Ameliorative Effect of Curcumin on Hepatic Oxidative Stress, Antioxidant Status, Cardiac Markers Enzymes and Inflammation in High Cholesterol Diet-Induced Hypercholesterolemia in Rats

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ABSTRACT

Hypercholesterolemia and hyperlipidemia has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases. The therapeutic effect of curcumin (CUR) administration on enzymatic antioxidant status, hepatic oxidative stress, cardiac marker enzymes and inflammation in high cholesterol diet-induced hypercholesterolemia in rats were evaluated. Sixty male rats were divided into four equal groups. Group I: (Control group) rats fed on normal diet. Group II: Rats fed hypercholesterolemic diet (HCD) [4% cholesterol (w/w) and 1% cholic acid]. Group III: rats fed HCD + curcumin (200 mg/kg b.wt./day, orally). Group IV: rats fed normal diet + curcumin (200 mg/kg b.wt. /day, orally). Blood and liver tissue samples were collected at 2, 4 and 6 weeks from the onset of treatment with curcumin. The obtained results showed marked increase in TNF-α, IL-6 concentrations, LDH, CK-MB, ALT, AST and GGT activities in addition to NO and L-arginine in liver tissue of hypercholesterolemic rats fed with high cholesterol diet. However, a significant decrease in liver tissues antioxidant enzymes (CAT, SOD and GPx) activities were observed in hypercholesterolemia induced in rats. Treatment with curcumin to hypercholesterolemic rats lowered serum TNF-α, IL-6, liver and cardiac marker enzymes, NO and L-MDA and ameliorate antioxidant enzymatic status in liver tissue. These results suggest that, treatment with curcumin has a powerful modulating effect on hypercholesterolemia induced inflammation and hepatic oxidative stress with enhanced the antioxidant defense system in liver tissues. Also, curcumin may be effective in controlling cholesterolemic status and has the potential in reducing cardiovascular complications due to hypercholesterolemia.

Keywords: Curcumin, Hypercholesterolemia, Antioxidant status, pro-inflammatory cytokines, oxidative stress, Cardiac marker enzymes.

1. INTRODUCTION

Hypercholesterolemia is a condition characterized by very high levels of cholesterol in the blood. Recently, hypercholesterolemia has been associated with enhanced oxidative stress related to increased lipid peroxidation. Increased generation of oxidized LDL is a major factor in the vascular damage associated with high cholesterol levels. Hence, the inhibition of oxidative stress under hypercholesterolemic conditions is considered to be an important therapeutic approach and efforts have been made to identify the antioxidative functions of various medicinal plants [1].

Hypercholesterolemia is one of the most important risk factors for atherosclerosis and subsequent cardiovascular disease [2]. Hypercholesterolemia and Hyperlipidemia has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases. In developing countries, the incidence of cardiovascular disease is increasing alarmingly especially, India is on the verge of a cardiovascular epidemic [3]. Feeding animals with cholesterol has often been used to elevate serum or tissue cholesterol levels to study the etiology of...
hypercholesterolemia-related metabolic disturbances [4]. Exogenous hypercholesterolemia causes fat deposition in the liver and depletion of the hepatocyte population, it can also cause malfunctioning of the liver, which apparently follows micro vesicular stenosis due to the intracellular accumulation of lipids [5]. In addition, feeding cholesterol-rich diets induces free radical production (ROS), followed by oxidative stress and hypercholesterolemia [6].

Oxidative stress results from an imbalance between the production of free radicals and their removal by the antioxidant defense system [7]. Excessive amounts of oxygen free radicals cause lipid peroxidation and the production of malondialdehyde (MDA), which lead to liver damage [8]. Recently, hypercholesterolemia has been associated with enhanced oxidative stress related to increased lipid peroxidation. Increased generation of oxidized LDL is a major factor in the vascular damage associated with high cholesterol levels. Hence, the inhibition of oxidative stress under hypercholesterolemic conditions is considered to be an important therapeutic approach and efforts have been made to identify the antioxidative functions of various medicinal plants [1].

Curcumin is the most active component of turmeric, which turmeric contains chemical constituents known as the curcuminoids, which composed of curcumin (curcumin I), de-methoxy-curcumin (curcumin II) and bis-demethoxycurcumin (curcumin III). Commercial curcumin contains curcumin I (~77%), curcumin II (~17%) and curcumin III (~3%) as its major components [10]. Also, Curcumin exhibits antioxidant, anti-inflammatory and anti-tumor properties [11]. Moreover, curcumin regulates the expression of genes involved in energy metabolism and lipid accumulation, decreasing the level of intracellular lipids [12]. Accordingly, the purpose of the present study was to investigate the effect of curcumin against high cholesterol diet induced hypercholesterolemia in rats. Also, to determine whether curcumin when administered to hypercholesterolemic induced-rats beneficial for prevention and treatment of hypercholesterolemia complications.

2. MATERIALS AND METHODS

2.1 Experimental animals

Sixty male albino rats, 12-16 weeks old and average body weight 180-220 g were used in the experimental investigation of this study. Rats were obtained from Laboratory Animals Research Center, Faculty of Veterinary Medicine, Moshtohor, Benha University. Animals were housed in separate metal cages, fresh and clean drinking water was supplied ad-libitum. Rats were kept at constant environmental and nutritional conditions throughout the period of experiment. The animals were left 14 days for acclimatization before the beginning of the experiment.

2.2 Curcumin

Curcumin (purity ~99%) was manufactured by Fluka Co. for chemicals and purchased from El-Goumhoria Co. for Trading Chemicals Medicines and Medical Appliances, Egypt. Curcumin was freshly prepared by dissolved in 7% DMSO solution then complete to 100 ml distilled water, and was administered every day orally at a dose of (200 mg/kg b.wt.) [13].

2.3 Induction of Hypercholesterolemia

Hypercholesterolemia was induced in rat by feeding high cholesterol diet [4% cholestrol (w/w) and 1% cholic acid (w/w)] for 8-weeks [14].

2.4 Experimental design

Rats were randomly divided into four main equal groups, 15 rats each, placed in individual cages and classified as follows:-

Group 1: Control Normal group: Rats fed an ordinary diet only without any treatment during the entire experimental period of 8-weeks.

Group 2: High cholesterol diet (HCD) group: Rats fed hypercholesterolemic diet (HCD) [4% cholestrol (w/w) and 1% cholic acid] and received no drug all over the periods of the experiment.

Group 3: High cholesterol diet (HCD) + curcumin treated group: Rats fed HCD and administered with curcumin (200 mg/kg, body weight/day, orally).

Group 4: Normal curcumin group: Rats fed normal diet and administered with curcumin (200 mg/kg, body weight/day, orally).

2.5 Sampling

Random blood sample and liver tissue specimens were collected from all animals groups (control and experimental groups) three times along the duration of experiment after 2 weeks, 4 weeks and 6 weeks from the onset of treatment with curcumin.

2.6 Blood samples

Blood samples for serum separation were collected by ocular vein puncture at the end of each experimental period in dry, clean, and screw capped tubes and serum were separated by centrifugation at 2500 r.p.m for 15 minutes. The clear serum was separated by automatic pipette and received in dry sterile samples tube, processed directly for determination of cardiac and hepatic marker enzymes (Alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH), CPK-MB, Gamma glutamyl transferase (GGT) activities), then kept in a deep freeze at -20°C until used for subsequent biochemical determination of Tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) concentrations.

2.7 Tissue samples (liver)

After blood samples collection five rats of each group were sacrificed by cervical decapitation. The livers specimens were quickly removed, rinsed in ice-cold 0.9% sodium chloride solution, quick frozen in a deep freeze at -20°C for subsequent biochemical analyses. All liver samples were analyzed for the determination of L-
malondialdehyde (L-MDA), Nitric oxide (NO), antioxidant enzymes (Catalase, superoxide dismutase, Glutathione peroxidase), and reduced Glutathione (GSH).

2.8 Preparation of liver tissues
For liver tissue, an appropriate portion, a 10% (w/v) of liver sample was homogenized in ice-cold Tris buffer, pH 7.4. The homogenates were centrifugated at 6000 rpm for 30 min, and the supernatants (cytosols) were used for measuring the activities of superoxide dismutase, catalase and glutathione peroxidase. Another part of homogenate, perfuse tissue with a PBS (phosphate buffered saline) solution, PH 7.4 Containing 0.16 mg /ml heparin to remove any red blood cells and clot. Homogenize the tissue in 5-10 ml cold buffer (i.e., 50 mM potassium phosphate, PH 7.5 0.1mM EDTA) per gram tissue, using tissue homogenizer, then centrifuge at 4000 rpm for 15 minutes at 4°C, the resulting supernatants were assayed for concentrations of reduced glutathione (GSH). Also, a 10% (w/v) homogenate of liver was prepared in ice-cold normal saline using a chilled glass-telon porter-Elvehjem tissue grinder tube, and then centrifuged at 3000 rpm for 15 min. The supernatant was used for estimation of liver L-malondialdehyde concentration (L-MDA).

2.9 Biochemical analysis
Serum ALT and AST, GGT, LDH, CPK-MB, TNF-α and IL-6 levels and liver tissue L-MDA, NO, GSH, CAT, SOD, Gpx activities were analyzed according to the methods described by (Murray, 1984) [15], (Beletti and Gella, 1990) [16], (Dito, 1979) [17], Rat Creatine Kinase MB Isoenzyme (CKMB) ELISA (Kamiya Biomedical Company, Cat. No. KT-12247), Beyaert and Fiers, (1998) [18], Chan and Perlstein, (1987) [19], (Mesbah et al., 2004) [20], Montgomery and Dymock, (1961) [21], Moron et al., (1979) [22], Luck, (1974) [23], Kakkar et al., (1984) [24], and Gross et al., (1967) [25], respectively.

2.10 Statistical analysis
The obtained data were statistically analyzed by one-way analysis of variance (ANOVA) followed by the Duncan multiple test. All analyses were performed using the statistical package for social science (SPSS, 13.0 software, 2009). Values of P<0.05 were considered to be significant.

Table 1: Effect of curcumin administration on serum TNF-α and IL-6 concentrations in normal and high cholesterol diet induced hypercholesterolemia in male rats.

<table>
<thead>
<tr>
<th>Exp.Groups</th>
<th>TNF-α (pg/ml)</th>
<th></th>
<th>IL-6 (pg/ml)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2Weeks</td>
<td>4 Weeks</td>
<td>6 Weeks</td>
<td>2Weeks</td>
</tr>
<tr>
<td>Normal control</td>
<td>16.46±3.44a</td>
<td>14.49±2.61a</td>
<td>13.97±1.43a</td>
<td>14.97±1.58b</td>
</tr>
<tr>
<td>High cholesterol diet</td>
<td>23.18±1.30a</td>
<td>29.97±4.96a</td>
<td>31.95±6.78b</td>
<td>17.18±1.44a</td>
</tr>
<tr>
<td>Curcumin Treated (200 mg/kg b. wt/day)</td>
<td>22.36±0.96a</td>
<td>24.88±2.85ab</td>
<td>23.23±1.18b</td>
<td>13.02±0.80a</td>
</tr>
<tr>
<td>Curcumin Normal (200 mg/kg b. wt/day)</td>
<td>22.85±3.77a</td>
<td>16.10±1.25bc</td>
<td>22.87±3.80ab</td>
<td>12.80±0.39a</td>
</tr>
</tbody>
</table>

Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at (P<0.05).

Table 2: Effect of curcumin administration on serum AST, ALT and GGT activities in normal and high cholesterol diet induced hypercholesterolemia in male rats.

<table>
<thead>
<tr>
<th>Exp.Groups</th>
<th>ALT (U/L)</th>
<th></th>
<th>AST (U/L)</th>
<th></th>
<th>GGT (U/L)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2Weeks</td>
<td>4 Weeks</td>
<td>6 Weeks</td>
<td>2Weeks</td>
<td>4 Weeks</td>
<td>6 Weeks</td>
</tr>
<tr>
<td>Normal control</td>
<td>71.24</td>
<td>90.47</td>
<td>65.54</td>
<td>120.63</td>
<td>165.59</td>
<td>143.78</td>
</tr>
<tr>
<td>High cholesterol diet</td>
<td>71.24</td>
<td>90.47</td>
<td>65.54</td>
<td>120.63</td>
<td>165.59</td>
<td>143.78</td>
</tr>
<tr>
<td>Curcumin Treated (200 mg/kg b. wt/day)</td>
<td>62.96</td>
<td>59.21</td>
<td>69.34</td>
<td>131.17</td>
<td>126.27</td>
<td>146.19</td>
</tr>
<tr>
<td>Curcumin Normal (200 mg/kg b. wt/day)</td>
<td>71.24</td>
<td>90.47</td>
<td>65.54</td>
<td>120.63</td>
<td>165.59</td>
<td>143.78</td>
</tr>
</tbody>
</table>

Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at (P<0.05).

3. RESULTS AND DISCUSSION
3.1 Effect of curcumin on serum pro-inflammatory cytokines in hypercholesterolemia rats
The obtained results in table (1) revealed that, a non-significant increase in serum TNF-α and IL-6 concentrations were observed in cholesterol fed rats after two weeks of the experiment. These increases become significant after four and six weeks as compared with rats fed normal control diet. Curcumin treatment in rats fed high cholesterol diet resulted in a non-significant decrease in serum TNF-α all over the period of the experiments. Also, a non-significant decrease in serum IL-6 level was observed after two weeks followed by a significant decrease after four and six weeks as compared to untreated high cholesterol-fed rats.

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3.2 Effect of curcumin on serum liver markers enzymes in hypercholesterolemic rats

The obtained results presented in table (2) showed a non-significant increase in serum ALT activity in cholesterol fed rats after two and four weeks of the experiment. These increases become significant after six weeks. Also, a significant increase in serum AST activity was observed in cholesterol fed rats all over the periods of the experiment. Moreover, a significant increase in serum GGT activity was observed in cholesterol fed rats after two and six weeks of the experiment. This increase become non-significant after four weeks when compared with rats fed normal control diet. Curcumin treatment in rats fed high cholesterol diet resulted in a non-significant decrease in serum ALT activity after two weeks followed by a significant decrease after four and six weeks. Also, a significant decrease in serum AST and GGT activities were observed in curcumin treated hypercholesterolemic rats all over the periods of the experiments as compared to untreated cholesterol -fed rats.

Table 3: Effect of curcumin administration on serum LDH and CK-MB activities in normal and high cholesterol diet induced hypercholesterolemia in male rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>LDH (nmol/ml)</th>
<th>CK-MB (nmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp.Groups</td>
<td>2 Weeks</td>
<td>4 Weeks</td>
</tr>
<tr>
<td>Normal control</td>
<td>333.59±126.85c</td>
<td>381.44±87.46b</td>
</tr>
<tr>
<td>High cholesterol diet</td>
<td>1120.47±92.39a</td>
<td>1096.99±113.23a</td>
</tr>
<tr>
<td>Curcumin Treated (200 mg/kg b.wt/day)</td>
<td>622.27±137.55b</td>
<td>790.07±261.11b</td>
</tr>
<tr>
<td>Curcumin Normal (200 mg/kg b.wt/day)</td>
<td>298.66±20.89c</td>
<td>463.61±108.56b</td>
</tr>
</tbody>
</table>

Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at (P≤0.05).

Table 4: Effect of curcumin administration on liver tissue NO, L-MDA and GSH concentrations in normal and high cholesterol diet induced hypercholesterolemia in male rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NO (ng/g tissue)</th>
<th>L-MDA (nmol/g tissue)</th>
<th>GSH (ng/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp.Groups</td>
<td>2 Weeks</td>
<td>4 Weeks</td>
<td>6 Weeks</td>
</tr>
<tr>
<td>Normal control</td>
<td>110.68</td>
<td>90.84</td>
<td>104.10</td>
</tr>
<tr>
<td>High cholesterol diet</td>
<td>±3.94 b</td>
<td>±7.78 b,c</td>
<td>±6.56 b-c</td>
</tr>
<tr>
<td>Curcumin Treated (200 mg/kg b.wt/day)</td>
<td>±5.68 a</td>
<td>±5.69 a</td>
<td>±1.64 a</td>
</tr>
<tr>
<td>Curcumin Normal (200 mg/kg b.wt/day)</td>
<td>45.30</td>
<td>35.20</td>
<td>89.44</td>
</tr>
</tbody>
</table>

Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at (P≤0.05).

Table 5: Effect of curcumin administration on liver tissue CAT, SOD and GPX activities in normal and high cholesterol diet induced hypercholesterolemia in male rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CAT(U/g tissue)</th>
<th>SOD (U/g tissue)</th>
<th>GPx (ng/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp.Groups</td>
<td>2 Weeks</td>
<td>4 Weeks</td>
<td>6 Weeks</td>
</tr>
<tr>
<td>Normal control</td>
<td>90.79</td>
<td>69.49</td>
<td>59.01</td>
</tr>
<tr>
<td>High cholesterol diet</td>
<td>±5.87 a</td>
<td>±4.28 b,c</td>
<td>±4.93 b,c</td>
</tr>
<tr>
<td>Curcumin Treated (200 mg/kg b.wt/day)</td>
<td>±5.49 a,b</td>
<td>±2.73 bc</td>
<td>±6.02 a</td>
</tr>
<tr>
<td>Curcumin Normal (200 mg/kg b.wt/day)</td>
<td>60.27</td>
<td>61.71</td>
<td>57.98</td>
</tr>
</tbody>
</table>

Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at (P≤0.05).

3.3 Effect of curcumin on serum cardiac markers enzymes in hypercholesterolemic rats

The obtained data in table (3) revealed that, a significant increase in serum LDH and CK-MB activities were observed in cholesterol fed rats all over the periods of the experiment when compared with rats fed normal control diet. Curcumin treatment in rats fed high cholesterol diet resulted in a significant decrease in serum LDH and CK-MB activities all over the periods of the experiments as compared to untreated high cholesterol -fed rats.

3.4 Effect of curcumin on liver oxidative stress biomarkers in hypercholesterolemic rats

The obtained results demonstrated in table (4) showed a significant increase in liver tissue NO concentration in...
cholesterol fed rats after two and four weeks followed by a non-significant increase after six weeks. Also, a significant increase in liver L-MDA concentration was observed all over the periods of the experiment. Meanwhile, a non-significant decrease in liver tissue GSH concentration was observed in cholesterol fed rats all over the periods of the experiment when compared with rats fed normal control diet. Curcumin treatment in rats fed high cholesterol diet resulted in a significant decrease in liver NO, L-MDA and GSH concentrations all over the periods of the experiment as compared to high cholesterol -fed non treated rats.

3.5 Effect of curcumin on liver enzymatic antioxidants status in hypercholesterolemic rats

The obtained results illustrated in table (5) revealed that, a significant decrease in liver CAT activity was observed in cholesterol fed rats after two weeks of the experiment. This decreases became non-significant after four and six weeks. Also, a non-significant decrease in liver SOD activity was observed in cholesterol fed rats after two weeks followed by a significant decrease after four and six weeks. Moreover, liver GPX activity showed a significant decrease after two and six weeks and non- significantly decreased after four weeks when compared with rats fed normal control diet. Curcumin treatment in rats fed high cholesterol diet resulted in a non-significant decrease in liver tissue CAT activity after two weeks and non-significantly increased after four and six weeks. Liver SOD activity was significantly decreased after two weeks and this decrease become non-significant after four weeks, followed by a non-significant increase after six weeks. Also, a non-significant increase in liver GPX activity was observed after two and four weeks of the experiment followed by a significant increase after six weeks as compared to untreated high cholesterol-fed rats.

Hypercholesterolemia is one of the greatest risk factors contributing to prevalence and severity of coronary heart diseases [26]. Alteration in oxidative stress induced by reactive oxygen species (ROS) and impairments of the antioxidant system play a critical role in the pathogenesis of hypercholesterolemia and subsequent cardiovascular diseases [27]. Liver is the organ essential for the maintenance of systemic lipid homeostasis, and easily susceptible to damage by ROS [28]. It has been shown that hyperlipidemia reduces the hepatic antioxidant defense system [29], a significant increase in serum TNF-α and IL-6 concentrations were observed in high cholesterol fed rats when compared with rats fed normal control diet. The present study exhibited that, high cholesterol diet also results in gross systemic inflammatory responses, as indicated by markedly elevated pro-inflammatory cytokines concentrations. Because of the in vivo nature of our experimental model, the elevated cytokine concentrations in serum could be derived either from circulating white blood cells or from any tissue source, such as endothelium [30]. The mechanisms underlying the different responses over time of different cytokines are not clear. It is possible that the high IL-6 and TNF-α response, which are mainly produced by macrophages and monocytes [31]. Prolonged increases in the concentrations of these cytokines are most likely associated with either severe damage of tissues after trauma or invasion of the body by pathogenic organisms [32].

Previous studies have found that dietary high cholesterol intake can increase the productions of atherogenic inflammatory cytokines such as IL-6 and TNF-α [33]. In addition, excessive intake of cholesterol may cause vascular inflammation, and pro-inflammatory cytokines such as TNF-α and IL-6 may stimulate the expression of adhesion molecules and chemokines such as Vascular Cell Adhesion Molecule-1 (VCAM-1), Intracellular Adhesion Molecule-1 (ICAM-1), and fibronectin in aorta tissue [34,35]. VCAM-1 and ICAM-1 are thought to play an important role in the process of atherosclerosis by recruiting inflammatory cells, and they are both are up-regulated by pro-atherogenic factors [35].

A single cell layer lining the vascular wall, called the vascular endothelium, plays an important role in maintaining the structure and function of vessels. Besides, being the vascular endothelium a mechanical barrier between blood and vessel wall, it is also the origin of production for different bioactive factors that regulate vascular tone, coagulation, cell proliferation, cell death, and inflammation [36]. The cytokines can be represented in two shapes including the anti-inflammatory markers such as IL-1β, IL-6 and TNF-α, the second shape is the adhesion molecules such as ICAM-1 and VCAM-1 [37]. Reactive oxygen species are well documented to function as signaling molecules, stimulating cellular activities ranging from cytokine secretion to cell proliferation, and at higher concentrations, they can induce cell injury and death by oxidative modification of proteins and carbohydrates, lipid peroxidation, and DNA [38]. This is combined with, oxidants lead to the activation of endothelial cells, the action that may results in a wide range of functional changes such as the increase in expression of VCAM-1, ICAM-1, and E-selectin, and the production of chemokines, such as monocyte chemo attracting peptide-1 (AP-1) [39]. In a parallel results, Cieri et al. [1998] [40] reported that, ICAM-1 is one of the most important intercellular adhesion molecules involved in atherogenesis and noticed increased circulating ICAM-1 plasma levels in NIDDM patients that may be resulted from the acute increase of plasma glucose which leads to produce an oxidative stress and induce cellular expression of ICAM-1. In addition, Ozawa et al., (2003) [41] illustrated the induction of ICAM-1 through pro-inflammatory cytokines (IL-1β, TNF-α, IFN-γ and IL-2) and also, VCAM-1 can be induced by IL-1β [42]. These cytokines have a probable anti-inflammatory action on the artery wall by producing an improvement in endothelial dysfunction in addition to their lipid-controlling action [43]. Curcumin treatment in rats fed high cholesterol diet resulted in a non-significant
decrease in serum TNF-α and IL-6 concentrations. Curcumin was able to modulate the production of various inflammatory cytokines, thereby exhibiting potent anti-inflammatory activity. It has been shown to down-regulate nuclear factor-kB (NF-kB), a transcription factor that plays a critical role in the induction of many pro-inflammatory mediators involved in chronic and acute inflammatory diseases and various cancers [44]. Moreover, Singh and Aggarwal, (1995) [45] establish that, curcumin suppressed NF-kB activation induced by different inflammatory stimuli, resulting in the suppression of NF-kB-dependent gene products that suppress apoptosis and mediate proliferation, invasion, and angiogenesis [44]. The down regulation of NF-kB by curcumin results in a decrease in the expression of TNF-α, IL-1 and IL-6 [47].

Serum ALT activity was significantly increased after six weeks of the experiment and the activity of serum AST was markedly increased in high cholesterol fed rats all over the periods of the experiment. Also, a significant increase in serum GGT activity was observed in cholesterol fed rats after two and six weeks when compared with rats fed normal control diet. Similarly, Alkhamees, (2013) [48] indicated that, high cholesterol diet significantly induced elevation in plasma liver enzymes ALT and AST. These parameters are known to be markers for hepatotoxicity. Who added that, histopathological findings revealed several impairments in liver sections from high cholesterol diet supplemented rats by showing moderate degree of fat accumulation, degeneration, fibrosis and inflammatory infiltrates. In the present study, the increase of ALT, AST and GGT activities in blood serum indicate that tissue impairment caused by dyslipidemia may be led to adverse effect by increasing lipid peroxidation which in turn produce damage to liver tissue so outflow of these enzymes from the liver cytosol to the blood stream which indicate that inability of liver to metabolize the ALT and AST [49]. Curcumin treatment in rats fed high cholesterol diet resulted in a significant decrease in serum ALT activity after four and six weeks. Also, curcumin treatment caused significant decrease in serum AST and GGT activities all over the periods of the experiment as compared to untreated cholesterol-fed rats. Curcumin caused reduction of plasma ALT activity, which is generally used as mark of liver injury [50]. This results for curcumin is consistent with work in experimental animals with various chemically induced liver injuries. In these animals, a rise in ALT activity can be limited by curcumin administration [51,52]. Also, Madkour, (2012) [53] recorded that, curcumin showed a protective effect against lambda cyhalothrin-induced hepatic dysfunction through decreasing the activities of ALT and AST towards their respective normal value that is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by lambda cyhalothrin.

The results of current study showed a significant increase in serum LDH and CK-MB activities in high cholesterol fed rats all over the periods of the experiment when compared with rats fed normal control diet. These results are nearly similar to those noted by Suana runswat et al., (2011) [54] who reported that, high cholesterol diet markedly suppressed hepatic and cardiac functions as expressed by an augmentation of serum levels of AST, ALT, LDH and CK-MB activities. Increased activities of these enzymes in serum are indicative of cellular damage, loss of functional integrity, and/or permeability of cell membrane [55]. Curcumin treatment in rats fed high cholesterol diet resulted in a significant decrease in serum LDH and CK-MB activities all over the periods of the experiment compared to untreated cholesterol-fed rats. Curcumin increases the cardiac glutathione content, suggesting that it may augment the action of these naturally occurring sulfhydryl groups to maintain membrane integrity with concomitant decrease of enzymes leakage from the cardiocytes, protection of cardiac tissue from damage, and improvement survival of rats [56]. Also, Dikshit et al., (1995) [57] observed the prevention of ischemia-induced biochemical changes by curcumin in the cat heart. Curcumin pretreatment prevented the ischemia-induced elevation in L-MDA contents and LDH release.

The obtained results showed a significant increase in liver tissue NO concentration in high cholesterol fed rats after two and four weeks of the experiment when compared with rats fed normal control diet. Similar results were previously reported in hypercholesterolemic rats [58], rabbits [59] and might be regarded as a defense mechanism to compensate for continuous inactivation of NO by oxygen-derived free radicals in hypercholesterolemia [60,61]. Another possible explanation for the obtained result is increased inducible nitric oxide synthase (iNOS) activity with cholesterol feeding [62]. Curcumin treatment in rats fed high cholesterol diet resulted in a significant decrease in liver tissue NO concentration all over the periods of the experiment as compared to untreated cholesterol -fed rats. These results are nearly similar to those recorded by Sharma et al., (2006) [63] who demonstrated that, curcumin inhibited TNF-α and NO release in a dose-dependent manner. These results indicate an anti-nociceptive activity of curcumin, possibly through its inhibitory action on NO and TNF-α release and point to its potential to attenuate diabetic neuropathic pain. Curcumin has been widely described to inhibit iNOS expression and NO production, at least in part via direct interference in NF-kB activation [64,65].

A significant increase in liver tissue L-MDA concentration was observed in cholesterol fed rats all over the periods of the experiment when compared with rats fed normal control diet. In the present study, hypercholesterolemia, a major cause for atherosclerosis was associated with increases in the levels of the lipid peroxidation product L-MDA, and decrease in the level of GSH in Liver tissue suggesting an increase in the levels or activity of oxygen radicals.
MDA and GSH have been considered as specific indicators of oxidative stress [66]. L-MDA level can be used as a marker of lipid peroxidation and its measurement gives a direct evidence for LDL oxidation and is leading in predicting free radical-induced injury, therefore, the observed elevation in tissue L-MDA may be attributed to hyperlipidemia that enhances the processes of lipid peroxidation. Hypercholesterolemia could increase the levels of ROS through stimulation of polymorphonuclear leukocytes (PMNls) and dysfunction of endothelial cells [67,68]. Curcumin treatment in rats fed high cholesterol diet resulted in a significant decrease in liver tissue L-MDA concentration all over the periods of the experiment as compared to untreated cholesterol-fed rats. In the present study the inhibited LPO in liver tissue of rats as a result of anti-LPO effect of curcumin may be attributed to its redox metal-binding activity [69], free radical scavenging properties [70] and anti-oxidative potential [71]. Also, the two electrophilic a, b-unsaturated carbonyl groups in the structure of curcumin may be involved in neutralization of the hydroxyl radicals. Hence, the observed anti-protein oxidation effect of curcumin could be due to its direct free radical scavenging potential [72].

A non-significant decrease in liver tissue GSH concentration was observed in cholesterol fed rats all over the periods of the experiment when compared with rats fed normal control diet. Intracellular reduction oxidation (redox) state is regulated in part by the presence of GSH [73]. The GSH/GSSG ratio may be sensitive indicator of oxidative stress. Significant decrease in the level of total glutathione observed on HCD feeding might be due to impaired GSH biosynthesis and constant on slaughter of ONOO-formed by reactions of O2- and NO, both of which increased in hypercholesterolemia. GSH plays critical role in the detoxification process against reactive nitrogen species e.g. NO, NO2 and ONOO- [74]. Oxidized glutathione (GSSG) is formed by the linking of two tripeptides by disulfide bridge. The generation of GSSG takes place during the oxidation of GSH by glutathione peroxides in the following reaction to maintain the sufficient level of GSH. The increased oxidized glutathione levels in hypercholesterolemic rats can be attributed to spontaneous non-enzymatic GSH oxidation [75]. Curcumin treatment in rats fed high cholesterol diet resulted in a significant decrease in liver tissue GSH all over the periods of the experiment. Reduced glutathione, a key antioxidant, is an important constituent of intracellular protective mechanisms against various noxious stimuli, including oxidative stress. On the other hand, reduced GSH, which constitutes the main component of the endogenous non-protein sulfhydryl pool, is known to be a major low molecular weight scavenger of free radicals in the cytoplasm [76]. Because of their exposed sulfhydryl groups, non-protein sulfhydryl bind electrophilic radicals and metabolites that may be damaging to cells [77]. In the present study, the observed decrease in GSH concentration effect curcumin administrations may be due to an increase in the free radical associated with hypercholesterolemia. Hypercholesterolemia induces not only atherosclerosis but also produces a lot of free radicals in blood and tissues [78].

The obtained results showed a significant decrease in liver tissue CAT activity in high cholesterol fed rats after two weeks of the experiment. Also, a significant decrease in liver tissue SOD activity was observed after four and six weeks. Moreover, a significant decrease in liver GPX activity was observed after two and six weeks of the experiment when compared with rats fed normal control diet. Oxidative stress is an imbalance between the free radicals production especially reactive oxygen species (ROS) and antioxidants systems and has been implicated in accelerated atherosclerosis [79]. The accumulation of cholesterol in erythrocytes, leukocytes, platelets and endothelial cells can lead to an increase in the concentration of reactive species [80, 81], and a reduction in the antioxidant defense systems, such as CAT, GPx and SOD enzyme activities [82]. The decrease in the activities of these enzymes could be attributed to the excessive utilization of these enzymes in inactivating the free radicals generated due to the high cholesterol diet [83]. Curcumin treatment in rats fed high cholesterol diet resulted in a non-significant decrease in liver tissue CAT activity after two weeks and non- significantly increased after four and six weeks. Liver SOD activity was significantly decreased after two weeks and this decrease become non-significant after four weeks, followed by a non-significant increase after six weeks. Also, a non-significant increase in liver GPX activity was observed after two and four weeks of the experiment followed by a significant increase after six weeks as compared to untreated high cholesterol-fed rats. Moreover, administration of curcumin improved the antioxidant status and thereby preventing the damage to the liver, mainly because of the antioxidant sparing action of curcumin. The antioxidant mechanism of curcumin may include one or more of the following interactions. Scavenging or free radicals [84], inhibition of oxidative enzymes like cytochrome P450 [85], oxygen quenching and making it less available for oxidative reaction, interacting with oxidative preventing its outcome and disarming oxidative properties of metal ions such as iron [86]. Thus, in this work, curcumin effectively prevented tissue damage by decreasing the oxidative stress and restoring the antioxidant status. Curcumin has two o-methoxy phenolic OH groups attached to the â-diketone moiety having methylene CH2 group. It is believed that the H abstractions from these groups are responsible for the remarkable antioxidant activity of curcumin. The free radical scavenging activity of curcumin can arise by the resonance stabilization of its radicals from two phenolic OH groups (mainly) or from the CH group 2 of the â-diketone moiety [87]. Moreover, Reddy et al., (2012) [88] reported that, curcumin not only is a phenolic antioxidant that mostly donates H atoms from the phenolic groups, but also is â-diketone radical chain-breaking substance that can give H atom from methylene CH2. Therefore, Treatment
with curcumin significantly reduced intracellular ROS production by increasing the activity of ROS scavenging enzymes (GPx, SOD and CAT) in liver tissue.

4. CONCLUSION

In conclusion, the present study demonstrated that curcumin administration produces potent antiatherogenic and an effective treatment against hypercholesterolemia induced by high cholesterol diet in rats, since curcumin was able to ameliorate serum biochemical parameters, cardiac marker enzymes and liver function. Moreover, administration of curcumin significantly reduced lipid peroxidation and help to modulate oxidative stress caused by hypercholesterolemia in rats as well as improved the antioxidant defense system. We recommended that, administration of diet rich in the natural antioxidant is very important for protection of different body tissue, against oxidative stress or hypercholesterolemia and cardiac vascular disease and may be beneficial for patients who suffer from hyperlipidemia, hypercholesterolemia and/or arteriosclerosis.

5. REFERENCES

molecule-1 and c-reactive protein following administration of aequous extract of piper sarmentosum on experimental rabbits fed with cholesterol diet. Lipid Health Dis, 10:2.


