CHAPTER-IV
Insulin Resistance and its Reversal Agents in Alcoholism
INTRODUCTION

Alcohol abuse is thought to be a risk factor for the cause of liver damage, hyperlipidemia and insulin resistance in human beings. Hazard of heavy alcohol intake induces hyperglycemia, hyperinsulinemia, glucose intolerance, increased insulin resistance, hypercholesterolemia and hypertriglyceridemia. In the postprandial state alcohol induces hyperglycemia by inducing glycogenolysis and accelerate the peripheral insulin resistance. During the ethanol metabolism, the aldehyde degradation produces reactive metabolites, which causes toxicity to almost all the organs. The coexistence of hyperglycemia, hyperlipidemia, hyperinsulinemia and insulin resistance in alcohol fed rats appears a good model for studying the effect of synthetic compounds/natural products for their combined or individual effect, if any, on antihyperglycemic, dyslipidemic and insulin resistance reversal property.

EXPERIMENTAL PROTOCOLS

Alcohol Fed Rat Model

Male Charles Foster strain of albino rats aged 40 ± 2 days and body weight 120 ± 20 g were used in the present study. The rats were randomly divided into two groups. Rats of group I, termed as control rats were given sucrose (10 g/l), this was done primarily to maintain isocaloric conditions for 45 consecutive days. Rats of group II termed as alcoholic rats received ethanol at dose of 3.76 g/kg body weight per day for 45 consecutive days. Oral glucose tolerance curve was made weekly after two weeks of sucrose and ethanol feeding. After three weeks of alcohol feeding rats was showing impairedness in their glucose tolerance. On 4th week animals of alcohol fed group showed a significant (p<0.05) level of glucose intolerance as depicted in the graph [Fig 23 (c-d)]. However, glucose tolerance curve of sucrose fed group was found to be normal.

Animals were selected from the alcohol fed group after 30 days consecutive feeding of ethanol and divided into groups each containing six animals, were termed as alcohol fed control and alcohol fed group + test compounds/standard drug groups. Test compounds/antidiabetic standard drug, was fed to alcohol fed + test compound/standard drug groups of rats at a dose of 100 mg/kg for two weeks once daily (p.o.). Alcohol fed control group was received same volume of vehicle (1 % gum acacia). Oral glucose
tolerance test was performed on day 7\textsuperscript{th} and day 14\textsuperscript{th} of test compounds/ standard drug feeding and on day 15\textsuperscript{th} blood glucose was measured by glucometer using glucostrips and blood was collected from retro orbital plexus to obtain serum for the measurement of serum insulin and lipid profile by the same protocol as mentioned in chapter I. Each biochemical parameter was expressed as mean ± standard deviation. Groups were compared by analysis of variance with the Student’s ‘t’ test.

**Measurement of Insulin Resistance**

The steady state basal plasma glucose and insulin concentrations are determined by their interactions in a feedback loop. A computer-solved method has been used to predict the homeostatic concentrations, which arise from varying degrees of \(\beta\)-cell deficiency and insulin resistance. Comparison of fasting values of glucose and insulin with model’s predictions allows a quantitative assessment of the contributions of insulin resistance and \(\beta\)-cell dysfunction to the fasting hyperglycemia (Mathews et al., 1985). A method was devised for the measurement of insulin resistance, known as Homeostasis Model Assessment (HOMA), which was based on the relationship between fasting blood glucose and fasting insulin level. This measure had been used in various population trials including UKPDS. According to this model, insulin resistance was measured by the formula:

\[
\text{Insulin resistance (HOMA)} = \frac{\text{Insulin}}{22.5} e^{-\ln \text{glucose}} = \frac{I_o \times \ln G_o}{22.5}
\]

\[
\beta\text{-cell dysfunction} = \frac{(20 \times I_o)}{(G_o- 3.5)}
\]

Where \(G_o\) was fasting glucose concentration (mM) and \(I_o\) was fasting insulin concentration (\(\mu\text{U/ml}\)).
Fig 23. Levels of Various Parameters in Serum of Sucrose and Alcohol Fed Rats

a. OGTT Before the Start of Sucrose and Alcohol Feeding (Day 0)

b. OGTT After Two Weeks of Sucrose and Alcohol Feeding (Day 14)

c. Oral Glucose Tolerance After Three Weeks of Sucrose and Alcohol Fed Rats (Day 21)

d. Oral Glucose Tolerance After Four weeks of Sucrose and Alcohol Fed Rats (Day 28)
RESULTS AND DISCUSSION

The present study shows the elevation in blood glucose profiles in Charles Foster albino rats following consecutive feeding of ethanol. Glucose tolerance was found largely impaired in these rats when fed with ethanol for four consecutive weeks. Elevated serum insulin levels were observed in the alcohol fed rats compared to sucrose fed rats. An increase in serum cholesterol and serum triglycerides was also observed in alcohol fed rats compared to normal control rats. Two synthetic compounds (S-001-471 and S-002-853) and a standard drug metformin which were found active in all the above said animal models for diabetes mellitus (Results of chapter II). Alcohol fed rats treated with S-001-471 and S-002-853 were showing significant improvement on their oral glucose tolerance curve after two weeks treatment with these antidiabetic molecules compared to vehicle control rats [Table 16, Fig 24 (a-d)]. Lowering in blood glucose profile and significant reduction in serum insulin levels was observed in alcohol fed rats when treated with S-001-471 and S-002-853 for two weeks. A significant improvement on lipid profiles was also observed in alcohol fed albino rats treated with S-001-471 and S-002-853 (Table 17). Metformin lowers all the above said elevated parameters significantly in alcohol fed rats at the same dose level and also having significant improvement on glucose tolerance curve after two weeks treatment [Table 7, Fig 24 (e-f)].

Table 16: Synthetic compounds of various class / series tested in alcohol fed rats

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sample code</th>
<th>Dose (mg/kg)</th>
<th>Alcohol Fed Rats (% Activity)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Day 14</td>
</tr>
<tr>
<td>Chalcone derivative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>S-001-471</td>
<td>100</td>
<td>13.3</td>
</tr>
<tr>
<td>Flavone derivative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>S-002-853</td>
<td>100</td>
<td>21.8</td>
</tr>
<tr>
<td>Standard drug</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Metformin</td>
<td>100</td>
<td>38.9*</td>
</tr>
</tbody>
</table>
Insulin Resistance and its Reversal Agents in Alcoholism

Fig 24. Effect of S-002-471, S-002-853 or Metformin during two weeks treatment on Oral Glucose Tolerance in Alcohol Fed Rats

(a) One week Treatment with S-002-471
- Sucrose Fed Rats
- Alcohol Fed Rats
- Alcohol+S-002-471 Fed Rats

(b) Two weeks Treatment with S-002-471
- Sucrose Fed Rats
- Alcohol Fed Rats
- Alcohol+S-002-471 Fed Rats

(c) One week Treatment with S-002-853
- Sucrose Fed Rats
- Alcohol Fed Rats
- Alcohol+S-002-853 Fed Rats

(d) Two weeks Treatment with S-002-853
- Sucrose Fed Rats
- Alcohol Fed Rats
- Alcohol+S-002-853 Fed Rats

(e) One week Treatment with Metformin
- Sucrose Fed Rats
- Alcohol Fed Rats
- Alcohol+Metformin Fed Rats

(f) Two weeks Treatment with Metformin
- Sucrose Fed Rats
- Alcohol Fed Rats
- Alcohol+Metformin Fed Rats
Table 17. Biochemical parameters in the serum of sucrose fed, alcohol fed, alcohol+S-001-471, alcohol+S-002-853 and alcohol+metformin fed group of rats after two weeks of treatment with these antihyperglycemic molecules

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose (mM)</td>
<td>4.40±0.27</td>
<td>5.37±0.23**</td>
<td>4.99±0.36*</td>
<td>4.58±0.39*</td>
<td>4.47±0.26**</td>
</tr>
<tr>
<td>Serum insulin (μ Unit/ml)</td>
<td>38.16±3.76</td>
<td>61.17±5.49***</td>
<td>56.4±5.90</td>
<td>50.3±4.85*</td>
<td>45.6±5.80***</td>
</tr>
<tr>
<td>Serum cholesterol (mg/dl)</td>
<td>118.34±5.14</td>
<td>149.32±7.61***</td>
<td>140.5±7.60</td>
<td>132.8±8.50</td>
<td>128.6±6.87+</td>
</tr>
<tr>
<td>Serum triglycerides (mg/dl)</td>
<td>53.06±3.53</td>
<td>97.47±7.89***</td>
<td>90.2±5.75</td>
<td>79.8±6.85*</td>
<td>75.5±7.10*</td>
</tr>
</tbody>
</table>

Alcohol fed rats was compared with sucrose fed rats. *p<0.05; **p<0.01; ***p<0.001
Test compound(s)/ drug treated rats were compared with alcohol fed rats. *p<0.05; **p<0.01; ***p<0.001

Blood glucose levels were found higher in alcohol fed rats but insulin levels were also remained high suggesting that these animals are resistant towards insulin. Hyperinsulinemia is caused by increased insulin secretion in response to glucose, which results from hyperactivity of pancreatic cells to glucose and an activation of sympathetic nerves (Pugazhenthi, et al., 1993). Antidiabetic agent S-001-471 and S-002-853 prevented and attenuated the increase in both blood glucose and plasma insulin suggesting that these molecules promote insulin sensitivity. Insulin sensitivity has been reported to reduce plasma insulin levels leading by the same occasion to a reduction of plasma glucose and blood pressure (Dai and McNeil, 1995). Therefore, seems that these molecules exert insulin resistance reversal activity by improving insulin sensitivity as observed with certain antidiabetic agent like metformin (Verma et al., 1994), thiazolidinedione (Suzuki et al., 1997; Verma et al., 1998) and angiotensin converting enzymes inhibitors (Limura et al., 1995). In conclusion, this study has shown the antihyperglycemic and insulin resistance reversal effect of S-001-471 and S-002-853 in alcohol fed rats. These results may land further support to mount up evidence that these molecules, if taken in sufficient quantities, could conceivably be beneficial in the attenuation and prevention of insulin resistance, hyperinsulinemia and hypertension induced by alcohol feeding and in genetic diabetes. Further studies are required to establish the mechanism(s) underlying the antihyperglycemic effect and insulin sensitizing activity of these agents.
Effect of S-001-471, S-002-853 and Metformin on Insulin resistance (HOMA)

The antidiabetic agents S-001-471, S-002-853 and metformin proved to be good insulin resistant reversal agents. Insulin resistance was observed in albino rats following feeding of ethanol at a dose of 3.76 g/kg for one month. For normal animals the median insulin resistance was reported to be 1.45 using HOMA index and for diabetic patients it was found to be > 2.69 (Mathews et al., 1985). Here in this case, animals of the alcohol fed rats insulin resistance was found to be around 4.56, which reduced significantly with the treatment of S-001-471, S-002-853 and metformin separately (Fig 25).

![Insulin Resistance Index (HOMA)](image)

Fig 25. Insulin resistance was calculated using a formula devised by Mathews et al., (1985), any group having value >2.69 was regarded as insulin resistant. Insulin resistance was developed in alcohol fed rats. S-001-471, S-002-853 and metformin treatment help in lowering insulin resistance in alcohol fed rats.

The basal hyperglycemia of diabetes mellitus may be considered as a compensatory response with sufficient insulin secretion, from a reduced 2-cell capacity, to control glucose efflux (Holman and Turner, 1979; Turner and Holman, 1976). In the present study investigations were made with a model of the interaction between insulin and glucose.

Treating insulin resistance is not a simple matter of either giving more insulin to push the signalling pathway harder, or reducing insulin concentrations to reduce the consequences of hyperinsulinemia. Also there is no simple quantitative clinical test for insulin resistance, and there are few therapeutic options. Giving more insulin can increase those actions of insulin that are impaired by the bottlenecks of signal
Transduction. However, creating the desired effect on a severely compromised pathway of insulin action (e.g. impaired glucose transports) can result in gross accentuation of other less desirable actions of insulin (e.g. lipogenesis, leading to hypertriglyceridemia and obesity, or sodium retention, promoting hypertension). Indeed, excess insulin exacerbates insulin resistance at the receptor and post-receptor levels. It is evidenced from the results that both antidiabetic agents S-001-471 and S-002-853 possess the property to reverse the insulin resistance and increases insulin sensitivity in ethanol fed albino rats. Insulin resistance was found to be established in this animal model with very high value as compared with predicted insulin resistance value i.e. 2.69. With these studies it is being reported for the first time that these agents were found to be active in making insulin more sensitive and one day it become reality that these agents may take the shape of a drug that could come to market with having antidiabetic and insulin resistant reversal potential.