Hypolipidemic and antioxidant effects of phytochemical compounds against hepatic steatosis induced by high fat high sucrose diet in rats

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ABSTRACT
The global feeding on Western diet, which is enriched in fat and fructose, has been proposed to be a cause of metabolic disorder, including hepatic steatosis which is associated with oxidative stress. In the present study, we investigated the influence of co-treatment with phytochemical compounds, quercetin (Q), α-coumaric (CA) and berberine (BB) on the hepatic lipid profile and oxidative stress markers of rats fed a HFS diet. Rats fed HFS diet showed hypertriglyceremia, hypercholesterolemia, and alteration of oxidative markers in hepatic tissues and serum and histological changes in liver tissues. Supplementing a HFS diet with CA and BB ameliorates hypertriglyceremia, hypercholesterolemia and oxidative stress biomarkers. However, Q improved the biochemical changes in lipid profile and oxidative stress, but failed to improve the histological changes in liver tissue. In conclusion, the present results evidences indicate that CA and BB could be considered as promising complementary treatments against hepatic steatosis development associated with HFS.

Key words: Liver; High fat high sucrose diet; Dyslipidemia; Oxidative stress; Phytochemical compounds.

1. INTRODUCTION
Energy balance in the body is controlled by a number of overlapping systems influencing both caloric intake and energy expenditure. It is well known that consumption both of a high-fat diet and/or high-sucrose diet may alter the homeostatic regulation of energy balance and cause obesity as well as some features of metabolic syndrome [1-4]. Obesity, diabetes and associated medical conditions have been increasing over the years, concomitantly with increased availability and consumption of hypercaloric and more palatable foods [5-7]. Moreover, the increased prevalence of excessive visceral obesity is associated with various diseases, particularly obesity-related cardiovascular diseases, diabetes mellitus type 2 and certain types of cancers [8]. In particular, increasing amount of fat in the diet have been shown to be associated with the risk of obesity and hyperlipidemia in human and rodents by altering total cholesterol (TC) and triglyceride (TG) levels in plasma and tissues. Recent evidence suggests that oxidative stress may be the mechanistic link between obesity and related complications. Hyperlipidemia enhances the risk of coronary heart disease and non-alcoholic fatty liver disease (NAFLD) which is associated with reactive oxygen species (ROS) formation [9].

Non-alcoholic fatty liver disease is the most common cause of chronic liver disease and encompasses a number of diseases, from steatosis (lipid deposition) and non-alcoholic steatohepatitis to cirrhosis (fibrosis) and liver failure [10].
Anatomically, steatosis can take one of two forms depending on the size of the lipid vesicles: microvesicular steatosis is the condition in which fat is stored in multiple small vesicles within the hepatocyte cytoplasm, whereas macrovesicular steatosis refers to the condition in which fat is stored in a single large vesicle [11]. Mitochondrial dysfunction and hyperglycaemia seem to be involved in NAFLD development through oxidative stress. At the beginning of steatosis, accelerated fatty acid catabolism causes excessive electron flux in the electron transport chain and ROS overproduction. The resulting oxidative stress alters mitochondrial morphology and function, thereby further increasing ROS generation [12]. Moreover, inadequacy of antioxidant defenses probably due to lower intake of antioxidant- and phytochemical-rich foods [13].

Many important bioactive compounds have been discovered from natural sources using bioactivity-directed fractionation and isolation [14]. Quercetin is an important dietary flavonoid found in red onions, apples, berries, citrus fruits, and tea [15]. Quercetin reduced systolic blood pressure in hypertensive human participants and in animal models of hypertension [16, 17], reduced serum TG and TC concentrations in high-fat diet-fed rabbits after 12 wk of treatment [18], and reduced body weight, plasma concentrations of TG, TC, and insulin in obese Zucker rats [19]. Coumaric acids are organic compounds that are hydroxy derivatives of cinnamic acid. They have three isomers: o-coumaric acid (CA), m-coumaric acid, and p-coumaric acid, which differ in the position of the hydroxy substitution of the phenyl group [20, 21]. Anti-adipogenic effect of CA appears to be mediated through the downregulated expression of adipogenic transcription factors (PPARγ and C/EBPα) and adipocyte-specific proteins (leptin), suppresses dyslipidemia, hepatosteatosis and oxidative stress in obese rats [22]. Alkaloid berberine (BB) is another type of phytochemicals has been reported to possess anti-obesity properties [23]. The inhibitory effects of BB on chemically induced cytotoxicity, lipid peroxidation, and oxidative stress in the liver has been reported [24, 25].

2. MATERIALS AND METHODS

2.1. Chemicals
Quercetin, o-coumaric acid, berberine chloride, DMSO, Folin-Ciocalteu, 5,5′-dithiobiobis-(2-nitro-benzoic) acid (DTNB), sodium dodecyl sulphate, thiobarbituric acid, epinephrine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of the highest purity commercially available.

2.2. Animals and experimental design
Fifty adult six weeks Wistar rats (80–120 g) were purchased from the animal house of Faculty of Medicine, Assiut University, Assiut, Egypt. Rats were housed in cages in the animal house of Zoology department, Faculty of Science, Assiut University. Rats were housed in cages and were kept in the room temperature about 30°C with normal light/dark cycle. They were allowed to acclimatize for one week before the experiment and they had ad libitum access to diet and water. All experiments followed protocols approved by the Institutional Animal Care and Life Committee, Assiut University.

After one week of acclimatization, rats were randomly divided into 2 main groups, control of 10 rats with standard diet (SD, 80% carbohydrates, 18% proteins and 2% fats) and 40 rats with high fat high sucrose diet (HFS, 55% chow diet, 15% beef tallow, 10% sucrose, 5% roasted peanut, 5% egg, 5% milk powder, 3% sesame oil and 2% NaCl salt) plus 10% sucrose in drinking water. After six weeks of feeding, the 40 rats were subdivided into four groups. The first group (HFS) was left as positive control group. The other three groups were daily treated orally for six weeks with Q (50 mg/kg b.w), CA (75 mg/kg b.w) were dissolved in 20% DMSO and BB (50 mg/kg b.w) dissolved in pre-warmed saline solution.

2.3. Collection and preparation of samples
Rats of different groups were killed by cervical dislocation after anesthesia with ether, liver was quickly removed and small piece fixed in 10% neutral buffered formalin for histopathological investigations. The other part was first frozen with liquid nitrogen then stored at -20°C to be used for biochemical studies. 10% w/v homogenates in 0.1
M phosphate buffer (pH 7.4) were prepared using IKA Yellow line DI homogenizer (18 Dispenser, Germany). The homogenates were centrifuged at 6000 rpm for 1 h at 4°C and the supernatant cytosols were kept frozen at -20°C for the subsequent biochemical assays.

2.4. Serum lipid profile

Serum TG, TC and high-density lipoprotein cholesterol (HDL–C) were determined using commercially available reagent kits (Egyptian Company for Biotechnology (S.A.E), Cairo, Egypt). In briefly, serum TG was determined enzymatically [26], TC was determined by a modification of the cholesterol oxidase method [27], HDL–C was determined by the precipitation method [28], and low-density lipoprotein cholesterol (LDL–C) was calculated as LDL = TC - (TG/5 + HDL) [29].

2.5. Histopathological examination.

Specimens from the liver were taken from all rat groups directly after scarification. They were fixed in 10% neutral buffer formalin, embedded in paraffin, sectioned at 5 microns and stained with hematoxylin and eosin stain (H&E). The sections were examined using the light microscope [30]. Stained sections were examined under light microscope (Olympus CX31, Japan) and photographed using a digital camera (Olympus, Camedia C-5060, Japan).

2.6. Biochemical determination

The total protein content in liver and serum was determined colorimetrically using the method of Lowry et al. [31]. Serum and hepatic malondehydride (MDA) levels as nmol/mg protein were estimated by the method of Ohkawa et al., [32] using thiobarbituric acid (TBA) which reacts with MDA to form a stable pink color. Serum and hepatic glutathione (GSH) levels as μg/mg were measured according to Beutler et al. [33] based on the reduction of DTNB with GSH to produce a yellow compound (reduced chromogen) that is directly proportional to GSH concentration. Super oxide dismutase (SOD) activity (Units/mg protein) was assessed by Misra and Fridovich [34] which is based on the inhibition of the autoxidation of epinephrine at alkaline medium. Catalase activity (CAT) as Units/mg protein was determined according to the procedure of Lück [35], based on its ability to decompose hydrogen peroxide.

2.7. Statistical analysis

The results were analysed using one way analysis of variance (ANOVA) followed by Newman-Keuls Multiple Comparison Test and graph-pad prism program for windows, version 3.0 (Graph pad software, Inc, San Diago CA. USA). Levels of significance between the groups were accepted at p < 0.05, 0.01 or 0.001, and the data were expressed as mean ± Standard error (SE).

3. RESULTS

Figure 1 shows the effects of HFS diet on serum lipid profile and the role of different treatments in modulation of these effects. High fat high sucrose diet (HFS)-fed rats showed a significant increase of serum LDL, TG and TC and a significant decrease of HDL. Treatment with CA and BB displayed a significant decrease of serum LDL, TG and TC and a significant increase of HDL, while treatment with Q modulated the changes of serum TG and TC.

Figure 2 shows that the concentration of LPO as TBARS (nmol MDA/mg protein) was increased in hepatic tissue and serum of HFS-fed rats but it was significantly increased in hepatic tissue. Treatment with Q, CA and BB showed a significant decrease of its concentration. In HFS-fed rats, a significant decrease in the activities of SOD and CAT was found in the hepatic tissue compared with that of control group fed on SD but it was no change in serum levels. Also, all treatments showed a significant recovery of SOD and CAT activities. Moreover, figure 2 shows no significant changes in hepatic and serum GSH in HFS-fed rats but it was significantly decreased in serum of HFS-fed rats treated with CA as compared with HFS-fed rats.

Figure 3 shows the histological changes in liver tissues from rats fed SD and HFS diets. As revealed by staining with H&E, we found that, in the livers of rats fed with SD, had no marked abnormalities (Fig. 3A), while those fed HFS (Fig. 3B) showed microvesicular steatosis characterized by variably enlarged hepatocytes with very fine fat vacuoles and macrovesicular
steatosis with well-defined fat vacuoles. It was surprising that treatment with Q exhibited macro-
vesicular steatosis with well-defined fat vacuoles (Fig. 3C).

Figure 1. High fat high sucrose diet (HFS)-fed rats displayed an increase of serum low density lipoproteins (LDL), triglycerides (TG) and total cholesterol (TC) and low level of high density lipoproteins (HDL). Treatment with quercetin (Q), o-coumaric acid (CA) and berberine (BB) modulate these changes. Data represent the mean ± SE. a: represent the significant difference between HFS group and control. b: represent the significant difference between treated groups and HFS group.

Figure 2. High fat high sucrose diet (HFS)-fed rats displayed an increase of hepatic and serum lipid peroxidation (LPO) and decrease of SOD and CAT activities in hepatic tissue. Treatment with quercetin (Q), o-coumaric acid (CA) and berberine (BB) modulate these changes. Data represent the mean ± SE. a: represent the significant difference between HFS group and control. b: represent the significant difference between treated groups and HFS group.
Treatment with CA revealed no marked changes in comparison to the other treatments (Fig. 3D). On the other hand, treatment with BB showed less changes represented by microvesicular steatosis as shown in (Fig.3E).

4. DISCUSSION

Obesity is a strong risk factor for developing dyslipidemia [36, 37] and fatty liver which can later progress to nonalcoholic fatty liver disease [38]. Nonalcoholic fatty liver disease (NAFLD) is usually caused by two hits, the first is induced by peripheral insulin resistance causing hepatic steatosis and the second is thought to be caused by reactive oxygen species inducing oxidative injury leading to fibrosis [39]. The model used in this study mimics Egyptian diets, which consist mostly of increased carbohydrate and/or fat intake. According to the literature, both high-fat and high-fructose regimens may cause metabolic disorders in rats [40]. In the present study, HFS diet resulted in metabolic disorder represented by changes in lipid profile as proven by increasing serum levels of TG,
TC and LDL and low level of HDL [41]. These findings may be due to high fat from beef tallow induced hypercholesterolemia. Moreover, dietary catabolism [42]. In the current experiment, treatment of rats fed HFS diet with Q, CA, and BB modulated the previous alterations in lipid profile. In the same aspect, Yeh et al., [43] found that CA treatment of rats fed with 3% cholesterol for 6 weeks caused significant decrease in plasma lipids and hepatic TC. Quercetin reduced insulin resistance, dyslipidemia, and hypertension in the experimental model of metabolic syndrome of obese Zucker rats [19]. Also, BB treatment of high fat diet induced obesity in mice resulted in decrease of serum TG and TC and concluded that BB had excellent potential as an effective anti-obesity agent with no obvious toxicity [44].

Lipid alterations have been considered as contributory factors to oxidative stress in obesity [45]. Increased production of ROS as well as reduced antioxidant defense mechanisms have been suggested to play a role in both humans and animal models of obesity [46]. Epidemiological data in conjunction with in vitro studies strongly indicate that foods containing phytochemicals have strong protective effects against major disease risks including cancer, diabetes and cardiovascular diseases due to their antioxidant potential [47]. In the present study, increased levels of hepatic and serum lipid peroxidation by-products were observed in the HFS-fed rats. This result was in accordance to Yamamoto and Oue [16, 48]. Noeman et al. found that high fat-induced obesity is accompanied by increased hepatic oxidative stress [49]. Lipid peroxidation by-products produced by the fatty liver caused cell damage by impairing membrane structure and function [50]. Oral administration of Q, CA and BB to HFS-fed rats decreased lipid peroxidation by-products level in hepatic tissue and serum. It has been shown that animal body had an effective mechanism to prevent the free radical induced tissue cell damage, this accomplished by a set of endogenous antioxidant such as SOD, CAT and GSH. The present data demonstrate that HFS-fed rats exhibited a significant decrease of hepatic SOD and CAT and non-significant changes in GSH content.

Supplementation of HFS-fed rats with Q, CA and BB recovered this decrease. Decrease of sucrose significantly produced hypertriglyceridemia across rats’ life span that had free food access or due to increased secretion of TG and decreased its antioxidant enzymes may be due to rapid consumption and exhaustion of storage of these enzymes in fighting free radicals.

Hepatic steatosis is a risk factor for liver cirrhosis and atherosclerosis. Both the liver and adipocytes play a major role in the regulation of cellular and circulating serum lipids, predominantly TG and TC [51]. Increased blood glucose due to insulin resistance (data not shown) results in increased release of free fatty acids from adipocytes causing steatosis [52]. Furthermore, peripheral insulin resistance can induce hepatic steatosis, which is characterized by reduced insulin-suppressing effect in hepatic glucose production that contributes to hepatic lipogenesis [53]. Histopathology of HFS-fed rats liver showed features of NAFLD such as microvesicular steatosis characterized by variably enlarged hepatocytes with very fine fat vacuoles and macrovesicular steatosis with well-defined fat vacuoles. This observation was in harmony with the finding of Nascimento et al. [54] mice fed HFS diet supplemented with fish oil. Treatment of HFS-fed rats with CA revealed no marked changes in comparison to the other treatments while BB showed partial prevention of steatosis which was manifested by microvesicular steatosis. Quercetin was not effective as a hepatic lipogenesis reducer. Accumulation of lipid in liver results from the defective triacylglycerol storage in adipose tissue that occurs with lipodystrophy [55], and is a key step in the initiation and progression of insulin resistance [56, 57].

In conclusion, the present study provides evidence that high fat high sucrose diet induce biochemical and histological changes led to hepatic steatosis. Oral supplementation of o-coumaric acid and berberine ameliorate the biochemical changes in the liver especially the hepatic lipid accumulation and oxidative stress markers, but Q ameliorates only the latter. These experimental evidences indicate that o-coumaric acid and berberine could be considered as promising complementary treatments against hepatic steatosis development associated with high fat high sucrose diet.
AUTHORS’ CONTRIBUTION

This paper is part of the Ph.D. of SMMR. HMO, SKAE and THE-M share in conception, design methodology, writing and revision of the manuscript. All authors are involved in drafting the manuscript, read and approved the final of the manuscript.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

REFERENCES


