Antioxidant, Hepatoprotective and Antiatherogenic Effects of Curcumin on High Fat Diet Induced Dyslipidemia in Rats

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Abstract: Present study is designed to investigate the atheroprotective potential of curcumin in rat models of diet induced dyslipidemia. Twenty four wistar rats of same age and sex were selected for the study and divided into four experimental groups with six animals per group. Group I was control untreated rats, group II rats received high fat diet for eight weeks, group III received 0.2 g/kg of body weight/day curcumin together with fat diet and group IV had only curcumin administration for up to four weeks. Results showed that curcumin treatment with or without diet administration significantly reduce plasma lipid levels, ALT, AST, ALP and glucose concentrations. High fat diet administration significantly suppresses levels of catalase, SOD and glutathione in these tissues. Whereas MDA levels were significantly raised. Curcumin treatment improve these tissue antioxidant enzyme status together with decreased MDA levels. These results suggests that daily dietary intake of curcumin in recommended doses may be helpful in restoring lipid disorders and maintaining body’s antioxidant status in patients of cardiovascular diseases.

Key words: Curcumin, atherosclerosis, high fat diet, dyslipidemia, rats

INTRODUCTION

Since last few years, lipid-inquisitive researchers are focusing on investigating the efficacy of use of functional foods and nutraceuticals (FFN) as a new genre of therapies to treat dyslipidemia (Marinangeli and Jones, 2010). FFN are natural conventional or purified food substances that improve physiological processes or provide protection from chronic pathologies. Curcumin, [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadien-3,5-dione] (Garcea et al., 2004), a yellow colored pigment and a natural phenol is the active component of Turmeric (Curcuma longa L.) which is a herbaceous, rhizomatous and perennial flowery plant belong to family Zingiberaceae (Chattopadhyay et al., 2004). Turmeric has long been known to possess medicinal properties and in folk and ayurvedic medicines its use as an antiseptic, antibacterial, antiviral, antipyretic and anaesthetic agent is common (Chaturvedi, 2009). Curcumin is thought to be the principle pharmacological agent as it has strong antioxidant potential and can directly interact with various cellular signaling molecules as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, C-reactive protein (CRP), glutathione (GSH), prostaglandin E2, endothelin-1, transforming growth factor (TGF)-β etc (Gupta et al., 2013). Considering curcumin as a highly pleiotropic molecule, research studies are now evaluating its role against complex multi-factorial, oxidative stress based human diseases as cancer, inflammation, obesity, diabetes mellitus and cardiac diseases (Mohanty et al., 2004; Kamat et al., 2007; Baum and Ng, 2004; Yeh et al., 2005).

Cancer is a highly oxidative state of genetic abnormalities and cell signaling alterations that must be controlled with multi targeted drugs. Various in vitro and in vivo studies have proved anticancer potential of curcumin and its extracts. A study of Garcea et al. (2005) reported that a daily dose of 3.6 g of curcumin in colorectal patients was effective in reducing MDA levels (marker of DNA adduct formation) in rectal tissues. Another study of Durgaprasad et al. (2005) showed significant reduction of red blood cells malondialdehyde (MDA) and glutathione (GSH) levels in patients of tropical pancreatitis when administered with 500 mg of curcumin together with piperine for six weeks. Curcumin administration in patients of prostate cancer also found to reduce serum levels of prostate specific antigens (Ide et al., 2010). Curcumin is a potent suppressor of nuclear factor (NF)-κB activation and therefore can inhibit inflammation. This anti-inflammatory activity of curcumin is by inhibition of IκB kinase and AKT pathways (Aoki et al., 2007). Curcumin also regulate (decrease) the genetic expression of several inflammatory mediators as IL-1, IL-6, IL-8, CRP, TNF and MIP-1α (Skommer et al., 2007). Cardioprotective role of curcumin have been proved in various studies. Single oral dose of curcumin was found to be effective in protecting rat myocardium from ischemic insult caused by isoprenaline induced myocardial tissues (Manikandam et al., 2004). Curcumin in these rats decrease myocardial tissue levels of lipid peroxides and superoxide anions and increase superoxide dismutase (SOD), GSH-peroxidase and...
catalase (CAT). Curcumin also improve cardiac function by decreasing blood levels of circulating lipids (cholesterol, triglyceride, LDL) and by preventing oxidative modification of LDL-C particles (Quiles et al., 1998). Curcumin can attenuates fatty streak development (Quiles et al., 2002) and atheroma formation in aortic walls through its platelet derived growth factor (PDGF) stimulated vascular smooth muscle cell (VSMC) migration and proliferation inhibition (Yang et al., 2006). Diabetes mellitus is the second most important promoter of cardiovascular complications and curcumin have been reported to increase blood insulin concentration (Murugan and Pari, 2006), pancreatic β cells antioxidant status and activation of PPAR-gamma ligand binding activity (Nishiyama et al., 2005) in various human and animal studies. Curcumin also inhibit streptozotocin (STZ) induced pancreatic islet damage through its islet cell free radical scavenging abilities (Meghana et al., 2007). In conjunction with these previous studies, present study is designed to investigate the atheroprotective efficacy of curcumin consumption in rat model of diet induced hyperlipidemia together with the assessment of its hepatoprotective, hypoglycemic and antioxidant effects.

MATERIALS AND METHODS
Animals: Female, wistar albino rats (n = 24) of same age and body weights (180-250 g) were purchased from International Center for Chemical and Biological Sciences (ICCBS), Karachi, Pakistan for the study. Animals were initially acclimatized for about one week and housed as two animals per cage (49x40 cm polypropylene cages with stainless steel top grill). Room temperature was maintained between 22-27°C with 50-60% humidified air currents and 12 h light dark cycles. Animals were provided standard laboratory diet and water ad libitum throughout the period of study. Special care was taken while selecting the animal for the study. Animals that appeared as hunched or scruffy, having skin infections, fight wounds and pregnancy were excluded.

Experimental protocol: After acclimatization, animals were randomly divided into four experimental groups.

Group I: Control-received normal laboratory diet. n = 6
Group II: Hyperlipidemic-received 12 g of high fat diet (HFD) (Khalifa et al., 2008) (Composition: Casein 30%, raw beef fat 40%, wheat flour 7%, bran 4%, glucose, 10%, salt 6%, vitamin mixture (vitamin A 5000 IU/g, vitamin D3 100 IU/g, Bi: 1 mg/g, B6: 1.25 mg/g, B12: 0.5 mg/g, B12: 5 µg/g, vitamin C 15 mg/g, vitamin E 4 mg/g and K2: 0.75 mg/g) in addition to methionine 25 mg/g and lysine 20 mg/g) for eight weeks. n = 6

Group III: Hyperlipidemic+curcumin treated-received high fat diet with 0.2 g/kg of body weight curcumin orally per day for about four weeks (Kapoor et al., 2008). n = 6
Group IV: Curcumin treated-animals received above mentioned dose of curcumin together with normal lab diet for four weeks. n = 6

Animals from all experimental groups were weighed and their daily food intake with normal activity was regularly monitored.

Sample collection: Heparinized blood samples were collected after 12 h of fast from all the animals by cardiac puncture. Rats were initially anesthetized with chloroform, then dissected to open the chest cavity so that heart can be visualized. A 5CC syringe was inserted in the right ventricle and about 4-5 mL of blood was collected through this procedure. A portion of blood sample was centrifuged in heparin coated glass tubes at 3000 g for 5 min to collect plasma. For serum, blood samples were allowed to stand undisturbed for about 1-2 h in clean glass tubes. When blood cells were clot and settled down at the bottom of tubes, samples were centrifuged at 3000 g for 5 min. Both plasma and serum samples were stored at -80°C till analysis.

Histological preparations: Organs (Liver, heart and aorta) were excised from the dissected animals, removed from adhering fats and tissues, washed with saline, dried over the filter paper and weighed. Tissues were stored at -80°C till homogenization.

Preparation of homogenates
Liver: Small pieces of tissue were sliced and weighed up to 1 gm. Tissue pieces were then mixed with 5 mL of cold KCl (1.17%) solution and homogenized. Homogenates were centrifuged initially at 800 g for 5 min (4°C) and the obtained supernatant again centrifuged at 10,500 g for 20 min (4°C) to have post mitochondrial supernatant (PMS) for biochemical analysis (Noori et al., 2009).

Heart: Four gram of tissue was homogenized with 16 mL of ice cold potassium phosphate buffer (pH-7, containing Na2/EDTA-1mM/L) using Ultra Turrax T-25 Polytron tissue homogenizer. This was then mixed with a(10 mL) solution of butylated hydroxy toluene (500 mM/L) prepared in acetonitrile to prevent formation of new peroxides during the assay. This mixture was then centrifuged at 200 g for 4 min (4°C), supernatant was removed and stored at -80°C till analysis (Noori et al., 2009).

Aorta: Aortic tissues (1 gm) were homogenized with 10 mL of KH2PO4 buffer (100 mM; pH-7, containing
Na<sub>2</sub>EDTA-1 mM/L). Centrifugation was done at 20000 g for 30 min (4°C) and separated supernatant was used for biochemical estimations.

**Biochemical analysis:** Serum total cholesterol (TC) concentration was determined by CHOD-PAP enzymatic end point method using enzymatic kit specified the method of Flegg (1973), triglyceride (TG) levels were determined with glycerol phosphate oxidase (GPO) method (Fossati and Prencipe, 1982) using enzymatic kit. Serum HDL-C levels were determined colorimetrically by phosphotungstate magnesium chloride method using commercial enzymatic kit with method of Seigler and Wu (1981). LDL-C levels were calculated using Friedwald's formula (Friedewald et al., 1972). VLDL-C concentration was calculated with formula TG/5 (Bairaktari et al., 2005). Atherogenic Index of Plasma (AIP) was determined using formula Log (TG/HDL) (Umeshchandra et al., 2012). Plasma glucose concentration was determined with glucose oxidase method using enzymatic kit (Global, UK). Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined with International Federation of Clinical Chemistry (IFCC) method using enzymatic kit by the method of Bergmeyer and Horder (1980). Serum alkaline phosphatase concentration was also determined with IFCC recommended kinetic method by Tietz et al. (1983).

**Estimation of tissue catalase levels:** Catalase levels in tissue homogenates were estimated by method of Sinha et al. (1972). The reagents were mixed as 1.96 mL of 0.01 M potassium phosphate buffer (pH-7), 1 mL of 0.2 M hydrogen peroxide solution with 0.04 mL of tissue homogenate making final volume of 3 mL. 2 mL of dichromate acetic acid reagent (50 mL of 5% dichromic acid mixed with 150 mL of glacial acetic acid) was added in 1 mL of above mixture. Preparation was then boiled for 10 min, cooled with tap water and read against reagent blank at Schimadzu-UV-spectrophotometer at 570 nm wavelength. Catalase concentration (mM/g of tissue) in tissue homogenates was then estimated from calibration curve obtained by running a series of standard solutions (0.05-0.6 mM).

**Estimation of tissue superoxide dismutase (SOD) levels:** Tissue SOD levels were estimated by the method of Kono (1978). Specifically, the reaction mixture contains 1.3 mL of EDTA- Na<sub>2</sub>CO<sub>3</sub> solution (0.1 mM EDTA mixed with 50 mM Na<sub>2</sub>CO<sub>3</sub>; pH-10), 0.5 mL of 90 μM nitroblue tetrazolium (NBT) solution; 0.1 mL of Triton X-100 EDTA solution (0.66% Triton X-100 prepared in EDTA-Na<sub>2</sub>CO<sub>3</sub> solution of pH-10) and 0.1 mL of 20 mM hydroxylamine hydrochloride solution (pH-6). Rate of NBT reduction was recorded per minute at Schimadzu UV spectrophotometer with wave length of 560 nm first for reaction mixture and then after adding 0.1 mL of tissue homogenate. Percent inhibition in rate of NBT reduction was recorded as U/g of tissue.

**Estimation of tissue glutathione (GSH) levels:** GSH levels in tissue homogenates were determined by the method of Carlberg and Mannervik (1985). 0.1 mL of tissue homogenate was mixed with 0.3 mL of 10% bovine serum albumin, 1.5 mL of 50 mM potassium phosphate buffer (pH-7.6), 0.35 mL of 0.8 mM β-NADPH and 0.1 mL of 30 mM oxidized glutathione. Absorbance of the mixture was then recorded on Kinetic spectrophotometer PRM 500 for 5 min at 25°C controlled temperature.

**Estimation of tissue malondialdehyde (MDA) levels:** Tissue malondialdehyde contents in form of thiobarbituric acid reacting substances was assessed by the method of Ohkawa et al. (1979). Reaction mixture was prepared by adding 0.2 mL of 8.1% sodium dodecyl sulphate, 1.5 mL of 20% acetic acid (pH = 3.5) and 1.5 mL of 0.8% solution of thiobarbituric acid to 0.2 mL of tissue homogenate. The mixture volume was brought up to 4 mL with distilled water, heat at 95°C for 60 min, then mixed with 5 mL (15:1 v/v) solution of n-butanol and pyridine. After centrifugation, organic layer of the mixture was separated and its absorbance was measured at 532 nm on Schimadzu UV 120 spectrophotometer.

**Statistical analysis:** Results were presented as mean±SEM. Statistically significant differences between experimental groups were calculated by Student’s t-test with p-values<0.05 considered as significant.

**RESULTS**

Results for the four experimental groups i.e., control (CONT), hyperlipidemic group (HYPL), Curcumin treated (CURT) and hyperlipidemic+curcumintreated (HYPL+CURT) groups of the present study were as under.

**Effects on body weights of rats:** Table 1 represent effects of HFD on body weights of rats in hyperlipidemic group, curcumin treated and curcumin with HFD supplemented group. Significant increase in body weights was observed in hyperlipidemic rats with dietary fat supplementation for eight weeks as compared to the control group (Table 1). Curcumin treatment (p<0.05) decrease body weights in HYPL+CURT rats as compared to HYPL group but these body weights were still significantly (p<0.05) higher than body weights of rats from CONT group (Table 1).

**Effects on plasma TC and TG levels:** Plasma TC and TG levels were significantly (p<0.05) increased after HFD administration for eight weeks in experimental animals
compared to CONT group (Table 2). Curcumin treatment together with HFD administration decreases (p<0.05) these values compared to HYPL group and this reduction was upto the base line values of CONT group (p<0.05). Curcumin administration in animals of CURT maintain baseline values of these two parameters (p<0.05) compared to HYPL and HYPL+CURT group (Table 2).

**Effects on plasma lipoprotein levels:** Plasma LDL-C and VLDL-C levels were significantly increased (p<0.05) in animals of HYPL group after eight weeks compared to CONT group (Table 2). Curcumin administration in these animals for four weeks significantly (p<0.05) decrease these values compared to HYPL group, but this decrease was not significant compared to CONT group. CURT group showed significant (p<0.05) regulation of base line values of LDL-C and VLDL-C compared to HYPL group (Table 2). However this regulation was not significant as compared to CONT group.

Plasma HDL-C levels were significantly decreased (p<0.005) with HFD administration in animals of HYPL group compared with CONT group (Table 2). Curcumin treatment in these animals significantly increase these values (p<0.05). In animals of CURT group plasma HDL-C levels were close to the control group and are significantly (p<0.05) higher than HYPL group (Table 2).

**Effects on atherogenic index of plasma (AIP):** Atherogenic index of plasma was significantly increased (p<0.01) in rats fed with HFD as compared to the CONT group (Table 2). Curcumin consumption in these animals decrease (p<0.05) these values compared to HYPL and CONT group. Animals from CURT group had AIP levels similar to the animals of CONT group (Table 2).

**Effects on liver enzymes:** HFD treatment in rats of HYPL group significantly raises (p<0.005) ALT, AST and ALP levels as compared to CONT group (Table 3). Curcumin treatment reduce (p<0.005) serum levels of these enzymes in HYPL+CURT (p<0.005) and CURT groups (p<0.005) as compared to HYPL group. This decrease in HYPL+CURT (p<0.005) group was significant as compared to CONT group but was non significant in CURT group (Table 3).

**Effects on blood glucose concentration:** Significant increase (p<0.005) was observed in blood glucose concentration in HYPL group compared to CONT group (Table 4). Curcumin supplementation decrease blood glucose levels in rats of both HYPL+CURT and CURT group (p<0.005) as compared to HYPL group (Table 5).

**Liver tissue catalase levels:** Catalase enzyme levels in liver tissues decrease significantly (p<0.01) in HYPL group compared to CONT group (Table 5). Curcumin consumption significantly prevent (p<0.01) this decrease in catalase levels in HYPL+CURT group. In animals of CURT group curcumin administration maintain base line values of liver catalase compared to HYPL and CONT group.

**Liver SOD levels:** Hyperlipidemic treatment non significantly decrease liver SOD levels in rats compared to CONT group (Table 6). In HYPL+CURT (p<0.005) and CURT groups liver SOD levels were increased compared to HYPL group. However this increase was not significant as compared to CONT group (Table 6).

**Liver MDA levels:** Liver MDA levels were significantly increased (p<0.005) in animals of HYPL group compared to CONT group after HFD administration for eight weeks (Table 7). Curcumin supplementation
animals of HYPL+CURT group significantly reduce these values. This reduction was not up to the base line values but significantly higher (p<0.05) than values of CONT group. Animals of CURT group showed liver MDA levels higher than CONT group but significantly (p<0.005) less than the HYPL+CURT group (Table 7).

Liver GSH levels: Liver tissue GSH levels were non significantly decreased with HFD diet administration in rats of HYPL group (Table 8). Curcumin supplementation in HYPL+CURT rats significantly (p<0.005) raised this value with in four weeks of treatment but this increase was non significant compared to the CONT group. Curcumin administration also increase (p<0.05) liver GSH levels in animal of CURT group compared to HYPL group (Table 8).

Heart tissue catalase levels: Significantly decreased (p<0.005) heart tissue catalase levels were observed in HYPL rats compared to CONT group (Table 5). These values were increased with curcumin in animals of HYPL+CURT group, however CURT group showed non significant change in tissue catalase levels as compared to HYPL but significant as compared to CONT group (Table 5).

Heart tissue SOD levels: SOD levels were decreased nonsignificant in heart tissue homogenates of HYPL animals compared to CONT group (Table 6). Curcumin treatment in HYPL+CURT and CURT groups (p<0.05) increase SOD levels which was non significant compared to HYPL and CONT group (Table 6).

Heart tissue MDA levels: Heart tissue MDA levels were significantly (p<0.05) increased with HFD diet administration for eight weeks in rats of HYPL group (Table 7). Curcumin supplementation for four weeks reduce this increase in rats of HYPL+CURT group compared to HYPL group and also maintain tissue MDA levels in animals of CURT group compared to CONT and HYPL (p<0.05) group (Table 7).

Heart tissue GSH levels: GSH levels in heart tissues of rats were significantly decreased (p<0.05) compared to CONT group (Table 8). With curcumin treatment in these animals tissue GSH levels were increased (p<0.05) compared to HYPL group but this increase was non significant. In animals of CURT group, GSH levels were maintained close to the GSH levels of CONT group animals (Table 8).

Aortic tissue catalase levels: Catalase levels in aortic tissue homogenates from HYPL rats were low (p<0.01) as compared to CONT group (Table 5). In HYPL+CURT and CURT groups, aortic catalase levels were non significantly increased with curcumin supplementation as compared to HYPL group (Table 5). However, compared to CONT group the values were significantly (p<0.05) different.

Aortic tissue SOD levels: Decreased SOD levels were observed in aortic tissue homogenates of rats from HYPL group (Table 6). This decrease was not significant as compared to CONT group. SOD levels were increased in HYPL+CURT and CURT groups (p<0.01)
with curcumin consumption compared to HYPL group. But this was a non significant (p>0.05) change as compared to CONT group (Table 6).

**Aortic tissue MDA levels**: Aortic MDA levels were significantly (p<0.05) high in tissue homogenates from rats of HYPL group compared to CONT group (Table 7). Curcumin administration in rats of HYPL+CURT group and CURT group control this increase compared to HYPL and CONT groups (p<0.05) (Table 7).

**Aortic tissue GSH levels**: Animals of HYPL group had decreased (p<0.01) aortic GSH contents compared to CONT group (Table 8). Curcumin treatment in HYPL+CURT and CURT groups increase GSH levels significantly compared to CONT (p<0.05) and HYPL groups.

**DISCUSSION**

In present study, 0.2 g/kg of body weight/day curcumin administration in hyperlipidemic animals for four weeks significantly decrease plasma TC, TG, LDL-C and VLDL-C levels (Table 2) showing suppression of HFD induced dyslipidemia in these animals together with significant decrease in their body weights. Increased blood LDL and (oxidized) Ox-LDL levels have been proved in various research studies as the core pathologic phenomenon of atherosclerosis (Kita et al., 2001; Tomkin and Owens, 2012) and it was estimated that for every 25 mg/dL reduction in blood LDL levels there could be 11% decrease in the CVD linked mortality rate (Delahoy et al., 2009). Curcumin supplementation in our study significantly regulates plasma levels of LDL with only four weeks of treatment in HFD fed rats. These liporegulatory effects of curcumin are also reported in various other studies (Quiles et al., 2002; Ramirez-Tortosa et al., 1999). This hypocholesterolemic effect of dietary curcumin may be attributed to its stimulatory effect on hepatic cholesterol 7α-hydroxylase enzyme, an enzyme that regulate cholesterol catabolism (Babu and Srinivasan, 1997). Curcumin also reported to modulate (decrease) HMG CoA reductase enzyme activity to decrease serum and liver cholesterol, triglycerides and free fatty acid levels (Murugan and Pari, 2006). Plasma HDL-C levels were significantly increased in animals of HYPL+CURT and CURT group in our study (Table 2) and this proves that curcumin not only regulate hyperlipidemia through decreased blood cholesterol and triglyceride levels but it also enhance the levels of lipid removing cholesterol i.e. HDL-C in blood as is reported by Jang et al. (2008) with increased apo A1 and paraoxonase enzyme activity (Jang et al., 2008).

Hepatoprotection is that desired property which is needed in many treatment regimens available for coronary artery disease (CAD). “Statins” are very well known hypolipidemic agents that are used to treat dyslipidemia in patients of CAD world wide and one of the most common side effect reported with them is the development of hepatotoxicity (Calderon et al., 2010). Our study also checked hepatoprotective potential of designed dose of curcumin and curcumin administration significantly regulate serum ALT, AST...
and ALP levels in animals of HYPL+CURT and CURT groups (Table 3). Similar results were reported by Weisberg et al. (2008) with decreased levels of hepatic markers of inflammation, hepatic NF-κB activity and increased hepatic enzyme levels after curcumin administration in high fat diet induced obese and leptin deficient ob/ob male C57BL/6J mice. Hyperglycemia is a disorder that not only affect renal and vascular physiology but functioning of other organs of the body as well. High fat diet fed rats from HYPL in our study develop significant hyperglycemia compared to the CONT group (Table 4) which was decreased with curcumin supplementation in HYPL+CURT and CURT groups (Table 4). Hyperglycemia relevant to hyperlipidemia usually develops as a consequence of insulin resistance. Curcumin has significant hypoglycemic activity reported in studies of Murugan and Pari (2005) and Weisberg et al. (2008). Decreased adipose tissue adiponectin production, macrophage infiltration of body's fat tissues, hyperlipidemia and increased blood insulin levels with antioxidant protection of pancreatic β cells are the mechanisms that lie behind hypoglycemic effects of curcumin. Increased systemic oxidative burden together with hyperlipidemia promote vascular wall lipid deposition and fatty streak formation. Therefore researchers are interested in discovering a multipotential therapeutic agent that not only control dyslipidemia but also can manage increased oxidative stress in physiological systems. In our study we also checked the efficacy of the designed dose of curcumin in controlling systemic oxidative stress in three important organs of the body i.e., heart, aorta and liver. Curcumin administration in animals of HYPL+CURT and CURT group increase the levels of tissue CAT, SOD and GSH and decrease MDA levels (Table 5-8). This effect of curcumin may be attributed to the inhibition of ROS generating cellular activities with inhibition of NADPH oxidase, lipoxygenase/cyclooxygenase, xanthine dehydrogenase and nitric oxide synthase enzyme activities (Deby-Dupont et al., 2005; Lin et al., 2007) thereby enhancing the bioavailability of cellular antioxidant enzymes.

Conclusion: Present study concludes that daily dietary consumption of curcumin in recommended doses is effective in reducing various CVD risk factors. However, further research studies are needed to explore the exact molecular mechanism of curcumin in fat diet induced dyslipidemic models.

REFERENCES


