Modulation of fructose-induced insulin resistance syndrome in rats by rosiglitazone and α-lipoic acid

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Abstract
Increased fructose intake has been linked to the epidemiology of insulin resistance (IR) syndrome, type 2 diabetes mellitus, renal damage and non-alcoholic steatohepatitis. As oxidative stress plays a pivotal role in the pathology of IR, the present study was conducted to investigate the effects of rosiglitazone alone or combined with α-lipoic acid, a potent antioxidant, on fructose-induced IR syndrome in rats. Markers chosen for assessment included effects on body weight gain, glucose and insulin levels, IR, β-cell function, lipid profile, nitric oxide (NO) metabolites and antioxidant status. Moreover, liver and kidney functions were assessed both biochemically and histologically. Male rats were fed with fructose-enriched diet (FED) or standard rat chow for 16 weeks. By the end of the 10th week, FED-fed rats were divided into three groups; one was left untreated (control group) and the other 2 groups were treated p.o. with rosiglitazone (4 mg/kg) and rosiglitazone plus α-lipoic acid (100 mg/kg), respectively. Treatments continued daily for 6 weeks, afterwards blood samples were collected, animals were sacrificed and their livers and kidneys isolated. Feeding rats with FED resulted in increased weight gain, hyperinsulinemia, hyperglycemia, IR and β-cell dysfunction. These changes were coupled with disturbances in lipid homeostasis, antioxidant status and alterations in NO metabolites as well as liver and kidney dysfunctions. Concomitant administration of α-lipoic acid with rosiglitazone potentiated the effects of the latter on most of the investigated parameters. In conclusion, the combination of rosiglitazone and α-lipoic could ameliorate most of the symptoms associated with IR syndrome in rats.

Key Words: Rosiglitazone, α-lipoic acid, fructose, oxidative stress, insulin resistance.

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1. INTRODUCTION
Insulin resistance (IR) syndrome, also referred to as the metabolic syndrome (MS), is characterized by IR, hyperinsulinemia, hyperlipidemia, obesity and increased risk for developing non-alcoholic fatty liver disease (NAFLD), type 2 diabetes mellitus (T2DM), cardiovascular and renal diseases (Reaven, 1995; Khosla et al., 2005; Ouyang et al., 2008).

Consumption of large amounts of dietary fructose is one of major factors that contribute to the development of obesity, MS and T2DM (Miller and Adeli, 2008; Dekker et al., 2010). Underlying factors for fructose-induced IR are varied. Fructose is more lipogenic than glucose, leading to greater elevations of triglycerides (TG) content in the skeletal muscle and in turn to IR (Basciano et al., 2005). Moreover, excessive fructose consumption can adversely affect the liver causing dysfunction and increased lipid peroxidation (Ouyang et al., 2008).

Rosiglitazone is an insulin sensitizer belonging to the thiazolidinediones (TZDs) group that acts by activation of nuclear peroxisome proliferator-activated receptor gamma (PPAR-γ) resulting in decreased IR in target tissues as well as increased expression of genes involved in fatty acid metabolism in adipocytes (Cuzzocrea et al., 2004; Basturk et al., 2012; Welters et al., 2012). In September 2010, the FDA initiated restricted access to rosiglitazone, to patients unable to obtain glycemic control with other agents, secondary to continued concerns about its cardiovascular safety. However, owing to its effects on PPAR-γ, rosiglitazone is still extensively used in different areas of research not only as an insulin sensitizer (Welters et al., 2012) but also as neuroprotective in Huntington's disease (Jin et al., 2013) and as anti-cancer agent (Bojkova et al., 2013). Interestingly, rosiglitazone was reported to protect cardiomyocytes against ischemia reperfusion injury and oxidative stress (Kim et al., 2012; Gao et al., 2013).
α-Lipoic acid is a sulfur-containing fatty acid antioxidant that, unlike most other antioxidants, functions in both water and fat sections of the cell (Kagan et al., 1992; Rochette et al., 2013) making it an unusually broad spectrum antioxidant. It has been used in the western world to treat complications associated with diabetes (Henriksen et al., 2006; Patel et al., 2011; Khalil, 2013; Wang et al., 2013). The benefits of combined therapy with both rosiglitazone and α-lipoic acid were shown in certain dermatologic disorders (Venkatraman et al., 2004).

As oxidative stress contributes largely to the development of insulin resistance (Evans et al., 2003), it seemed interesting to investigate the effects of using rosiglitazone alone or combined with a unique antioxidant as α-lipoic acid in attenuating some of the features associated with fructose-induced IR syndrome in rats. Parameters chosen to fulfill these attributes included effects on body weight gain, blood glucose and insulin levels, insulin resistance, β-cell function and lipid profile. Fructose-enriched diet (FED)-induced liver and kidney damage was assessed by determination of serum transaminases, uric acid and creatinine levels as well as histological examination of tissue sections from both organs. Moreover, the collected blood and tissue samples were used for the estimation of total antioxidant capacity (TAC), lipid peroxides and nitric oxide (NO) metabolites.

2. MATERIALS AND METHODS

2.1. Animals

Adult male Wistar rats, weighing 120-150 g, were used in the present study. They were purchased from the animal house of the National Cancer Institute (Cairo, Egypt). During the study, animals were kept in the animal house of Faculty of Pharmacy, Cairo University under the appropriate conditions of controlled humidity, temperature and light. The study was carried out according to the international guidelines of care and use of laboratory animals and was approved by the Ethics Committee for Animal Experimentation at Faculty of Pharmacy, Cairo University. All animals except the normal group were kept on especially prepared high fructose diet designed to induce IR syndrome, according to the method described by Storljen et al. (2000), with free access to water.

2.2. Drugs and chemicals

Rosiglitazone and α-lipoic acid were purchased from Memphis Pharmaceutical Company (Egypt) and Eva Pharmaceutical Company (Egypt), respectively. As for composition of rat diet: fructose was purchased from Winlap (UK); casien and cellulose from Lobachemie (India); cholesterol as well as mineral and vitamin mixtures from Sigma (USA); L-lysine and DL-methionine from SD-fine-chem Limited (India) and choline chloride from Avocado Organic (UK). Insulin reagent kit was purchased from Diagnostic Product Corporation (USA), total cholesterol kit from Audit Diagnostics (Ireland), TG kit from Biocon Diagnostik (Germany) and kit for TAC from Biodiagnostic (Egypt). Reagent kits for glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), HDL-cholesterol, creatinine and uric acid were purchased from Stanbio (Italy).

2.3. Experimental design

Rats were divided into four groups each consisting of 12 rats. Rats in the first group were fed with normal laboratory chow whereas rats in the remaining three groups were fed with FED composed of 66% fructose, 20% casine, 3% cellulose, 1% cholesterol, 5% sheep fats, 1% choline chloride, 1% DL-methionine, 1% L-lysine, 1% mineral mixture and 1% vitamins mixture. After 10 weeks of diet initiation, animals in the four groups were treated as follows: groups I and II received 1% Tween 80 daily for 6 weeks and served as normal- and FED-control groups, respectively; group III received rosiglitazone (4 mg/kg; p.o.) and group IV received both rosiglitazone (4 mg/kg; p.o.) and α-lipoic acid (100 mg/kg; p.o) daily for 6 weeks. All animals remained on their diet for the whole duration of the treatment period and their weights were recorded weekly.

At the end of the study, blood samples were withdrawn from the retro-orbital sinus of all rats. Serum was separated by centrifugation at 3000 rpm for 15 minutes and divided into small aliquots that were stored at -80°C. Animals were sacrificed by cervical dislocation, livers and kidneys were rapidly excised, washed with saline, weighed and homogenized in ice-cold saline to prepare 10% homogenates that were divided into several aliquots and stored at -80 °C. Parts of the livers and kidneys from each group were preserved in 10% formalin for histopathologic examination.

2.4. Biochemical and histological assays

Percentages of body weight gain, liver index and kidney size were calculated. Serum samples were used for estimation of the levels of fasting blood glucose, insulin, TG, total cholesterol, HDL-cholesterol, creatinine and uric acid as well as the activities of AST and ALT using commercial reagent kits.

Homeostasis model assessment of insulin resistance (HOMA-IR) and β-cell function (HOMA-BCF) were calculated according to the equations provided by Matthews et al. (1985) where HOMA-IR = serum glucose (mmol/l) X serum insulin (μIU/ml) / 22.5 and HOMA-BCF = serum insulin (μIU/ml)/ serum glucose (mmol/l)-3.5. LDL-cholesterol was calculated from the formula described by Friedewald.
et al. (1972) where LDL-C = total cholesterol – (HDL-C + TG/5).

In addition, the prepared homogenates and sera were used for estimation of TAC, NO metabolites measured as total nitrate/nitrite (NO₃⁻) and thiobarbituric acid reactive substances (TBARS) as an index of lipid peroxidation.

TAC was determined using commercial reagent kits. Determination of TBARS was done according to the method described by Mihara and Uchiyama (1978) whereas NO₃⁻ was determined according to the method described by Miranda et al. (2001).

Tissue samples preserved for histopathology were fixed in 10% formalin and used to prepare paraffin blocks. Sections of 5 μm were obtained and stained with Hematoxylin and Eosin (H & E). Images were captured and processed using Adobe Photoshop (version 8).

2.5. Statistical analysis

Data were expressed as means ± standard error (SE). Results were analyzed using one-way-analysis of variance test (ANOVA) followed by Tukey Kramer multiple comparison's test. For all statistical tests, the level of significance was set at p < 0.05. Graph Pad Software InStat (version 2) was used to carry out these statistical tests.

3. RESULTS

Feeding rats with FED for 10 weeks resulted in significant increases in body weight gain, liver index and kidney size (Table 1) coupled with increased serum levels of fasting blood glucose and insulin (Table 2). These changes were accompanied by an increase in HOMA-IR and a decrease in β-cell function (Table 2). In addition, activities of serum transaminases and levels of creatinine and uric acid were significantly increased by fructose feeding (Table 3). Moreover, FED-fed rats showed increased serum levels of TG, total cholesterol and LDL-cholesterol (Figure 1) as well as TAC and TBARS (Figure 2). On the other hand, serum levels of HDL-cholesterol and NO₃⁻ were significantly reduced as compared with that of normal control rats (Figures 1 and 2). In addition, fructose feeding resulted in a significant increase in TBARS and NO₃⁻ contents of liver and kidney coupled with a decrease in TAC of both tissues (Figures 3 and 4).

Oral treatment of FED-fed rats with rosiglitazone alone for 6 weeks was associated with a significant decrease in body weight gain, liver index and kidney size (Table 1) as well as improvements in serum levels of insulin, HOMA-IR, NO₃⁻, TG, total cholesterol, LDL-cholesterol and HDL-cholesterol (Table 2; Figure 1). Increased activities of serum AST and ALT as well as elevated serum levels of TBARS and uric acid were also reduced by rosiglitazone treatment (Table 3; Figure 2). Similarly, reduction in the formation of TBARS in liver and kidney tissues with a parallel increase in TAC of kidney was also noted (Figures 3 and 4).

Combination of rosiglitazone with α-lipoic acid further reduced body weight gain, HOMA-IR, ALT activity and serum TBARS level as compared to rosiglitazone-treated group (Tables 1, 2 and 3; Figure 2). Similarly, the same combination significantly increased liver and kidney TAC and reduced NO₃⁻ and TBARS contents, as compared to the group treated with rosiglitazone alone (Figures 3 and 4). Moreover, the previous combination improved β cell function and serum levels of glucose and creatinine which were not significantly improved by rosiglitazone monotherapy (Tables 2 and 3).

Examination of liver sections taken from FED-fed rats revealed notable fibrosis and fatty degeneration of hepatocytes especially in the zone surrounding the portal area as compared with the normal group (Figure 5a&b). Sections taken from rats treated with rosiglitazone revealed decreased fibrosis although dilatation and congestion of portal vein were still observed (Figure 5c). Combination of rosiglitazone with α-lipoic acid resulted in marked decrease in fibrosis; however, fatty degeneration at the periphery of the hepatic lobules was still observed (Figure 5d).

Kidney damage by fructose feeding appeared in the form of fibrosis coupled with dilatation and extravasations of blood into the lumen of most tubules as compared to the normal rats. An increase in the space between the tuft of capillaries and the wall of Bowman capsule, denoting increased amount of filtrate, was also noted (Figure 6a&b). Sections taken from FED-fed rats treated with rosiglitazone alone or combined with α-lipoic acid showed lack of improvements at the histological level as fibrosis, cellular infiltration in-between the glomeruli and dilatation of most tubules were still observed (Figure 6c&d).
Table 1: Effects of rosiglitazone (4 mg/kg) alone or combined with α-lipoic acid (100 mg/kg) on FED-induced changes in body weight gain, liver index and kidney size.

<table>
<thead>
<tr>
<th>Groups</th>
<th>BW gain (%)</th>
<th>Kidney size (%)</th>
<th>Liver index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>43.44 ± 1.94</td>
<td>0.54 ± 0.01</td>
<td>2.66 ± 0.09</td>
</tr>
<tr>
<td>FED</td>
<td>58.19 ± 3.97</td>
<td>0.72 ± 0.05</td>
<td>3.80 ± 0.09</td>
</tr>
<tr>
<td>RGZ</td>
<td>37.30 ± 3.01</td>
<td>0.59 ± 0.02</td>
<td>3.30 ± 0.18</td>
</tr>
<tr>
<td>RGZ + α-lipoic acid</td>
<td>21.20 ± 2.80</td>
<td>0.57 ± 0.01</td>
<td>3.04 ± 0.08</td>
</tr>
</tbody>
</table>

FED: fructose enriched diet, RGZ: rosiglitazone, BW: body weight. Treatments were given daily (p.o.) for 6 weeks after induction of insulin resistance syndrome by feeding rats with FED for 10 weeks. Rats remained on their diet for the whole period of treatment. Values are means of 6-10 rats ± SE. *Significantly different from control group at p<0.05. †Significantly different from FED group at p<0.05. ‡Significantly different from rosiglitazone group at p<0.05.

Table 2: Effects of rosiglitazone (4 mg/kg) alone or combined with α-lipoic acid (100 mg/kg) on FED-induced changes in blood glucose and insulin levels as well as insulin resistance and β-cell function.

<table>
<thead>
<tr>
<th>Groups</th>
<th>FBG (mg/dl)</th>
<th>HOMA-BCF</th>
<th>HOMA-IR</th>
<th>FINS (µIU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>91.35 ± 5.39</td>
<td>358.1 ± 30.20</td>
<td>2.69 ± 0.18</td>
<td>11.93 ± 1.47</td>
</tr>
<tr>
<td>FED</td>
<td>152.83 ± 13.24</td>
<td>137.63 ± 11.80</td>
<td>11.91 ± 0.47</td>
<td>31.73 ± 2.78</td>
</tr>
<tr>
<td>RGZ</td>
<td>119.42 ± 9.19</td>
<td>153.42 ± 19.60</td>
<td>5.85 ± 0.30</td>
<td>19.84 ± 1.31</td>
</tr>
<tr>
<td>RGZ + α-lipoic acid</td>
<td>86.51 ± 4.53</td>
<td>395.40 ± 42.78</td>
<td>3.97 ± 0.27</td>
<td>18.57 ± 1.30</td>
</tr>
</tbody>
</table>

FED: fructose enriched diet, RGZ: rosiglitazone, FBG: fasting blood glucose FINS: fasting insulin, HOMA-IR: homeostasis model assessment of insulin resistance, HOMA-BCF: homeostasis model assessments of β-cell function. Treatments were given daily (p.o.) for 6 weeks after induction of insulin resistance syndrome by feeding rats with FED for 10 weeks. Rats remained on their diet for the whole period of treatment. Values are means of 6-10 rats ± SE. *Significantly different from control group at p<0.05. †Significantly different from FED group at p<0.05. ‡Significantly different from rosiglitazone group at p<0.05.
Table 3: Effects of rosiglitazone (4 mg/kg) alone or combined with α-lipoic acid (100 mg/kg) on FED-induced changes in the activities of serum transaminases as well as serum levels of creatinine and uric acid.

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST (U/l)</th>
<th>ALT (U/l)</th>
<th>Creatinine (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>57.10 ± 0.63</td>
<td>33.40 ± 0.65</td>
<td>0.73 ± 0.04</td>
<td>1.58 ± 0.14</td>
</tr>
<tr>
<td>FED</td>
<td>91.01 ± 2.70</td>
<td>75.15 ± 2.80</td>
<td>1.51 ± 0.09</td>
<td>3.67 ± 0.20</td>
</tr>
<tr>
<td>RGZ</td>
<td>65.19 ± 1.30@</td>
<td>46.85 ± 1.07@</td>
<td>1.36 ± 0.08</td>
<td>2.50 ± 0.20@</td>
</tr>
<tr>
<td>RGZ + α-lipoic acid</td>
<td>65.00 ± 1.50@</td>
<td>37.80 ± 1.30@</td>
<td>1.19 ± 0.06@</td>
<td>2.56 ± 0.25@</td>
</tr>
</tbody>
</table>


Treatments were given daily (p.o.) for 6 weeks after induction of insulin resistance syndrome by feeding rats with FED for 10 weeks. Rats remained on their diet for the whole period of treatment.

Values are means of 6-10 rats ± SE.

*Significantly different from control group at p<0.05.
@Significantly different from FED group at p<0.05.
#Significantly different from rosiglitazone group at p<0.05.

Fig. 1: Effects of rosiglitazone (4 mg/kg) alone or combined with α-lipoic acid (100 mg/kg) on FED-induced changes in serum levels of TG (a), T. cholesterol (b), LDL-cholesterol (c) and HDL-cholesterol (d). Treatments were given daily (p.o.) for 6 weeks after induction of insulin resistance syndrome by feeding rats with FED for 10 weeks. Animals remained on their diet for the whole period of drug treatment.

Each bar with vertical line represents mean of 6-10 rats ± SE.

*Significantly different from control group at p<0.05.
@Significantly different from FED group at p<0.05.
#Significantly different from rosiglitazone group at p<0.05; FED: fructose enriched diet, TG: triglycerides, T. cholesterol: total cholesterol, RGZ: rosiglitazone, lip: α-lipoic acid.
Fig. 2: Effect of rosiglitazone (4 mg/kg) alone or combined with α-lipoic acid (100 mg/kg) on FED-induced changes in serum levels of TBARS (a), NOx (b) and TAC (c). Treatments were given daily (p.o.) for 6 weeks after induction of insulin resistance syndrome by feeding rats with FED for 10 weeks. Animals remained on their diet for the whole period of drug treatment. Each bar with vertical line represents mean of 6-10 rats ± SE. *Significantly different from control group at p<0.05, @Significantly different from FED group at p<0.05, #Significantly different from rosiglitazone group at p<0.05; FED: fructose enriched diet, TAC: total antioxidant capacity, TBARS: thiobarbituric acid reactive substance, NOx: total nitrate/nitrite, RGZ: rosiglitazone, lip: α-lipoic acid.

Fig. 3: Effect of rosiglitazone (4 mg/kg) alone or combined with α-lipoic acid (100 mg/kg) on FED-induced changes in liver contents of TBARS (a), NOx (b) and TAC (c). Treatments were given daily (p.o.) for 6 weeks after induction of insulin resistance syndrome by feeding rats with FED for 10 weeks. Animals remained on their diet for the whole period of drug treatment. Each bar with vertical line represents mean of 6-10 rats ± SE. *Significantly different from control group at p<0.05, @Significantly different from FED group at p<0.05, #Significantly different from rosiglitazone group at p<0.05; FED: fructose enriched diet, TAC: total antioxidant capacity, TBARS: thiobarbituric acid reactive substance, NOx: total nitrate/nitrite, RGZ: rosiglitazone, lip: α-lipoic acid.
Fig. 4: Effect of rosiglitazone (4 mg/kg) alone or combined with α-lipoic acid (100 mg/kg) on FED-induced changes in kidney contents of TBARS (a), NOx (b) and TAC (c). Treatments were given daily (p.o.) for 6 weeks after induction of insulin resistance syndrome by feeding rats with FED for 10 weeks. Animals remained on their diet for the whole period of drug treatment. Each bar with vertical line represents mean of 6-10 rats ± SE. *Significantly different from control group at p<0.05, †Significantly different from FED group at p<0.05, ‡Significantly different from rosiglitazone group at p<0.05; FED: fructose enriched diet, TAC: total antioxidant capacity, TBARS: thiobarbituric acid reactive substance, NOx: total nitrate/nitrite, RGZ: rosiglitazone, lip: α-lipoic acid.

Fig. 5: Photomicrograph of sections of liver tissue of (a) control rat showing the normal structure of liver composed of cords of polyhedral hepatocytes radiating from the central vein (C) some of them have large rounded vesicular nuclei (arrow head) while others are bi-nucleated (arrow); (b) fructose enriched diet fed rat showing severe fibrosis (arrow head) and notable fatty degeneration (arrow) of hepatocytes especially in zone which surrounds the portal area (P); (c) fructose enriched diet fed rat treated with rosiglitazone (4 mg/kg) showing minimal fibrosis in the form of fine strands of connective tissue extending from the portal area (arrow) in addition to dilatation and congestion of portal vein and (d) fructose enriched diet fed rat treated with rosiglitazone (4 mg/kg) plus α-lipoic acid (100 mg/kg) showing marked decrease in fibrosis, however, fatty degeneration at the periphery of the hepatic lobules was still observed (arrow) (H & E X 100, 200).
Fig. 6: Photomicrograph of sections of kidney tissue of (a) control rat showing the normal structure of the renal tissue composed of glomerulus (G) located in between many types of tubules, (b) fructose enriched diet fed rat showing marked dilatation and extravasations of blood into the lumen of the tubules (arrow) with an increase in the space between the tuft of capillaries and the wall of the capsule denoting increased amount of filtrate (arrow head), (c) fructose enriched diet fed rat treated with rosiglitazone (4 mg/kg) showing marked cellular infiltration (arrow) in between the glomeruli and (d) fructose enriched diet fed rat treated with rosiglitazone (4 mg/kg) plus α-lipoic acid (100 mg/kg) showing marked dilatation of almost all the tubules (H & E X 100, 200).

4. DISCUSSION

Feeding rats with FED in the current study resulted in hyperglycemia, hyperinsulinemia and increased weight gain. Fructose does not stimulate insulin secretion in the short-term, however, IR and obesity induced by long-term fructose feeding results in compensatory hyperinsulinemia (Elliott et al., 2002). At the beginning, increased insulin secretion helps to maintain normal blood glucose level as it compensates for the body’s reduced responses to insulin. Yet, over time, the increase in hepatic glucose production coupled with reduced glucose disposal from the circulation, leads to elevated blood glucose level (DeFronzo, 1988).

Moreover, fructose feeding was shown to reduce phosphorylation of insulin receptor substrates in liver and muscle of rats (Ueno et al., 2000) leading to IR as noted by the observed elevation of HOMA-IR. IR eventually leads to β-cell deterioration as a decrease in insulin sensitivity must be balanced by a compensatory increase in insulin secretion; thus β-cell failure occurs when islets are unable to compensate for IR (Prentki and Nolan, 2006), making the observed decrease in HOMA-BCF value by fructose feeding an expected consequence.

Treatment of FED-fed rats with rosiglitazone alone or combined with α-lipoic acid decreased serum insulin level and improvement insulin sensitivity reflected by decreased HOMA-IR value when compared to FED-fed group. In accordance with these results, treatment of obese rats with rosiglitazone and/or α-lipoic acid lowered elevated plasma glucose level and reduced the associated IR (Muurling et al., 2003; Ozdoğan et al., 2012; Castro et al., 2013).

Improvement of glucose homeostasis by rosiglitazone may be a consequent of insulin sensitization or a direct action of PPAR-γ on the transcription of genes involved in glucose disposal (Kalofoutis et al., 2007). In addition, rosiglitazone and α-lipoic acid increase fatty acid oxidation and decrease accumulation of TG in skeletal muscle; the latter is considered one of the major factors contributing in IR (Koh et al., 2005). Hence the beneficial effects of rosiglitazone and α-lipoic acid on insulin sensitivity in the current study can be explained.

Feeding rats with FED, in the present study, was associated with disturbances in lipid profile manifested by increased serum levels of TG, total cholesterol and LDL-cholesterol parallel to a decrease in HDL-cholesterol level. Long-term fructose feeding can cause dyslipidemia by down regulation of PPAR-α, which in turn leads to a reduction in TG catabolism and reduced expression of HDL components (Roglans et al., 2002; Basciano et al., 2005).

Treating rats with rosiglitazone alone or combined with α-lipoic acid, in the present experiments, improved FED-induced changes in lipid profile. In agreement with the present results, it was
reported that rosiglitazone and α-lipoic acid significantly decreased levels of TG, LDL-C and total cholesterol in T2DM (Mimura et al., 1994; Udapa et al., 2012). Such effects could be attributed to increased fat oxidation in muscle and liver (Koh et al., 2005). Rosiglitazone was reported to improve vasculature architecture of adipose tissues of normal subjects (Gealekman et al., 2012), which may add to its therapeutic benefits in IR subjects. Moreover, α-lipoic acid can improve hypertriglyceridemia by down-regulating genes that express major liver enzymes involved in the de novo synthesis of fatty acids and TG (Butler et al., 2009).

In the present study, feeding rats with FED resulted in significant increases in activities of serum transaminases and liver index. Histological examination of liver sections of FED-fed group correlated with the observed biochemical changes where severe fibrosis and fatty degeneration of hepatocytes were observed. Fructose-induced liver dysfunction could be regarded as an expected consequence owing to the close association between NAFLD and IR (Ouyang et al., 2008).

Fructose-induced biochemical and histological changes in the liver were largely improved by treatment with rosiglitazone alone or combined with α-lipoic acid. In accordance with the present results, Tahan et al. (2007) reported that rosiglitazone reduced liver index, improved ALT serum activity and attenuated liver inflammation in a rat model of NAFLD. Effects of rosiglitazone could be explained by its anti-inflammatory effect mediated by activation of PPAR-γ expressed on macrophages (Cuzzocrea et al., 2004) resulting in decreased production of pro-inflammatory cytokines implicated in the pathogenesis of NAFLD (Copaci et al., 2006).

In a similar fashion, fructose feeding increased kidney size and elevated serum levels of kidney function tests. This was supported by histological examination of kidney sections of FED-fed group that revealed marked fibrosis. The observed elevation in uric acid level by fructose feeding was previously reported (Nakagawa et al., 2006). Fructose enters hepatocytes and is metabolized through a series of reactions to uric acid (Hallfrisch, 1990). In addition, urinary excretion of uric acid decreases in FED-fed rats, as hyperuricemia causes endothelial dysfunction and renal vasoconstriction (Khosla et al., 2005), which can impair excretion of urate and other substances normally cleared by the kidney as creatinine.

Concomitant treatment of FED-fed rats with rosiglitazone and α-lipoic acid resulted in a significant decrease in serum uric acid and creatinine levels. Using insulin sensitizers is associated with a decrease in serum uric acid level of overweight hypertensive patients (Tsunoda et al., 2002). Moreover, Somani et al. (2000) reported that α-lipoic acid prevented cisplatin-induced increase in plasma creatinine level.

Parallel to the observed hyperuricemia, fructose feeding was associated with lower serum NOx level whereas tissues NOx contents were increased. Serum NOx is reduced in hyperuricemic rats as uric acid induces endothelial dysfunction by inhibiting NO production (Khosla et al., 2005). On the other hand, the reported increased expression of inducible nitric oxide synthase (iNOS) in kidneys of FED-fed rats (Cosenzi et al., 2002) and in NAFLD (Solís Herruzo et al., 2006) could account for the increased liver and kidney NOx contents.

The combination of rosiglitazone and α-lipoic acid, in the current study, resulted in a significant reduction in NOx contents of livers and kidneys of FED-fed rats. This effect was not achieved by rosiglitazone monotherapy; hence it could be explained by the reported down-regulation of iNOS expression by α-lipoic acid (Demarco et al., 2004).

Feeding rats in the current experiment with FED was associated with marked increases in lipid peroxide products (TBARS) in serum, livers and kidneys that were attenuated by treatment with rosiglitazone alone or combined with α-lipoic acid. The observed antioxidant effect of rosiglitazone depends on its ability to induce AMP-activated protein kinase which, in turn, prevents the activation of NAD(P)H oxidase, a major source of ROS production during hyperglycemia (Ceolotto et al., 2007). α-Lipoic acid appears to be an ideal antioxidant owing to its ability to scavenge multiple reactive species (Kagan et al., 1992; Rochette et al., 2013) and to regenerate other antioxidants such as vitamin E or C and glutathione plus its metal chelating activity (Bast and Haenen, 2003).

As oxidative stress leads to activation of a number of cellular stress-sensitive pathways linked to IR (Evans et al., 2003), the noted improvement of IR by rosiglitazone alone or combined with α-lipoic acid could also be attributed to their observed antioxidant properties.

Parallel to the noted increase in lipid peroxidation, fructose feeding decreased liver and kidney TAC which could be explained by exhaustion of tissue antioxidants owing to fructose-induced lipid peroxidation. Consequently, treatment of FED-fed rats with rosiglitazone plus α-lipoic acid elevated liver and/or kidney TAC owing to their documented antioxidant properties (Kagan et al., 1992; Wang et al., 2011; Castro et al., 2013).

On the other hand, fructose feeding resulted in an increase in serum TAC that was not reduced by any of the treatment regimens. This could be regarded as a defense up-regulatory mechanism to counteract
fructose-induced oxidative stress. Moreover, the observed increased serum uric acid concentration by fructose feeding could explain the noted increase in serum TAC in FED-fed group considering that uric acid is one of the endogenous antioxidants (Rizzo et al., 2010).

In conclusion, feeding rats with FED resulted in a group of symptoms that simulates the MS in humans. Treatment of rats with rosiglitazone and α-lipoic acid could attenuate most of the consequences of IR or MS.

5. ACKNOWLEDGEMENT

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6. REFERENCES


