**Rauwolfia serpentina** improves altered glucose and lipid homeostasis in fructose-induced type 2 diabetic mice

Muhammad Bilal Azmi and Shamim A Qureshi
1Department of Biochemistry, University of Karachi, Karachi, Pakistan
2Department of Biochemistry, Dow Medical College, Dow University of Health Sciences, Karachi, Pakistan

**Abstract:** *Rauwolfia serpentina* is well-reported in traditional medicines for the treatment of hypertensive and neurological disorders. However, its antidiabetic potential has been currently described in both alloxan-treated and normoglycemic mice. Present effort was carried out to investigate the effect of methanol root extract (MREt) of *R. serpentina* in fructose-induced type 2 diabetic mice. Experimental mice were grouped into normal control (distilled water 1ml/kg) and fructose-induced type 2 diabetic groups (10% fructose 1 ml/kg). The second group sub-divided into negative (0.05% DMSO 1ml/kg) control, positive (pioglitazone 15mg/kg) control and three test groups (MREt 10, 30 & 60 mg/kg). Each treatment was given orally for 14 days consecutively then mice were sacrificed in order to collect serum and liver samples to analyze physical, biochemical as well as hematological markers. MREt significantly improved percent body weight and glycemic change along with serum insulin, total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL-c), very low-density lipoprotein (VLDL-c), high-density lipoprotein-cholesterols (HDL-c), total hemoglobin, glycosylated hemoglobin, hepatic glycogen, coronary risk and fasting insulin resistance indices while suppressed down the activity of 3-hydroxy-3-methylglutaryl Coenzyme A reductase enzyme in test groups when compared with diabetic controls. The present findings conclude that MREt of *R. serpentina* can effectively betters the carbohydrate and lipid homeostasis by either inhibiting fructose absorption in intestine or decreasing insulin resistance in fructose-induced type 2 diabetic mice.

**Keywords:** Fructose, insulin resistance, 3-hydroxy-3-methylglutarylcoenzyme A, *Rauwolfia serpentina*.

**INTRODUCTION**

Type 2 or non-insulin dependent diabetes mellitus (T2D) becomes health problem globally which affects both genders in every country especially developing countries like Pakistan (Ijaz and Ajmal, 2011). It is a hormone-linked metabolic disorder associated with relative insulin deficiency or insulin resistance (Kadowaki, 2000). Insulin resistance reflects the incompatibility of insulin with its receptors on target tissues including liver, muscle and adipose tissues (Patel et al., 2013) which results in persistent hyperglycemia by impairing carbohydrate, lipid and protein metabolism (Hsu, 2013). Genetic and acquired factors are increasing the risk of insulin resistance worldwide (Singh, 2011). Among the acquired factors, diet with high-sugar (sucrose/fructose) content plays a significant role in the growth of insulin resistance by producing hypercholesterolemia and hypertriglyceridemia (Salas-Salvadó et al., 2011; Adeneye, 2012; Khitan and Kim, 2013) which in turn increases the number of patients with vascular complications (Ahmed et al., 2010).

Fructose is an obesity inducer ketohexose that produced dihydroxy acetone phosphate (DHAP) and glyceraldehyde-3-phosphate (GA3P) without passing through the step catalyzed by phosphofructokinase, one of the rate-regulatory enzymes of glycolysis, thereby accelerating the synthesis of glucose via gluconeogenesis and triglycerides (TG) that lead to hyperglycemia and hypertriglyceridemia (Feinman and Fine, 2013; Bray, 2007). This increased amount of TG not only mask the insulin receptors on target tissues and lead to hyperinsulinemia but also act as an alternate source of energy for the body results in increased production of acetyl coenzyme A that cannot be easily handled by tricarboxylic acid cycle (TCA) and induced to cholesterol biosynthesis that leads to hypercholesterolemia (Feinman and Fine, 2013; Bray, 2007; Johnson et al., 2009). In spite of these harmful effects, high fructose corn syrup (HFCS) is one of the widely used sweeteners in commercial food stuffs where it acts as a slow poison for increasing the risk of metabolic disorders especially T2D globally (Bray et al., 2004; Gorana et al., 2012). Many hypoglycemic agents like biguanides and thiozolidinediones have been used for the management of this health hazard but possess few side effects (Masuda and Terauchi, 2010). However, literature also witnesses the importance of herbal remedies with no or negligible side effect in the treatment of T2D (Chang et al., 2013).

The well-known antihypertensive medicinal plant *Rauwolfia serpentina* Benth (family Apocynaceae) is also famous for its variety of ethno-medicinal effectiveness like in the treatment of snake bite, gastrointestinal tract disorders, breast cancer, skin problems, etc (Azmi and Qureshi, 2012a). Currently its short- and long-term antidiabetic activities have been reported in alloxan-
induced diabetic mice where it was found to improve the atherogenic, arteriosclerosis and cardioprotective indices (Qureshi et al., 2009; Azmi and Qureshi, 2012b; Azmi et al., 2015). Another investigation has explored the antioxidant and haematinic properties of methanolic root extract of *R. serpentina* in alloxan-induced (type 1 diabetic) mice (Azmi and Qureshi, 2013). However, antidiabetic activity of same plant has not been reported in insulin resistance diabetes so far. Therefore, for the first time, present effort was designed to evaluate the antidiabetic and lipid lowering efficiencies of methanol root extract of *R. serpentina* in fructose-induced type 2 diabetic mice.

**MATERIALS AND METHODS**

**Plant material and preparation of methanol extract**
The roots of *R. serpentina* were procured, authenticated and kept (KU/BCH/SAQ/02) at Biochemistry Department, University of Karachi (UoK), Karachi-75270, Pakistan. The methanol roots extract (MREt) was prepared as described by Azmi and Qureshi in 2012 and stored in refrigerator below 10°C until used (Azmi and Qureshi, 2012a).

**Induction of fructose-induced type 2 diabetes**
It was done by giving 10% fructose solution (1ml/kg) orally once a day for 14 days connectively in overnight fasted mice (Neeharika et al., 2012).

**Antidiabetic medicine and vehicle for MREt**
Commercially available pioglitazone (Zolid, 15mg/kg) of Getz Pharma, Pakistan Ltd. and 0.05% dimethyl sulfoxide (DMSO) of Fisher Chemicals (United Kingdom) were used as positive control and vehicle for MREt in present study.

**Experimental mice and their grouping**
Wistar male albino mice (n = 42) from 25 to 35 grams in weight were procured from the commercial breeding center of Dow University of Health Sciences (DUHS), Karachi, Pakistan and kept in animal house of same university according to the international guidelines of animal care and handling. These mice were provided standard laboratory diet with easy access to water *ad libitum* and divided in different groups on the basis of treatments (fig. 1). The present research protocol was approved by Institutional Ethical Review Board (IERB – Authority Reference Number: IRB-186/DUHS-10 ) and Board of Advance Studies and Research (BASR) of DUHS and UoK respectively. All treatments were given to their respective groups orally once per day for consecutive 14 days. At the end of 14th day, mice were sacrificed; whole blood, serum and liver samples were collected and used to analyze biochemical markers.

**Determination of physical parameter**
Percent change in body weights of all mice of each group was calculated (Azmi and Qureshi, 2012b) after measuring their weights on initial and final day of trial with the help of weighing balance.

\[
\text{Body weight change (\%)} = \left( \frac{\text{Final day weight} - \text{Initial day weight}}{\text{Initial day weight}} \right) \times 100
\]

**Determination of biochemical parameters**
Percent glycemic change of all mice was calculated after measuring fasting blood glucose (FBG) at initial and final day of trial by using glcomemeter (Abbott Laboratories, Pakistan) from tail vein (Azmi and Qureshi, 2012b).

\[
\text{Glycemic change (\%)} = \left( \frac{\text{Final day FBG} - \text{Initial day FBG}}{\text{Initial day FBG}} \right) \times 100
\]

Insulin level in serum was determined by the help of cobas c411 analyzer, Hitachi (Roche Diagnostics GmbH, Mannheim, Germany) whereas fasting insulin resistance index (FIRI) was calculated by using following formula (Duncan et al., 1995).

\[
\text{FIRI} = \frac{\text{Fasting Insulin (\muU/ml)} \times \text{Fasting glucose (mg/dl)}}{25}
\]

Serum total cholesterol (TC), triglycerides (TG) and high-density lipoprotein-cholesterol (HDL-c) were determined by enzymatic kits (Randox, United Kingdom). However, low density lipoprotein-cholesterol (LDL-c), very low density lipoprotein-cholesterol (VLDL-c) and coronary risk index (CRI) were calculated through following formulae (Azmi and Qureshi, 2012b).

\[
\begin{align*}
\text{LDL-c (mg/dl)} & = \text{TC} - \left( \frac{\text{TG}}{5} \right) - \text{HDL-c} \\
\text{VLDL-c} & = \frac{\text{TG}}{5} \\
\text{CRI} & = \left( \frac{\text{Total Cholesterol}}{\text{HDL-c}} \right)
\end{align*}
\]

**Determination of hematological parameters**
Total hemoglobin (Hb) and glycosylated hemoglobin (HbA1c) levels were estimated by Automated Analyzer, Sysmex (XS-1000i) and Nycocard Kit of USA, respectively.

**Determination of hepatic parameters**
Estimation of glycogen content in liver homogenate was performed by colorimetric method (Dubois et al., 1956). Whereas 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity was determined in terms of HMG-CoA/Mevalonate ratio (Rao and Ramakrishnan, 1975).

**STATISTICAL ANALYSIS**
Data of present research was analyzed by using one-way ANOVA, followed with least significant difference (LSD) test, through SPSS version 18. Results are mentioned as mean ± SEM (Standard Error Mean). All values are considered significant at *p*<0.0001, *p*<0.01 & *p*<0.05.
RESULTS

Effect of MREt on physical parameter
Pioglitazone and two doses (10 and 30 mg/kg) of MREt induced 1.6, 4.9 and 2.09% increase in body weights of mice in positive control and test (IV, V & VI) groups when compared with diabetic control groups (II and III) which showed prominent gain (6-9%) in their body weights. However, dose of 60 mg/kg of same extract (\(p<0.01\) \(\&\) \(p<0.05\)) effectively prevent gain in body weights of mice of group VII (fig. 2).

Effect of MREt on FBG and other biochemical parameters
Three of the doses of MREt showed 25.38, 18.64 and 17.40% gain (\(p<0.01\), \(p<0.0001\)) in FBG respectively in group V, VI and VII which was compared to diabetic control groups that depicted 76.8-80% increase in same parameter (fig. 2). A gradual decrease (\(p<0.01\) \&\(p<0.0001\)) in serum insulin levels (6.01 to 3 pmol/l) and FIRI (4.35-1.93) was observed in test groups when compared with diabetic controls which showed increased insulin level and insulin resistance while pioglitazone showed more significant effect on both of these parameters (fig. 3). Similarly, MREt (10, 30 & 60 mg/kg) showed significant decrease in serum levels of TC, TG, LDL-c, VLDL-c and increase in HDL-c level in their respective test groups whereas completely opposite picture of lipid profile was observed in diabetic group II \& III. On contrary, pioglitazone was only found effective (\(p<0.0001\) \&\(p<0.05\)) in decreasing the levels of TG and VLDL-c (table 1). Prominent decrease (\(p<0.05\) \&\(p<0.01\)) in CRI from 3.5 to 1.48 in MREt-treated test groups while diabetic control groups showed much increase (i.e., up to 4.09) in the same ratio. Beside this, positive control group showed high value of CRI (fig. 4).

Effect of MREt on hematological parameters
High HbA1c (7.13%) and low total Hb (7.55%) levels were observed in diabetic control groups. However, three of the doses of MREt gradually improved (\(p<0.01\) \&\(p<0.0001\)) the magnitude of total Hb and HbA1c levels in their respective test groups. Similarly, positive control also improved HbA1c but not total Hb level in group IV (fig. 5).

Table1: Effect of MREt on biochemical parameters

<table>
<thead>
<tr>
<th>Groups</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL-c (mg/dl)</th>
<th>LDL-c (mg/dl)</th>
<th>VLDL-c (mg/dl)</th>
<th>Glycogen (g/g of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>139.74±14.32</td>
<td>140.21±7.32</td>
<td>54.52±7.59</td>
<td>65.22±9.71</td>
<td>28.04±1.46</td>
<td>1.39±0.27</td>
</tr>
<tr>
<td>II</td>
<td>199.63±21.35</td>
<td>261.51±13.61</td>
<td>58.16±14.09</td>
<td>94.64±29.49</td>
<td>52.30±2.72</td>
<td>0.49±0.06</td>
</tr>
<tr>
<td>III</td>
<td>237.80±21.16</td>
<td>219.54±31.43</td>
<td>61.25±5.28</td>
<td>132.64±18.30</td>
<td>43.91±6.29</td>
<td>0.56±0.13</td>
</tr>
<tr>
<td>IV</td>
<td>193.50±17.21</td>
<td>167.74±7.72</td>
<td>43.37±7.40</td>
<td>115.47±19.39</td>
<td>35.55±1.54</td>
<td>0.79±0.06</td>
</tr>
<tr>
<td>V</td>
<td>176.73±8.35</td>
<td>178.14±10.58</td>
<td>50.82±1.54</td>
<td>90.30±4.75</td>
<td>35.63±2.12</td>
<td>0.52±0.11</td>
</tr>
<tr>
<td>VI</td>
<td>163.50±12.78</td>
<td>144.46±12.28</td>
<td>77±5.55</td>
<td>57.61±5.73</td>
<td>28.89±2.45</td>
<td>0.74±0.10</td>
</tr>
<tr>
<td>VII</td>
<td>165.75±3.88</td>
<td>146.66±6.24</td>
<td>113.17±3.49</td>
<td>23.25±2.81</td>
<td>29.33±1.25</td>
<td>0.86±0.11</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=8). *\(p<0.05\), **\(p<0.01\) and ***\(p<0.0001\), when compared with respective group II (a) and III (b).
Rauwolfia serpentina improves altered glucose and lipid homeostasis in fructose-induced type 2 diabetic mice

Effect of MREt on hepatic parameters
MREt (10-60mg/kg) and pioglitazone improved liver glycogen content from 0.5-0.86g/g of hepatic tissue in test and positive control groups while diabetic control groups showed decreased glycogen content in liver tissues (table 1). HMG-Co A reductase activity was found inhibited by observing improved \((p<0.0001 \& \ p<0.01)\) HMG-CoA/Mevalonate ratio in MREt-treated test groups especially group VI & VII as compared diabetic control groups (fig. 4).

Therefore, the preliminary and most reported effect of T2D is the weight gain (Bray, 2007). The same feature was observed in present study that all mice of diabetic control groups showed percent gain from 6-9% in their body weights after consuming 10% fructose solution (1ml/kg) consecutively for 14 days. Whereas gradual improvement in body weights of mice in three test groups was observed in dose-dependent manner. In this respect, the highest dose (60 mg/kg) of MREt completely prevents gain in body weights of mice in its respective group. Similarly, all three doses of MREt found effective in controlling fructose-induced hyperglycemia in experimental groups when compared to diabetic control groups which showed extreme increase in FBG level. Pioglitazone, a well-known medicine used in T2D also found effective in preventing fructose-induced percent glycemic and weight gain in positive control group. The percentage of Hb\(_{A1c}\) is about 4-6% in non-diabetic condition whereas in T2D persistent hyperglycemia induced non-enzymatic glycation of total Hb thus elevates the amount of Hb\(_{A1c}\) from 6% to onwards and creates an unrecognized mild anemia which gradually increases with the age of diabetes (Minshawy and El-Bassuoni, 2010; Cederberg et al., 2010). Similar situation was observed in present study where high Hb\(_{A1c}\) and low total Hb levels were found in diabetic control groups. On contrary, three of the doses of MREt gradually improved the magnitude of total Hb and Hb\(_{A1c}\) in their respective test groups by showing good control of Hb\(_{A1c}\) from 4.4-4.8%. Interestingly, pioglitazone was only found effective in improving Hb\(_{A1c}\) but did not show any effect on decreased total Hb concentration in positive group.

DISCUSSION

Insulin resistance is the classical feature of T2D and well-accepted as the initiator of disability and death worldwide (Patel et al., 2013; Hsu, 2013). Excessive intake of HFCS is also reported as one of the leading causes of insulin resistance which slowly and gradually induce obesity by producing hypertriglyceridemia that eventually decrease the sensitivity of receptors for its agonist insulin, this situation leads to hyperinsulinemia, hyperglycemia, hyperlipidemia and many other important signs of T2D (Khitan and Kim, 2013; Ahmed et al., 2010; Johnson et al., 2009; Bray et al., 2004; Gorana et al., 2012).

The hypoglycemic effect of MREt observed in present study may be due to its extra-pancreatic action via inhibiting fructose absorption in intestine or reducing insulin resistance in fructose-induced T2D mice. The last possibility was clarified by observing a significant gradual decrease in serum insulin levels in MREt-treated groups which also lowers FIRI in same experimental groups when compared to the both diabetic controls.
which showed hyperinsulinemia and increased insulin resistance (FIRI). Therefore, MREt may be effective in improving insulin resistance which improves glucose uptake in target tissues and stimulate anabolic processes of insulin like glycogenesis, lipogenesis, etc, thereby decrease fructose-induced hyperglycemia and HbA1c level in test groups. It was also confirmed by noticing the dose-dependent increased in hepatic glycogen in three MREt-treated test groups. However, the possibility of inhibiting the fructose absorption in intestine by MREt cannot be overlooked as the same extract was found to improve glucose tolerance in glucose-fed mice earlier (Azmi and Qureshi, 2012a).

High dietary fructose is reported as a lipogenic agent, which after intestinal absorption enters in liver cells through insulin-independent glucose transporters (GLUT-5) and stimulates the synthesis of glycerol-3-phosphate, acyl coenzyme A (acyl-Co A) and acetyl coenzyme A (acetyl-Co A). The first two components accelerate the formation of triglycerides (fats) and its transporting vehicle VLDL-c which enhance the deposition of newly synthesized triglycerides on membranes of peripheral tissues, thereby possibly involve in masking of insulin receptors and induce insulin resistance (Rutledge and Adeli, 2007). Likewise, acetyl-Co A speeds up the synthesis of cholesterol and cholesterol transporting protein, LDL-c thus encouraging the hypercholesterolemia and discouraging the role of HDL-c that leads in cholesterol efflux from peripheral tissues (Lateef and Qureshi, 2014). Both of these hyperlipidemic effects of fructose provoke the risk of life-threatening heart problems in insulin resistance diabetes (Hsu, 2013; Khitan and Kim, 2013; Ahmed et al., 2010). In such type of diabetes, normally oral hypoglycemic agent is prescribed in combination with hypocholesterolemic agent in order to minimize the risk of heart problems (Gomez et al., 2005). In the present effort, MREt (10, 30 & 60mg/kg) showed significant decrease in serum levels of TC, TG, LDL-c, VLDL-c and increment in HDL-c quantity in their respective experimental groups while pioglitazone was only found effective in decreasing TG and VLDL-c levels. The hypotriglyceridemic effect of MREt may be due to its ability of enhancing insulin sensitivity for its receptor. The hypocholesterolemic effect of MREt in test groups may be associated with inhibition of HMG-CoA reductase activity, the rate-limiting enzyme involved in the cholesterol biosynthesis (Lateef and Qureshi, 2014). This possibility was also evidenced by observing gradually improved HMG-CoA/Mevalonate ratio in all extract-treated groups as compared to decrease values of same ratio found in diabetic control groups. These findings are more confirmed by observing a significant decrease in CRI of test groups while diabetic control groups showed marked increase in the same index. Despite this, positive control (group IV) showed high value of CRI. CRI reflects the susceptibility towards cardiovascular problems (Azmi and Qureshi, 2012b). Therefore, MREt of R. serpentina strongly minimized the risk of cardiovascular problems in fructose-induced diabetic mice as same as it was efficiently improved cardio-protective indices in alloxan-induced (type 1 diabetic) mice (Azmi and Qureshi, 2012b). A significant amount of alkaloids and polyphenolic compounds have been estimated in MREt previously (Azmi and Qureshi, 2012b) which could be involve in improving the glucose and lipid homeostasis in fructose-induced diabetic mice.

**CONCLUSION**

Results from present effort concluded that MREt of *R. serpentina* improves hyperinsulinemia, hyperglycemia, hypertriglyceridemia and hypercholesterolemia in fructose-induced T2D mice either by inhibiting fructose absorption in intestine or reducing insulin resistance.

**REFERENCES**


Rauwolfia serpentina improves altered glucose and lipid homeostasis in fructose-induced type 2 diabetic mice


