

Beneficial Effects of Curcumin and *Ruta Chalepensis* on the Antioxidant System and Inflammation in Hypercholesteromic Rats

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Abstract: Background: Curcumin (*Curcuma longa*) and *Ruta Chalepensis* are extensively used as a household remedy for various diseases. The components of these plants are of great interest in medicinal chemistry. Aim: This study was designed to evaluate and compare the efficacy of infusion of either curcumin or ethanolic extract of *Ruta chalepensis* in improving the liver function, reducing oxidative damage and inflammation in hypercholesteromic rats. Materials and Methods: Rats were divided into four groups; control group, hyper-cholesteromic control group and two groups were fed on high-cholesterol diet and were treated with either curcumin or ethanolic extract of *Ruta chalepensis* for 90 days. Results: In hypercholesteromic rats, the plasma levels of total cholesterol, HDL-C, LDL-C, TG, ALT, AST, CRP, TBARS plus enzyme activity of cyclooxygenase (COX) in monocytes were significantly increased, while the levels of GSH, SOD and GPx were significantly decreased. Treatment with either curcumin or ethanolic extract of *Ruta chalepensis* could modulate above mentioned biochemical parameters to their normal levels. Conclusion: These results suggested that treatment by curcumin or ethanolic extract of *Ruta Chalepensis* could reduce oxidative stress as well as inflammation in hypercholesteromic rats.

Key words: Curcumin, *Ruta chalepensis*, superoxide dismutase, reduced glutathione, Cholesterol, C-reactive protein, hypercholesteromic rat.

INTRODUCTION

Hypercholesterolemia, high-cholesterol diet, and oxidative stress increase serum total cholesterol and low density lipoprotein (LDL)-cholesterol levels, resulting in increased risk for atherosclerosis development (Assamann *et al.*, 1999). Raised serum lipid levels, particularly of cholesterol along with generation of reactive oxygen species (ROS) play a key role in the development of coronary artery disease and atherosclerosis (Vincent *et al.*, 2010). The need to decrease plasma lipids has been recognized and dietary control of blood cholesterol has been explored (Hakimoglu *et al.*, 2007). Several lines of evidence indicate that oxidative stress may play an important role in various pathological conditions (Sherazede *et al.*, 2010). To protect tissues from these damaging effects, the organism possesses enzymatic and non-enzymatic antioxidant systems (Valko *et al.*, 2007). Hypercholesterolemia is among the most common health problems treated with traditional remedies (Manjunatha and Srinivasan 2007). However, it has been reported that medicinal plants have high antioxidant capacity and also the potential to reduce lipid and cholesterol in the body due to their bioactive compounds (Araújo and Leon 2001). Numerous epidemiologic studies have evaluated several inflammatory markers for their clinical usefulness in predicting risk of cardiovascular disease (Willerson and Ridker 2004). Prostaglandins (PGs) biosynthesis has been implicated in the pathophysiology of cardiovascular processes and a variety of inflammatory diseases (Dubois *et al.*, 1998). The rate-limiting enzyme in the biosynthesis of PGs is cyclooxygenase (COX). COX enzyme is essential in the inflammatory process and controls the down stream regulation of immune cell activation and inflammatory cytokine induction (Hoozemans and O'Banion 2005).

In recent years, there has been renewed interest in the treatment of different diseases using herbal drugs as the World Health Organization (WHO) has also recommended evaluation of the effectiveness of plants in conditions where we lack safe modern drugs (Ayyanar *et al.*, 2008). One of these herbal plants is curcumin (diferuloylmethane). The latter is a natural polyphenol compound isolated from *Curcuma longa* Linn. (Zingiberaceae) and widely used as spice (Araújo and Leon 2001) and as anti-inflammatory, anticancer, anti-diabetic, anti-dementia, and antioxidant (Raghvendra *et al.*, 2011). Another herbal plant is commonly called Rue from the family *Rutaceae* and genus; *Ruta*. There are two main species of *Ruta* used in traditional medicine; *Ruta chalepensis* and *Ruta graveolens* (Iauk *et al.*, 2004). Traditionally, *Ruta* is also used as remedy for many inflammatory diseases [Browner 1985; Chávez *et al.*, 2003].

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases. However, relatively little knowledge about their mode of action is available. Based on their traditional use, the present study is carried out to evaluate the efficacy of curcumin and *Ruta chalepensis* as anti-

inflammatory and as anti-hyperlipidemic agents in addition to their ability to induce the various antioxidant parameters in experimental animal models.

MATERIAL AND METHODS

Preparation of *Ruta Chalepensis* Extract:

The collected aerial part of the plant material was washed thoroughly and dried in shade. The powdered plant material (200 g) was soaked in 70 % ethanol and kept for three days. The extract was concentrated to dryness by rotary evaporator in low pressure yielding the dried extract. The extract was cleared of low polarity contaminants such as fats, chlorophyll, xanthophyll by repeated extraction with petroleum ether (60–80 °C). This is used as *Ruta chalepensis* ethanolic extract (Kim and Kim, 2010).

Experimental Animals:

Forty adult male albino rats weighing 90 - 105 g were housed in wire mesh cages at room temperature. Veterinary care was provided by the Laboratory Animal House Unit of the Faculty of Medicine, Biochemistry Department, Cairo University and used with the approval of Animal Ethics Committee. Rats were housed with normal light dark cycle, and were allowed to acclimatize to their environment for five days before the start of the experiment. The body weights were measured twice per week. All rats were fed with a commercial rat feed. The experimental animals in groups II, III and IV in the present study were fed on diets contained 45% high fat in order to induce hypercholesterolemia. Rats in groups III and IV were fed by oral injection with curcumin (300mg/kg) or ethanolic extract of *Ruta chalepensis*.

The animals were divided into the following groups (each group consisted of 10 rats):

Group I (Gr-I): Control group were fed on normal laboratory diet and orally administered an equivalent volume of vehicle (distilled water).

Group (II) (Gr.II): Hypercholesteromic control rats. (Normal laboratory diet+1.5 % Cholesterol+0.5% cholic acid).

Group III (Gr-III): Treated group (cholesterol diet+ curcumin at a dose of 300 mg/kg body weight dissolved in saline given orally by gastric intubation everyday for 90 days.

Group IV (Gr-IV): Treated group (cholesterol diet+ ethanolic extract of *Ruta chalepensis* at a dose of 40 mg/kg body weight dissolved in saline given orally by gastric intubation everyday for 90 days.

The rats were maintained on their respective diet for 90 days. At the end of the experimental period, the rats were sacrificed after overnight fasting by euthanasia. Blood was kept in ice cold containers for various biochemical analyses

Sample Collection:

Animals' Blood samples were withdrawn from all rats through the retro-orbital route using heparinized capillary tubes. The blood samples were delivered into centrifuge tubes and centrifuged at 2000 rpm for 20 minutes and plasma as well as packed red blood cells were separated and stored at -80°C until used.

Measurement of Plasma Parameters:

Serum concentrations of triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and activities of aspartate aminotransferase enzyme (AST) and Alanine transaminase enzyme (ALT) and C-reactive protein (CRP) were measured by an enzymatic colorimetric method of Kim and Kim. (2010). using a commercial enzymatic kit (Bio-Diagnostic, Cairo, Egypt). Also, thiobarbituric acid reactive substances (TBARS) were measured as a marker of the lipid peroxidation according to the method of Ohkawa *et al.*, (1979). The plasma level of low density lipoprotein –cholesterol (LDL-C) concentration was calculated according to the formula' $LDL-C = TC/1.19 + TG/1.9 - HDL-C/1.1 - 38$ (mg/dl)' that was described by Ahmadi *et al.*, (2008).

Assay of Antioxidants:

Superoxide dismutase enzyme (SOD) was assayed in the erythrocytes as described by Elstner *et al.*, (1983). Glutathione peroxidase (GPx) enzyme was determined by the method of Paglia and Valentine, (1967) using cumene hydroperoxide as substrate. Glutathione (GSH) was measured according to the procedure of Anderson, (1985).

Activity of Cyclooxygenase (COX) in Peripheral Blood Mononuclear Cells (PBMC):

Peripheral blood mononuclear cells were separated from heparinized blood by the Ficoll-Hypaque density gradient centrifugation method and after two washes, the peripheral blood mononuclear pellets were frozen at -80°C. COX activity was assayed by the method of Shimizu *et al.*, (1984).

Results:

Table-1 shows that the activities of ALT and AST in plasma were significantly increased in hypercholesteromic rats (Gr-II) by 13.8%, 20.8% respectively, when compared to their control counterparts in Gr-I. On the other hand, when hypercholesteromic rats were treated with curcumin (Gr-III) or ethanolic extract of *Ruta chalepensis* (Gr-IV), a significant decrease in the plasma levels of ALT and AST was noticed, $p < 0.01$, comparing to their control counterparts in GII. Furthermore, the percentage decrement of the plasma levels of total cholesterol, LDL-C, HDL-C and TG in hypercholesteromic rats by curcumin were 40 %, 63.8%, 7%, 41% respectively, ($P < 0.01$) (Table 2).

Table 1: Plasma levels of some liver enzymes among different groups.

	Gr-I	Gr-II	Gr-III	Gr-IV	*p-value
ALT (U/L)					
Mean \pm SEM	36.2 \pm 1.36 ^a	41.2 \pm 6.2 ^b	31.3 \pm 7.9 ^c	32.2 \pm 4.1 ^d	<0.01
AST (U/L)					
Mean \pm SEM	60.1 \pm 6.2 ^a	72.2 \pm 12.5 ^b	62.4 \pm 5.2 ^c	68.5 \pm 2.1 ^d	<0.01

*P-value \leq 0.05 is considered significant. For each significant test, mean groups sharing subscript are not different from each other, $p > 0.05$

Also, in the present study ethanolic extract of *Ruta chalepensis* is used as another hypocholesteromic agent. In this regard, a significant decrease in the plasma levels of the previously mentioned lipid parameters by 34.3%, 58.7%, 11% and 38.5, respectively, $p < 0.01$ (Table 2).

Table 2: Plasma levels of total cholesterol (TC), LDL-C, HDL -C and triglyceride (TG) among different groups:

	Gr-I	Gr-II	Gr-III	Gr-IV	*p-value
TC (mg/dl) Mean \pm SEM	111 \pm 1.1 ^a	125 \pm 7.6 ^b	75 \pm 6.3 ^c	82.1 \pm 8.4 ^d	<0.01
LDL-C (mg/dl) Mean \pm SEM	66 \pm 2.34 ^a	127 \pm 5.3 ^b	46 \pm 3.1 ^c	52.4 \pm 3.7 ^a	<0.01
HDL-C (mg/dl) Mean \pm SEM	44.3 \pm 1.2 ^a	46.3 \pm 2.54 ^b	43.1 \pm 3.35 ^a	41.2 \pm 4.51 ^a	<0.01
TG (mg/dl) Mean \pm SEM	97 \pm 7.34 ^a	195 \pm 8.12 ^b	115 \pm 7.53 ^c	120 \pm 4.34 ^d	<0.01

*P-value \leq 0.05 is considered significant. For each significant test, mean groups sharing subscript are not different from each other, $p > 0.05$

Activities of antioxidant enzymes SOD, and GPx as well as GSH concentration (Table 3) were decreased significantly associated with significant increase in the plasma levels of TBARS, a lipid peroxidation product (Table 4) in the hypercholesteromic rats as compared to their control counterparts in Gr-I. These results were modulated when hypercholesteromic rats were supplemented with curcumin (Gr-III) or ethanolic extract of *Ruta chalepensis* (Gr-IV). In addition, the increment in the enzymatic and non-enzymatic antioxidants levels were more pronounced in Gr-III than in Gr-IV.

Table 3: Levels of antioxidant enzymes among different groups:

	Gr-I	Gr-II	Gr-III	Gr-IV	p-value
SOD (U/gHb)					
Mean \pm SEM	12.4 \pm 0.58 ^a	6.2 \pm 0.8 ^b	11.2 \pm 0.5 ^a	10.3 \pm 0.34 ^d	<0.01
GSH (mmol/L)					
Mean \pm SEM	16.2 \pm 1.1 ^a	11.5 \pm 1.3 ^b	15.6 \pm 0.9 ^c	13.5 \pm 2.1 ^d	<0.01
GPx (U/gHb)					
Mean \pm SEM	220 \pm 10.6 ^a	180 \pm 9.2 ^b	378 \pm 10.7 ^c	383 \pm 11.2 ^d	<0.01

*P-value \leq 0.05 is considered significant. For each significant test, mean groups sharing subscript are not different from each other, $p > 0.05$

COX activity in PBMC was significantly increased in hypercholesteromic rats in comparison with their control counterparts in Gr-I. Treatment with curcumin or ethanolic extract of *Ruta chalepensis* showed a significant decrease in COX activity when compared to hypercholesteromic rats in Gr-II. Plasma CRP level was significantly increased in hypercholesteromic rats compared to normal rats in Gr-I. Supplementation with either curcumin or ethanolic extract of *Ruta chalepensis* significantly decreased the plasma CRP level (Table 4).

Table 4: Effect of curcumin and ethanolic extract of *Ruta Chalepensis* on the plasma levels of C-reactive protein (CRP), thiobarbituric reactive substances (TBARS) and activity of cyclooxygenase (COX) enzyme among different groups:

	Gr-I	Gr-II	Gr-III	Gr-IV	p-value
CRP (mg/ml)					
Mean \pm SEM	51.7 \pm 0.35 ^a	68.4 \pm 0.87 ^b	52.2 \pm 0.32 ^a	58.2 \pm 0.25 ^c	<0.01
TBARS (mmol/L)					
Mean \pm SEM	0.46 \pm 0.01 ^a	0.76 \pm 0.03 ^b	0.45 \pm 0.09 ^a	0.51 \pm 0.01 ^d	<0.01
COX (U/mg)					
Mean \pm SEM	3.2 \pm 0.18 ^a	8.6 \pm 0.85 ^b	4.2 \pm 0.42 ^c	5.4 \pm 0.23 ^a	<0.01

*P-value \leq 0.05 is considered significant. For each significant test, mean groups sharing subscript are not different from each other, $p > 0.05$

Discussion:

Measuring liver enzymes activities in the serum are used as indicators of chemically induced liver damage (Drotman and Lawhorn 1978). Injuries to the liver associated with marked alteration in liver chemistry have variously been treated using various crude extracts of plants (Bhandarkar and Khan 2004; Raja *et al.*, 2007). In the present study, the effect of either curcumin or ethanolic extract of *Ruta chalepensis* on liver function was evaluated by measuring the activities of serum GPT and GOT. These enzymes leak into the circulation when hepatocytes are damaged. It is believed that high serum cholesterol level can cause liver damage (Srinivasan and Sambaiah 1991). Indeed, our results show that high-fat diet caused significant increase in serum GOT and GPT levels. However, rats treated with either curcumin or ethanolic extract of *Ruta chalepensis* had lower serum GOT and GPT levels. The reduction in the serum liver enzyme activities indicates that curcumin and ethanolic extract of *Ruta chalepensis* may protect against liver injury.

High blood cholesterol is one of the greatest risk factors contributing to the prevalence and severity of coronary heart disease (Wilson *et al.*, 1998). In the present study, rats fed on a high fat diet revealed a significant increase in the plasma level of total cholesterol, LDL-C, HDL-C and TG. These results are in agreement with those obtained by Sherazede *et al.*, (2010) and Alisi *et al.*, (2008).

The present study postulated that when using curcumin as a hypolipidemic agent, the biochemical parameters of lipid profile were significantly decreased. These results are consistent with the results obtained by Rao *et al.*, (1970); Srinivasan and Sambaiah (1991). These authors have reported that curcumin can cause an increased excretion of cholesterol and bile acid in the rat feces. In addition, it has been reported that curcumin can decrease the serum cholesterol via enhanced cholesterol 7 α -hydroxylase (CYP7A1) enzyme activity, which controls cholesterol homeostasis (Babu and Srinivasan 1997; Banerjee *et al.*, 2003).

Another antihyperlipidemic agent tested in the present study is *Ruta chalepensis*. This is one of the family members of Rutaceae. Most of the previous studies have been focused on the spice known as *Ruta graveolens* which can act as a hypo-lipidemic agent (Ratheesh *et al.*, 2011; Ratheesh, and Helen 2007). In the present study, the ethanolic extract of *Ruta chalepensis* showed a promising effect as an anti-hyperlipidemic agent according to the data that are presented in Table 2.

Increased serum lipid levels, particularly of cholesterol along with the generation of reactive oxygen species (ROS) play a key role in the development of coronary artery disease and atherosclerosis (Ross, 1999). The body has evolved a complex defense strategy to minimize the damaging effects of various oxidants. Central to this defense are the non-enzymatic and enzymatic antioxidants. These include reduced glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) respectively, which act in concert to protect the organism from oxidative damage (Gutteridge and Halliwell 1996). Lipid peroxidation is a fundamental process in atherogenesis. The present study postulated that hypercholesteromic rats escorted a significant increase in the plasma level TBARS associated with decrease in both enzymatic and non-enzymatic antioxidants. Our results are in agreement with those obtained by Das *et al.*, (2000) and Ratheesh *et al.*, (2011). Those authors have suggested that high fat diet increased the plasma level of TBARS in the serum of hypercholesteromic rabbits and guinea pigs.

The decrease in the enzymatic antioxidants could be due to a feedback inhibition or oxidative inactivation of the enzyme protein due to increased ROS generation (Ratheesh *et al.*, 2011). It has been reported that several polyphenol compounds isolated from the turmeric plant (*Curcuma longa*) such as curcumin possess strong antioxidant properties, which reduce the formation of ROS by directly inhibiting the reactive oxygen generating enzymes. In addition, it has been reported that curcumin can induce expression of several antioxidant enzyme genes including SOD and catalase (CAT) (Suphim *et al.*, 2010; Aggarwal and Sung 2009). Indeed, our results show a significant reduction in the formation of TBARS in parallel with improvement in the levels of both enzymatic and non-enzymatic antioxidants. Furthermore, the ethanolic extract of *Ruta chalepensis* has a great positive effect on the antioxidant system in hypercholesteromic rats as well as on the plasma level of TBARS.

CRP is an acute-phase protein that has been identified as an important biomarker for various inflammatory and degenerative diseases (Ratheesh *et al.*, 2011). In addition, the rate-limiting enzyme in the biosynthesis of prostaglandins (PG) is prostaglandin-H₂-synthase or cyclooxygenase (COX). COX enzymes are essential in the inflammatory process. Prostaglandin (PG) biosynthesis has been implicated in the pathophysiology of cardiovascular processes and a variety of inflammatory diseases (Dubois *et al.*, 1998). In the present study, supplementation with curcumin or ethanolic extract of *Ruta chalepensis* decrease the activity of COX in the monocyte extracted from hypercholesteromic rats. These results suggest that each of curcumin and ethanolic extract of *Ruta chalepensis* may protect against the inflammation induced by high fat diet.

To conclude, this study revealed that curcumin and ethanolic extract of *Ruta chalepensis* having both anti-hyperinflammatory and anti-hyperlipidemic efficacy as well as augment the antioxidant system. However, further clinical studies are required to assess the efficacy and safety of *Ruta chalepensis* and curcumin in hyperlipidemic humans as well as to elucidate the exact mechanism of action of the active ingredient in the ethanolic extract of *Ruta chalepensis* that mediates the protective effect.

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