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Rice Bran Oil and Pumpkin Seed Oil Alleviate Oxidative Injury and Fatty Liver in Rats Fed High Fructose Diet

Sahar Y. Al-Okbi[1], Doha A. Mohamed[1], Thanaa E. Hamed[1], Reham S.H. Esmail[2]*

[1]Food Sciences and Nutrition Department, National Research Centre, Dokki, Cairo, Egypt
[2]Pathology Department, National Research Centre, Dokki, Cairo, Egypt

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Nonalcoholic steatohepatitis (NASH) and nonalcoholic fatty liver disease are increasing in adults and are likely to be increasing in children. The aim of the present research was to evaluate the protective effect of rice bran oil and pumpkin seed oil against high fructose diet (HFD) inducing nonalcoholic steatohepatitis (NASH). The results showed significant elevation of plasma total and direct bilirubin, transaminases activities, total cholesterol (T-Ch), triglycerides (TG), low density lipoprotein-cholesterol (LDL-Ch), tumor necrosis factor-α (TNF-α) and malondialdehyde (MDA) with significant increase in liver TG, T-Ch and MDA along with significant reduction in plasma high density lipoprotein cholesterol (HDL-Ch) and increase in T-Ch/HDL-Ch in rats fed HFD compared to rats fed on balanced diet. Histopathology of liver of rats fed on HFD confirmed the induction of NASH. Rice bran oil and pumpkin seed oil produced improvement in the biochemical parameters with different degrees. Pumpkin seed oil reversed all histopathological changes that occur in liver tissue which became comparable to normal in some rats. In conclusion, rats fed high fructose diet are a good model for studying NASH. Rice bran oil and pumpkin seed oil afford hepato protection against NASH in rat model.

INTRODUCTION

Fructose is a monosaccharide that is widely available in natural food sources such as fruits and honey. However, in most countries the main source of fructose is from sucrose, a disaccharide composed of equal portions of fructose and glucose. Fructose intake has increased markedly over the last 2 centuries, primarily due to the increasing intake of sucrose and high-fructose corn syrup [Tappy & Le, 2010; Ha *et al*., 2013]. Fructose is known to stimulate fat accumulation in the liver by both increasing synthesis and blocking fat oxidation [Ackerman *et al*., 2005]. Therefore, fructose and sugar-sweetened beverages have been related to the risk of non-alcoholic fatty liver disease (NAFLD). Plasma triglycerides are increased by sugar-sweetened beverages, and this increase appears to be due to fructose moiety [Bray, 2012]. NAFLD is the most common hepatic manifestation of obesity, affecting 20%–30% of adults [Neuschwander-Tetri, 2005; Vos & Lavine, 2013]. NAFLD is defined as the accumulation of lipid, primarily triacylglycerols, in the liver [McCullough, 2004]. Soft drink consumption was associated with NAFLD independent of metabolic syndrome [Abid *et al*., 2009] or in the absence of traditional risk factors, including obesity, diabetes or hyperlipidemia [Assy *et al*., 2008]. In fact, an increasing body of evidence indicates that fructose in the diet itself causes NAFLD [Nomura *et al*., 2012]. NAFLD is the most common cause of chronic liver injury worldwide. It has a broad pathologic spectrum which ranges from simple fatty infiltration of the liver or steatosis, to non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis and to liver failure [Assy *et al*., 2000]. NASH is fatty liver with inflammation and elevated oxidative stress and it is a progressive form of NAFLD that is diagnosed by histopathological features [Brunt *et al*., 1999]. NAFLD and NASH have been recently recognized as hepatic manifestations of metabolic syndrome [Day & Saksena, 2002]. It has been shown previously that experimental NASH is accompanied by liver dysfunction and dyslipidemia and that NASH is a risk factor for cardiovascular diseases [Al-Okbi *et al.*, 2013]. So, it can be hypothesized that therapy which improves liver function and produces antioxidant, anti-inflammatory, hypolipidemic and lipotropic effect might prevent progression of NASH and its cardiovascular risk. Based on lack of effective therapies for NASH, natural bioactive constituents from plant materials especially plant foods might be useful in this respect. From such plant foods; rice bran oil and pumpkin seed oil are reported to contain bioactive constituents that might possess the aforementioned activities. Rice bran oil (RBO) is a rich source of bioactive constituents such as oryzanols, phytosterols, tocopherols, tocotrienols, squalene, policosanols and ferulic acid [Khatoon & Gopalakrishna, 2004; Ardiansyah *et al*., 2006]. These bioactive constituents have been reported to possess multiple health benefits including reduction of cholesterol levels [Chen & Cheng, 2006], antioxidant [Xu *et al*., 2001] and anti-inflammatory activity [Aki-

* Corresponding Author: Tel.: 01222357571; Fax: (202) 33370931;
E-mail: dohamohamed@yahoo.com (Prof. Dr. Doha A. Mohamed)

TABLE 1. Composition of experimental diets (g/100 g).

Ingredients	High fructose diet	High fructose diet containing rice bran oil	High fructose diet containing pumpkin oil	Balanced diet
Casein	17.0	17.0	17.0	12.0
Corn oil	-	-	-	10.0
Rice bran oil	-	5.5	-	-
Pumpkin oil	-	-	5.5	-
Butter fat	5.5	-	-	-
Fructose	70.0	70.0	70.0	-
Starch	-	-	-	70.5
Salt mix.	3.5	3.5	3.5	3.5
Vitamin mix.	1.0	1.0	1.0	1.0
Cellulose	3.0	3.0	3.0	3.0

hisa *et al*., 2000]. Pumpkin seed oil has been reported to possess anti-inflammatory, hypolipidemic and antioxidant effect [Suresh & Das, 2003; Makni *et al*. 2008, 2010]. These reported bioactivities of both RBO and pumpkin seed oil could render them potential health benefit as fatty liver preventives. So the aim of the present study was to evaluate the beneficial effect of rice bran oil and pumpkin seed oil administration in rats fed high fructose diet inducing NASH. NASH-related biochemical parameters planned to be assessed included liver fat, plasma lipids, TNF-α, MDA in both plasma and liver and liver function tests along with histopthological examination of the liver.

MATERIAL AND METHODS

Plant materials

Egyptian rice bran was stabilized after milling by heating at 125°C for 15 min and supplemented by Dr. Amr M. Helal, International Trade & Marketing Managing, Cairo, Egypt. Pumpkin seed oil (*Cucurbita pepo* L., Family Cucurbitaceae var. styria) was obtained from Graz, Austria.

Animals

Male Sprague Dawley rats of body weight equal to 160.3±15.13 g as mean±SD were used in the present study. Animals were obtained from Animal House of National Research Centre, Cairo, Egypt. Animals were kept individually in stainless steel cages; water and food were given *ad-libtium*.

Preparation of plant extracts

Rice bran was subjected to continuous extraction by petroleum ether (40–60°C) using Soxhlet apparatus to prepare the oil. The solvent was completely removed by evaporation under reduced pressure at a temperature not exceeding 40°C. The oil yield was 18.4%.

Diets

Experimental diets were prepared in powder form and their composition was shown in Table 1. High fructose diet was prepared similarly to Kawasaki *et al*. [2009] to induce NASH (nonalcoholic fatty liver with inflammation).

Experimental procedures

Twenty-four rats were divided into four groups, each of six rats. The first was normal group where rats received a balanced diet. The second group was control where rats were fed on high fructose diet (for induction of NASH). Rats of group three and four were fed on high fructose diet containing rice bran oil and pumpkin seed oil and also given an oral daily dose of 200 mg/rat/day of rice bran oil and pumpkin seed oil, respectively. During the experiment, body weight and food intake were recorded weekly. After thirty-five days (end of the study) total food intake, body weight gain and food efficiency ratio (body weight gain/total food intake) were calculated. Blood samples were collected from eye orbital of anaesthetized rats after an overnight fast. Heparin was added to blood and plasma was separated by centrifugation at 3000 rpm. Plasma total cholesterol (T-Ch) [Watson, 1960], high density lipoprotein cholesterol (HDL-Ch) [Burstein *et al*., 1970], low density lipoprotein cholesterol (LDL-Ch) [Schriewer *et al*., 1984] and triglycerides (TG) [Megraw *et al*., 1979] were determined. The T-Ch / HDL-Ch ratio was calculated. Malondialdehyde (MDA) was determined as an indicator of lipid peroxidation [Satoh, 1978]. Plasma tumor necrosis factor-α (TNF-α) (an inflammatory biomarker) [Stepaniak *et al*., 1995] was determined. The activity of aspartate transaminase (AST) and alanine transaminase (ALT) [Reitman & Frankel, 1957] was estimated as indicators of liver function and/or damage. Liver was immediately removed, weighed and stored at -20°C till analyzed. Total hepatic lipids were extracted and weighed according to the procedure of Folch *et al*. [1957] and the concentration of triglycerides and cholesterol assessed [Megraw *et al*., 1979 and Watson, 1960, respectively]. MDA was determined in the liver according to Ohkawa *et al*. [1979]. For histopathological study, part of liver was removed, placed in 10% formaldehyde, dehydrated in graded alcohol and embedded in paraffin. Fine sections were prepared, mounted on glass slides and counter-stained with hematoxylin and eosin for light microscopic analysis [Ekor *et al*., 2010]. Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre, Cairo, Egypt, and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85–23, revised 1985).

Statistical analysis

The results of animal experiments are expressed as the mean±SE and they are analyzed statistically using the one-way analysis of variance ANOVA followed by Duncan's test. In all cases $p<0.05$ was used as the criterion of statistical significance.

RESULTS

Table 2 showed different biochemical parameters of different experimental groups. Plasma levels of total and direct bilirubin and plasma activities of AST and ALT were increased significantly in HFD-fed rats, indicating liver dysfunction. Treatment with rice bran oil or pumpkin seed oil with HFD resulted in significant reduction of AST and ALT activity and decreased levels of total and direct bilirubin compared to

TABLE 2. Biochemical parameters of normal and fatty liver rats (Mean±SE).

Parameters	Normal control	High fructose control	Rice bran oil	Pumpkin oil
Plasma				
T-Cholesterol (mg/dL)	85.7±2.299[a]	161.0±5.512[b]	145.7±3.826[c]	143.4±3.988[c]
Triglycerides (mg/dL)	93.2±1.539[a]	113.1±2.095[b]	101.6±3.019[c]	102.3±1.145[c]
HDL-Ch (mg/dL)	41.6±0.611[a]	23.6±0.757[b]	33.2±1.077[c]	31.5±1.057[c]
LDL-Ch (mg/dL)	20.9±0.562[a]	103.8±3.487[b]	72.8±2.414[c]	71.2±2.414[c]
T-Ch/ HDL-Ch	2.1±0.061[a]	6.8±0.169[b]	4.4±0.213[c]	4.6±0.094[c]
MDA (nmol/mL)	4.9±0.281[a]	7.8±0.214[b]	5.8±0.314[c]	6.2±0.278[c]
TNF-α (pg/mL)	19.5±0.566[a]	33.0±0.52[b]	22.9±0.500[c]	23.3±0.492[c]
ALT (U/L)	54.3±1.429[a]	85.2±2.441[b]	71.8±0.792[c]	73.0±0.816[c]
AST (U/L)	43.5±0.763[a]	83.3±1.706[b]	66.2±0.872[c]	65.7±0.715[c]
T. Bilirubin (mg/dL)	0.346±0.008[a]	0.505±0.005[b]	0.436±0.008[c]	0.425±0.005[c]
D. Bilirubin (mg/dL)	0.147±0.006[a]	0.247±0.010[b]	0.203±0.003[c]	0.198±0.005[c]
Liver tissue				
Total fat (mg/g tissue)	22.5±0.563[a]	45.9±1.278[b]	30.8±0.749[c]	31.2± 0.601[c]
T-Cholesterol (mg/g tissue)	2.0±0.138[a]	6.8±0.159[b]	3.4±0.153[c]	3.9± 0.380[c]
Triglycerides (mg/g tissue)	4.9±0.159[a]	13.6±0.752[b]	5.1±0.366[c]	4.7± 0.207[c]
MDA (nmol/g tissue)	9.4±0.325[a]	17.3±0.403[b]	13.4±0.326[c]	13.5± 0.326[c]

In each row, different letter means significant difference at 0.05 probabilities.

TABLE 3. Nutritional parameters of different experimental groups (Mean±SE).

Groups	Initial body weight (g)	Final body weight (g)	Body weight gain (g)	Total feed intake (g)	Food efficiency ratio	Liver weight/ body weight %
Normal control	160.3±8.352[a]	251.3±9.463[a]	91.0±5.017[a]	481.5±6.162[a]	0.189±0.008[a]	2.4±0.074[a]
High fructose control	160.3 ±5.419[a]	255.7±3.693[a]	95.4±2.666[a]	555.3±3.963[b]	0.172±0.001[a]	3.1±0.168[b]
Rice bran oil	160.3±6.425[a]	245.7±9.393[a]	85.4±5.116[a]	559.7±12.779[b]	0.154±0.011[b]	2.8±0.120[b]
Pumpkin oil	160.3±6.524[a]	241.2±7.559[a]	80.9±5.185[a]	561.2±11.887[b]	0.144±0.008[b]	2.8±0.052[b]

In each column, different letter means significant difference at 0.05 probabilities.

the HFD-fed rats without treatment. HFD-fed rats exhibited a significant increase in plasma total cholesterol, triglycerides, LDL-Ch and the ratio of T-Ch/HDL-Ch compared to control rats. In addition, significant increases were observed in total fat, T-Ch and TG in the liver tissue of HFD-fed rats compared to control normal. Rats treated with rice bran oil or pumpkin seed oil showed significant improvement in plasma lipid profile and the contents of liver total fat, T-Ch and TG with different degrees. Plasma and liver levels of MDA increased significantly in HFD rats compared to normal control. Rats fed on HFD and treated with rice bran oil or pumpkin seed oil showed significant reduction in MDA levels but still higher than normal rats. Plasma level of TNF-α was significantly higher in HFD group than in normal rats. This elevation was reduced significantly in rats fed on HFD and treated with rice bran oil or pumpkin seed oil.

Nutritional parameters of normal and fatty liver rats are shown in Table 3. The results revealed that non-significant changes were noticed in final body weight and body weight gain, whereas the total food intake was significantly higher in fatty liver rats, with or without treatment with the tested oils, in comparison with the normal rats. Rats fed on HFD and treated with rice bran oil or pumpkin seed oil showed significant reduction in the food efficiency ratio compared with normal control and high fructose control. Liver weight/ body weight % showed significant reduction when the control group fed the balanced diet was compared with the other groups.

Liver histopathology

Figure 1 (a-h) showed sections of liver of different experimental groups. It can be noticed that liver of rats fed on balanced diet (Figure 1 a) has normal appearance, the hepatocytes run in trabeculae of normal thickness, without inflammatory cell collections or other changes.

Figure 1 (b, c) showed that liver of rats fed on high fructose diet was characterized by fatty changes or steatosis with variable sized intracellular fat vacuoles (macro and microvesicular steatosis). Also collections of necro-inflammatory foci within the liver lobules were noticed. The effect was variable within the rats, with most of them showing severe changes. The accumulations of fat along with inflammation in the liver reflect the occurrence of NASH.

Liver of rats given rice bran oil (Figure1 d, e) during feeding with the high fructose diet showed that the fat vacuoles and the inflammation were generally lesser than in the fatty

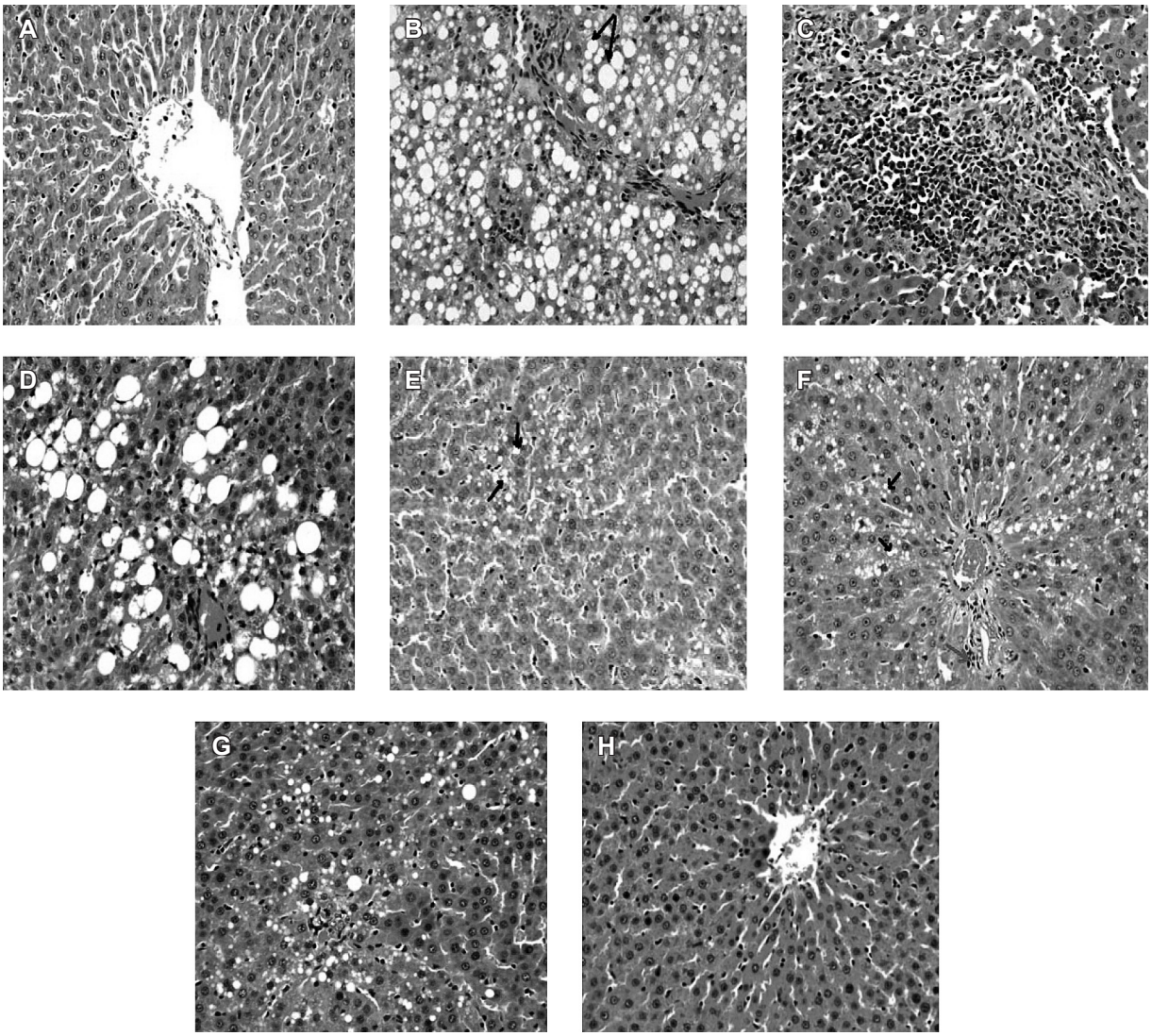

FIGURE 1. Section of rat liver (H & E, x400): (a) normal rats; (b, c,) fatty liver control rats which are HFD fed rats; (d, e) rats fed HFD with rice bran oil; and (f, g, h) rats fed HFD with pumpkin seed oil.

Figure 1 (A) Liver of control normal rats showed no pathological changes. Figure 1 (B): In rats fed HFD, the liver showed parenchyma with variable sized fat vacuoles (black arrows). Figure 1 (C): In rats fed HFD, the liver showed dense collection of inflammatory cells within the liver lobules. Figure 1 (D): In rats fed HFD with rice bran oil, the liver showed less fatty changes compared to control rats fed HFD (moderate fatty changes). Figure 1 (E): Fewer and smaller fat vacuoles in the hepatocytes (black arrows) with mild fatty changes were noticed in the liver of rats fed HFD with rice bran oil reflecting prominent improvement. Figure 1 (F): In the liver of rats fed HFD with pumpkin seed oil, fatty changes and inflammation was less than the control fed HFD. The fat vacuoles were almost of the micro vesicular type (black arrows, upper arrows) and minimal inflammation was seen (green arrow, lower arrow). Figure 1 (G): In the liver of rats fed HFD with pumpkin seed oil; reduction in the fatty changes and inflammation was noticed compared to the control fed HFD. Fat vacuoles of variable sizes are seen. Figure 1 (H): In the group fed HFD with pumpkin seed oil; some rats showed liver with almost normal appearance, no fatty changes and no inflammatory cells collections.

liver of control group, however the changes were variable ranging from moderate to mild.

Liver of rats given pumpkin seed oil (Figure1 f, g & h) during feeding with the high fructose diet showed fatty changes and inflammation much lesser than the control fatty liver group, the changes ranged from moderate to mild, moreover some rats showed liver with almost normal features.

DISCUSSION

Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver injury in many countries around the world. It has a broad pathologic spectrum which ranges from simple fatty infiltration of the liver or steatosis, to nonalcoholic steatohepatitis (NASH), fibrosis, cirrhosis and to liver failure. NASH is considered as one of the risk factors for cardiovascular diseases including arteriosclerosis where elevation of oxidative stress, inflammation and levels of plasma TG, LDL-Ch and total cholesterol result from the increased synthesis and accumulation of cholesterol and fat in liver during NASH [Al-Okbi *et al*., 2013]. Studies have shown that the intake of high fructose diet results in insulin resistance (IR), hepatic steatosis, excessive generation of reactive oxygen species (ROS), malfunctioning of the liver and depletion of the hepatocyte population [Jaya & Amuradha, 2010]. There is evidence that oxidative stress contributes to the development of steatohepatitis from steatosis induced by high-energy diet [Barbuio *et al*., 2007].

Administration of high fructose diet (HFD) induces the development of metabolic syndrome characterized by obesity, IR and liver steatosis [Angulo & Lindor, 2002; Rippe & Angelopoulos, 2013]. A body of evidence indicates that accumulation of fat in the liver increases the susceptibility to other insults such as oxidative stress and subsequent inflammation that results in the progression of steatosis to steatohepatitis, fibrosis and cirrhosis [Koteish & Diehl, 2001]. Induction of oxidative stress in the present study was evident from the increased plasma and liver MDA, the lipid peroxidation biomarkers in the control rats fed HFD. Also, the HFD-fed rats showed increased level of inflammatory biomarker TNF-α. HFD feeding was associated with hepatocellular damage and microvesicular steatosis and inflammation as shown from the histopathological results of the present study. The increases in plasma levels of total and direct bilirubin and activities of AST and ALT confirmed the induced liver injury by histopathology. Rice bran oil and pumpkin oil could effectively protect against the hepatic oxidative stress and inflammation induced by HFD which is manifested by reduction of MDA and TNF-α. Treatment with rice bran oil and pumpkin seed oil notably prevented the elevation of liver enzymes as shown from the results and which is supported by prevention of liver cell damage and preserving cell integrity as seen from liver histology possibly leading to maintenance of the functionally of active cells.

The contents of total lipids, cholesterol and TG were significantly elevated in liver of HFD fed rats, which is a serious risk factor for the development of steatohepatities and liver injury. Results of the histopathological examination of liver of HFD-fed rats showed widespread deposition of lipid droplets inside the parenchymal cells which are consistent with the result of the biochemical analysis of fat. Evidence of lipid accumulation in liver exposed to HFD has been reported previously [Aragno *et al*., 2009]. Fructose is highly lipogeneic, so HFD used in this study might result in the increased delivery of fatty acids through the portal circulation together with increasing liver fat synthesis resulting in fatty liver. Lipid dysregulation in fructose-fed rat model has been associated to the activation of oxidative stress and inflammatory pathways in the liver which favors the progression to NAFLD [Basciano *et al*., 2005]. An evolving hypothesis is that metabolic disease, ROS formation and inflammation create a progressive cycle leading to disease progression and NAFLD [Raval *et al*., 2006]. Treatment of HFD-fed rats with rice bran oil and pumpkin seed oil showed considerable reduction of liver fats which together with the reduction of oxidative stress shown by malondialdehyde inhibition may lead to the prevention of NASH.

Recent studies have demonstrated that ingestion of vegetable polyunsaturated fatty acids is inversely related to the incidence of heart disease by decreasing plasma cholesterol and triacylglycerol [Vijaimohan *et al*., 2006]. The present results indicated that both rice bran oil and pumpkin seed oil rich in polyunsaturated fatty acids produced reduction in triglycerides and cholesterol in both plasma and liver of rats with a reduction of plasma LDL-Ch and an increase in HDL-Ch. Furthermore, the atherogenic index markedly decreased due to significant reduction in T-Ch/HDL-Ch ratio in both groups fed HFD supplemented with either rice bran oil or pumpkin seed oil. Feoli *et al*. [2003] stated that the increase in HDL-Ch is one of the most important criteria of anti-hypercholesterolemic activity.

The insignificant change in body weight gain between rats given high fructose diet without treatment and these given the balanced diet agreed with the work of Kawasaki *et al*. [2009] that proved induction of fatty liver without change in body weight. Final body weight and body weight gain reduced insignificantly in rats fed on HFD and treated with rice bran oil or pumpkin seed oil compared to control rats fed HFD. It has been reported previously that there is a positive correlation between body weight and plasma triglycerides [Howard *et al*., 1983]. It can be noticed that administration of rice bran oil or pumpkin seed oil induced significant reduction in TG which may explain the reduced body weight. The significant increase of Liver weight/body weight% in rats fed on HFD compared to those fed balanced diet might be due to increased fat deposition in the liver in those rats as shown from the present results. The reduction of Liver weight/body weight% in rats fed on HFD and treated with rice bran or pumpkin seed oil might be ascribed to the significant reduction of liver fat compared to those fed HFD.

The protective effect of rice bran oil and pumpkin seed oil against fatty liver progression may be attributed to the presence of biologically-active compounds. Pumpkin seed oil contains phenolic compounds, tocopherol, β-carotene, unsaturated fatty acids and sterols [Al-Okbi *et al*., in press]. The major total fatty acids present in rice bran oil and pumpkin oil are unsaturated fatty acids such as oleic acid and linoleic acid [Chopra & Sambaiah, 2009; Al-Okbi *et al*., in press]. These unsaturated fatty acids play a crucial role in reducing blood cholesterol in humans and rats [Barakat & Mahmoud, 2011] which might be related to reduction of cholesterol synthesis and/or increased cholesterol catabolism in the liver.

Rice bran oil is rich in an array of bio-active phytochemicals such as γ-oryzanols, phytosterols, tocopherols, tocotrienols, squalene, policosanols ferulic acid and unsaturated fatty acids [Khatoon & Gopalakrishna, 2004; Ardiansyah *et al*., 2006]. It was reported by Al-Okbi *et al*. [in press] that Egyptian rice bran oil, used in the present study, contains stigmasterol, campesterol and β-sitosterol as 12% of unsaponifiable matter. It also contains β-carotene at 225 μg/100g oil, α-tocopherol at 665 μg/g, γ-tocopherol at 70 μg/g, while δ-tocopherol was 172 μg/g oil. Alpha, γ and δ- tocotrienol concentrations were found to be 119, 183 and 8.24 μg/g in rice bran oil, respectively. Gamma-oryzanol content of the same rice bran oil was 3.33 g/100 g. Total policosanol was present in rice bran oil at 69.62 mg/100 g [Al-Okbi *et al*., in press]. So, rice bran is a rich natural source of vitamin E [Saunders, 1985]. Gamma-oryzanol, which is a mixture of 10 ferulate esters of triterpene alcohol [Lloyd *et al*., 2000], has been reported to contribute to multiple health benefits, including, reduction of cholesterol levels [Chen & Cheng, 2006], antioxidant functions [Xu *et al*., 2001] and anti-inflammatory activity [Akihisa *et al*., 2000]. The high contents of γ-oryzanol and γ-tocotrienols in rice bran oil can lead to increased fecal neutral sterol and bile acid excretion, *via* upregulation of cholesterol synthesis and catabolism [Chen & Cheng,

2006; Wilson *et al*., 2007] in the liver. Nagasaka *et al*. [2007] reported that γ-oryzanol suppressed NF-κB activation, inhibited inflammatory responses of macrophage cell line and that γ-oryzanol increased adiponectin secretion from adipocyte [Ohara *et al*., 2009]. So, these effects might be responsible for reduction of fatty liver and its progression to steatohepatitis. In a recent study, Fang *et al*. [2010] established that tocotrienol from rice bran oil functioned as PPAR (peroxisome proliferator activated receptors) modulators and improved whole body glucose utilization and insulin sensitivity of diabetic Db/Db mice by selectively regulating PPAR target genes. Hence, this effect may also contribute to the inhibition of the incidence of fatty liver.

Pumpkin seed is a rich natural source of proteins, phytosterols, polyunsaturated fatty acids, antioxidant vitamins such as carotenoids and tocopherol [Stevenson *et al*., 2007], which possess significant antioxidant activity, anti-inflammatory and hypolipidemic effects [Suresh & Das, 2003]. Pumpkin seed oil used in the present study showed previously to contain 3.5% of oleic acid, 38.9% of linoleic acid and 5.8% of palmitic acid [Al-Okbi *et al.*, 2014]. The same pumpkin seed oil contains stigmasterol and campesterol as 43.6% of unsaponifiable portion. It also contains α and δ- tocopherol in addition to 28.5μg of β-carotene/100 g oil [Al-Okbi *et al*., 2014]. Phenolic compounds were present in the same pumpkin seed oil at 14.033 mg GAE/g [Al-Okbi *et al*., 2014]. The hypolipidemic, antioxidant, anti-inflammatory and liver lipid lowering effects of pumpkin seed oil in the present study are certainly attributed to the presence of the aforementioned bioactive constituents. These components might also reduce the progression of fatty liver to NASH. Makni *et al*. [2008, 2010] demonstrated that flax and pumpkin seed mixture supplemented to diet of hypercholesterolemic rats had a significant anti-atherogenic, hypolipidemic and antioxidant potency.

CONCLUSIONS

Rats fed the high fructose diet are a good model for studying NASH. Rice bran oil and pumpkin seed oil afford hepato protection against NASH in rat model.

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