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Effects of Local Curcumin on Oxidative Stress and Total Antioxidant Capacity *in vivo* Study

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Abstract: Plants have been one of the important sources of medicine even since the dawn of human civilization. Curcumin has been found to possess tremendous therapeutic potency as antiinflammatory, antioxidant and antimicrobial agent. The present study was designed to examine possible potential therapeutic and protective effect of curcumin from oxidative stress and on total antioxidant capacity in liver damage. The study was conducted using H_2O_2 as inducing agent of oxidative stress *in vivo*. Rats were randomly divided into five groups, where n = 20 for each group. Group 1 (G1) rats served as control group. Group 2 (G2) rats subjected to experimentally induced oxidative stress by the *ad libitum* supply of drinking water containing 0.5% H_2O_2(v/v) was prepared daily over entire 60 days. Group 3 (G3) rats received H_2O_2 for sixty days followed by giving 200 mg kg^{-1} of curcumin for 30 days. Group 4 (G4) was simultaneously given curcumin (200 mg kg^{-1}) for 15 days then followed by receiving H_2O_2 with curcumin for sixty days. Group 5 (G5) rats was received H_2O_2 for sixty days followed by giving 200 mg kg^{-1} of N-acetyl 1-cystine as standard drug for 30 days. Levels of marker enzymes (ALT, AST and ALP), uric acid, Total Protein (TP) and tumor necrosis factor (α-TNF) were assessed in serum for all studied groups. Malondialdehyde (MDA), 8-hydroxy-2-deoxyguinosine, Total Antioxidant Capacity (TAC), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) were assayed in liver homogenates for all studied groups.The results revealed significant increase ($p<0.05$) in levels of ALT, AST, ALP, uric acid and α-TNF while there are significant decrease ($p<0.05$) in levels of TP in G2 comparing to G1. Also there are significant differences ($p<0.05$) between G3 and G4 comparing to G2 and between G3, G4 and G5 which curcumin elicited a significant hepatoprotective activity by lowering the levels of serum marker enzymes and lipid peroxidation. The results also revealed a significant increase ($p<0.05$) in levels of MDA and 8-H-2-deoxy guinosine while there was significant reduction ($p<0.05$) in TAC, GSH, SOD and catalase in G2 comparing to G1. Also there are significant differences ($p<0.05$) between G3 and G4 comparing to G2 and between G3, G4 and G5. The conclusion could be drown from this study that the ability of curcumin as therapeutic agent and hepatoprotective against liver damage from oxidative damage and on TAC more than N-acetyl 1-cystine related to its antioxidant and free radical scavenger activity.

Key words: Curcumin, liver damage, oxidative stress, total antioxidant capacity

INTRODUCTION

Oxidative stress can cause irreversible cellular damage because intracellular defense mechanisms are depleted and therefore cannot protect cells against Reactive Oxygen Species (ROS) which are a group of highly reactive zmolecules that are produced through sequential reductions of O_2 (Rani *et al*., 2010). There is a balance between ROS generation (pro-oxidants, i.e., 8-hydroxy-2-deoxy-guinosine) and ROS degradation (antioxidants e.g., Super Oxide Dismutase (SOD) and catalase) in the cells. Both excess of ROS or antioxidants can result in an abnormal oxidative stress state (Akinrinmade and Akinrinde, 2013). Most of the chemicals such as hydrogen peroxide (H_2O_2) damage liver cells mainly by inducing lipid peroxidation and other oxidative stress in liver (Zhou *et al*., 2012). Exogenously administered hydrogen peroxide (H_2O_2) can mimic the physiological effects of oxidative stress which H_2O_2 induces both apoptosis and necrosis in a concentration-dependent manner (Choi *et al*., 2009). In the absence of a reliable liver protective drug and severe undesirable side

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effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for traditional herbal medicines that are claimed to possess hepatoprotective activity (Jain *et al*., 2007). Curcumin obtained from *Curcuma longa* or turmeric is a member of Zingiberaceae family which is a perennial herb with short and thick rhizomes (Tushar *et al*., 2010). Curcumin considered as a potent scavenger of reactive oxygen and nitrogen species (Hamid *et al*., 2011) Some of these activities are responsible for the ability of curcumin to protect DNA against free radical-induced damage and to protect hepatocytes against various toxins (Rao, 1996). Other study showed that curcumin has ability to protect lipid, hemoglobin and DNA against oxidative degradation (Cekmen *et al*., 2009). Aim of the present study is to examine possible potential therapeutic and protective effect of curcumin on oxidative stress and TAC in liver damage which may pave the way for possible therapeutic application.

MATERIALS AND METHODS

Uric acid, ALT, AST, ALP, α-TNF were determined in serum by using standard kits. Uric acid was determined by colorimetric methods according to Artiss (Fossati *et al*., 1980). The ALT and AST were determined by colorimetric methods according to Reitman and Frankel (1957) using kit from Randox Laboratories, England. The ALP was determined by kit from Bio Merieux, France according to Kind and King (1954) method. The α-TNF was determined by ELISA according to the manufactures protocol. The MDA, 8-hydroxy-2-deoxy guinosine, TAC, GSH, SOD and CAT were determined in liver tissue. MDA levels were assessed for products of lipid peroxidation. MDA which is referred to as thiobarbituric acid-reactive substance, was measured with thiobarbituric acid at 532 nm in a spectrophotometer according to the method of Buege and Aust (1978). Another oxidative stress marker, 8-hydroxy-2-deoxy-guinosine was measured by Enzyme-Linked Immuno Sorbet Assay (ELISA) method by the using kit according to the manufactures protocol. Total Antioxidant Capacity (TAC) was measured using Randox Total Antioxidant Capacity (cat No. Nx 2331) according to the method of Miller *et al*. (1993). The GSH was determined by the specterophotometric method. The which was based on the use of Ellman's reagent (Ellman, 1959). The SOD activity was measured by determining the reduction of nitroblue tetrazolium by superoxide anion produced with xanthine and xanthine oxidase (Sun *et al*., 1988). Catalase activity was determined according to the method of Aebi (1982), by monitoring the initial rate of the disappearance of hydrogen peroxide at 240 nm in a specterophotometer. Crude curcumin was purchased from local wet market in Baghdad, Iraq. The rhizomes were powdered and macerated in 95% ethanol for 48 h using Soxhlet apparatus. The extract was filtered by Whatman filter paper and dryness by a rotary evaporator. Male Wistar rats (180-220 g) were used in this study. The animals were housed in plastic cages fed on standard diet and given tap water *ad libitum*. The temperature and humidity controlled facility with a constant 12 h light/dark cycle.

Study design: The animals were randomly divided into five groups, each consisting of twenty animals. Group 1 (G1) rats served as control and received regular diet without any treatment. Group 2 (G2) rats subjected to experimentally induced oxidative stress by the *ad libitum* supply of drinking water containing 0.5% H_2O_2 (v/v) was prepared daily over entire 60 days. The choice of the dose of H_2O_2 was based on the results of previous studies (Al-Shammry and Al-Okaily, 2009). Group 3 (G3) rats received H_2O_2 for sixty days followed by giving (200 mg kg^{-1}) of curcumin for 30 days. The dose of curcumin also based on previous study (Chuang *et al*., 2000). Group 4 (G4) rats was simultaneously given curcumin (200 mg kg^{-1}) for 15 days then followed by receiving H_2O_2 with curcumin for sixty days. Group 5 (G5) rats received H_2O_2 for sixty days followed by giving (200 mg kg^{-1}) of N-acetyl 1-cystine for 30 days as standard drug. Blood samples were collected from all rats then centrifuged at 3000 g for 20 min. Serum samples were analyzed for determination of uric acid, ALT, AST, ALP, α-TNF. Animals were killed by cervical dislocation and then livers were rapidly removed. A part of each liver was weighed and rinsed with 0.15 M Tris-HCl (PH-7.4). A 10% w/v of liver homogenate was prepared in 0.15 M Tris-Hcl buffer and processed for the estimation of MDA, 8-hydroxy-2-deoxy guinosine and TAC. From part of the homogenate, after precipitating proteins with 20% trichloro-acetic acid containing 1 mM EDTA, the supernatant was used for estimation of glutathione. The rest of the homogenate was centrifuged at 2000 rpm for 10 min at 4°C. The cell free supernatant which obtained was used for the estimation of SOD and catalase activity.

Statistical analysis: The data was expressed as Mean±SD. The comparison between patients group and control group were analyzed by using student t-test. The p-value of <0.001 was considered to be significant.

RESULTS

The results in Table 1 shows the levels of ALT, AST, ALP, uric acid, TP and α-TNF in serum of all studied groups (G1, G2, G3, G4 and G5) of rats. The results revealed significant increase ($p<0.001$) in levels of ALT, AST, ALP, uric acid and α-TNF while there are significant

Table 1: Levels of ALT, AST, ALP, uric acid, TP and α-TNF in sera of all studied groups (G1, G2, G3, G4 and G5) of rats

Groups (n = 20)	ALT (U L^{-1})	AST (U L^{-1})	ALP (U L^{-1})	Uric acid (μmol L^{-1})	TP (mg dL^{-1})	α-TNF (g mL^{-1})
G1	34.36±0.2	38.20±0.1	33±0.64	270±12.67	6.24±0.34	64.70±0.1
G2	94.02±0.5	97.00±0.15	110±0.51	500±19.4	4.62±0.24	84.02±0.12
G3	46.00±0.7	47.00±0.1	51±0.79	299±14.1	6.02±0.79	69.20±0.82
G4	35.00±0.4	40.00±1.23	37±0.40	285±24.2	6.19±1.43	65.86±0.2
G5	54.87±0.92	48.22±0.8	57±0.91	300±14.7	5.86±0.88	74.80±0.89

Table 2: Levels of MDA, 8-h-2-deoxy guinosine, TAC, GSH, SOD and CAT in liver tissue of all studied groups (G1, G2, G3, G4 and G5) of rats

Groups (n = 20)	MDA nmol/g wet tissue	8-h-2-deoxy guinosine (ng mL^{-1})	TAC (mmol L^{-1})	GSH (μg mg^{-1})	SOD (U/mg protein)	CAT (U/mg protein)
G1	370.00±0.49	0.06±0.009	3.26±0.11	6.34±0.11	102.50±0.46	370±0.49
G2	92.19±2.02	0.59±0.12	0.79±0.023	0.91±0.20	57.09±0.25	233±0.84
G3	37.83±1.27	0.14±0.02	3.31±0.09	4.61±0.29	89.00±0.54	344±0.15
G4	31.84±1.45	0.082±0.01	2.10±0.081	5.44±0.13	97.00±0.63	365±0.58
G5	54.99±4.99	0.35±0.64	1.01±0.98	3.99±0.93	77.90±0.69	299±0.87

decrease ($p<0.001$) in levels of TP in G2 comparing to G1. Also there are significant differences ($p<0.001$) between G3 and G4 comparing to G2 and significant differences between G5 ($p<0.001$) comparing to G3 and G4. The levels of AST, ALT, ALP, uric acid and α-TNF were elevated after H_2O_2 administration in G2 comparing to control group while TP level was lowered in response to H_2O_2 administration in G2 comparing to control group. The levels of AST, ALT, ALP, uric acid and α-TNF were significant lowered after administration of curcumin at a dose of 200 mg kg^{-1}. The results indicate stabilization of plasma membrane as well as repair of hepatic tissue damage caused by curcumin.

Table 2 shows the levels of MDA, 8-h-2-deoxy guinosine, TAC, GSH, SOD and catalase in liver tissue for all studied groups (G1, G2, G3, G4 and G5) of rats. There was significant increase ($p<0.001$) in levels of MDA and 8-H-2-deoxy guinosine while there was significant reduction ($p<0.001$) in TAC, GSH, SOD and catalase in G2 comparing to G1. Also there were significant differences ($p<0.001$) between G3 and G4 comparing to G2 and significant differences between G5 ($p<0.001$) comparing to G3 and G4.

DISCUSSION

The elevated levels of serum marker enzymes are indicative of cellular leakage and loss of functional integrity of cellular membrane and liver. The ALP activities on the other hand are related to functioning of hepatocytes, its increase in serum is due to increased synthesis in the presence of increased biliary pressure (Aniya *et al*., 2005). The increased level of uric acid in G2 is probably due to that uric acid acts as extracellular antioxidants because of its ability to remove singlet oxygen, hydroxyl and peroxyl radical (Vrca *et al*., 2004). Uric acid exerts antioxidant activity as a chain breaking agent by scavenging oxygen radicals once they are produced and by forming a stable complex with iron ions as a preventive antioxidant so that uric acid founded to be a particularly strong serum antioxidant (Ogbera and Azenabor, 2010). The results from the present study suggests that altered uric acid level to nearly normal in curcumin treated animals could be due to strong antioxidant property of the extract.

Tumor Necrosis Factor (α-TNF) was markedly increased after H_2O_2 administration in the current study which agreement with previous study of Farghaly and Hussein (2010) who revealed that the hepatocytes treated with toxic agent release factors activate cytokines like α-TNF. Lipid peroxidation has been postulated to be the destructive process in liver injury due to H_2O_2 administration and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals (Min *et al*., 2005). Treatment with curcumin significantly reduced the levels of lipid peroxidation markers which may be due to its potential antioxidant activity.

A significant decrease in TAC and GSH were found in G2 comparing to G1. It has been reported that different antioxidants can be generated depending on which ROS are produced Total antioxidant capacity can, therefore, vary according to which antioxidant quantified (Al-Aubaidy and Jelinek, 2011). The synergistic effect of antioxidants in human known to provide greater protection against free radical aggression than any single antioxidant alone (Savu *et al*., 2012). However, TAC takes into account all antioxidant and the synergistic effects between them (Valkonen and Kunsi, 1997). Glutathione (GSH) is one of the most abundantly naturally occurring tri peptide, non-enzymatic biological antioxidant present in liver (Ramani *et al*., 2011). Its functions are concerned with the removal of free radicals such as H_2O_2 and superoxide radicals, maintenance of membrane protein, detoxification of foreign chemicals and biotransformation of drugs (Yanadaiah *et al*., 2011). The decreased levels of TAC and GSH have been associated with an enhanced level of lipid peroxidation in G2 of rats. Pretreatment and

Post treatment with curcumin significantly increased the level of TAC and GSH in a dose dependent manner. Thus curcumin may act by inducing the detoxifying enymes and these enzymes may detoxify the Reactive Oxygen Species (ROS) following ministration of toxicants. Serum activities of SOD and CAT are the most intracellular sensitive enzymatic index in liver injury caused by ROS and oxidativestress (Loki and Rajamohan, 2003). Therefore, reduction in the activities of these enzymes may indicate the toxic effects of ROS produced by H_2O_2. In the present study, it was observed that pretreatment with curcumin caused a significant rise in hepatic SOD and CAT activitieswhichsuggests that curcumin can reduce ROS that may lessen the oxidative damage to the hepatocytes and improve the activities of the liver antioxidant enzymes thus protecting the liver from oxidative stress.

CONCLUSION

The conclusion could be drown from the present study indicates that curcumin is safe, nontoxic and effectiveness herbal in inhibiting oxidative stress and increasing TAC. The study thus provided more insight into the mechanism of the hepatoprotective action of curcumin extract and also provides a scientific basis for its usage in the traditional systems of medicine, for the management of hepatotoxicity, since, curcumin exhibit more potency than N-acetyl 1-cystine in hepatoprotective and prevention of liver damage. Also the results showed that pretreatment with curcumin (G4) exhibit more antioxidant ability in liver damage than treated groups (G3, G5) with curcumin and N-acetyl 1-cystine, respectively.

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