducing glutathione and as a result recovering the scavenger enzyme activity.

Key words: curcumin, obstructive jaundice, hepatic damage.

Introduction

Obstructive jaundice (OJ) is an important clinical problem that may lead to serious sequelae, namely the growing incidence of endotoxaemia that results from a defective host immune response [1]. The crucial factors playing a role in this setting are that nitric oxide (NO) formation and the increased expression of inducible nitric oxide synthesis (iNOS) react with free oxygen radicals, which leads to the formation of the most harmful peroxynitrite anion causing lipid peroxidation, cellular damage and

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apoptosis [2]. Although the fundamentals of OJ are known in the literature, there is no specific current treatment applied.

Curcumin (diferuloyl methane; 1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is the major constituent in the rhizome of *Curcuma longa*, commonly known as turmeric [3, 4], which is a spice used extensively in curries and mustards as a colouring and flavouring agent. Earlier studies have shown that curcumin inhibits reactive oxygen species (ROS) production as well as calcium entry [3]. It can also affect other cellular processes such as the activation of apoptosis, the inhibition of platelet aggregation, the inhibition of inflammatory cytokine production and the inhibition of cyclooxygenase and lipoxygenase isoenzymes [4, 5]. Concerning the liver, a recent study has revealed that curcumin, by increasing Hsp70, produces protective effects on hepatic ischaemic reperfusion injury [6]. In the present study, we investigated the therapeutic effect of curcumin against biliary obstruction-induced hepatic fibrosis and oxidative injury in rats.

Material and methods

Animals

Thirty male Wistar-albino rats weighing 250-300 g (Istanbul University, Institute of Experimental Medicine and Research, Turkey) were used in the study. All animals were housed in wire-mesh bottomed cages in a 12-h light/dark cycle. Rats were kept in a room at constant temperature, 22 ±2°C, and were fed a standard chow diet and water. The study was approved by the ethics committee of Istanbul University, Istanbul Medical School.

Chemicals and reagents

The curcuminoid mixture purchased from Sigma (Sigma, C1386, St. Louis, MO, USA) was identified as curcumin. The authentic curcuminoids were dissolved in corn oil at a concentration of 1 mg/ml in brown glass vials and stored at 4°C. Reagents were obtained from Merck (Darmstadt, Germany).

Study groups

Rats were randomly divided into 3 groups each including 10 animals: group I, sham-operated; group II, our control group, ligation and division of the common bile duct (BDL); group III curcumin-treated and BDL ligated group. In group III, rats were administered curcumin at a dosage of 20 mg/kg once a day by gastrogavage, starting from 15 days pre-BDL to 7 days post-BDL, and to the control group (group II) an equal volume of corn oil was administered instead. Animals were killed on postoperative day 7 by high-dose ketamine injection. Blood and liver samples were taken for examination.

Operative procedure

Animals were anesthetized by intramuscular injection of 30 mg/kg ketamine hydrochloride (Ketalar; Parke-Davis, Istanbul, Turkey) and 5 mg/kg xylazine (Rompun; Bayer, Istanbul, Turkey). Midline laparotomy was performed under sterile conditions. In the sham-operated group (group I) the common bile duct (CBD) was freed from the surrounding soft tissue and was manipulated without ligation and transection. In group II and III, CBDs of the rats were identified, double ligated with 4-0 silk, and divided between the ligatures. The same surgeon performed all procedures. The abdominal incisions were closed in 2 layers with continuous 3-0 silk sutures. Animals were allowed to feed after the operation. On the seventh day, all of the rats were sacrificed. After the laparotomy, the liver was resected for histopathological examination. The pathologist was blinded as to which group an animal belonged. In addition liver tissue malondialdehyde MDA, NO, SOD, glutathione and catalase levels were detected using biochemical methods.

Biochemical analyses

Blood samples obtained from cardiac puncture were analyzed for serum bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyltransferase (GGT) and alkaline phosphatase (ALP) levels. Aspartate aminotransferase and ALT activities in serum were measured according to the Reitman-Frankel colorimetric transaminase procedure, whereas colorimetric determination of ALP activity was done according to the method of Belfield and Goldberg, using commercially available kits (BioMérieux, France) [7, 8]. Assay of serum gamma-glutamyltransferase activity was done according to Szasz based on the hydrolysis of the substrate -γ-glutamyl-3-carboxy-*p*-nitroanilide + glycyl-glycine, using a commercially available kit (Quimica Clinica Aplicada, Spain).

Evaluation of oxidative stress

Postmortem liver samples were taken and kept on an ice bath until homogenization. The tissues were homogenized in (20 wt/vol) serum physiological solution (0.9% NaCl), then centrifuged at 4,000 g for 15 min and upper clear supernatants were used in the assays. All the procedures were performed at 4°C throughout the experiments. Protein level of the clear supernatants was studied by Lowry's method. Malondialdehyde (MDA) levels (nmol/mg) and glutathione-peroxidase (Px) (mIU/mg) enzyme activities were measured in the supernatants. The levels of MDA in tissue samples were determined as an indicator of lipid

peroxidation. MDA levels were measured by thiobarbituric acid reactive substances method [9]. After the samples were pre-incubated with fish oil and xanthine–oxidase system at room temperature for 1 h, MDA level was determined. GSH-Px activity was measured by following changes in NADPH absorbance at 340 nm. Plasma levels of MDA and GSH were determined by the same method [10].

Superoxide dismutase activity

Superoxide dismutase activity was assayed according to the method of Kono *et al.*, wherein inhibition of the reduction of nitroblue tetrazolium (NBT) by the superoxide dismutase is measured at 560 nm using a Shimadzu UV/visible spectrophotometer. Briefly, the reaction was initiated by the addition of hydroxylamine hydrochloride to the reaction mixture containing NBT and post-nuclear fraction of forebrain homogenate. The results were expressed as units/mg protein, where one unit of enzyme is defined as the amount of enzyme inhibiting the rate of reaction by 50% [10].

Catalase activity

Catalase activity was assayed by the method of Luck *et al.*, wherein the breakdown of H_2O_2 is measured at 240 nm. Briefly, the assay mixture consisted of 3 ml of H_2O_2 phosphate buffer (1.25×10^{-2} H_2O_2 m) and 0.05 ml of supernatant of forebrain homogenate (10%) and the changes in absorbance were recorded at 240 nm using a Shimadzu UV/visible spectrophotometer. Enzyme activity was calculated using the millimolar extinction coefficient of H_2O_2 (0.07). The results were expressed as µmol H_2O_2 decomposed/min/mg protein [10].

Glutathione level

Glutathione levels in the liver homogenates were determined by the method described previously [10]. After precipitation with metaphosphoric acid, supernatant were reacted with 5,5'-ditiobis-2-nitrobenzoic acid (DTNB). Absorbance was read spectrophotometrically at 412 nm.

Nitric oxide level determination

Nitric oxide is deactivated very rapidly by oxidation to stable end products such as nitrite and nitrate in biological fluids. Measurements of nitrite and nitrate were made by using a procedure based on the Griess reaction [11]. Blood samples were separated to their serums and stored at –80°C until they were used for assay. Nitrates were quantitatively converted to nitrites for analyses. Enzymatic reduction of nitrate to nitrite was carried out by using coenzymes (NADPH and FAD).

N-1-(naphthyl)-ethylenediamine dihydrochloride, sulfanilamide and incubation solutions were mixed. These mixtures were incubated for 5 min at room temperature in dimmed light and measured at 550 nm. A total of 1.00 mM sodium nitrite was used as standard for determination of nitrite and 80 mM potassium nitrate was used as standard for determination of nitrate (Nitric Oxide Colorometric Assay; Boehringer Mannheim, Cat. No. 1756281, Mannheim, Germany).

Histopathological examination

The histopathological analyses were performed in the Biochemistry Department of Istanbul University School of Medicine. Excised liver specimens were fixed in 4% PBS-buffered paraformaldehyde and embedded in paraffin. Five-micrometer liver sections stained with haematoxylin and eosin were evaluated at × 200 magnification by a point counting method for severity of hepatic injury using the ordinal scale: grade 0 = minimal or no evidence of injury; grade 1 = mild injury with cytoplasm vacuolization and focal nuclear pyknosis; grade 2 = moderate to severe injury with extensive nuclear pyknosis, loss of intercellular borders, and mild to moderate neutrophil infiltration; grade 3 = severe injury with disintegration of hepatic cords, haemorrhage, and severe PMN infiltration. Statistical evaluation was made using this scale. An average of 100 adjacent points on a 1 mm^2 grid were graded for each specimen [12].

Statistical analysis

Statistical analyses were performed using Sigma Stat software version 3.0 (SPSS Inc., Chicago, USA). Results are expressed as mean ± SEM. Overall comparisons of the treatment groups were performed by using the one-way analysis of variance (ANOVA) method that applies post-hoc multiple Holm-Sidak tests, and by using the nonparametric Kruskal-Wallis (failing normality) or post-hoc multiple Dunn tests. Correlation analysis in groups between parameters were performed by Spearman rank order correlations. *p* Values < 0.05 were considered statistically significant.

Results

BDL resulted in extrahepatic cholestasis, and this was confirmed by simultaneous increase of total serum bilirubin (from 0.6 ±0.1 to 7.875 ±0.175 mg/dl, at day 0 and day 7, respectively), alkaline phosphatase, and ALT. The results are as presented by Table I. Moreover, acute biliary obstruction determined an oxidative stress status in the liver, characterized by a significant increase of malondialdehyde in the liver tissue and a significant fall in reduced glutathione obtained in the liver.

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Effects of curcumin administration

Direct bilirubin increased significantly in group II and group III compared to the sham group. On the other hand, when group II and group III were compared with each other, the level of bilirubin was lower in group III ($p < 0.05$). Malondialdehyde, NO, SOD, MPO and catalase levels in the jaundiced rats treated with curcumin were even lower than those in the control group ($p < 0.05$) (Tables I, II). As observed with reduced glutathione, the increase in the activity of scavenger enzymes after curcumin administration was higher than in the control group.

Pathological evaluation

The photographs of the specimens were then scored by a blinded observer unaware of the treatment. In the curcumin-administered group, the levels of fibrosis in the tissues of the liver had decreased (Figure 1). This fall is significantly different when compared with those of the control group ($p < 0.05$). The histological score of the organ was calculated using the semi-quantitative scale outlined in Table III.
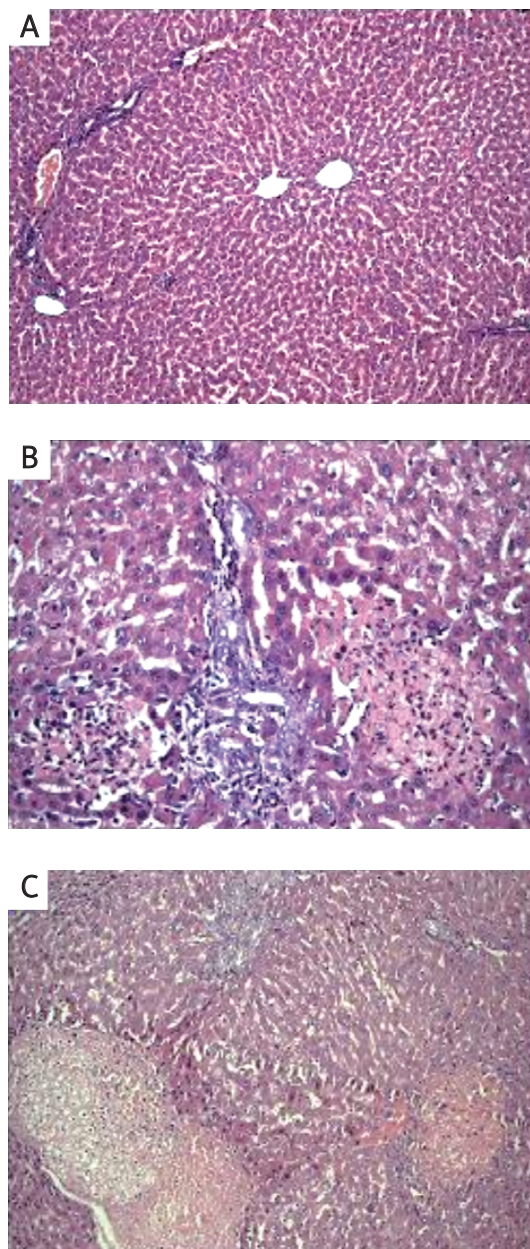
Table I. Comparison between group II (control group, bile duct ligation group) and group III (curcumin nutrition group)

Comparison	Group 2 $n = 10$	Group 3 $n = 10$	Value p
MDA	35.125	22.625	< 0.05
Glutathione	1.550	4.600	0.01
ALT	120.375	98.250	< 0.05
GPX	50.987	47.750	< 0.05
D. Bil	7.875	6.638	< 0.05
CAT	15.588	34.313	0.001
AST	1292.500	445.000	< 0.05
NO	0.400	0.320	< 0.05
ALP	1562.500	945.000	< 0.05
MPO	48.000	36.500	< 0.05
SOD	3.000	2.800	< 0.05

This table shows the difference between the curcumin-nourished group (group 3) and the control group (group 2)

Table II. Comparison between group 1 (sham) and group 2, 3. The table shows a significant difference between the sham group and the other groups 2 and 3

Comparison	Group 1 $n = 10$	Group 2 $n = 10$	Group 3 $n = 10$	Value p
MDA	13.938	35.125	22.625	< 0.001
Glutathione	11.212	1.550	4.600	< 0.001
ALT	56.625	120.375	98.250	0.037
GPX	40.475	50.987	47.750	< 0.05
D. Bil	0.660	7.875	6.638	< 0.001
CAT	47.125	15.588	34.313	< 0.001
AST	239.000	1292.500	445.000	< 0.001
NO	0.750	0.400	0.32	0.01
ALP	417.500	1562.500	945.000	< 0.05
MPO	35.500	48.000	36.500	< 0.05
SOD	2.000	3.000	2.800	< 0.05

The table shows a significant difference between the sham group and the other groups 2 and 3

Figure 1. A) Group 1 histopathological view of the normal rat liver, 100× HE. B) Group 2 Histopathological view of the bile duct ligated group, high grade cirrhotic rat liver, 200× HE. C) Histopathological view of the curcumin nutrition group (Group 3), moderate cirrhotic rat liver, 200× HE

Discussion

In obstructive jaundice models in the literature, a lot of substances have been tried for hepatic damage but in clinical practice there is no effective substance found yet that is nutritious and directly preventive. Curcumin, which we used in our study, is an effective scavenger of reactive oxygen species and reactive nitrogen species in the test tube [13, 14]. However, it is not clear whether curcumin acts directly as an antioxidant *in vivo*. Due to its limited oral bioavailability in humans, plasma and tissue curcumin concentrations are likely to be much lower than those of other fat-soluble antioxidants, such as α-tocopherol (vitamin E). However, the finding that 7 days of oral curcumin supplementation (3.6 g/day) decreased the number of oxidative DNA adducts in malignant colorectal tissue suggests that curcumin taken orally may reach sufficient concentrations in the gastro-intestinal tract to inhibit oxidative DNA damage [14, 15]. In addition to direct antioxidant activity, curcumin may function indirectly as an antioxidant by inhibiting the activity of inflammatory enzymes or by enhancing the synthesis of glutathione, an important intracellular antioxidant. Chan *et al.* [16] reported that in vivo oral treatment of curcumin reduces iNOS mRNA expression in the liver of lipopolysaccharide (LPS)-injected mice by 50-70%. It was reported that low concentrations of curcumin could inhibit NO production in activated macrophages, demonstrating that curcumin significantly reduces the levels of mRNA and 130-kDa protein of inducible NOS expressed in activated macrophages [17].

Additionally, Shen SQ *et al.* [18] reported that curcumin pre-treatment protects the liver from warm I/R injury through multiple pathways, regulates multiple bioactive molecule expression and activity, and inhibits cell apoptosis and neutrophil infiltration. Overexpression of Hsp70 and antioxidant enzymes after pre-treatment with curcumin may play an essential role in protecting the liver against warm I/R injury [19, 20]. Another study on the positive effects of curcumin in the liver has recently been conducted by Bruck *et al.* [21], who demonstrated that curcumin inhibited hepatic fibrosis in a rodent model by reducing oxidative stress and inhibiting HSC (hepatic stellate cells) activation and collagen a1(I) gene expression. *In vitro*, curcumin induces apoptosis and inhibits activation and proliferation of HSCs. In addition, it prevents formation and development of the extracellular matrix by inhibiting collagen a1(I), fibronectin and α-smooth muscle actin gene expression, by enhancing matrix metalloproteinase-2 and -9 expression and suppressing CTGF expression (Xu *et al.*, 2003; Zheng and Chen, 2006; Cheng *et al.*, 2007). Thanks to these multiple biological

Table III. Histopathological changes in liver

Animal (*n* = 10)	Group I	Group II	Group III
Animal 1	1	2	1
Animal 2	0	3	1
Animal 3	0	3	1
Animal 4	0	3	1
Animal 5	1	3	2
Animal 6	0	3	2
Animal 7	0	3	1
Animal 8	0	3	3
Animal 9	0	3	1
Animal 10	0	2	1
Mean	0.2	2.8	1.4

Grade 0 = minimal or no evidence of injury, grade 1 = mild injury with cytoplasm vacuolization and focal nuclear pyknosis, grade 2 = moderate to severe injury with extensive nuclear pyknosis, loss of intercellular borders, and mild to moderate neutrophil infiltration, grade 3 = severe injury with disintegration of hepatic cords, haemorrhage, and severe PMN infiltration

effects, curcumin may be of therapeutic benefit in several diseases. In animal models, curcumin prevents development of several cancers (Sharma et al., 2005; Shishodia *et al.*; 20,21) Curcumin also reduces risk factors or symptoms associated with cardiovascular disease, type II diabetes, Alzheimer's disease, rheumatoid arthritis, multiple sclerosis, cataract formation, infection and pulmonary disease (Shishodia et al., 2007). In phase-I clinical studies, oral administration of curcumin is generally well tolerated at pharmacological concentrations (3600–8000 mg/day for 4 months). Preliminary phase-I clinical trials in patients with cancer and various inflammatory disorders also support the use of curcumin in these diseases, although these are small studies and warrant further investigation (reviewed in Sharma *et al.*, 2005; Hsu and Cheng, 2007).

As to our current study, the first study in the literature on the effects of curcumin on OJ, BDL caused significant increases in the hepatic malondialdehyde levels, end products of lipid peroxidation, while curcumin treatment abolished the increase in malondialdehyde, probably in part by scavenging the very reactive oxygen derived from free radicals. We also used MPO as an index of the accumulation of activated neutrophils in the venous blood because MPO activity is directly proportional to the neutrophil count in the blood and the tissue injury caused by OJ. In our control group, we found that MPO activity in the liver tissue increased from 35.5 to 48 U/g on the 7th day of bile duct ligation. However, in the curcumin treated group, MPO activity had significantly reduced to 36 U/g when compared with that of the control group. Moreover, in our study, histopathological analyses showed that

curcumin as a liver protector was very useful for downregulating hepatic fibrosis, and when mean histopathological grades were compared, the histopathological grades of the curcumin treated group were significantly lower than those of the control group ($p < 0.05$).

By far the most important problem in patients with obstructive jaundice is some failure in their general condition in the preoperative period due to the problems accompanying the illness. This downfall in patients' condition may lead to life-threatening consequences. In our study, it was observed that nutrition with curcumin, reducing oxidative damage in experimental obstructive jaundice, also diminished liver damage. Another factor worth mentioning is that one of the main problems concerning curcumin treatment, which is also one of the missing elements in our study, is the present non-existence of intravenous or intramuscular forms of this drug while applying it to clinical practice. Because of this, we had to dissolve curcumin in corn oil for gastrogavage. On the other hand, in the study of Shen SQ *et al.* [6], curcumin, having been dissolved in DMSA and applied directly into the mesenteric veins, shows, as a result, positive effects on hepatic ischaemic reperfusion, which, along with our study, can be considered very encouraging in coping with obstructive jaundice since it saves time while preparing the patient for the definitive treatment.

Briefly, during the preparatory phase until the definitive treatment, adding curcumin into nutritional supplementary liquids or using it alone as an intravenous infusion in patients with jaundice may save time for the surgeon and the patient. With regard to this issue, more wide-ranging clinical studies are required.

References

1. Pastor A, Collado PS, Almar M, Gonzales-Gallego J. Antioxidant enzyme status in biliary obstructed rats: effects of N-acetylcysteine. J Hepatol 1997; 27: 363-70.
2. Nychytailo MIu, Malyk SV. Biochemical markers in diagnosis and prognosis of obturative jaundice. Klin Khir 2004; 8: 13-8.
3. Aggarwal BB, Shishodia S, Takada Y, et al. Curcumin suppresses the paclitaxelinduced nuclear factor-kappaB pathway in breast cancer cells and inhibits lung metastasis of human breast cancer in nude mice. Clin Cancer Res 2005; 11: 7490-8.
4. Hussain AR, Al-Rasheed M, Manogaran PS, et al. Curcumin induces apoptosis via inhibition of PI3'-kinase/AKT pathway in acute T cell leukemias. Apoptosis 2006; 11: 245-54.
5. Yeh CH, Chen TP, Wu YC, Lin YM, Jing Lin P. Inhibition of NFkappaB activation with curcumin attenuates plasma inflammatory cytokines surge and cardiomyocytic apoptosis following cardiac ischemia/reperfusion. J Surg Res 2005; 125: 109-16.
6. Shen SQ, Zhang Y, Xiang JJ, Xiong CL. Protective effect of curcumin against liver warm ischemia/reperfusion injury in rat model is associated with regulation of heat shock protein and antioxidant enzymes. World J Gastroenterol 2007; 13: 1953-61.
7. Moss DW, Henderson AR, Kachmar JF. Enzymes. In: Tietz NW (eds). Fundamentals of Clinical Chemistry. WB Saunders Company Philadelphia 1987; 372-3.
8. Martinek RG. A rapid ultraviolent spectrophotomeetric lactic dehydrogenase assay. Clin Chem Acta 1972; 40: 91-9.
9. Winsten S, Cehelyk B. A rapid micro diazo technique for measuring total bilirubin. Clin Chim Acta 1969; 25: 441-6.
10. Beuge JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol 1978; 52: 302-11.
11. Hillegas LM, Griswold DE, Brickson B, Albrightson-Winslow C. Assesment of myeloperoxidase activity in whole rat kidney. J Pharmacol Methods 1990; 24: 285-95.
12. Senturk H, Uzunalimoglu O, Batur Y, et al. Long-term efficacy of interferon-alpha and ursodeoxycholic acid in treatment of chronic type C hepatitis. Dig Dis Sci 1997; 42: 1438-44.
13. Biswas SK, McClure D, Jimenez LA, Megson IL, Rahman I. Curcumin induces glutathione biosynthesis and inhibits NF-kappaB activation and interleukin-8 release in alveolar epithelial cells: mechanism of free radical scavenging activity. Antioxid Redox Signaling 2005; 7: 32-41.
14. Gaddipati JP, Sundar SV, Calemine J, Seth P, Sidhu GS, Maheshwari RK. Differential regulation of cytokines and transcription factors in liver by curcumin following hemorrhage/resuscitation. Shock 2003; 19: 150-6.
15. Chan MM, Huang HI, Fenton MR, Fong D. In vivo inhibition of nitric oxide synthase gene expression by curcumin, a cancer preventive natural product with anti-inflammatory properties. Biochem Pharmacol 1998; 55: 1955-62.
16. Pan MH, Lin-Shiau SY, Lin JK. Comparative studies on the suppression of nitric oxide synthase by curcumin and its hydrogenated metabolites through down-regulation of IkappaB kinase and NFkappaB activation in macrophages. Biochem Pharmacol 2000; 60: 1665-76.
17. Ghoneim AI, Abdel-Naim AB, Khalifa AE, El-Denshary ES. Protective effects of curcumin against ischaemia/reperfusion insult in rat forebrain. Pharmacol Res 2002; 46: 273.
18. Madan B, Ghosh B. Diferuloylmethane inhibits neutrophil infiltration and improves survival of mice in high-dose endotoxin shock. Shock 2003; 19: 91-6.
19. Bruck R, Ashkenazi M, Weiss S, et al. Prevention of liver cirrhosis in rats by curcumin. Liver Int 2007; 27: 373-83.
20. Sharma RA, Gescher AJ, Steward WP. Curcumin: the story so far. Eur J Cancer 2005; 41: 1955-68.
21. Shishodia S, Chaturvedi MM, Aggarwal BB. Role of curcumin in cancer therapy. Curr Probl Cancer 2007; 31: 243-305.