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Biochemical effects of Resveratrol and Curcumin combination on obese diabetic rats

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Abstract

Obesity is a global health problem associated with various metabolic disorders as type-2 diabetes, cardiovascular diseases, and oxidative stress. Therefore, utilization of nontoxic natural products represents a logical preventive and/or therapeutic approach for it. The aim of the present article is to evaluate anti-obesity and antioxidant effects of curcumin (50 mg/kg.b.w) and resveratrol (25 mg/kg.b.w) and their combinatorial formulation in obese diabetic rats feed high fat diet (HFD) for 8 consecutive weeks prior. The results of this study also showed that curcumin and resveratrol, both individually and in combination showed antioxidant and anti-obesity actions in experimental diabetic rats for 8 consecutive weeks prior. Indeed, them combination was significantly prevented the increase in the levels of body weight gain, blood glucose, triacylglycerols, total cholesterol, LDL-C, HDL-C, free fatty acids, atherogenic index, thiobarbituric acid reactive substances (TBARS) in the liver tissue and improved the insulin resistance index, reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR) and superoxide dismutase (SOD) when compared to the high fat diet (HFD) control than either agent alone. The results clearly suggest that the combination of dietary curcumin and resveratrol produced a higher anti-obesity, anti-atherogenic, anti-diabetic and antioxidant activities on experimental obese diabetic rats than their individual influences.

Keywords: Curcumin; resveratrol; anti-obesity; type 2 diabetes mellitus; glucose; insulin resistance index; atherogenic index.

Introduction

There is a worldwide epidemic of obesity, which is associated with a number of pathologies including dyslipidemia, glucose intolerance, insulin resistance and diabetes mellitus, all of which are risk factors for cardiovascular disease and mortality (Eckel et al., 2004). Obesity, type 2 diabetes, hypertension, and dyslipidemia are closely linked to insulin resistance,

and this cluster of diseases is called metabolic syndrome. The chronic inflammation observed in obesity has been reported in the development of atherosclerosis, another proinflammatory disease (Pongchaidecha et al., 2009). Polyphenols have been reported to possess various pharmacological actions, including anti-obesity (Ohta et al., 2006), antidiabetic (Ruzaidi et al., 2005) and anti-cancer (Peng et al., 2006). Among these polyphenols; curcumin and resveratrol. The polyphenolic substance, curcumin (diferuloylmethane), is the major yellow pigment extracted from turmeric, the powdered rhizome of the herb, *Curcuma longa*. Turmeric is extensively used as a spice and also as a food preservative in the Far East (Grant and Schneider, 2000). Curcumin exhibits multiple anticarcinogenic effects and its role in the chemoprevention of cancer is being assessed in clinical trials (Leu and Maa, 2002). It has also been shown to be protective in rat models of diverse diseases such as atherosclerosis, ischemia-reperfusion injury, cystic fibrosis (Egan et al., 2004) and diabetes mellitus (Murase et al., 2002). Sharma et al., (2006) suggested that curcumin has potential in the prevention and treatment of obesity, diabetes, atherosclerosis, and metabolic syndrome and it has been reported to modulate numerous targets that have been linked to obesity and insulin resistance. Resveratrol (3, 5, 4- trihydroxystilbene), have been getting increasing attention lately, as one of the phytochemicals found particularly in grapes and mulberries. It is involved in numerous cellular responses including cell cycle arrest, apoptosis and differentiation, and has anti-inflammatory, anticancer, anti-oxidant properties (Brisdelli et al., 2009). On the other hand, implications for resveratrol in obesity have discovered recently and there are limited studies showing anti-obesity effects of resveratrol in vivo. A dietary supplementation of mice with 400 mg/kg/day in high fat diets increased their resistance to obesity by causing diminished total body fat content and decreasing depots of epididymal, inguinal and retroperitoneal white adipose tissue (Lagouge et al., 2006). Although there are reports of synergy between resveratrol and other phytochemicals in vitro there is no previous evidence for synergy *in vivo* (Lai et al., 2011). There are no reports of the anti-obesity of curcumin when combined with resveratrol in one formula in obese diabetic rats fed high-fat diet. Our interested research program to explain the anti-obesity, anti-diabetic and antioxidant effects of curcumin and resveratrol in the form of combinatorial formulation in obese diabetic rats fed high fat diet, which may pave the way for possible therapeutic application.

Materials and Methods

Chemicals

Curcumin (1,7-bis[4-hydroxy-3-methoxyphenyl]-1,6-heptadiene-3,5-dione), SB203580, a p38 MAPK inhibitor, were obtained from BIOMOL International LP (Butler Pike, PA). Resveratrol was purchased from Nutrabio Co. (USA). All other chemicals used in this study were of the analytical grade, preserved under standard situation and were provided from standard commercial suppliers.

Experimental design

This experiment was conducted in accordance with guidelines established by the Animal Care and Use Committee of October 6th University. Adult albino rats weighing around 200 ± 20 gms were purchased from Faculty of Veterinary Medicine, Cairo University. They were individually housed in cages in an air-conditioned room with a temperature of 22±2 ^{o}C,

a relative humidity of 60%, and an 8:00 to 20:00 light cycle. During the acclimatization period, each animal was raised on a regular diet (Dyets Inc., Bethlehem, PA) *ad libitum*.

A suspended solution of 3g % of curcumin, resveratrol and their combinatorial were prepared for intragastric intubation of rats. The animals were randomly divided into five groups 8 rats in each, two controls groups and four treatment groups. Control group-I: (was received a regular diet + 1 ml tween 80 for 8-week period). Control group-II: (was received a high fat diet + 1ml tween 80 for 8-week period). Group III: Was fed a high-fat diet with curcumin (50 mg/kg bw/ml tween 80) suspended in tween 80 orally in a single daily dose for an 8-week period. Group IV: Was fed a high-fat diet with resveratrol (25 mg/kg bw/ml tween 80) suspended in tween 80 orally in a single daily dose for an 8-week period (Majid et al., 2011). Group V: Was fed a high-fat diet with curcumin (50 mg/kg bw/ml tween 80) + resveratrol (25 mg/kg bw/ml tween 80) suspended in tween 80 orally in a single daily dose for 8-week period.

Hyperglycemia induction diet was purchased from Dyets Inc. (diet, Bethlehem, PA, USA). The nutrition contents of the high fat diet were similar to those of the regular diet except for the addition of 200 g of fat/kg and 1% (w/w) cholesterol (Assinewe et al., 2003). Body weights were measured weekly, and every other week, blood was collected for blood glucose analysis. At the end of the study, blood was also collected for the determination of plasma insulin, insulin resistance index, atherogenic index and lipid levels. In addition, *in vivo* antioxidant and lipid peroxidation parameters in tissue of liver after which they were killed.

Blood sampling and Biochemical assays

Blood was withdrawn from the orbital venous plexus every other week, using a heaprinized capillary tube without anesthesia. The blood samples, centrifuged, and plasma was used freshly for estimation of plasma glucose (Assinewe et al., 2003). Homeostatic index of insulin resistance (HOMA-IR), calculated by glucose (mM) x insulin (mU/ml)/22.5(Sasaki et al., 1972). Triacylglycerols (Matthews et al., 1985), total cholesterol (Fossati and Prencipe, 1982), HDL- cholesterol (Allain et al., 1974) and free fatty acid concentrations (Burnstein et al., 1970), LDL-cholesterol (Falholt et al., 1973) formula (LDL-cholesterol = total cholesterol – triacylglycerols/5 – HDL-cholesterol). VLDL-cholesterol concentration (Friedewald, 1973) formula (vLDL-cholesterol = triacylglycerols/5). The atherogenic index [log (TG/ HDL-C)] was also calculated (Dobiasova and Frohlich, 2001). Then after ethanol anesthesia all rats were surfeited and the liver were removed directly used for estimation of triacylglycerols and cholesterol content were measured as described previously (Han et al., 1999 and Park et al., 2005). Briefly, a portion (100 mg) of liver tissue was homogenized in phosphate buffer saline (pH 7.4, 1 ml). The homogenate (0.2 ml) was extracted with isopropyl alcohol (1 ml), and the extract was analyzed using a Triacylglycerols E-Test (Wako Pure Chemical Industries) to determine liver triacylglycerols content. The homogenate (0.2 ml) was extracted with chloroform methanol (2: 1, 1 ml), and the extract was concentrated under a nitrogen stream. The residue was dissolved in isopropyl alcohol and analyzed using a Cholesterol E-Test (Folch et al., 1957). Finally, another portion from liver was blotted, weighed and homogenized with methanol (3 volumes). The lipid extract obtained by the method of Folch et al. (1957). It was used for the estimation of TBARS (Nichans and Samulelson, 1968). Another

Table 1. Changes in body weight of control and experimental groups of rats.

Groups	Numbers of weeks (Body weights of rats in g)				
	0	2	4	6	8
Control group-I Regular diet (RD)	220.4±5.8	232.5±7.3	240.2±10.5	252.9±9.8	260.3±11.4
Control group-II High-fat diet (HFD)	222.7±6.5	260.6±10.3**	274.8±5.8**	296.3**±7.9	310.2**±8.4
HFD+ Curcumin	218.5±11.5	252.4±8.6*	260.7±120.4*	270.6*± 10.2	279.4*±7.5
HFD + Resveratrol	223.6± 8.4	240.6±9.3*	250.5±6.5*	258.4*±7.9	265.9*±6.8
HFD + Curcumin + Resveratrol	215.6±9.2	235.5±6.6**	246.8± 10.4**	251.4±8.1**	258.4±5.6**

*Significantly different from control group at $p< 0.05$. **Significantly different from control group at $p< 0.01$.

Table 2. Effect of curcumin, resveratrol and their combinatorial formulation on weight gain, food intake and feed efficiency.

Groups	Initial	Final	Weight gain (g/8 wk)	Food intake (g/8 wk)	Feed efficiency ($\times 10^{-3}$)
Control group-I Regular diet (RD)	220.4±5.8	260.3±11.4	39.9±4.6	11545	3.46
Control group-II High-fat diet (HFD)	222.7±6.5	310.2±8.4	87.5±6.6	14265	6.13**
HFD+ Curcumin	218.5±11.5	279.4±7.5	60.9±5.2	13635	4.46*
HFD + Resveratrol	223.6±8.4	265.9±6.8	42.3±4.7	12870	3.29**
HFD + Curcumin + Resveratrol	215.6±9.2	258.4 ± 5.6	42.8±6.3	13155	3.25**

*Significantly different from control group at $p< 0.05$; **Significantly different from control group at $p< 0.01$; Feed efficiency = [weight gain (g/8 wk)]/[food intake (g/8 wk)]

portion of the tissues was homogenized with phosphate buffer saline and used for the estimation of GSH (Ellman, 1959), GPx (Rotruck et al., 1973), GR (Horn, 1965) and SOD (Kakkar et al., 1984).

Statistical analysis

The data were analyzed using the one-way analysis of variance (ANOVA) (Roa et al., 1985).

Results

Body weight and food intake were determined once every 2 weeks. The body weight of the normal rats in the regular diet group gradually increased as the rats grew during the 8-week trial (Table 1). Curcumin-resveratrol combination fed rats were significantly reduced despite the even larger increase in food intake compared to the high fat diet control rats (Table 2). Feed efficiency, calculated by weight gain divided by total food intake during the 8-week period, was compared in order to figure out the relationship between food intake and weight gain. Feed efficiency curcumin-resveratrol combination fed group was lower than the value shown for the high fat diet control group and similar to that of regular diet fed group (Table 2).

Table 3. Effect of curcumin, resveratrol and their combinatorial formulation on plasma insulin, plasma glucose and (HOMA-IR).

Groups	Plasma insulin (mU/ml)	Plasma glucose (mM)	Homeostatic insulin resistance index (HOMA-IR)
Control group-I Regular diet (RD)	85.64±5.3	7.1±1.4	27.02
Control group-II High-fat diet (HFD)	156.17±8.6**	13.3±2.1**	92.31**
HFD+ Curcumin	135.77±10.22*	9.8 ±0.9*	59.13**
HFD + Resveratrol	122.51±8.15**	8.2*±1.08	44.64**
HFD + Curcumin + Resveratrol	98.06±6.47**	7.6±1.3**	33.12**

*Significantly different from control group at $p< 0.05$; **Significantly different from control group at $p< 0.01$.

Homeostatic index of insulin resistance (HOMA-IR)

Plasma glucose was determined every other week and was compared between groups in table 3. Plasma glucose levels were barely increased in the regular diet fed control group, while a marked increase after the 8 weeks was observed for rats only fed with the high fat diet. Curcumin-resveratrol combination fed rats showed a significant decrease in blood glucose levels when compared to the high fat diet control group (Table 3). The insulin resistance of the high fat diet control group was higher than that of the regular diet group, while insulin resistance indices of curcumin-resveratrol combination fed rats were significantly reduced when compared to the high fat diet control group.

Plasma lipid levels

Plasma lipid levels in high fat diet fed rats were dramatically increased with compared to the levels in regular diet fed rats except for the HDL-cholesterol level (Table 4). In the high fat diet control group, plasma triacylglycerols (TG), total cholesterol, LDL-cholesterol, vLDL-cholesterol, free fatty acid and total cholesterol (TC) were increased as compared to the regular diet group. Curcumin-resveratrol combination fed rats showed considerably reduced levels of TG, TC, LDL-C, v LDL-C, free fatty acid and also, showed an increased level HDL-C with compared to that in high fat diet fed control group.

Table 4. Effect of curcumin, resveratrol and their combinatorial formulation on plasma TG, TC, HDL-C, LDLC, vLDLC, FFA and atherogenic index.

Groups	TG (mg/dl)	TC (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	vLDL-C (mg/dl)	FFA (mg/dl)	Atherogenic index
Control group-I Regular diet (RD)	80.7±8.6	109.8±104	47.14± 4.57	46.56± 8.35	16.1± 3.25	72.44± 8.24	0.2334± 0.009
Control group-II High-fat diet (HFD)	210.35±14.*	310.47± 16.4**	25.96± 6.1**	241.81± 12.64**	42.7± 5.11**	153.36± 10.4**	0.9086± 0.028**
HFD+ Curcumin	186.92±9.7*	290.76± 15.24 *	35.61± 3.89*	217.77± 15.39*	37.38± 5.82*	120.93± 13.4*	0.7200± 0.021*
HFD + Resveratrol	164.82±14.*	257.82*± 13.61	39.44± 5.38*	185.4± 9.35*	32.96± 6.22*	113.57± 7.89*	0.6210 ±0.019*
HFD + Curcumin + Resveratrol	149.80±10.75**	238.63± 17.09**	45.11± 5.1**	163.56± 11.08**	29.96± 4.65**	97.44 ±9.16**	0.5212± 0.018**

*Significantly different from control group at $p< 0.05$; **Significantly different from control group at $p< 0.01$; Feed efficiency = [weight gain (g/8 wk)]/[food intake (g/8 wk)]

Table 5**.** Effect of curcumin, resveratrol and their combinatorial formulation on liver triacylglycerols and cholesterol content.

Groups	Liver triacylglycerols (mg/100g liver)	Liver cholesterol (mg/ 100g liver)
Control group-I Regular diet (RD)	285.4± 8.63	310.6±13.27
Control group-II High-fat diet (HFD)	436.9±15.28**	562.5±17.4**
HFD+ Curcumin	372.4±12.95**	488.7±14.68**
HFD + Resveratrol	332.7±10.49**	359.26±19.35**
HFD + Curcumin + Resveratrol	310.29±18.04	344.6±13.55**

*Significantly different from control group at $p< 0.05$; **Significantly different from control group at $p< 0.01$.

Table 6. Effect of curcumin, resveratrol and their combinatorial formulation on liver TBARS, GSH, GPx, GR and SOD.

Groups	TBARS (mol of MDA formed /g tissue)	GSH (mg/g tissue	GPx (mg of GSH Consumed /min/mg protein)	GR (mg of GSH consumed/min/mg protein)	SOD (unit / min /mg/protein)
Control group-I Regular diet (RD)	13.32± 2.11	8.14± 1.65	13.22± 2.17	4.15± 1.52	35.11± 4.20
Control group-II High-fat diet (HFD)	24.65± 4.63**	4.22± 0.84**	6.74± 2.33**	2.36± 1.65**	22.90± 3.64**
HFD+ Curcumin	19.26± 3.68*	5.69± 1.49*	8.48*± 1.48	2.85± 1.16*	27.44± 4.06*
HFD + Resveratrol	15.11± 2.77	6.27± 2.53*	9.05± 1.65*	3.25± 1.43*	33.47± 3.48*
HFD + Curcumin + Resveratrol	13.66± 3.18	7.44± 1.82**	9.86± 2.34**	4.10± 1.22**	36.27± 5.16**

*Significantly different from control group at $p< 0.05$; **Significantly different from control group at $p< 0.01$; Feed efficiency = [weight gain (g/8 wk)]/[food intake (g/8 wk)]

Liver triacylglycerols and cholesterol content

Table 5 shows liver lipid content. At the end of administration, liver triacylglycerols and cholesterol levels were significantly higher for the high fat diet fed group when compared to the regular fed group. For high fat diet/ curcumin-resveratrol combination fed rats, triacylglycerols and cholesterol accumulation were significantly suppressed when compared to the high fat diet control group.

Liver oxidative stress biomarkers

Table 6 showed that liver TBARS level was significantly higher and GSH, GPx, GR and SOD were significantly lower for the high fat diet fed group when compared to the regular fed group. For all high fat diet/ curcumin-resveratrol combination fed rats TBARS accumulation was significantly suppressed and Liver GSH, GPx, GR and SOD levels were markedly increased when compared to the high fat diet control group.

Discussion

Obesity is the most common nutritional disorder in the developed world and it is considered a risk factor associated with the development of major human diseases, including cardiovascular disease, diabetes, and cancer. Increased intake of high caloric (energy and fat)

food promotes body fat storage and greater body weight and adiposity in humans and animals (Bray et al., 2002). Over-the-counter remedies based on nutritional supplements are extremely popular, especially with respect to obesity and body composition. Inhibition of the digestion and absorption of dietary fat has been used as targets in obesity treatment (Estadella et al., 2004). The anti-obesity effects of curcumin, resveratrol and their combinatorial were inves-tigated using obese diabetic rats fed high-fat diet as a model of obese type-II diabetes. When fed a high-fat diet, rats develop obesity and type-II diabetes by 12-weeks old and these rats are thus widely used for research into obesity and diabetes (Hayashi and Ito, 2002). Curcumin, resveratrol and their combinatorial were found to significantly suppress increases in body weight, showing anti-obesity actions (Table 1). Plasma glucose and insulin levels were significantly higher for the high-fat diet group than for the regular diet group, and severe type II diabetes was induced. Curcumin, resveratrol and their combinatorial suppressed these incr-eases in plasma glucose and insulin levels. The insulin resistance index, a simpler method to measure insulin sensitivity usually used in clinical and animal studies. This results were came in accordance with the recorded data by Sasaki et al., (2009). HOMA-IR has been suggested as a biomarker to assess insulin resistance and secretion and is a useful clinical index for insulin sensitivity and pancreatic β-cell functions in epidemiological studies. Although HOMA-IR has several limitations in terms of accuracy and reliability, it expresses essentially hepatic insulin resistance as confirmed by Bonora et al., (2005). We also observed curcumin, resveratrol and their combinatorial significantly improved the IRI within high-fat diet group. Although, we cannot provide direct evidence for the effect of tested compounds on insulin release in obese diabetic rats, recently curcumin was reported to enhance insulin release by induction of β -cell electrical activity. This result is similar to the observed data by Best et al., (2007). The combinatorial formulation of curcumin and resveratrol improve IRI more than their individual influences.

Curcumin, resveratrol and their combinatorial were found to significantly suppress increases in plasma lipids content, showing anti-obesity actions (Table 4). Also, lowered fat accumulation, clarifying that the tested compounds suppresses TG, TC, HDL-C, LDL-C, vLDL-C and free fatty acid (Table 4). Most of the reduction in plasma cholesterol occurred in the fraction of LDL, because of apo-B containing lipoprotein fractions are through to be responsible for cholesterol deposition in atherosclerotic plaques, (Schaefer and Asztalos, 2006) this change could be attributed to a reduction in LDL would be advantageous clinically extract had an improving effect on the hypercholesterolemia induced by a high fat diet. Moreover, dietary supplementation of curcumin decreased the plasma cholesterol, free fatty acids and triacylglycerols concentrations in rats as (Kamal-Eldin *et al*. 2000), reported reduced low plasma density lipoprotein and very low-density lipoprotein and total cholesterol concentrations in the livers rats by dietary supplementation of curcumin. Also, Soni and Kuttan, (1992), observed a significant decrease in serum lipid peroxides, an increase in HDL-C and a decrease in total serum cholesterol concentrations upon curcumin administration. Furthermore, feeding the turmeric extract along with saturated fats and cholesterol decreased the plasma cholesterol level and the susceptibility of LDL to oxidation in rabbits. Reduced plasma cholesterol concentrations of curcumin, resveratrol and their combinatorial-fed rats are possibly related to the altered activity of two effective enzymes in cholesterol metabolism, HMG-CoA reductase and cholesterol 7α-hydroxylase. Although the activity of HMG-CoA reductase was not investigated in the current experiment and in other published studies on curcumin and resveratrol, as Babu and Srinivasan, (1997) suggested that a cholesterol-lowering effect

of curcumin could be mediated by the stimulation of hepatic cholesterol-7α-hydroxylase activity. Also, Jung *et al*., (2006) who state that polyphenols decrease liver HMG-CoA reductase activity in type-2 diabetic mice.

In the present study, liver TBARS level was significantly higher in the high fat diet control group, whereas GSH, GPx, GR and SOD levels were lower. Due to hyperglycemia increases oxidative stress through the overproduction of ROS. These ROS contribute to organ injury in systems such as the heart and liver, and oxidative damage is generally increased in diabetes (West, 2000). Curcumin, resveratrol and their combinatorial normalized the antioxidant enzymes activities of liver in high fat diet rats. It is plausible that the tested compounds supplementation seemed to alter these enzymes activities in liver toward maintaining antioxidant homeostasis in high fat diet rats. Several in vivo and in vitro studies have reported that in a variety of cancerous diseases of different body organs, curcumin inhibits lipid peroxidation, and augments the activities of anti-oxidants such as SOD, GSH, GST and GPx (Sharma et al., 2001). The activity of hepatic GSH, GPx, GR and SOD was generally higher as a result of dietary curcumin, resveratrol, and curcumin + resveratrol in high-fat-fed rats. Curcumin has been reported to exert a protective effect against nicotine-induced lung toxicity by modulating the extent of lipid peroxidation and augmenting antioxidant defense system by a significant countering of the decrease in the levels of ascorbic acid, vitamin E, GSH, GPx, SOD, and CAT (Kalpana and Menon, 2004). Thus, the present study, which examined the effect of feeding two hypolipidemic compounds, curcumin and resveratrol, both individually and in combination along with a high-fat diet on lipid profile and antioxidant enzymes in liver has revealed that a high-fat diet compromises the endogenous antioxidant defense mechanisms. Dietary curcumin and resveratrol, which brought about significant anti-obesity influence, were also found to effectively reduce this oxidant stress in hypolipidemic animals as indicated by countering of the depleted antioxidant molecules and decreased activity of antioxidant enzymes. Also, the effect of the combination was certainly more pronounced their individual influences. Anti-obesity of curcumin and resveratrol in combinatorial formulation in obese diabetic rats fed high-fat diet has not been reported earlier to our knowledge and this study is might be the first observation of that kind.

In conclusion, the present study showed that that the combination of dietary curcumin and resveratrol produced a higher anti-obesity anti-atherogenic, anti-diabetic and antioxidant activities on obese diabetic rats than their individual influences.

Conflict of interest

There is no conflict of interest associated with the authors of this paper.

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