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Amelioration of Insulin Resistance in Rats Treated with Rice Bran Oil

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ABSTRACT

Background: insulin resistance (IR) is a pathological condition characterized by inadequate peripheral tissue metabolic response to circulating insulin. It plays pathophysiological role in type 2 diabetes mellitus (T2DM). High dosage of fructose in the diet (60 g/100 g diet) may induce insulin resistance accompanied by deleterious metabolic consequences including hyperglycemia and hyperinsulinemia. Rice bran oil (RBO), is a rich source of antioxidants especially γ-oryzanol, α-tocopherols and tocotrienols which contribute to high oxidative stability, longer shelf life than other edible oils and high antioxidant property against free radicals. The present work was undertaken to study if the addition of rice bran oil in rat's diets ameliorate the insulin resistance.

Materials and methods: to achieve this target, plasma fasting glucose, serum insulin and calculated HOMA-IR, which assesse the presence of insulin resistance, was evaluated. Serum lipid profile (cholesterol, triglycerides, high-density lipoprotein- cholesterol (HDL) and low-density lipoprotein-cholesterol (LDL) was also evaluated. In addition, the oxidative stress was assessed through hepatic malondialdehyd (MDA) as an oxidative biomarker and the antioxidant enzyme superoxide dismutase (SOD) was also estimated.

Results: RBO ameliorated HOMA-IR, oxidative biomarker (MDA) and increased SOD activity.

Conclusion: high fructose diet induced oxidative stress which lead to insulin resistance, this was ameliorated by addition of RBO.

Keywords: Insulin resistance, Oxidative stress, Rice Bran Oil

INTRODUCTION

Insulin resistance (IR) is a major underlying mechanism responsible for the 'metabolic syndrome' which is also known insulin resistance syndrome. The incidence of metabolic syndrome is increasing at an alarming rate, due to increase in the consumption of high-fructose corn syrup (HFCS). Consumption of fructose, in the form of added sugars such as high fructose corn syrup or sucrose, has increased markedly in the last few years, which is strongly correlated with the prevalence of metabolic syndrome[(1)]. High dosage of fructose in the diet (60g/100g diet) may induce insulin resistance accompanied by deleterious metabolic consequences including hyperglycemia and hyperinsulinemia[(2)]. Rice bran oil (RBO) is an important product of rice bran extracted during rice processing industry. It is widely popular edible oil in east Asia countries such as China, Korea, Japan, Taiwan and Thailand[(3)]. RBO has mild flavor, high smoke point, good stability and no adverse effect, that make it a suitable alternative for other used oils for industrial and culinary purposes[(3)]. Crude rice bran oil is rich in unsaturated linoleic and oleic fatty acids and bioactive compounds such as γ-oryzanol, phytosterols, tocopherols, and tocotrienols[(4)]. Many studies have shown that, bioactive compounds in RBO have therapeutic effects against diabetes mellitus by reducing oxidative stress[(5)]**.** The present work was undertaken to study if the addition of rice bran oil in rat's diets ameliorate the insulin resistance.

MATERIALS AND METHODS

Experimental Animal Design

A total 50 adult female albino rats weighting 140-220 g were used throughout this study. The animals were purchased from the breeding unit of the Egyptian Organization for Biological Products and Vaccines (Helwan, Egypt). Rats were divided into five groups (10 rats each), housed in steel cages (5/cage) at constant environmental temperature (25°C±5) and humidity (50%±10) with dark and light cycle (12hrs). Animals were maintained for a week on a standard diet, before starting the experiment as an acclimatization period. Food and water were provided *ad libitum*. Animals were allocated to their groups according to the following scheme: **Group I**: **Normal Control (NC)**, this group fed

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standard diet and left intact without any treatment. **Group II**: **Normal control with Rice Bran Oil (RBO),** rats in this group fed standard diet contains rice bran oil as a source of fat. **Group III**: **High Fructose Diet (HFD),** this group was further divided into 2 sub-groups. Rats in each sub- group fed high fructose diet (60%) either for only one month **(HFD1)** or for 2 months **(HFD2)** serving as reference groups for the corresponding treated groups. **Group IV: High Fructose Diet1 + Rice Bran Oil (HFD1+ RBO),** this group fed high fructose diet containing rice bran oil as a source of lipids for one month (prophylactic group). **Group V**: **High Fructose Diet 2 + Rice Bran Oil (HFD2+ RBO),** this group fed high fructose diet for 30 days, and then fed HFD with rice bran oil for another 30 days, (therapeutic group).

Diets preparation: the standard and high fructose (60 g/100 g) diets were prepared as described previously by **Rajasekar *et al.*** [6] while diet containing RBO (10%) was prepared according to **Wang *et al.*** [7]**.**

Biological Evaluation:

Body weights of rats in all groups were recorded weekly throughout the experimental period, and body weight gain was calculated at the end of the feeding period.

Blood Sampling and tissue processing

At the end of the experimental period, rats were weighed then anesthetized with Urithane (99%, Aldrich) at a dose of 1g/kg B. W. intraperitoneally and blood samples were collected from the retro-orbital venous plexus after overnight fasting. Liver was quickly removed and washed with phosphate buffer saline and stored at -20°C in PBS.

Biochemical Assay: Fasting plasma glucose level was determined by the enzymatic colorimetric method of **Sharp** [8]**,** while serum insulin was measured using the method described by **Dhahir *et al.*** [9]**.** Homeostasis model assessment insulin resistance index (HOMA-IR) was calculated according to the formula of **Pickavance *et al.*** [10]**:** HOMA-IR= [Fasting insulin (µIU/ml) × fasting glucose (mmol/L)] ⁄ 22.5. Serum cholesterol and triglycerides were assayed according to the method of **Trinder** [11] and **Bucolo & David** [12] respectivly. Serum high-density lipoprotein-cholesterol (HDL-C) was determined according to the method of **Trinder** [11]. While serum low density lipoprotein-cholesterol (LDL-C) was calculated according to the formula of **Adebayo *et al.*** [13]: LDL-C (mg/dl) = Total cholesterol ─ [HDL-C+ Serum triacylglyceride/5]. Atherogenic index (AI) was calculated also using equation of **Adebayo *et al.*** [13]: Atherogenic index= Total cholesterol/ HDL-C. Tissue homogenate was prepared according to the method of **Nishikimi *et al.*** [14]**.** Exactly 0.5 g of hepatic tissue was weighed and homogenized using automatic homogenizer in 10ml of ice-cold 0.05 mM potassium phosphate buffer solution (pH 7.4) to yield ultimately 5% (w/v) whole liver homogenate. The homogenates were centrifuged at 5000 rpm for 15 min at 4°C then the supernatant was used for determination of malondialdehyde (MDA), superoxide dismutase (SOD). Hepatic malondialdehyde (MDA) concentration and SOD activity were estimated according to methods of **Ohkawa *et al.*** [15] and **Nishikimi *et al.*** [14] respectivly**.**

Statistical analysis

The data are presented as mean ± SE. One way analysis of variance (ANOVA) followed by post- hoc test [least significant difference analysis (LSD)] was performed using the statistical package for social science (SPSS) version 16 to compare all the treated groups .The value of $p \leq 0.05$ was considered statistically significant**.**

RESULTS

Data in table (1) illustrated that the body weight gain was reduced significantly ($p<0.02$) after addition of RBO from the first day (HFD1+RBO), compared to control group. In addition, HFD2 showed significant elevation ($p<0.03$) in body weight gain, compared to HFD1 group.

Table (2) revealed significant elevation ($p<0.001$) in plasma glucose levels in rats fed HFD for one month with percent changes reached 50.33 and 53.4%, compared to NC and RBO groups, respectively. Addition of RBO to the HFD diet improves the plasma glucose level ($p<0.001$), with % change reached 20.9 %, as compared to HFD1. On the other hand rats fed HFD diet for 2 months revealed significant reduction in plasma glucose($p<0.01$), compared to rats fed HFD for only one month.

As regard to serum insulin, high fructose diet induced hyperinsulinemia in rats (HFD1 and HFD2) ($p<0.001$) with % changes reached 96.25 and 188.73%, respectively, compared to normal rats. Moreover, rats fed HFD for 2 months showed highly significant elevation in serum insulin ($p<0.001$), compared to rats fed HFD for one month. RBO treated groups improved

hyperinsulinemia ($p<0.01$ and $p<0.001$, respectively) compared to its respective control groups (HFD1 and HFD2) (table 2).

Table (2) revealed significant elevation in HOMA-IR ($P<0.001$) in both HFD1 and HFD2 with %change reached 139.73 and 220.1% as compared to control group. Rats fed HFD for 2months showed pronounced elevation in HOMA-IR ($P<0.001$) compared to rats fed HFD for only one month. Addition of RBO to the HFD either from the 1st day or after one month on the HFD, HOMA-IR was decreased significantly ($P<0.02$, 0.001) as compared to its respective control group. Rats fed HFD for 2 months showed pronounced elevation in HOMA-IR ($P<0.001$), compared to rats fed HFD for only one month.

Figure (2) showed non-significant differences in serum cholesterol (sCHO) levels in RBO, HFD1, and HFD1+RBO (prophylactic) groups, compared to NC group. However, highly significant increase (79.81and 43.31, % $P<0.001$) in serum cholesterol was recorded in HFD2 and HFD2+RBO (therapeutic) groups, compared to NC group. Feeding high fructose diet for 2 months elevated serum cholesterol level significantly ($P<0.001$), compared to that feeding only for one month. Rice bran oil, however, reduced significant ($P<0.001$) serum cholesterol level as compared to its corresponding control (HFD2) group. On the other hand, serum triglyceride (TG) level in HFD1 was significantly elevated ($P<0.006$) as compared to RBO. While both HFD1, HFD1+RBO were non-significant when compared with NC group. Rats fed fructose for 2 months revealed highly significant elevation in serum triglyceride (TG) level ($P<0.001$) as compared to NC and rats fed fructose for one month. Although the therapeutic group (HFD2+RBO) revealed reduction in serum triglyceride (TG) level, compared to HFD2, but this reduction was statistically non-significant.

Significant elevations were observed in Atherogenic index (AI), in all studied groups except RBO group, compared to control group. Dramatic increase in AI value ($P<0.001$) was observed in rats fed HFD for 2months, compared to rats fed for only one month. Significant reduction in AI ($P<0.001$) was recorded in therapeutic group, compared to HFD2 (table 2).

Table (2) revealed significant reductions (23.25, 17.49 and 35.10%, $P<0.004$, $P<0.027$ and $P<0.001$, respectively) in serum HDL-C levels in HFD1, HFD1+RBO (prophylactic) and HFD2 groups, respectively, compared to NC group. Both HFD1and HFD1+RBO (prophylactic) groups exhibited significant reduction in HDL-C level ($P<0.001$and $P<0.002$, respectively), compared to RBO group. HFD2+RBO (therapeutic) group revealed highly significant elevation ($P<0.001$) in serum HDL-C level as compared to HFD2 group. Serum levels of (LDL-C) were significantly elevated in all studied groups except RBO, compared to NC group. In addition, highly significant elevation was observed in HFD1, prophylactic and therapeutic groups ($P<0.031$, $P<0.004$ & $P<0.001$, respectively), compared to RBO group. Rats in HFD2 group showed remarkable elevations ($P<0.001$), in serum LDL-C, compared to HFD1 and HFD2+RBO.

Significant elevation ($P<0.001$) in hepatic MDA was observed in HFD1 (46.58%), compared to NC group. Addition of RBO to the HFD reduced the concentration of MDA ($P<0.001$), compared to HFD1. Rats fed HFD for 2 months reduced the hepatic MDA concentration ($P<0.002$), compared to rats fed HFD for one month. Moreover, addition of RBO after feeding HFD for one month reduced the hepatic MDA significantly ($P<0.02$), compared to rats fed HFD for 2 months (table 3).

Unlikely, hepatic activity of superoxide dismutase (SOD) was decreased in all experimental groups except RBO, compared to NC group. Addition of RBO to the two diet regimens improved the activity of antioxidant enzyme ($P<0.005$), compared to HFD1. Hepatic SOD was significantly elevated in therapeutic (HFD2+ RBO) group ($P<0.02$), compared to HFD2 (table 3).

DISCUSSION

The development of insulin resistance in high fructose fed rats is well documented in the previous studies [2, 16] and has been established in the present study. The degree of insulin resistance was higher in fructose-fed rats (HFD1and HFD2) as indicated by significant elevation of serum insulin levels and HOMA-IR. HOMA-IR was (5.37) for HFD1 and (7.17) for HFD2 which were higher than the proposed cut-off point (2.29), indicating severe insulin resistance [17]. The present study demonstrated that, RBO reduced the level of insulin and HOMA-IR, compared to normal control and fructose fed rats (HFD1and HFD2). These results are in line with **Abd El-Wahab *et al.*** [18], who reported that supplementation of rice bran oil to fructose-fed rats improves insulin

resistance and reduces lipo- and glucotoxicity. The present work illustrated that, feeding rats with HFD for 4 weeks did not alter serum cholesterol (sCHO) and TG levels, while feeding rats for 8 weeks elevated its level, compared to NC and those fed HFD for one month. The increase in serum cholesterol level in rats fed HFD for 8 weeks may be resulted from the increased LDL-C, since insulin is the main factor that increases the binding of LDL particle to the liver cells [19]. **Bieger *et al.*** [20] have shown that, the increase in blood triglyceride concentration can decrease the number of insulin receptors, thereby reducing insulin sensitivity. **Nandhini *et al.*** [21] reported that, hypertriglyceridemia may be due to reduction of lipoprotein lipase activity which is an important enzyme responsible for hydrolysis of triglyceride, this leads to hypertriglyceridemia. According to the post hoc test, LDL-C and atherogenic index (AI) levels in rats fed HFD for 8 weeks showed highly significant elevation, compared to rats fed the HFD for only 4 weeks. Administration of RBO to rats fed HFD for 8 weeks decreased significantly serum cholesterol as compared to its corresponding control (HFD2). On the other hand, RBO decreased significantly serum LDL-C, while HDL-C was significantly increased as compared to their corresponding controls (HFD2). These results agree with **Abd El-Wahab *et al.*** [18]**.** These reduction effects on cholesterol and LDL-C may be due to the presence of antioxidant phytochemicals present in RBO such as γ-oryzanol, tocopherols and tocotrinols, which prevent the formation of oxidized LDL particles and improve its binding to their receptors, especially after improving the insulin resistance state. In the present study, Fructose loaded rats, displayed an imbalance between peroxidation process and antioxidant system. There was impairment in the antioxidant defense system in rats fed high-fructose diet for 4 and 8 weeks indicated by highly significant reduction in hepatic SOD activity accompanied by significant elevation in hepatic malondialdehyde (MDA), compared to NC group. The current results agree with those of **Hussein *et al.*** [22]and **Abd El-Wahab *et al.*** [18]**.**Moreover, tocotrienols and γ-oryzanol, which are two major antioxidants in RBO, may play important roles against oxidative stress [5,23]. So, it is explained the increase of SOD activity in treated groups with RBO. The present study concluded that, high fructose diet induced oxidative stress due to insulin resistance, which was normalized by addition of RBO.

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Table 1 Statistical significance of body weight gain (%) in the different experimental groups expressed as Mean ± SE**.**

Groups	% change of B.W gain
NC	7.40±1.2
RBO	7.60±1.32
HFD1	6.57±0.87
HFD1+RBO	3.52±1.06[ab]
HFD2	10.34±1.08[c]
HFD2+RBO	9.8±1.35

- a: sig. vs NC, b: sig. vs RBO, c: sig. vs HFD1
- change % from normal control
- LSD at $p<0.05$

Table 2. Plasma glucose (mg/dl), serum insulin (µIU/ml) and HOMA-IR value as well as serum cholesterol (mg/dl), triglyceride (mg/dl), atherogenic index and lipid profile (mg/dl) in different experimental groups expressed as Mean±SE.

Groups parameters	NC	RBO	HFD1	HFD1+RBO	HFD2	HFD2+RBO
Glucose (mg/dl) **% change**	120 ±3.95	117.6±4.372 50.33	180.4±10.13[ab] 18.92	142.7±2.48[abc] 11.83	134.2±4.93[c] 4.59	125.5±7.23
Insulin (mU/L) **% change**	7.46±0.36	8.85±0.36 18.63	14.64±0.83[ab] 96.25	11.79±0.75[abc] 58.04	21.54±1.16[ac] 188.74	11.55±0.73[abd] 54.82
HOMA-IR **% change**	2.24±0.11	2.56±0.13 14.29	5.37±0.56[ab] 139.73	4.14±0.26[abc] 84.82	7.17±0.55[ac] 220.09	3.53±0.26[ad] 57.59
sCHOL (mg/dl) **% change**	54.88±1.99	63.7±4.21 16..07	60.68±3.85 10.57	65.04±2.16 18.51	98.68±6.75[ac] 79.81	78.65±3.47[abd] 43.31
sTG (mg/dl) **% change**	77.73±3.76	67.76±3.87 12.83	96.54±6.31[b] 24.2	71.5±3.11[c] 8.01	131.66±2.5[ac] 69.38	125.66±14.92[ab] 61.66
Atherogenic Index (AI) **% change**	2.26±0.19	2.34±0.20 3.54	3.18±0.28[ab] 40.71	3.15±0.20[ab] 39.38	5.91±0.32[ac] 161.5	3.32±0.26[abd] 46.9
HDL-C **(mg/dl) % change**	25.84±2.29	27.69±1.05 7.16	19.83±1.33[ab] 23.25	21.32±1.41[ab] 17.49	16.77±0.84[a] 35.1	24.31±1.01[cd] 5.92
LDL-C **(mg/dl)% change**	15.31±1.13	17.04±2.28 11.3	26.25±2.90[ab] 71.46	29.47±1.50[ab] 92.49	62.53±4.34[ac] 73.29	34.32±4.03[abd] 124.17

- a: sig. vs NC, b: sig. vs RBO, c: sig. vs HFD1, d: sig. vs HFD2
- change % from normal control
- LSD at $p<0.05$

Table 3: Hepatic malondialdehyde (MDA) (µmol/g) and antioxidant activity of SOD **(U/g)** in different experimental groups expressed as Mean±SE.

Groups parameters	NC	RBO	HFD1	HFD1+RBO	HFD2	HFD2+RBO
MDA **(nmol/g. tissue)** **% change**	53.54± 2.88	47.96±4.61 10.42	78.48±1.99[ab] 46.58	40.34±3.57[c] 24.65	60.92±3.05[c] 13.78	40.94±3.30[d] 23.5
SOD **(U/g tissue)** **% change**	2.27±0.14	2.68±0.26[ab] 18.06	0.66±0.10[ab] 70.93	1.24±0.09[abc] 45.37	0.42±0.08[a] 81.5	0.90±0.08[abd] 60.35

- a: sig. vs NC, b: sig. vs RBO, c: sig. vs HFD1, d: sig. vs HFD2
- change % from normal control
- LSD at $p<0.05$