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Rice Bran Oil Ameliorates Hepatic Insulin Resistance in Fructose Fed-Rats

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ABSTRACT

Background: Insulin resistance is a pathological condition characterized by inadequate peripheral tissue metabolic response to circulating insulin. High dietary fructose causes insulin resistance syndrome, primarily due to simultaneous induction of genes involved in glucose, lipid and mitochondrial oxidative metabolism. Rice bran oil (RBO) is a rich source of antioxidants which contribute to higher oxidative stability and longer shelf life than other edible oils. **Aim of the work**: The current study investigated the effects of the daily intake of RBO on insulin resistant rat liver, as a central organ in carbohydrate metabolism.

Materials and methods: Rats were allocated in 5 groups. Animals in groups 1 and 2 received standard diet and standard diet containing RBO, respectively. Group 3: animals fed high fructose diet (HFD), which was categorized into: rats fed HFD either for one month (HFD1) or for 2 months (HFD2). Group 4, rats were fed HFD containing RBO for one month (HFD1+RBO), while rats in group 5 were fed HFD for 30 days then RBO was added to the diet for another 30 days (HFD2+RBO). **Results and conclusion:** addition of RBO to this model improved insulin sensitivity in liver.

Keywords: insulin resistance, rice bran oil, glycogen.

INTRODUCTION

Insulin resistance is increasing at an alarming rate, becoming a major public and clinical problem worldwide. Insulin resistance is defined as impaired ability of insulin to promote glucose uptake and it exerts its metabolic effects in liver, skeletal muscle and adipose tissue[(1,2)]. Experimental studies in animals documented that the general increase in fructose consumption is correlated with hyperglycemia, dyslipidemia and insulin resistance[(3-5)].

Fructose, a simple sugar found in honey, fruit and high-fructose corn syrup, has a unique metabolism that results in oxidative stress and lipogenesis[(6,7)]. Fructose intake has increased markedly due to the increasing intake of beverages sweetened with sucrose (50% fructose) and high fructose corn syrup (55–90% fructose)[(8)].

Rice bran oil (RBO) is unique among edible oils as a result of its nutritional and functional properties such as γ-oryzanol, phytosterols and tocopherols[(9)]. These bioactive compounds reduce oxidative stress which causes many diseases such as diabetes, cancers and neurodegenerative diseases[(10)]. Several studies have demonstrated that RBO possesses hypoglycemic activity[(5,11)] since chronic exposure to hyperglycemia may induce dysregulation of gene expression that converge on impaired insulin secretion and increased apoptosis[(12)].

This study was designed to monitoring the effects of the daily intake of RBO on insulin resistant rat liver, as a central organ in carbohydrate metabolism.

MATERIALS AND METHODS

Animals: A total of 60 adult female Albino rats weighing 140-220 g were used throughout this study. Animals were purchased from the breeding unit of the Egyptian Organization for Biological Products and Vaccines (Helwan, Egypt) and they were housed in steel mesh cages (4/cage). Rats were maintained for a week (Acclimatization period), they were fed on a commercial pellet diet. Food and water were provided *ad libitum.*

Preparation of diets

The standard, high fructose (60g/100g) diets and diet containing 10% RBO were prepared as previously described by **Rajasekar *et al.*** [(13)] and **Wang *et al.*** [(14)].

Study design

Rats were allocated into 5 groups. Normal control group (**NC**): rats were fed on a standard diet. Rice Bran Oil group (**RBO)**: rats were fed on a standard diet contained 10% RBO as the sole source of fat. High Fructose Diet group (**HFD**): this group was subdivided into 2 sub-groups: rats were fed on HFD for only one month (**HFD1)** and rats which were fed on HFD for 2 months (**HFD2)** and served as reference groups for the corresponding treated groups. Rats were fed on HFD which contained 10% RBO for one month (**HFD1+ RBO).**

Rats in this group were fed on HFD for 30 days, and then received HFD with 10% RBO for another 30 days (**HFD2+ RBO).** Animals were maintained in their designed groups for 4 weeks except group 5.

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Body weight of the animals in all groups was recorded weekly. All animal experiments were carried out in accordance with the principles outlined in the Declaration of Helsinki (Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964).

Blood collection and tissue sampling:

Rats were anesthetized and then blood samples were taken from the retro-orbital venous plexus after overnight fasting. Blood was immediately centrifuged. Serum samples were aliquoted and stored at -20°C. Liver was quickly excised and rinsed from blood in phosphate buffer saline (PBS, pH 7.4), dried and weighed.

Biochemical assay

Fasting serum glucose level was assayed by the enzymatic colorimetric method[15], while serum insulin was assayed using the enzyme linked immunoassay (Rat insulin ELISA kit, Glory science Co., USA) according to the method of **Dhahir *et al.*[16]**. Homeostasis model assessment insulin resistance index (HOMA-IR) was calculated: HOMA- IR= [Fasting insulin (μIU/ml) × fasting glucose (mmol/L)] / 22.5[17]. Hepatic glycogen was determined by the colorimetric method of **Carroll *et al.*[18]**.

Histological analysis

Liver sections (Three independent rats from each group) were fixed in 10% neutral-buffered formalin then they were paraffin embedded.

The paraffin embedded sections were cut into 4-μm slices and stained with hematoxylin and eosin, while examination of hepatic glycogen was performed using Best's carmine stain.

Statistical analysis

Data were expressed as means ± standard error of mean. Differences between the mean values were assessed with one way analysis of variance (ANOVA) and followed by post-hoc test (least significant difference analysis, LSD). A p-value ≤ 0.05 was considered significant. The statistical analyses were applied using computer-based software (SPSS) version 16.

The study was approved by the Ethics Board of Al-Azhar University.

RESULTS

Non-significant changes in the body weight were observed in HFD-fed rats, compared to the control group **(Table 1).**

Histopathological observations of H&E stain of livers were performed as supporting evidence in biochemical analysis.

Figure (1-A) showed the normal morphological characteristics of the hepatic cells, whereas the hepatic cells of HFD-fed rats (For 1 and 2 months) showed cytoplasmic vacuolation and focal hepatic necrosis associated with mononuclear cells infiltration as illustrated in **figure 1 (B and C,** respectively).

Slight cytoplasmic vacuolation was realized in hepatocytes of **HFD1+RBO group**, in addition to activation of Kupffer cells of **HFD1+RBO and HFD2+RBO** groups were observed in **figure 1 (D and E**, respectively).

Our results showed moderate insulin resistance in the fructose-fed rats, as demonstrated by hyperinsulinemia and increased HOMA-IR value in **HFD1 and HFD2 groups**. Beside, hyperglycemia was observed in rats fed HFD for 4 weeks, while rats fed HFD diet for 8 weeks revealed significant reduction in serum glucose ($p<0.01$), compared to those fed HFD for 4 weeks (**Table 2).**

Table 1: statistical significance of body weight change (%) in all the experimental groups.

Groups	Percentage of body weight change%
NC	3.25
RBO	7.25
HFD1	4.06
HFD1+RBO	0
HFD2	10.58
HFD2+RBO	10

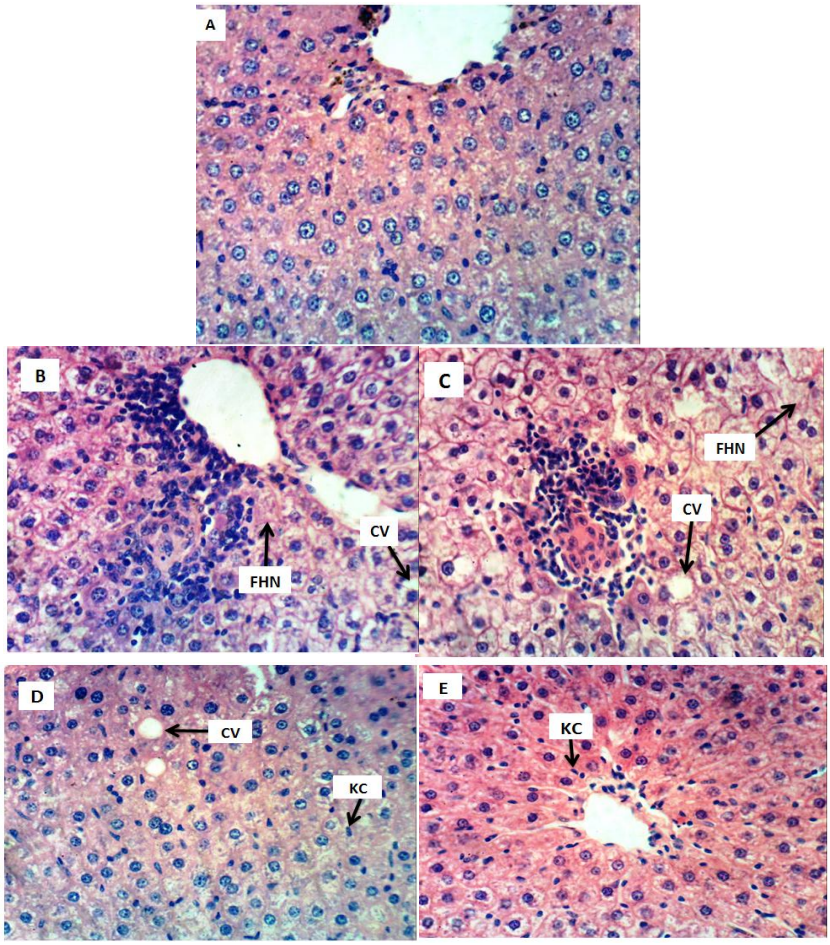
Table 2: statistical significance of plasma glucose, serum insulin in addition to calculated HOMA-IR and hepatic glycogen concentration in all the experimental groups.

Groups	Glucose (mg/dL)	Insulin (mU/L)	HOMA-IR	Glycogen (g/100g liver
NC				
Mean±SE	120 ±3.95	7.46±0.36	2.24±0.11	0.82±0.26
Range	(108-136)	(6.50-10.1)	(1.81-275)	(0.53-1.09)
RBO				
Mean±SE	117.6±4.37	8.85±0.36	2.56±0.13	0.81±0.57
Range	(90-134)	(7.50-10.5)	(1.67-3.00)	(0.26-1.78)
HFD1				
Mean±SE	180.4±10.13[ab]	14.64±0.83[ab]	5.37±0.56[ab]	2.51±0.88[ab]
Range	(134-230)	(10.6-16.7)	(2.24-7.33)	(1.76-4.07)
HFD1+RBO				
Mean±SE	142.7±2.48[abc]	11.79±0.75[abc]	4.14±0.26[abc]	1.47±0.74[abc]
Range	(135-159)	(9.03-14.90)	(3.05-5.33)	(0.55-2.86)
HFD2				
Mean±SE	134.2±4.93[c]	21.54±1.16[ac]	7.17±0.55[abc]	1.37±0.25[abc]
Range	(121-161)	(17.6-25.5)	(5.51-10.13)	(1.15-1.78)
HFD2+RBO				
Mean±SE	125.5±7.23	11.55±0.73[abd]	3.53±0.26[ad]	1.02±0.36
Range	(99-148)	(8.90-15.7)	(2.64-5.58)	(0.62-1.43)

-a: significance vs NC, b: significance vs RBO, c: significance vs HFD1, significant at $p<0.05$

In spite of the improvement in the serum insulin level in **HFD1+RBO and HFD2+RBO groups** ($p<0.01$ and $p<0.001$, respectively), compared to their respective control groups (**HFD1 and HFD2)** insulin level was still highly elevated than the control group. Addition of RBO to the HFD diet (**HFD1+RBO)** improved serum glucose ($p<0.001$), as compared to **HFD1**. Moreover, **HFD** contained RBO (**HFD1+RBO and HFD2+RBO)** reduced HOMA-IR significantly ($p<0.02$ and $p<0.001$, respectively) as compared to their respective control group.

Rats fed on HFD for 4 and 8 weeks showed a significant elevation in hepatic glycogen concentration especially in **HFD1 group**. However, rats fed on the diets containing RBO revealed reduced levels (**Table 1& figure 2).**



CV: cytoplasmic vacuolation, FHN: Focal hepatic necrosis, KC: Kupffer cells, CV: cytoplasmic vacuoles

Figure 1: photomicrophotograph of rat hepatic tissue of control group (A), (HFD fed groups) for one month (B) and two (C) months and (RBO groups) [HFD1+RBO group (D) and HFD2+RBO group (E)]. (X400, H & E).

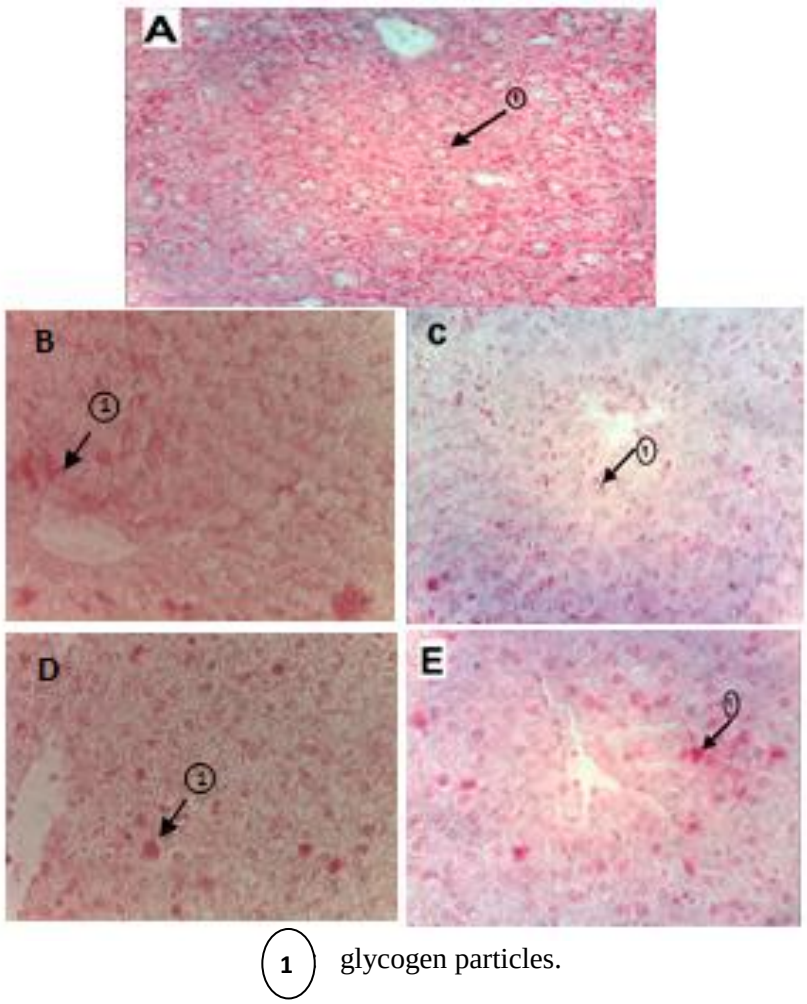


Figure 2. Microphotograph of rat liver tissue of control (A), (HFD fed groups) for one month (B) and 2 (C) months and (RBO groups) [HFD1+RBO group (D) and HFD2+RBO group (E)] (Best's carmine stain, X400)

DISCUSSION

Insulin resistance syndrome is a cluster of related variables that included resistance to insulin-induced glucose uptake and hyperinsulinemia[19,20]. Insulin resistance occurs at multiple levels in cells, from the cell membrane to the nucleus[21].

Feeding rats with HFD either for one or two months revealed non-significant change in the body weight gain, compared to the control group. These results agree with those of **Al-Okbi *et al.***[22] and **Palavicino-Maggio & Kuzhikandathil**[23].

However, **Hsieh *et al.***[24] and **Rajarshi *et al.***[25] showed that feeding a high-fructose diet (60%), significantly increased body weight compared to the control group. **Toop & Gentili**[26] also reported that consumption of a 10%–21% fructose beverage resulted in increased body weight in adult male rodents. On the other hand, **Bantle *et al.***[27] observed reduction in the body weight after feeding rats with a diet contained 17% fructose.

Based on the histopathological examination, HFD groups revealed pathological changes in the liver architecture, as indicated by cytoplasmic vacuolation, hepatocytes necrosis and mononuclear cells infiltration. These results confirm the induction of liver dysfunction by HFD. However, RBO administration reduced these pathological changes and showed somewhat normal appearance, which proved the protective and therapeutic effects of RBO.

Previous studies confirmed that the high-fructose diet induced elevation in the serum levels of ALT and AST, the specific markers of hepatocellular injury[22,28,29]. These elevations were significantly reduced in groups fed HFD containing RBO, compared to their respective control rats[11].

Insulin resistance induced by high fructose diet in rats was well documented[3,5,30,31] and has been established in the present study. The degree of insulin resistance was higher in HFD1 and HFD2 groups as indicated by the significant elevation of serum insulin levels and HOMA-IR. The development of hyperglycemia in HFD1 group may be due to the formation of glucose from fructose by

gluconeogenesis and impaired utilization of glucose by tissues, due to insulin resistance[32].

Addition of RBO restored insulin sensitivity and reduced HOMA-IR, compared to the normal control and fructose fed rats (HFD1and HFD2). Diminishes in insulin level along with the reduction in glucose and HOMA-IR suggested that RBO acts as a hypoglycemic agent through improving insulin action rather than insulin secretion. These results agree with those of **Abd elbast *et al.*[11] and Abd El-Wahab *et al.*[5],** who reported that addition of RBO to high fructose diet-fed rats, improved insulin resistance. The appreciable amount of oleic acid and tocotrienols in RBO may be the causes of glucose reduction and insulin sensitivity in rats fed HFD containing RBO[33,34].

Because of the absence of glucose in our fructose diet, so the substrate for glycogen synthesis in the fructose-fed groups likely came from dietary fructose through the gluconeogenic pathway due to the induction of fructose-1,6-bisphosphatase as reported by **Koo *et al.*[35]**.

Rats fed HFD for 30 days had elevated levels of hepatic glucose-6-phosphatase which catalyzes the terminal reaction of both glycogenolysis and gluconeogenesis[36]. Moreover, phosphoenolpyruvate carboxykinase is another regulatory enzyme in gluconeogenesis and its activity is greater in animals fed high fructose diets[37]. Together with the current results, these findings suggest that the reduction in IRS-1/PI3-kinase association, due to impaired insulin signaling in the liver of rats fed HFD, can reduce the effects of insulin on glucose-6-phosphatase and phosphoenolpyruvate carboxykinase and consequently increases the hepatic glycogen as observed in HFD1 and HFD2 groups. In addition, feeding fructose for 8 weeks (HFD2) increased the hepatic glucose release which promotes hyperinsulinemia and insulin insensitivity. As a result of insulin sensitivity improvement due to addition of RBO, the hepatic glycogen content was reduced significantly as compared to HFD groups.

CONCLUSION

The present results indicated that rats fed high fructose containing RBO alleviated insulin impairment originating from high fructose feeding for 4 weeks.

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