Amelioration of the Possible Adverse Effects of Rosiglitazone by Fenugreek and Vitamin E in STZ-induced Diabetes in Rats

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ABSTRACT

The combination therapy could be a new and highly effective therapeutic strategy to manage hyperglycemia. Combination of commercial drugs with phyto-chemicals may reduce the side effects caused by these synthetic drugs. This study was designed to investigate the protective effect of fenugreek seed extract and/or vitamin E against the side effects of rosiglitazone streptozotocin induced diabetes in Sprague-Dawley rats. Mature male Sprague-Dawley rats were divided into 6 equal groups (8 rats each) and treated as follows: Group 1, kept as control group and orally given buffer citrate groups 2 were injected intraperitoneally (i.p.) with STZ (50mg/Kg) induce hyperglycemia, kept as control positive; groups (3) was administered daily intraperitoneal doses of roriglitazone (10 mg/kg) while groups (4-6) were administered combined treatments of vitamin E (100 mg/kg) or fenugreek (30mg/kg), respectively. Our results indicated that STZ caused significant elevation of serum glucose level, LDL-c ,total-cholesterol, CK-MB, LDH, and significant decrease in serum HDL-c. While, rosiglitazone treatment alone significantly decreased serum total -cholesterol, LDL-c, and serum glucose level. On the other hand, the diabetic group treated with rosiglitazone showed elevation in the CK-MB, LDH levels in compared to the diabetic control. The combination group(rosiglitazone+ vitamin E+fenuugreek) showed more potent group with marked reduction in CK-MB ,LDH ,LDL-c ,total cholesterol and serum glucose levels. Hepatic and elevation in HDL-c level. Histopathological study of heart section showed focal myocarditis in a group treated with rosiglitazone. Concomitant treatment with vitamin E (100 mg/kg) and/or fenugreek (30mg/kg) significantly improved histopathological structure of heart tissue in variable degrees. In conclusion, the combined effect of vitamin E (100 mg/kg) with fenugreek (30mg/kg)and rosiglitazone (10mg/kg) had powerful effect than any other studied doses.

Key words: Combination therapy, rosiglitazone, vitamin E, fenugreek, ck-mb, anti-oxidant.

Introduction

Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia. Depending on the etiology of diabetes mellitus, factors contributing to hyperglycemia include reduced insulin secretion, decreased glucose utilization, and increased glucose production (Alam et al., 2013).

Genetic factors, inactive lifestyle and obesity play an important role in the development of type 2 diabetes mellitus. (Deacon et al., 2007). Oxidative stress, also, is one of the main factors playing a role in the development of diabetic complications, ROS impair β-cell glucose – mediated insulin secretion and the regulation of mass regeneration via detrimental effects on mitochondrial function. (Li, et al., 2008)

Long term complications of diabetes include retinopathy with potential loss of vision, nephropathy leading to renal failure, peripheral neuropathy and autonomic neuropathy causing gastrointestinal, genitourinary and cardiovascular symptoms. Hypertension and abnormalities of lipoprotein metabolism are often found in people with diabetes (American Diabetes Association, 2014).

All the drugs are associated with side effects. Though it is important that glycaemic control should be achieved as rapidly as possible to minimize the impact of glucose toxicity, it is also necessary to provide therapy to control other related risk factors, including oxidative stress, dyslipidemia, mitochondria dysfunction and vascular complications (Duckworth 2009; Jain and Saraf, 2010). Drug combinations have been used for treating diseases and reduce suffering (Bijnsdorp et al., 2011). Two drugs that produce almost similar effects when used together may sometimes produce enhanced, same or diminished effect (Wagner and Ulrich – Merzenich 2009).

Thiazolidindiones are oral antidiabetic drugs which provide good glycemic control along with beneficial effects on dyslipidemia (Haberbosch, 2007). However, some studies have indicated that some of these agents are associated with risk of myocardial infarction (Nissen and Wolski, 2007) and heart failure (Granberry et al., 2007).

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Accordingly, the present study aimed to ameliorates the possible adverse effect of rosiglitazone by fenugreek and vitamin E in STZ-induced diabetes in rats.

Materials and Methods

Animals

Adult male albino Wistar rats, weighing 120–150g each were used in the current study. They were purchased from the National Research Centre (NRC; Giza, Egypt). Animals received human care in compliance with the guidelines of the animal care and use committee of the NRC. The animals were kept in a quiet place and were allowed free access to water and standard food pellets throughout the period of investigation. Experiments were performed according to the National Regulations of Animal Welfare and Institutional Animal Ethical Committee (IAEC).

Chemicals

Streptozotocin was obtained from Sigma (Egypt)

Plant

The seed of Trigonella foenum-Graecum were purchased from Horticulture Department, Ministry of Agriculture, Dokki, Giza. The plant were dried, crushed into a powder and then weighed and prepared for further extraction

Drugs

Rosiglitazone tablets was obtained from Glaxo Smithklein

Vitamin E capsules was obtained from Farco.

Experimental Design

Experimental diabetes was induced in overnight fasted rats by single intraperitoneal injection of streptozotocin (50 mg/kg b.wt) dissolved in freshly prepared 0.1 M of cold buffer (Ph4.5) (Rakieten et al., 1963). Rats were randomly assigned to six groups of eight rats per group and that were treated as follows:

- Group 1: Normal control rats receiving buffer citrate.
- Group 2: Diabetic control rats by STZ
- Group 3: Diabetic rats receiving 10 mg/kg rosiglitazone.
- Group 4: Diabetic rats receiving 5mg/kg rosiglitazone + 30mg/kg alcoholic extract of fenugreek.
- Group 5: Diabetic rats receiving 5mg/kg rosiglitazone +100mg/kg vitamin E.
- Group 6: Diabetic rats receiving 5mg/kg rosiglitazone + 30mg/kg alcoholic extract of fenugreek + 100 mg/kg vitamin E.

Methods

Preparation of blood samples

After the last dose of drug administration, animals were fasted for 6 hrs, but allowed free access to water. The venous blood samples were collected from the retro-orbital sinus of each animal using heparinized capillary tubes (Bjornson et al., 1983) Each samples was divided into 2 portion. The first aliquot of blood was placed in heparinized tube for plasma separation, the second portion of blood was placed in non – heparinized tube for serum separation.

Biochemical measurements

Diabetic biomarker : Glucose level was determined in serum samples according to Trinder, (1969) using Biodiagnostic kits, Egypt. Cardio vascular biomarkers: Determination of serum CK – MB level according to Panteghini et al. (1974) using Oxis Research,CA,USA and serum LDH level according to Lorentz et al. (1993) using Eli Tech, Puteux, France. Lipid profile: Determination of serum Cholesterol, LDL and HDL levels according to Demacker et al. (1983) using Biodiagnostic kits, Egypt

Statistical analysis:

The data were analyzed SPSS statistical software, comparison of the mean and standard deviation(SD)of the above -mentioned, biochemical and histological parameters among diabetic and treated groups ,diabetic and control groups was done .The significant level was considered at p value<0.05.

Results

Data presented in Table (1) showed that there was a significant rise in serum glucose level,CK-MB and LDH throughout the period in diabetic group. On the other hand the diabetic group treated with rosiglitazone showed
reduction in glucose level and significant elevation in CK-MB and LDH. As observed from results, the diabetic group treated with combination (rosiglitazone + fenugreek + vitamin E) showed better results as compared by diabetic control.

**Table 1:** Effects of fenugreek alcoholic extract, rosiglitazone and vitamin E, alone or in combination on serum glucose level, CK-MB and LDH levels in diabetic rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Parameters</th>
<th>Glucose</th>
<th>CK-MB</th>
<th>LDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td></td>
<td>91.3^a</td>
<td>90.1^b</td>
<td>250.4^c</td>
</tr>
<tr>
<td>Diabetic control</td>
<td></td>
<td>419.3^d</td>
<td>189.5^e</td>
<td>609^f</td>
</tr>
<tr>
<td>Rosiglitazone</td>
<td></td>
<td>143.3^g</td>
<td>218.3^h</td>
<td>761.4^i</td>
</tr>
<tr>
<td>Rosiglitazone + Fenugreek</td>
<td></td>
<td>134.4^j</td>
<td>164.9^k</td>
<td>424.8^l</td>
</tr>
<tr>
<td>Rosiglitazone + Vit E</td>
<td></td>
<td>130.9^m</td>
<td>167^n</td>
<td>396.5^o</td>
</tr>
<tr>
<td>Rosiglitazone + Vit E + Fenugreek</td>
<td></td>
<td>116.6^p</td>
<td>140.3^q</td>
<td>336^r</td>
</tr>
</tbody>
</table>

*Values are means of 6-8 rats ± SEM. As compared with normal control (a)p<0.01, diabetic control (b)p<0.05, one way ANOVA followed by tukey post hoc test)*

-CK-MB: Creatine Kinase Mono Enzyme-B
-LDH: Lactate Dehydrogenase

**Fig. 1:** Effects of fenugreek alcoholic extract, rosiglitazone and vitamin E, alone or in combination on serum glucose level, CK-MB and LDH levels in diabetic rats.

Data in Table (2) cleared that there was a significant rise in serum LDL, Cholesterol and reduction in HDL level throughout the period in diabetic group. On the other hand the diabetic group treated with rosiglitazone showed reduction in LDL, Cholesterol and significant elevation in HDL levels. As observed from results, the diabetic group treated with combination (rosiglitazone + fenugreek + vitamin E) showed better results as compared by diabetic control.

**Table 2:** Effects of fenugreek alcoholic extract, rosiglitazone and vitamin E, alone or in combination on serum HDL, LDL and cholesterol levels in diabetic rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Parameters</th>
<th>HDL</th>
<th>LDL</th>
<th>Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td></td>
<td>42.1^a</td>
<td>27.6^b</td>
<td>68.5^c</td>
</tr>
<tr>
<td>Diabetic control</td>
<td></td>
<td>37.9^d</td>
<td>43.03^e</td>
<td>215.5^f</td>
</tr>
<tr>
<td>Rosiglitazone</td>
<td></td>
<td>42.7</td>
<td>38.9^g</td>
<td>171.6^h</td>
</tr>
<tr>
<td>Rosiglitazone + Fenugreek</td>
<td></td>
<td>45.6^i</td>
<td>30.8^j</td>
<td>127.5^k</td>
</tr>
<tr>
<td>Rosiglitazone + Vit E</td>
<td></td>
<td>45.2^l</td>
<td>34^m</td>
<td>131.3^n</td>
</tr>
<tr>
<td>Rosiglitazone + Vit E + Fenugreek</td>
<td></td>
<td>46.3^o</td>
<td>29.3^p</td>
<td>109.5^q</td>
</tr>
</tbody>
</table>

*Values are means of 6-8 rats ± SEM. As compared with normal control (a)p<0.01, diabetic control (b)p<0.05, one way ANOVA followed by tukey post hoc test), HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein*
Fig. 2: Effects of fenugreek alcoholic extract, rosiglitazone, and vitamin E, alone or in combination on serum HDL, LDL, and Cholesterol levels in diabetic rats.

**Histological results**

**Group (1):** (Control group): (H&E) stained section of the control group showed normal histological structure of the heart.

**Group (2):** (H&E) stained section of the positive control group (STZ) showed congestion of myocardial blood vessels and focal myocarditis.
Group (3): Received rosiglitazone: (H&E) stained section received rosiglitazone showing myolysis of myocytes, intermyocardial edema and focal myocarditis

Group (4): Received rosiglitazone +fenugreek+ vitamin E: (H&E) stained section received rosiglitazone, fenugreek and vitamin E showing no histological changes

Discussion

The results presented in this study clearly indicated that treatment of diabetic rats with rosiglitazone (10mg/ kg b.wt) significantly decreased the blood glucose level, and increase in plasma insulin levels, whereas, significantly elevated HDL-C level in compared to diabetic non treated group.

Rosiglitazone treatment in our study alone or in combination improved the overall glycemic control. This results was observed by Osei et al., (2004). In addition, rosiglitazone ameliorated metabolic dysfunction, β-cell function in patients with T2DM (Pflützner et al., 2006; Hussein et al., 2011).

In Rosiglitazone there was a significant decrease in serum cholesterol, LDL-C as well as a significant increase in serum HDL-C as compared to diabetic control group (positive control).

In contrary to John et al. (2002) who said that rosiglitazone causes an increase in low density lipoprotein (LDL). A favourable finding observed in this study is the significant decrease of LDL-C with rosiglitazone.

Trigonellafoenum-graecum has shown antihyperlipidemic effects it delays the digestion and absorption of carbohydrates and enhances insulin action owing to presence of sparogenins which increase biliary cholesterol excretion as well Basch et al. (2003).

As implied from the obtained results, the diabetic group treated with fenugreek was significant increase in HDL level, significant decrease in cholesterol, LDL-C and TG.

As implied from the obtained results, there was significant increase in serum CK-MB and LDH in the diabetic group treated with rosiglitazone in compared with diabetic non treated group.

Serum CK-MB is considered to indicate myocardial injury. On the other hand, tissue break down elevates levels of LDH therefore its estimation could be used as a marker of tissue damage as acute myocardial information, heart failure, cancer (Ahmed et al., 2004). Hence, the CK-MB and LDH activities in serum reflect the alterations of membrane integrity and the degree of myocardial injury (Liu et al., 2012).

Guan et al. (2012) confirmed the STZ-induced cardiomyopathy represented by elevated levels of LDH and CK as well as myocardial apoptosis and hypertrophy.

Rosiglitazone treatment increased CK-MB and LDH serum levels. These results are in agreement with results obtained by Rabbani et al., (2009) who studied the effect of chronic STZs, therapy on increase the risk of myocardial infarction and congestive heart failure.

CK-MB and LDH serum levels was elevated as a potential injury biomarkers in rosiglitazone treated group.
Vitamin E has been observed reduction in serum LDL-c, cholesterol level and slightly elevation in serum HDL-C level.

This results agreement with Robby et al. (2011) who said the antioxidant supplementation are recommended to reduce damage by increased free radical toxicity in diabetes mellitus.

In our investigation diabetic group treated with vitamin E shows a significant improvement in cardiovascular biomarkers(CK-MB & LDH).

The results are supported by those obtained by Milman et al. (2008) who mentioned that the supplementation with vitamin E showed reduced cardiovascular events.

In the obtained results the diabetic group who treated with a combination drugs (rosiglitazone + vitamin E + fenugreek) shows the improvement in all diabetic biomarker, lipid profile and CK-MB, LDH in compare with diabetic non treated group.

Bijnsdorp et al. (2011) suggested that drug combination have been used for treating disease and reduce suffering.

The use of herbal with medicine enhances the effect of medicine and reduces its adverse effects (Lin and Sun 2010).

**Conclusion**

-Rosiglitazone is an oral antidiabetic drug, improves glucose and lipid metabolism, ameliorate insulin resistance, but also associated with an increased risk of myocardial infarction and cardio vascular mortality.

-Fennegreek has been shown exhibit antioxidant properties, anti diabetic and antihyperlipidemic effects owing to their phenolic constituents.

-Vitamin E supplementation as an antioxidant might lower the risk of developing type 2 diabetes and reduce cardiovascular events.

-Combination of oral administration of alcoholic extract of fennegreek which is rich in antioxidants nutrient and oral supplementation of vitamin E can ameliorate the adverse effects of rosiglitazone and gliclazide.

**References**


