Abstract:
The renin angiotensin system (RAS) was involved in various metabolic processes including insulin secretion, carbohydrate absorption and proliferation of adipose tissue. This study aims to evaluate the effects of aliskiren on the absorption of glucose in rats with fructose-induced metabolic syndrome. The results showed that treatment with aliskiren attenuates the increase in body weight and improves fasting serum glucose, insulin, triglycerides and cholesterol levels. Moreover, absorption of glucose was significantly decreased after oral glucose load in high-fructose diet fed rats. In conclusion, aliskiren significantly reduces body weight, fasting serum glucose, insulin, triglyceride and total cholesterol levels, and the rate of glucose absorption after oral glucose load in high fructose-fed rats.

Introduction:
Many reports have already confirmed that all of the renin-angiotensin system (RAS) components were expressed in many organs, which support the idea that modulation of locally expressed RAS produces a well-recognized impact on tissue homeostasis [1,2]. In addition to its involvement in the regulation of fluid and electrolyte balance [3] and regulation of blood flow [4], RAS was potentially involved in various metabolic processes including insulin secretion [5], carbohydrate absorption [6] and proliferation of adipose tissue [7]. Maintenance of experimental animals on a high-fructose diet was reported to dysregulate the intestinal RAS, with consequent pathological changes, including hypertension, glucose intolerance and hyperlipidemia [8,9]. The direct renin inhibitor, aliskiren, reduces blood pressure in rat models of spontaneous hypertension and in patients with essential hypertension and type 2 diabetes mellitus [10-12]. Beyond the antihypertensive activity, aliskiren also improves hyperglycemia and the impaired lipid profile in fructose-fed hypertensive rats [13,14]. Meanwhile, it has been recently
reported that aliskiren improves insulin sensitivity, hepatic steatosis and peripheral adiposity in experimental animal model of metabolic syndrome \cite{15,16}. However, the influence of blocking renin with aliskiren on the intestinal absorption of carbohydrates was poorly investigated in fructose-induced metabolic syndrome model. Therefore, we evaluated the effects of aliskiren, the direct renin inhibitor, on the intestinal absorption of glucose in rats with fructose-induced metabolic syndrome.

**Materials and Methods:**

**Animals**

Twenty-four male Wistar rats weighing 150-200g were utilized in the present study. They were housed in polypropylene cages and maintained under the standard laboratory conditions (temperature 25±2°C, 12:12 h light-dark cycle with free access to water and food). The rats were acclimatized to laboratory conditions for 5 days before starting experiments. They were allocated into 3 groups (8 rats each). The study protocol was carried out in accordance with the international guidelines of experiments on animals reported elsewhere, and approved by the Research Ethics Committee, College of Pharmacy, University of Sulaimani.

**Fructose-induced Metabolic Syndrome in Rats**

Feeding rats with a fructose-enriched diet, results in insulin resistance and hypertriglyceridemia \cite{17}. The rats were randomly assigned to 3 groups of 8 each and were treated as follows: first group (negative control), maintained on normal chow and treated with vehicle (0.5% Carboxy Methyl Cellulose), 0.5 ml/100 g, p.o, for 8 weeks; second group (positive control), maintained on 65% fructose rich diet and treated with vehicle as in first group; third group (aliskiren treated group), maintained on fructose rich diet and treated with aliskiren suspended in 0.5% CMC (25 mg/kg, p.o for 8 weeks).

**Oral Glucose Tolerance Test**

This test was performed after 8 weeks and before euthanization of the animals. After 12-hour fasting and determination of fasting blood glucose level (zero time), all animal groups had received glucose solution (2 g/kg; p.o.). Blood glucose level was measured after 15, 30, 60, 90 and 120 minutes after the glucose loading using a glucometer (Beurer Medical™ GmbH, Germany). The changes in blood glucose from the basal level after glucose load were calculated and represented as delta blood glucose.

**Biochemical analysis**

At zero time and after 8 weeks of treatment, fasting blood samples were collected from all animals, after mild anesthesia with i.p. injection of 50mg/kg thiopental sodium, through cardiac puncture and kept in ordinary tubes. Blood samples were centrifuged at 4000 rpm at 4°C, and serum was collected for biochemical analysis. Serum levels of glucose, insulin, triglycerides and total cholesterol were assayed using ready-made kits and according to the methods specified by the manufacturer.

**Statistical analysis**

All results were presented as mean±SD. The data were evaluated using Graph Pad Prism 5.1 software (Graph Pad Software Inc, California, US); the changes were analysed by repeated measures ANOVA. One-way ANOVA, followed by Bonferroni’s post hoc test, was performed when appropriate. A probability value of p<0.05 was considered statistically significant.

**Results:**

Table 1 showed that body weight of rats fed fructose-rich diet was significantly greater than that of negative controls, and treatment with aliskiren attenuates the fructose diet induced excessive increase in body weights, although body weight in this group was still significantly higher compared with negative control. Moreover,
fructose-rich diet significantly elevates fasting serum glucose level in positive control group after 8 weeks, which was significantly greater than that reported in negative control. Although treatment with aliskiren attenuates significantly the increase in serum glucose level compared with positive control group, it was still significantly greater than that reported in negative control group. As shown in table 1, the rats in positive control group were significantly hyperinsulinemic compared with both negative control and aliskiren-treated groups, where treatment with aliskiren completely normalizes fasting insulin levels after 8 weeks of fed with fructose-rich diet. Meanwhile, concurrent 8-week aliskiren treatment improved the rise of serum triglycerides and total cholesterol levels in the fructose-rich diet fed rats, compared with those challenged with fructose and vehicle treated (positive control).

Table-1: Effects of aliskiren treatment on serum glucose, insulin, triglyceride and total cholesterol in a high-fructose diet fed rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative Control</td>
</tr>
<tr>
<td>Body weight (gm)</td>
<td></td>
</tr>
<tr>
<td>Zero time</td>
<td>171.0±18.1</td>
</tr>
<tr>
<td>After 8 weeks</td>
<td>268.5±24.7*a</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td></td>
</tr>
<tr>
<td>Zero time</td>
<td>5.78±0.49</td>
</tr>
<tr>
<td>After 8 weeks</td>
<td>5.76±0.61*a</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td></td>
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<tr>
<td>Zero time</td>
<td>106.4±10.9</td>
</tr>
<tr>
<td>After 8 weeks</td>
<td>100.3±7.4*a</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td></td>
</tr>
<tr>
<td>Zero time</td>
<td>1.16±0.10</td>
</tr>
<tr>
<td>After 8 weeks</td>
<td>1.18±0.07*a</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td></td>
</tr>
<tr>
<td>Zero time</td>
<td>2.07±0.14</td>
</tr>
<tr>
<td>After 8 weeks</td>
<td>2.05±0.12*a</td>
</tr>
<tr>
<td>Fasting insulin/Fasting glucose</td>
<td></td>
</tr>
<tr>
<td>Zero time</td>
<td>18.4±1.92</td>
</tr>
<tr>
<td>After 8 weeks</td>
<td>17.5±1.97*a</td>
</tr>
</tbody>
</table>

Values were presented as mean±SD; number of rats= 8 in each group; * significantly different compared with zero time values within the same group; values with different superscripts (a,b,c) were significantly different among different groups after 8 weeks (P<0.05).

Figure-1 showed that in positive control group, blood glucose level was increased by an average of 12.4 mmol/L at 30 min after the glucose load, which is significantly greater than that reported in negative control group. In the animal group treated with aliskiren and challenged with glucose load, the 30-min post-load glucose level was increased by 6.9 mmol/L only (Figure 1), which was significantly lower than that reported in positive control group. Based on the AUC data after glucose load, the amount of glucose absorbed in positive control animals was significantly greater than that reported in negative control group. Meanwhile, treatment with aliskiren significantly decreased the amount of glucose absorbed
due to glucose challenge compared with vehicle-treated rats (positive control); however, it was still significantly higher than that of negative control rats (Figure 2).

![Graph](image1.png)

Figure-1: Effect of aliskiren on blood glucose after glucose loading in rat’s model of fructose diet-induced insulin resistance. Values with non-identical letters (a,b,c) were significantly different ($P< 0.05$).

![Graph](image2.png)

Figure-2: Effect of aliskiren on the incremental blood glucose AUC$_{0-120}$ min in rat’s model of fructose-rich diet induced insulin resistance after glucose load; values with non-identical letters (a,b,c) were significantly different ($P < 0.05$).

**Discussion:**
The major findings of the present study revealed that high-fructose diet caused increased serum glucose, insulin, TG and TC, in tune with the previous findings reported by others [18,14]. Moreover, concurrent administration of aliskiren improves the glycemic status, insulin resistance and the lipid profile in fructose-fed rats. It has been reported that maintenance of rats on high fructose diet in rats was associated with activation of local RAS in various types of tissues, including the GIT and adipose tissues [19]. Excessive consumption of high fructose diet was considered as lipogenic stimuli, predisposing to hypertriglyceridemia, pro-inflammatory state, and increase in ectopic fat deposition, especially in the hepatic tissue and skeletal muscle [20]. Meanwhile, the increased glycemic response curve in fructose-fed rats compared with negative control during the glucose tolerance test, along with elevated levels of serum glucose and insulin and increased insulin resistance index, indicated that fructose-fed rats showed inadequate response of insulin-sensitive tissues, which indicates a
state of insulin resistance. In contrast, aliskiren-treated rats had all those parameters normalized, suggesting a dramatic beneficial effect of decreasing GIT renin levels in preventing the development of insulin resistance. This finding was in tune with many previously reported data concerned with utilizing many approaches for blocking RAS activity \[21,19\]. In the present study, the possibility that blocking renin with aliskiren may decrease oral absorption of glucose was evaluated, and the results indicated significant decrease in the amount of glucose absorbed after oral dose of glucose. This could be attributed to the blocking effect of aliskiren on the locally liberated renin in the small intestine. Renin has been detected in the intestinal tissue \[22\], and renin over expression in the intestine was associated with enhanced glucose absorption in the jejunum \[23\]. Furthermore, the decrease in Ang II production due to aliskiren may improve the expression of glucose transporters in insulin-sensitive tissues and attenuate oxidative stress, which consequently lead to improve insulin resistance \[24\]. Such amelioration of systemic insulin resistance may subsequently reduce body weight and accumulation of lipids in peripheral adipose tissues. Based on \textit{in vitro} experiments using enterocytes from human jejunal mucosa, it has been reported that intestinal absorption of glucose was mainly mediated through the sodium-glucose transporter 1 (SGLT1) at the apex of the enterocytes, while activation of angiotensin type-2 receptor (AT2R) enhances intestinal glucose absorption through this transporter \[25\]. This in part may explain the decrease in the glucose level after oral glucose challenge due to treatment with aliskiren, which may decrease the available Ang II required for activation of SGLT1 in the apical part of the enterocytes. However, this idea needs to be confirmed using more specific approach. Moreover, mucosal release of many pro-inflammatory mediators, including IL-6 and IL-1α, due to long-term maintenance of rats on high-fructose diet promotes glucose absorption through direct action on the serosal side of the enterocytes \[26\]; while excessive epidermal growth factor release enhances SGLT1-mediated glucose transport \[27\]. In the present study, the use of aliskiren to block renin release decreases the Ang II levels and attenuates inflammatory response of the GIT \[28\]. In conclusion, the direct renin inhibitor, aliskiren, significantly reduces the increase in body weight, serum glucose levels, serum insulin levels, triglyceride levels and total cholesterol levels, and the rate of glucose absorption after oral glucose load in high fructose-fed rats.

References:


5- Surapongchai, J.; Prasannarong, M.; Bupha-Intr, T. and Saengsirisuwat, V. Angiotensin II induces differential insulin action in rat skeletal


