BIOCHEMICAL EFFECT OF CURCUMIN, GARLIC EXTRACT AND OLIVE OIL ON HYPERLIPIDEMIA INDUCED IN RATS

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A B S T R A C T

This study was performed to investigate the effect of oral supplementation of curcumin, garlic extract and olive oil on lipid profile, nitric oxide, adiponectin, endothelin-1, blood glucose and some inflammatory markers in normal, diabetic and hyperlipidemic rats supplementing high fat and cholesterol-enriched diet. Eighty female adult albino rats were divided into eight equal groups of 10 rats each. Group (1): negative control received normal diet only, group (2): rats fed on normal diet and received curcumin orally, group (3): rats fed on normal diet and receive garlic extract (1 ml/100g b.w.) orally, group (4): rats fed on normal diet and receive olive oil (0.5 ml/100g b.w.) orally, group (5): positive control received hyperlipidemic diet, group (6): rats fed on hyperlipidemic diet and received curcumin (350 mg/ 1 kg b.w.) orally, group (7): rats fed on hyperlipidemic diet and received garlic extract orally, group (8): rats fed on hyperlipidemic diet and received olive oil orally. The obtained results revealed that, curcumin, garlic extract and olive oil supplementations to hyperlipidemic rats showed a significant increase in serum HDL-cholesterol, nitric oxide, adiponectin and Endothelin-1 concentrations and significantly decrease in serum total cholesterol, triacylglycerols, LDL-cholesterol, Fasting blood glucose, Glycated Hemoglobin (HbA1C), high sensitive C-reactive protein and Interleukin-6 levels. These results suggest that, curcumin, garlic extract and olive oil supplementations may have some benefits in patients suffering from dyslipidemia and diabetes.

Key words: hyperlipidemia, diabetes, curcumin, olive oil, garlic, lipid profile, inflammation, Interleukin-6.

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1. INTRODUCTION

Hyperlipidemia is a common disorder caused by lifestyle habits in developed countries and is the major cause of coronary heart disease. It results from abnormalities in lipid metabolism or plasma lipid transport or a disorder in the synthesis and degradation of plasma lipoproteins (Jang *et al*., 2008). The consequence of hyperlipidemia can cause atherosclerosis, and thus the risk of coronary heart disease and stroke is increased. Diabetes mellitus is associated with hyperlipidemia, which is a significant risk factor for cardiovascular diseases (El-Moselhy *et al*., 2011). The incidence of type 2 diabetes mellitus is rapidly increasing worldwide. Type 2 (formerly called non-insulin dependent) diabetes mellitus accounts for over 90% of the diagnosed cases of diabetes (Pillarisetti and Saxena 2004). Diabetes is a well-recognized risk factor for atherosclerotic and cardiovascular disease that confers a markedly increased risk of coronary heart disease (CHD). The altered lipid profile characterized by elevated levels of circulating free fatty acids (FFAs) and triacylglycerols, as well as a reduction in high-density lipoprotein cholesterol (HDL-C) along with excess fat deposition in

various tissues including the liver (Banerjee *et al*., 2004). An abnormal accumulation of fat in the liver and muscle elicits insulin resistances that culminate in beta cell reduction in type 2 diabetes (Seo *et al*., 2008). Curcumin has been widely used in traditional medicine in Southeast Asia. It prevents many diseases including biliary disorders, anorexia, cough, diabetes, hepatic disorders, rheumatism, sinusitis, cancer, and Alzheimer's (Aggarwal and Harikumar 2009). Several studies have indicated that curcumin plays a beneficial role in terms of being an antioxidant, anti-tumorigenic and anti-inflammatory agent (Suzuki *et al*., 2005; Kurup *et al*., 2007; Ansari *et al*., 2007; Kurup and Barrios, 2008). On the other hand, garlic has been the focus of serious medical and clinical attention because of its beneficial effects on several cardiovascular risk factors like reduction of serum lipids, blood pressure and plasma viscosity (Ashraf *et al*., 2005). The active constituents are several complex sulfur-containing compounds that are rapidly absorbed, transformed and metabolized (Rizwan *et al*., 2005). Also, olive oil improves the major risk factors for cardiovascular disease, such as the lipoprotein profile, blood pressure, glucose metabolism and antithrombotic profile (Abdel-Aal *et al*., 2008). Some of these effects were attributed beside the monounsaturated fatty acids (MUFA) to the minor components of virgin olive oil (Al Jamal and Ibrahim 2011). Accordingly, the aim of this work was to investigate the antihyperlipidemic effects of oral supplementation of curcumin, garlic extract and olive oil on some inflammatory markers, blood glucose, lipid profile, adiponectin and endothelin-1 in serum of female rats feeding high fat diet.

2. Material and method:

2.1. Experimental animals:

A total number of (80) adult female albino rats of (4 – 6) weeks weighting (140-160) gm were used in the experimental investigation of this study. Rats were obtained from the Research Institute of Ophthalmology, Giza, Egypt.

Animals were housed in separated metal cages and kept at constant environmental and nutritional conditions and allowed free access to standard pellet diet and water was supplied ad-libtum.

2.2. Diet:

Diets supplied to rats according to NRC (1995).

2.3. Induction of Diabetes:

Streptozotocin powder manufactured by Sigma chemical Co. (USA) was used for induction of diabetes. According to (Mrudula *et al*., 2007). Streptozotocin is an analogue of N-acetyl glucosamine which is readily transported into pancreatic beta cells by Glut2 and cause β-cell toxicity, resulting in insulin deficiency (Mrudula *et al*., 2007).

2.4. Preparation of curcumin, garlic extract:

Curcumin was dissolved in (7%) of Di-methyl sulfoxide solution (DMSO) (Rong *et al*., 2012). Fresh garlic bulbs were obtained from the local market and cut into small pieces. 50g of garlic was homogenized in 100 ml of cold distilled water and crushed in a mixing machine. The resultant slurry was squeezed and filtered through a fine cloth and the filtrate was quickly frozen until used (Emmanuel and James 2011).

2.5. *Dosage of curcumin, garlic extract and olive oil:*

Curcumin was dissolved in (7%) of Di-methyl sulfoxide solution (DMSO) and administrated orally at a dose of (350 mg / kg bw/day) for 6 weeks (Aggarwal *et al*., 2003). However, Garlic extract was supplied orally to female rats at a dose of (1 mg / 100g bw/ day) for 6 weeks (Kim *et al*., 2011). Olive oil was given to female rats orally at a dose of (0.5 ml / 100g bw/ day) for 6 weeks (Nandakumaran *et al*., 2012).

Table (1): Composition of the basal and fat-enriched diets for rats:

Feed Ingrdients	Level (%) in basal diet	Level (%) in fat-enriched diet
Oil	15.00	13.00
Yellow corn	44.15	44.15
Soya bean meal (44%)	20.51	20.51
Wheat Bran	12.33	12.33
Cholesterol	0.00	1.00
Coconut Oil	0.00	2.00
Molasses	3.00	3.00
Common Salts	0.50	0.50
Lysine	0.18	0.18
DL-Methionine	0.74	0.74
Min.-Vit. Premix	2.00	1.50
Ground Limestone	1.59	1.09
Total	100.00	100.00

2.6. Experimental design:

Rats were randomly divided into (8) main equal groups, 10 rats each, placed in individual cages and classified as follow: group (1): negative control, group (2): rats fed on normal diet and receive curcumin orally, group (3): rats fed on normal diet and receive garlic extract (1 ml/100g bw) orally, group (4): rats fed on normal diet and receive olive oil (0.5 ml/100g bw) orally, group (5): positive control, group (6): rats fed on hyperlipidemic diet and receive curcumin (350 mg/ 1 kg b.w.) orally, group (7): rats fed on hyperlipidemic diet and receive garlic extract orally, group (8): rats fed on hyperlipidemic diet and receive olive oil orally.

2.7. Sample collection:

Blood samples were collected from all animal groups after 2, 4 and 6 weeks from the onset of curcumin, garlic and olive oil administration. The samples were collected in the morning after overnight fasting from the retro-orbital plexus of eyes.

Serum was separated by centrifugation at 3000 rpm for 10 minutes. The clear serum was aspirated and transferred into sterile labeled tubes and kept in a deep freeze at (-70° C) until used for subsequent biochemical analysis. Total cholesterol according to (Schettler, 1975). Triacylglycerols (Schettler, 1975). HDL-cholesterol (Gordon *et al*, 1977). LDL-cholesterol (Friedewald, 1972). Blood glucose (Trinder, 1969). High sensitive C-reactive protein (Kimberly et al, 2003). Nitric oxide (Montgomery and Dymock, 1961). Endothelin-1 (Rolinski, 1994) and Interleukin-6 (Hirano, 1990).

2.8. Statistical analysis:

The obtained data were statistically analyzed using one way analysis of variance (ANOVA) followed by the Duncan multiple test. All analysis were performed using SPSS (statistical package for social sciences, 1999; ver.10.0), values of $P \leq 0.05$ were considered to be significant.

3. RESULTS:

The obtained results presented in table (2) revealed that, Hyperlipidemia and diabetes caused significant increase in serum total

Table (2) : Effect of curcumin, garlic extract and olive oil on serum total cholesterol, serum triglyceride, LDL-cholesterol, blood glucose , glycated hemoglobin, high sensitive C-reactive protein, interleukin-6, endothelin-1 HDL-cholesterol, adiponectin and nitric oxide level in the blood.

Parameters	Groups							
	G1	G2	G3	G4	G5	G6	G7	G8
FBS	92.79a ±0.63	93.89ab ±0.61	94.33ab ±0.71	95.47b ±0.52	177.11e ±2.25	106.68d ±1.57	104.6c ±1.11	103.91c ±1.22
Adiponectin	5.93d ±0.05	4.52b ±0.06	6.40 ±0.06	5.68c ±0.08	2.77a ±0.08	8.42f ±0.06	9.81g ±0.07	8.15e ±0.05
hs-CRP (high sensitive C-reactive protein)	0.52b ±0.02	0.46ab ±0.02	0.42a ±0.01	0.48ab ±0.01	1.22d ±0.04	0.91c ±0.04	0.86c ±0.04	0.93c ±0.04
IL-6 (interleukin-6)	67.26e ±0.69	61.16cd ±0.37	66.73e ±0.59	62.28d ±0.45	83.35f ±0.54	54.17a ±0.6	60.48c ±0.55	57.45b ±0.56
NO (Nitric oxide)	76.24d ±0.47	94.48e ±0.46	73.73c ±0.48	71.18b ±0.78	66.63a ±0.47	111.70g ±0.41	98.79f ±0.39	110.96g ±0.7
Cholesterol	142.49c ±0.40	125.10b ±0.51	121.74a ±0.51	126.39b ±0.40	241.18g ±2.35	155.59d ±1.26	159.74e ±1.31	161.39f ±1.73
Triglyceride	99.27c ±0.38	89.94b ±0.47	87.12a ±0.39	89.33b ±0.39	199.48 ±1.55	135.27d ±1.27	136.28d ±1.45	141.18e ±1.29
HDL-C (high density lipoprotein cholesterol)	40.72d ±0.48	43.36e ±0.45	40.89d ±0.85	41.80de ±0.35	27.71a ±0.55	34.85c ±0.54	33.80c ±0.48	31.91b ±0.38
LDL (low density lipoprotein cholesterol)	130.31e ±0.71	114.12a ±0.81	114.58a ±1.11	117.85b ±0.76	195.81f ±0.80	114.07a ±1.47	119.57c ±2.71	121.71d ±1.28
HBA1C	5.02a ±0.11	5.05a ±0.08	5.09a ±0.06	5.04a ±0.07	7.02c ±0.11	5.63b ±0.07	5.70b ±0.10	5.70b ±0.09
Endothelin-1	0.96bc ±0.04	0.87ab ±0.04	0.77a ±0.04	0.85ab ±0.03	1.67d ±0.05	1.02c ±0.04	0.95bc ±0.04	0.94bc ±0.04

S.E.: Standard Error. a, b, c: Mean values with different superscript letters in the same row are significantly different at ($P \leq 0.05$).

cholesterol, Triacylglycerols, LDL-cholesterol, glucose, glycated hemoglobin, high sensitive C-reactive protein, interleukin-6 and endothelin-1 can confirm as compared with normal control group. Meanwhile, Oral administration of curcumin, garlic extract and olive oil cause significant reduction in all of parameters as compared to that of positive control group. Hyperlipidemia and diabetes induced significant decrease in serum HDL-cholesterol, adiponectin and nitric oxide can confirm as compared to that of normal control group.

4. DISCUSSION

The obtained results demonstrated that curcumin garlic extract and olive oil supplementation have potential effects in preventing hyperlipidemia, diabetes and on cardiovascular protection.

Interestingly, the results showed that curcumin, garlic extract and olive oil supplementation significantly improved serum lipid profile, as revealed by marked increase in HDL level and decrease serum total Cholesterol, triacylglycerols and LDL-C level. These results are nearly similar to these reported by (Karthikesan *et al*., 2010), who investigate the effect of curcumin on serum glucose level and lipid profile in hyperlipidemic rats through the inhibition of the generation of superoxide radicals. Also (Kim *et al*., 2011), declared the beneficial effects of garlic extract on diabetes and hyperlipidemia in rats fed a high-fat diet. They suggest that, the unsaturated side chains of garlic oil might have oxidized the reduced pyridine nucleotide which are necessary for fatty acid synthesis or might have inactive thiol grouping. It was found that olive oil improve lipid profiles and blood glucose in type-2 diabetic patients through the effect of monounsaturated fatty acids (MUFA), the minor components of virgin olive oil which prevents central fat redistribution and the postprandial decrease in peripheral adiponectin gene expression and insulin resistance induced by a carbohydrate-rich diet in insulin-resistant subjects (Al Jamal and Ibrahim 2011). The obtained results also indicate that curcumin garlic extract and olive oil supplementation were increased the serum NO level. This was matching with the study of (Patumraj *et al*., 2006), who showed that curcumin can increase serum NO level in diabetic rats. Also curcumin could significantly increase sodium nitroprusside SNP-induced vasodilatation in diabetes and could enhance smooth muscle cell relaxation when activated by NO donor. Other study of (Ginter and Smiko 2010), indicate the effect of garlic extract for improving serum NO level in rats with cardiovascular diseases. As garlic-derived organic polysulfides are converted by erythrocytes into hydrogen sulfide gas (H_2S), which has been shown to relax vascular smooth muscle, induce vasodilation of isolated blood vessels and reduce blood pressure. Moreover, the study of (Song *et al*., 2013) showed that olive oil improve NO level in serum of patients with hypertriglyceridemia through the reaction of oleic acid with free radical $NO^{\bullet}$ - and nitrite (NO^{-}_{2})-derived species yields nitrated oleic acid. Although the mechanisms of biological fatty acid nitration remain incompletely characterized, recent studies reveal that during oleic acid nitration, vinyl nitro regioisomers represent a component that displays distinctive chemical reactivity and receptor-dependent signaling actions. Regarding serum adiponectin level similar results was reported by (Wongcharoen and Phrommintikul 2009), who showed that, curcmiun improve serum adiponectin level in hyperlipidemic rats as curcumin inhibits the independent mitogen activated protein kinase (MAPK) pathways which are the pathways activated by most inflammatory stimulation. Also, (Amitai *et al*., 2013), showed that, garlic improve serum adiponectin level in spontaneously hypertensive rats treated for 6 weeks with a daily dose of 80 mg/kg/day of garlic that

is mediated via up-regulation of cellular glutathione levels in vascular endothelial cells and possibly preventing or remedying endothelial dysfunction. Moreover, (Hafida *et al*., 2013) reported that olive oil increases serum adiponectin level in obese rats due to reduced fat mass could be explained mechanistically by increased β-oxidation and reduced lipogenesis in adipose tissue. It was confirmed that curcumin, garlic and olive oil to have anti-inflammatory effect and lower levels of hs-CRP and Interleukin-6 in obese rats and this suggestion was supported by the finding of (Juha *et al*., 2007) who revealed that curcumin lowers serum hs-CRP and Interleukin-6 levels in obese individuals. They explain the rule of curcumin that, down regulates the expression of the NF-κB-regulated gene products such as tumor necrosis factor (TNF), interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8), macrophage interferon protein-1α (MIP-1α), adhesion molecules, C-reactive protein (CRP). Also (Diego *et al*., 2013) showed that garlic lowers hs-CRP and Interleukin-6 levels in serum of patients with hyperlipidemia. The 1,2-vinyldithiin (1,2-DT), a garlic-derived organosulfur compound decrease in peroxisome proliferator-activated receptor γ2 (PPARγ2) expression is associated with reduced peroxisome proliferator-activated receptor-γ (PPARγ) activity, suggesting that the negative effect of 1,2-DT on preadipocytes differentiation could be mainly due to an inhibitory effect on peroxisome proliferator-activated receptor-γ (PPARγ), the master regulator of adipogenesis. The role of these mechanisms of action of 1, 2-DT in the beneficial effects of garlic extract increasing the levels of adiponectin. However, (Sarda *et al*., 2012) investigate the effect of olive oil on serum hs-CRP and Interleukin-6 levels in subjects at high cardiovascular risk. They attributed the effect of olive oil to the components such as phenolic compounds, α-tocopherol, and carotenoids and to the high unsaturated/saturated fatty acid ratio with oleic acid (MUFA) as its main fatty acid. The obtained results revealed that curcumin garlic extract and olive oil supplementations increased serum level of endothelin-1 level and our results were similar to the study of (Chiu *et al*., 2009), who showed that, curcumin could improve serum endothelin-1 level in diabetic rats as vasoactive factors such as ET-1 that may act as an upstream mediator of fibronectin (FN) expression in diabetes by transcription factor NF-κB. It has been demonstrated that curcumin exerts one of its beneficial effects through the inhibition of NF-κB. Moreover (Rahman, 2003) showed that, garlic improve serum endothelin-1 level in serum of hyperlipidemic patients. He declared that, garlic extract and its main constituents have been reported to scavenge the t-butyl hydroperoxide radical and hence prevent lipid peroxidation of liver microsomes. Furthermore, (Pontiroli *et al*., 2004), showed the effect of olive oil for improving serum Endothelin-1 level in morbidly obese subjects. They explained that, olive oil might modulate the production of endothelin-1 (ET-1) and nitric oxide by arterial vessel, limiting the development of atheroma plaques. Moreover, they reported that eicosapentaenoic acid inhibited the ET-1 production stimulated by oxidized LDL.

Conclusion and recommendation

In conclusion, the present study demonstrated that, curcumin, garlic extract and olive oil supplementations showed positive effects on lipid profile, blood glucose level and serum inflammatory markers that may be developed by hyperlipidemia and diabetes. The antioxidant effects of curcumin, garlic and olive oil have been shown to attenuate Streptozotocin-induced diabetes and may prevent diabetic cardiovascular complications. Also, the present study demonstrated that curcumin, garlic and olive oil are potent vasorelaxants as well as

reduce the atherogenic properties of cholesterol. So we recommended that, curcumin, garlic extract and olive oil are useful in treatment of hyperlipidemia, cardiovascular disorders and insulin resistance. It must be used carefully and under medical supervision to get its therapeutic benefits and avoid their side effects.

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التأثير الكيميائي الحيوي للكركمين وخلاصة الثوم وزيت الزيتون على زيادة دهون الدم المحدث فى الفئران

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الملخص العربي

تعتبر السمنة الناتجة من ارتفاع نسبة الدهون فى الدم من أخطر الأمراض التى تنتشر فى الأيام الحالية نظرا لما تسببه من أمراض للقلب مثل تصلب الشرايين والجلطات وارتفاع ضغط الدم والإصابة بمرض السكر وذلك مع التغير الحادث فى النظام الحالى للحياة من زيادة استهلاك الوجبات الدسمة التى تحتوي على نسبة عالية من الدهون والتى بدورها تسبب ارتفاع نسبة الكوليستيرول فى الدم مع غياب الإقبال على ممارسة الرياضة التى من الممكن أن تقلل من التأثير الحادث لهذا النظام الغذائي المضر بصحة الإنسان. لذلك استهدفت هذه الدراسة إلى إظهار التحولات البيوكيميائية المحتملة التى قد تنتج عن المعالجة المستمرة بالكركمين وخلاصة الثوم وزيت الزيتون للفئران التى تمت زيادة الدهون فى دمها تجريبيا عن طريق تغذيتها على مدى طويل بعليقه غنية بالدهون. وتم إجراء الدراسة على عدد (80) ثمانين من إناث الفئران البيضاء والتى تتراوح أعمارها بين شهر وشهر ونصف وأوزانها بين (140-160 جرام) ووضعت فى أقفاص حديدية مفصولة وتعايشت فى نفس الظروف البيئية وظروف التربية والتغذية لمدة أسبوع قبل بدء التجربة حيث تم تغذيتها على نفس نوع العليقة دون تمييز. قسمت الفئران إلى ثمانى مجموعات كل منها مكونة من (10) فئران قسمت إلى: مجموعة ضابطة سلبية، مجموعة العليقة مع الكرمين، مجموعة العليقة مع خلاصة الثوم، مجموعة العليقة مع زيت الزيتون، مجموعة ضابطة إيجابية، مجموعة العليقة الغنية بالدهون مع الكرمين، مجموعة العليقة الغنية بالدهون مع خلاصة الثوم، مجموعة العليقة الغنية بالدهون مع زيت الزيتون. أظهرت النتائج تأثير التجريع بالكركمين والثوم وزيت الزيتون تحسن مستوى البروتينات الدهنية عالية الكثافة وأكسيد النيتريك والأديبونيكتين والإندوثيلين-1، فيما أظهرت نقصا فى مستوى الكوليستيرول الكلى والدهون الثلاثية والبرتينات الدهنية منخفضة الكثافة والسكر الصائم والهيموجلوبين السكرى وبروتين سي النشط عالي الحساسية والإنترلوكين-6. لذا ننصح باستخدام الكركمين وخلاصة الثوم وزيت الزيتون لتخفيض مستوى الدهون والسكر ولتفادى أخطار الإصابة بأمراض القلب والشرايين والسكرى الناتجة عن زيادة الدهون في الدم.

(مجلة بنها للعلوم الطبية البيطرية: عدد 26(2):109- 118, يونيو 2014)