RESULTS
&
DISCUSSION
GENERAL OBSERVATIONS

No mortality was observed in any experimental group throughout the experimental period of 60 days. No toxic effects of OSAE treatment were observed when normal animals were treated with OSAE during the experimental period. No visible side effects and variation in animal behavior (respiratory distress, abnormal locomotion and catalepsy) were evident in group-C+OSAE indicating the non-toxic nature of OSAE. The ancient systems of medicine including Ayurveda, Greek, Roman, Siddha and Unani have mentioned its therapeutic applications in cardiovascular disorders, diabetes and asthma without any adverse effects or toxicity. No significant variation was observed in the intake of food and water among the four experimental groups. Fructose fed rats (group-F) seems to be obese when compared with the remaining three groups. After 60 days of experimental period the animals were sacrificed and dissected for organ collection. Group-F animals showed huge accumulation of white adipose tissue throughout the body especially at abdominal region when compared with group-C. Group-F+OSAE showed relatively low accumulation of white adipose tissue as compared to group-F but still relatively higher than that of group-C. No physical variation was observed in the abdominal adipose tissue between group-C and -C+OSAE.

The body weights and relative organ weights (liver, kidney and heart) of four experimental groups at the end of experimental period are represented in Table 4a. Group-F showed significantly higher body weight, higher relative organ weight of liver and heart (but no change in relative kidney weight) when compared with group-C. The liver of these animals has shiny appearance and showed characteristic features of fatty liver. Administration of OSAE along with the fructose feed (group-F+OSAE) prevented fructose induced increase in body weight and relative organ weight. Statistically significant variation was not observed in the body weight and relative organ weights among groups C, C+OSAE and F+OSAE.

BODY WEIGHT

The body weights of four groups of animals at 15 day interval during the experimental period are represented in Table 4 and Figure 4. No significant variation in body weight of groups C+OSAE and F+OSAE was observed when compared with group-C. Group-F also showed no
significant variation up to 15 days, but a significant increase in weight gain was observed from 30 days onwards till the end of experimental period when compared with corresponding values of group-C. There was a gradual increase in the body weight within the group of group-C by 15, 29, 43.5, 58 %, group-F by 17.5, 37, 50, 64 %, group-F+OSAE by 14.4, 30, 43.7, 57.6%, group-C+OSAE by 14.4, 30, 43.7, 57.6 % respectively at 15, 30, 45 and 60 days of experimental period when compared with corresponding initial weights. Even though there was no variation in the initial body weights among four groups of animals, group-F showed significantly higher body weight by the end of experimental period when compared with group-C. However, at the end of experimental period, body weight of group-F+OSAE did not deviate from group-C. Thus, fructose induced abnormal weight gain was completely prevented with the OSAE treatment. Further, the body weight of group-C+OSAE did not deviate from group-C rats.

There are reports indicating an increase in energy intake, body weight, and adiposity with the consumption of high fructose diets both in humans (Kanarek and Orthen-Gambill, 1982; Kasim-Karakas et al., 1996) and animals (Tordoff and Alleva, 1990; Astrup et al., 2002; Reddy et al., 2008).

Insulin is involved in the regulation of body adiposity via its actions in the central nervous system (CNS) to inhibit food intake and increase energy expenditure (Woods et al., 1996; Schwartz et al., 2000). Insulin does not enter the brain, but is transported into the CNS via a saturable receptor-mediated process. Insulin receptors are localized in CNS areas involved in the control of food intake and energy homeostasis. Insulin administration into the CNS inhibits food intake in animals including non-human primates. Impairment of CNS insulin transport was inversely related to an increase in body weight (Kaiyala et al., 2000). Especially, knocking out the insulin receptor in neurons results in hyperphagia and obesity in mice (Bruning et al., 2000). Thus, reduced insulin delivery into the CNS or disruption of the insulin-signaling pathways in the CNS may result in weight gain and development of obesity. Fructose cannot provide satiety signals because it is transported in less quantity to the brain. Changes in diet have been studied as contributing factors to the development of obesity. The digestion, absorption, and metabolism of fructose differ from those of glucose. Hepatic metabolism of
TABLE 4: Effect of OSAE treatment on body weight in four groups of animals.

<table>
<thead>
<tr>
<th>Groups \ (n=8)</th>
<th>Body weight in grams</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 days</td>
</tr>
<tr>
<td>C</td>
<td>194.8 ± 3.9^a</td>
</tr>
<tr>
<td></td>
<td>(+15.09)</td>
</tr>
<tr>
<td>F</td>
<td>193.3 ± 3.1^a</td>
</tr>
<tr>
<td></td>
<td>(+17.43)</td>
</tr>
<tr>
<td>F + OSAE</td>
<td>195.2 ± 3.2^a</td>
</tr>
<tr>
<td></td>
<td>(+15.0)</td>
</tr>
<tr>
<td>C + OSAE</td>
<td>194.2 ± 4.3^a</td>
</tr>
<tr>
<td></td>
<td>(+14.42)</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M
Values with different superscripts within the column are significantly different at P<0.05 (Duncan's multiple range test).
Values in parenthesis indicate the percent change in body weight within group compared to initial weights.
Table 4a: Effect of OSAE treatment on relative organ weights in four groups of animals

<table>
<thead>
<tr>
<th>Groups ↓ (n=8)</th>
<th>Body weight (g)</th>
<th>Relative liver weight (g/100g body wt.)</th>
<th>Relative kidney weight (g/100g body wt.)</th>
<th>Relative heart weight (g/100g body wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>313.2 ± 4.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.73 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>626.34 ± 3.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>345.27 ± 2.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>F</td>
<td>315.2 ± 3.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.64 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>630.37 ± 2.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>425.26 ± 2.84&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>F + OSAE</td>
<td>307.2 ± 3.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.32 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>629.24 ± 2.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>346.62 ± 3.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C + OSAE</td>
<td>303.2 ± 3.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.25 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>632.45 ± 4.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>378.42 ± 2.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., (n=8 animals).
Values with different superscripts within the row are significantly different at P<0.05 (Duncan’s multiple range test).
Figure 4: Body weights of Control (C), Fructose fed (F), Fructose treated (F+OSAE) and Control treated (C+OSAE) rats during the experimental period.

Figure 5: Percent gain in body weights of four experimental groups during experimental period.
fructose favors de novo lipogenesis, and this may be linked with both hyperlipidemia and increased body fat stores (Herman et al., 1970; Kok et al., 1996).

In addition, insulin may modify food intake by its effect on leptin secretion which is mainly regulated by insulin-induced changes in glucose metabolism in fat cells (Muller et al., 1998; Havel, 2002). Leptin, the protein product of ob gene secreted from adipose tissue, functions as a circulating signal from body fat stores to the CNS, where it acts to limit adiposity by inhibiting food intake and increasing energy expenditure (Caro et al., 1996; Rohner-Jeanrenaud and Jeanrenaud, 1996). Decreases in circulating leptin concentrations correlate with increased sensations of hunger, and leptin administration can reduce appetite in humans (Westerterp-Plantenga et al., 2001). Plasma leptin concentrations are strongly correlated with adiposity in rodents (Maffei et al., 1995; Ahren et al., 1997), non-human primates (Havel, 1997) and humans (Weigle et al., 1997). Leptin secretion is dependent on insulin-mediated adipocyte glucose transport and metabolism (Mueller et al., 1998). Insulin increases leptin release (Saad et al., 1998) with a time delay of several hours. Thus, a low insulin concentration after ingestion of fructose (short-term feeding) would be associated with lower average leptin concentrations than would be seen after ingestion of glucose. But long-term feeding of high fructose diet develops hyperinsulinemia and insulin resistance which may lead to decreased leptin concentrations and its consequences.

Furthermore, during consumption of meals accompanied by glucose beverages, circulating concentrations of the orexigenic gastric hormone ghrelin clearly decreased 1-3 h after each meal, whereas ghrelin was much less suppressed after meals with fructose-containing beverages (Tschop et al., 2001; Havel et al., 2002). Thus insulin, leptin, and possibly ghrelin, function as key signals to the CNS in the long-term regulation of energy balance. The decreased insulin sensitivity may also be associated with decreased leptin and increased ghrelin levels, which could lead to increased energy intake and thereby contribute to weight gain, obesity, and its metabolic consequences during long-term consumption of diets high in fructose. It is noteworthy that OSAE treatment prevented weight gain and fat accumulation in visceral adipose tissue of fructose-fed rats despite increased energy intake. Obesity is almost
invariably associated with insulin resistance and any reduction of excess body fat contributes to an improvement of insulin sensitivity (Hauner, 1999). Thus, the beneficial effect of OSAE might be due to the improvement of insulin sensitivity.

In order to understand the effect of fructose feeding on plasma glucose, insulin and triglycerides and to know the preventive effect of OSAE if any, against fructose diet induced alterations in these parameters, studies were further extended to measure plasma glucose, insulin and triglycerides at 15 days interval till the end of experimental period in four groups of animals.

FASTING PLASMA GLUCOSE

The data showed in table 5 and figure 6 represents the fasting plasma glucose values of all four experimental groups throughout the experimental period at 15 days interval. The initial plasma glucose values of four experimental groups did not vary significantly. During the experimental period the plasma glucose level of group-F increased gradually from 81.3±3.3 to 102.6±1.5 mg/dl and the percent increase was 3.32, 13.16, 15.9 and 26.2 at 15, 30, 45 and 60 day intervals compared to initial level. Group-F showed a gradual increase in plasma glucose level during the experimental period with a significant higher glucose levels at 30 days (92.0±1.5), 45 days (94.2±2.3), and 60 days (102.6±1.5) with 13.16, 15.87 and 26.2% rise when compared with corresponding values of group-C. Group-F+OS showed no significant variation in plasma glucose level during the experimental period except at 30 days of experimental period when compared with group-C, confirming preventive effect of OS against fructose induced hyperglycemia. Group-C and group-C+OS remained persistently euglycemic throughout the course of the study.

Leaves of OS have been used in traditional remedies to control diabetes since antiquity. Chattopadhyay (1993) has reported a well defined role of alcoholic extract of OS leaves in suppressing blood glucose levels in glucose fed hyperglycemic, insulin treated and diabetic rats as compared to control animals. Agrawal et al., (1996) have suggested that OS leaves improved the β-cell function and enhanced insulin secretion. Sarkar et al., (1989) have reported that on dry weight basis, leaves are apparently more effective in lowering blood sugar levels as...
TABLE 5: Effect of OSAE treatment on fasting plasma glucose in four groups of animals.

<table>
<thead>
<tr>
<th>Groups ↓ (n=8)</th>
<th>Fasting plasma glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 days</td>
</tr>
<tr>
<td>C</td>
<td>81.5 ± 2.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(-0.98)</td>
</tr>
<tr>
<td>F</td>
<td>81.3 ± 3.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(+3.32)</td>
</tr>
<tr>
<td>F + OSAE</td>
<td>80.7 ± 3.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(+0.87)</td>
</tr>
<tr>
<td>C + OSAE</td>
<td>82.2 ± 2.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(-3.3)</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M
Values with different superscripts within the column are significantly different at P<0.05 (Duncan's multiple range test).
Values in parentheses indicate the percent change in body weight within group compared to initial weights.
Figure 6: Plasma glucose levels of Control (C), Fructose feed (F), Fructose treated (F+OSAE) and Control treated (C+OSAE) rats during the experimental period.

Figure 7: Percent change in plasma glucose levels of four experimental groups during the experimental period.
compared to dry seeds. There is a report that tulsi leaves inhibit absorption of glucose from the intestines, but the nature of the active principle and exact mode of its action remain unclear (Giri et al., 1987).

It is well known that high fructose feeding leads to insulin resistance, hyperinsulinemia, dyslipidemia and hypertension in animal models (Elliott et al., 2002). However, the effect of fructose feeding on plasma glucose levels as reported by earlier researchers varied from no change (Sambandam et al., 1997), transient elevation (Lee et al., 1994) to a significant elevation (Rosen et al., 1997). In the present study, fructose fed animals showed significantly enhanced plasma glucose levels from 30 day onwards till the end of experimental period. Dietary fructose metabolism leads to production of FFA in the liver of fructose fed rats increases hepatic glucose production. The plasma glucose level of group-C+OS and F+OS during the experimental period clearly indicates that OS does not exhibit hypoglycemic activity; instead, it shows antihyperglycemic activity. The hyperglycemic plants act through a variety of mechanisms such as improving insulin sensitivity, augmenting glucose dependent insulin secretion and stimulating the regeneration of islets of langerhans in pancreas of diabetic rats.

**Fasting plasma insulin**

The fasting plasma insulin levels at 15 days interval period of four groups of animals during the experimental period are represented in the table 6 and figure 8. There was a slight increase in the plasma insulin levels of group-C during the experimental period; this might be due to increase with the age and body weight. Control rats treated with OSAE showed a gradual decrease in the plasma insulin level during the experimental period with significantly lower value at 60 days when compared with corresponding values of group-C. Thus OSAE treatment maintained normoglycemia with lower insulin levels in group-C+OS, indicating enhanced insulin sensitivity. Group-F showed a gradual increase in plasma insulin during the experimental period, showing a significantly higher concentrations of insulin at 30 days (62.6±3.7 μIU/ml), 45 days (99.3 ±6.7), and 60 days (140.5 ± 8.7) with 38.8%, 109.1%, and 174.4 % increase, respectively, when compared with corresponding values of group-C. The plasma insulin
TABLE 6: Effect of OSAE treatment on fasting plasma insulin in four groups of animals.

<table>
<thead>
<tr>
<th>Groups \ (n=8)</th>
<th>Fasting plasma insulin (µL.U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 days</td>
</tr>
<tr>
<td>C</td>
<td>43.3 ± 3.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(-1.14)</td>
</tr>
<tr>
<td>F</td>
<td>44.6 ± 3.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>F + OSAE</td>
<td>43.0 ± 3.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C + OSAE</td>
<td>44.5 ± 2.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M.

Values with different superscripts within the column are significantly different at P < 0.05 (Duncan's multiple range test).

Values in paranthesis indicate the percent change in body weight within group compared to initial weights.
Figure 8: Plasma insulin levels of Control (C), Fructose feed (F), Fructose treated (F+OSAE) and Control treated (C+OSAE) rats during the experimental period.

Figure 9: Percent change in plasma insulin levels of four experimental groups during the experimental period.
concentrations of group-F+OSAE were 46.6 ± 4.5, 65.2 ± 5.6, and 68.8 ± 6.4 at 30, 45, and 60 days, respectively, with 25.56, 34.34, and 51 % lower levels when compared with group-F. The insulin levels of group-F+OSAE at the end of experimental period were significantly lower than group-F, but still significantly higher than group-C.

Insulin plays a central role in the regulation of glucose homeostasis. Fructose consumption, however, does not directly promote insulin secretion from pancreatic cells; chronic exposure seems to indirectly cause hyperinsulinemia and obesity through other mechanisms. Dietary fructose promotes gluconeogenesis by providing gluconeogenic substrates leading to hyperglycemia. Glucose produced as a result of fructose metabolism stimulates insulin release, but the fructose induced insulin resistance prevents the insulin from effectively metabolizing glucose, resulting in hyperglycemia (Bezerra et al., 2000; Teff et al., 2004). Insulin resistance also led to compensatory hyperinsulinemia, where the body attempts to balance the reduced effect of insulin by producing and releasing more insulin (Zavaroni et al., 1980; Suga et al., 2000).

Prominent insulin secretory effect of ethanol extract of OS was noted in perfused rat pancreas. Similar effects were found in acute insulin release studies using rat islets and the clonal rat cell line BRIN-BD11. In these experiments, insulinotropic effects were observed in the more polar compared with hexane and chloroform fractions. Ethanol extract and the aqueous, butanol and ethyl acetate fractions showed dose dependent stimulatory effects on insulin secretion in BRIN-BD11 cells (Hannan et al., 2006).

Non toxic concentrations of OS extract and partition fractions were used to study mechanisms underlying stimulation of insulin secretion. The exact stimulated basal insulin secretion with an action enhanced by increasing the glucose concentration over the range 2.8-16.7 mM. The effects of tolbutamide and a membrane depolarizing concentration of KCl (30Mm) were tested in the absence and presence of the extract. The extract insulin secretion by stimulating voltage gated calcium and other ion channels. Insulin secretion may be enhanced by the possible intracellular actions such as stimulation of adenylate cyclase or phosphotidyl inositol pathway or direct effect on exocytosis (Boyd, 1988).
Changes in plasma glucose and insulin after an oral glucose load at the end of experimental period (on day 60) of four experimental groups are shown in the Table 8 and Figure 12. Group-F showed a significantly higher basal (0 min of OGTT) glucose and insulin values when compared to remaining three groups. Both the plasma glucose and insulin values of four experimental groups reached maximum extent by 30 min after glucose challenge and returned to their corresponding basal/near basal levels by 120 min. The plasma glucose and insulin levels of group-F were higher than those of the remaining groups at 30, 60 and 120 min of oral glucose load.

The area under the curve of glucose (AUC\text{glucose}) and insulin (AUC\text{insulin}) during OGTT of four experimental groups are represented in Table 10 and Figure 13. The AUC\text{glucose} of group-F was elevated by 37 % when compared with group-C, whereas group-F+OSAE showed only 2% elevation when compared with group-C. The AUC\text{glucose} of group-C+OSAE were significantly lower than group-C showing improved glucose tolerance.

The AUC\text{insulin} of group-F was enhanced by 98% increase than group-C, whereas group-F+OSAE showed only a 3.7% increase when compared to group-C. However, OSAE administration to group-C+OSAE rats caused no change in AUC\text{insulin} from group-C.

The analysis of the glucose tolerance test and the comparison between areas under the curve of glycemia during 120 min from control and experimental groups reveals that fructose fed rats developed glucose intolerance but co-administration of OSAE along with fructose diet prevented development of glucose intolerance.

The glucose-insulin index as represented in Table 10 and Figure 16 showed no significant variation among three experimental groups of C, C+OSAE and F+OSAE. But glucose-insulin index of group-F is significantly greater (169.5%) than group-C. Administration of OSAE along with fructose diet for 60 days resulted in only 7.62% increase when compared with group-C. Thus co-administration of OSAE along with fructose diet prevented development of insulin resistance.
TABLE 8: Effect of OSAE treatment on Oral glucose tolerance test (OGTT) in four groups of animals.

<table>
<thead>
<tr>
<th>Groups</th>
<th>plasma glucose (mg/dl)</th>
<th>0 min</th>
<th>30 min</th>
<th>60min</th>
<th>90min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>81.8 ± 2.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>104.2 ± 2.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>93.3 ± 2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>88.2 ± 2.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>81.3 ± 2.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td>102.7 ± 1.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>136.7 ± 3.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>128.3 ± 2.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>120.4 ± 1.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>112.2 ± 2.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>F + OSAE</td>
<td></td>
<td>80.6 ± 1.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>107.2 ± 2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>97.1 ± 2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>91.3 ± 1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.4 ± 1.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C + OSAE</td>
<td></td>
<td>77.7 ± 2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>101.8 ± 2.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>92.8 ± 2.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86.1 ± 2.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>81.4 ± 2.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M

Values with different superscripts within the column are significantly different at P<0.05 (Duncan's multiple range test).

Values in paranthesis indicate the percent change in body weight within group compared to initial weights.
TABLE 9: Effect of OSAE treatment on plasma insulin during OGTT in four groups of animals.

<table>
<thead>
<tr>
<th>Groups \ (n=8)</th>
<th>Fasting plasma insulin (µL.U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>C</td>
<td>49.1 ± 2.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>F</td>
<td>138.4 ± 8.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>F + OSAE</td>
<td>66.7 ± 6.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C + OSAE</td>
<td>41.4 ± 3.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M.

Values with different superscripts within the column are significantly different at P<0.05 (Duncan's multiple range test).

Values in paranthesis indicate the percent change in body weight within group compared to initial weights.
TABLE 10: Effect of OSAE treatment on Area Under the Curve (AUC) of glucose, insulin during OGTT and Glucose-Insulin Index in four groups of animals.

<table>
<thead>
<tr>
<th>Groups</th>
<th>AUC&lt;sub&gt;glucose&lt;/sub&gt; (mg/dl x min)</th>
<th>AUC&lt;sub&gt;insulin&lt;/sub&gt; (μIU/ml x min)</th>
<th>G-I Index (units x 10⁶)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>11023.66 ± 122&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9596.34 ± 111&lt;sup&gt;a&lt;/sup&gt;</td>
<td>105.97 ± 6.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>F</td>
<td>14785.03 ± 272&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19019.15 ± 245&lt;sup&gt;b&lt;/sup&gt;</td>
<td>283.51 ± 7.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>F + OSAE</td>
<td>11236.82 ± 140&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9957.49 ± 77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>113.15 ± 5.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C + OSAE</td>
<td>10419.51 ± 133&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9315.52 ± 93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>97.14 ± 5.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M
Values with different superscripts within the column are significantly different at P<0.05 (Duncan's multiple range test). G-I index (Glucose-Insulin Index) is calculated as the product of AUC<sub>glucose</sub> and AUC<sub>insulin</sub> during OGTT.
Figure 12: Oral glucose tolerance test of Control (C), Fructose fed (F), Fructose treated (F+OSAE) and Control treated (C+OSAE) rats

Figure 13: Area under the curve of glucose of four experimental groups during OGTT

Plasma glucose (mg/ml) AUC of glucose (Unit × 1000)
Figure 14: Plasma insulin levels during oral glucose tolerance test of Control (C), Fructose feed (F), Fructose treated (F+OSAE) and Control treated (C+OSAE) rats during the experimental period.

Figure 15: Area under the curve of insulin of four experimental groups during OGTT.
Figure 16: Glucose-Insulin Index of Control (C), Fructose fed (F), Fructose treated (F+OSAE) and Control treated (C+OSAE) rats during the experimental period.

Figure 17: Percent recovery from fructose diet induced alterations in plasma TG, glucose, insulin and AUCglucose, AUC insulin and G-I index by OSAE treatment.
The treatment with OSAE attenuated the impairment of insulin stimulated glucose disposal in rats with insulin resistance. The development of insulin resistance in fructose-fed rats is well documented in the literature (Yagi et al., 1995; Thorburn et al., 1989) and has been established in our laboratory (Reddy et al., 2008). The ability of insulin to stimulate glucose disposal is markedly impaired as evidenced by increased glucose-insulin index in fructose fed rats thus indicating a decline in insulin sensitivity in peripheral tissues associated with insulin resistance by fructose feeding. OSAE treatment in fructose fed rats prevented glucose intolerance and abnormal increase in glucose-insulin index indicating the ability of plant extract in promoting the insulin sensitivity.

The classic relation between insulin resistance, increased fasting plasma insulin concentration, and glucose intolerance has been hypothesized to be mediated by changes in ambient FFA concentrations. Elevated FFA concentrations are one of the metabolic consequences of a chronic positive energy balance and increased body adiposity (Farooqi et al., 2001). The exposure to increased concentrations of FFA may reduce insulin sensitivity by increasing the intramyocellular lipid content (Virkamaki et al., 2001). Increased portal delivery of FFA, particularly from visceral adipose tissue, could also lead to impaired carbohydrate metabolism, because elevated portal FFA concentrations increase hepatic glucose production (Rebrin et al., 1995; Steil et al., 1998). In addition, over time, increased FFA concentrations may have a deleterious effect on β-cell function (Bergman and Ader, 2000).

An increased supply of FFA in the liver also leads to an increase in the production of VLDL triacylglycerol (Arner, 2001). Fructose consumption has been shown to induce hypertriacylglycerolemia. Because insulin resistance and reduced insulin binding have been reported in hypertriacylglycerolemic persons (Bieger et al., 1984), this may be one mechanism by which fructose diets promote insulin resistance. Another potential mechanism leading to insulin resistance could involve decreased production of the adipocyte protein, adiponectin, because reduced circulating concentrations of this hormone are associated with insulin resistance independently of body adiposity (Weyer et al., 2001).
Another theory explaining how chronic fructose over nutrition can lead to type-2 diabetes is the hexosamine hypothesis, where hexosamine flux is thought to regulate glucose and satiety-sensing pathways. With over expression of glutamine:fructose-6-phosphate amidotransferase, the key regulatory enzyme in hexosamine synthesis, the liver produces excess fatty acids, skeletal muscle becomes insulin resistant, and hyperinsulinemia results. This pathway of excess hexosamine flux leads to long-term storage of energy, and eventually obesity and type-2 diabetes (McClain, 2002).

In addition to above reasons, there is evidence that reactive oxygen species, such as superoxide anion and hydrogen peroxide, are involved in the pathogenesis of insulin resistance (Lum and Roebuck, 2001). There is also evidence that free radical scavengers, such as vitamin E (Jain and Wise, 1995), glutathione (Faure et al., 1997) and superoxide dismutase (Nakazono et al., 1991) are depressed in patients and experimental animals with insulin resistance. Furthermore, treatments with antioxidants have been shown to reduce insulin resistance in rats (Song et al., 2005). Moreover, treatments of diabetic animals with probucol (lipid-lowering and antioxidant) (Kaul et al., 1995) or with vitamin E or with N-acetylcysteine (free radical scavenger and glutathione donor) have been shown to reduce oxidative stress and insulin resistance (Paolisso et al., 1993; Faure et al., 1997; Lacy et al., 1998). It is plausible to speculate that antioxidants have a protective role against the development of hyperinsulinemia and insulin resistance.

Literature survey revealed that there are many plant products, extracts and herbal formulations that successfully prevented fructose induced insulin resistance in animal models. Preventive effect of diabegon, a polyherbal preparation, during progression of diabetes induced by high fructose feeding in rats was reported by Yadav et al. (2007). Hsu et al. (2007) showed Dioscorea as the principal herb of Die-Huang-Wan, a widely used herbal mixture in china, for improvement of insulin resistance in fructose rich chow-fed rats. Feeding soluble nondigestible saccharides with a high viscosity, such as guar gum and pectin, modifies glucose tolerance because it delays gastric emptying and flattens increases in glucose (Tabatabai and Li, 2000; Ou et al., 2001). Other plant extracts and herbal formulations that are reported to
prevent fructose diet induced abnormalities are Synsepalum dulcificrum (Chen et al., 2006), Zingiber officinale Roscoe (Kadnur and Goyal, 2005), Stevioside (a glycoside of Stevia rebaudiana) (Chang et al., 2005), Green tea leaf extract (Li et al., 2006), Acanthopanax senticosus root (Liu et al., 2005), Pterocarpus marsupium extract (Grover et al., 2005), Panax ginseng (Liu et al., 2005a), Leaf methanol extract of Momordica charantia (Vikrant et al., 2001) and Dorstenia psilurus extract (Dimo et al., 2001).

FASTING PLASMA TRIGLYCERIDES

The fasting plasma triglyceride levels of all the four experimental groups throughout the experimental period at 15 day interval are represented in table 7 and figure 10. No significant alterations in the levels of plasma triglycerides were observed during the experimental period of 60 days in both group-C and C+OS. In group-F, there was no significant change in plasma triglycerides up to 15 days, but from then there was a gradual and significant increase till the end of experimental period. There was 35%, 124.5% and 142.5% increase in triglyceride levels in group-F at 30, 45 and 60 days of experimental period respectively when compared with corresponding values of group-C. The plasma triglyceride levels of group F+OS showed no significant variation except at 45 days with 24.45% (95.7±4.6 mg/dl) increase when compared with corresponding value of group-C. Whereas significant lower TG levels at 30, 45 and 60 days were obtained in group-F+OS when compared with group-F. The result clearly shows that treatment with OSAE prevented fructose induced hypertriglyceridemia in group-F+OSAE which is in agreement with previous reports that the plasma TG levels increased in rats fed with fructose diet (Thorburn et al., 1989; Anurag and Anuradha, 2002). It is obvious that this phenomenon could be due to ability of fructose to modulate either VLDL-TG secretion or catabolism. Fructose fed rats has higher VLDL-TG secretion rates than glucose-fed rats, and the magnitude (extent) of the fructose-induced hypertriglyceridemia was comparable with the increment in VLDL-TG secretion rates (Zavaroni et al., 1982). Carbons from dietary fructose are found in both the FFAs and glycerol moieties of plasma TGs. Excess dietary fructose can be converted to pyruvate, enter the Krebs cycle and emerges as citrate directed toward FFA synthesis in the cytosol of hepatocytes. The DHAP formed during fructolysis can also be
TABLE 7: Effect of OSAE treatment on fasting plasma triglycerides in four groups of animals.

<table>
<thead>
<tr>
<th>Groups ↓ (n=8)</th>
<th>Fasting plasma triglycerides (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 days</td>
</tr>
<tr>
<td>C</td>
<td>75.2 ± 3.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(+1.06)</td>
</tr>
<tr>
<td>F</td>
<td>75.1 ± 3.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(+1.46)</td>
</tr>
<tr>
<td>F+OSAE</td>
<td>76.9 ± 3.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(-6.63)</td>
</tr>
<tr>
<td>C+OSAE</td>
<td>75.4 ± 2.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(+3.18)</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M
Values with different superscripts within the column are significantly different at P<0.05 (Duncan's multiple range test).
Values in paranthesis indicate the percent change in body weight within group compared to initial weights.
Figure 10: Plasma triglyceride levels of Control (C), Fructose feed (F), Fructose treated (F+OSAE) and Control treated (C+OSAE) rats during the experimental period.

Figure 11: Percent change in plasma triglyceride levels of four experimental groups during the experimental period.
converted to glycerol and then glycerol 3-phosphate for TG synthesis. Thus, fructose can provide trioses for both the glycerol 3-phosphate backbone, as well as the FFAs in TG synthesis. Indeed, fructose may provide the bulk of the carbohydrate directed toward de novo TG synthesis (Parniak, 1988; Segebarth et al., 1991).

Feeding rats on fructose, stimulated FAS, and created a 56% increase in TG secretion rate, and 86% increase in plasma TGs. Feeding glucose, however, did not affect TG production, nor did it affect induction of FAS. This is likely because glucose stimulates both TG production, and TG removal, maintaining homeostasis. Fructose stimulates TG production, but impairs removal, creating the known dyslipidemic profile (Kazumi et al., 1997). In these scenarios, where there is excess hepatic fatty acid uptake, synthesis and secretion, 'input' of fats in the liver exceed 'outputs', and hepatic steatosis occurs (Koteish and Diehl, 2001). The hepatic steatosis caused by stimulated lipogenesis has been illustrated by fructose fed animal models showing how aberrant leptin signaling, hyperinsulinemia, and dyslipidemia are related to TG induction (Koteish and Diehl, 2001).

In the fructose fed hamster model, animals showed decreased glucose disappearance rates, increased plasma FFAs and increased plasma and liver TGs (Kasim-Karakas et al., 1996). Taghibiglou et al. (2000) further characterized the fructose fed hamster model demonstrating the development of a metabolic dyslipidemic state characterized by high plasma levels of VLDL-TG and apolipoprotein B (apoB) due to hepatic lipoprotein overproduction. Serum TGs are elevated via both an increased secretion, and decreased clearance of VLDL (Kelley et al., 2004). Also, high rates of lipolysis in visceral adipose depots can increase availability of FFAs and promote hepatic TG synthesis. The TG is then packaged with apoB, and secreted as VLDL particles (Fried and Rao, 2003). Evidence has shown that there is a complex interplay of cellular enzymes regulating lipid synthesis and uptake, as well as export and oxidation. Observations of the actions of insulin affecting lipid secretion as well as inhibition of TG has brought research interests towards the effects of chronic insulin stimulation on VLDL secretion and transport. Excess VLDL secretion has been shown to deliver increased FFAs and TGs to muscle and other tissues, further inducing insulin resistance (Zammit et al., 2001).
Increased delivery of triglycerides or nonesterified fatty acids to the muscle interferes with the utilization of glucose, through the principles of Randle cycle (Randle, 1993), impairing insulin action. The hypertriglyceridemia and insulin resistance observed in metabolic syndrome are related to the decreased expression of lipoprotein lipase (Shibasaki et al., 2006). OSAE treatment prevented the fructose induced hyperglycemia and hypertriglyceridemia in fructose fed rats (Reddy et al., 2008).

**PLASMA LIPIDS**

Abnormalities of plasma lipid and lipoprotein metabolism are very common in diabetes and insulin resistance and have long been thought to play a role in atherogenesis under these two clinical conditions. The plasma lipid profile at 0 and 60 days of six experimental groups are represented in table 18.

No significant variation was observed in the plasma lipid profile i.e. total cholesterol (TC), LDL-C VLDL-C, HDL-C and atherogenic index of C, C + OSAE, F and F + OSAE-groups at 0 days of experimentation. At the end of experimental period of 60 days C-group showed significantly increased plasma TC (10.6 %), LDL-C (9.5 %), VLDL-C (38.7%) and a significantly decreased HDL-C (15%) when compared to corresponding initial values. These changes in the lipid profile during aging process were further intensified in group-F animals. *O.sanctum* treatment for 60 days prevented fructose diet intensified alterations in the plasma lipid profile during ageing process. After completion of 60 days of experimental period insulin resistance (F-group) rats showed significantly enhanced plasma TC (63%) LDL-C (112%) and VLDL-C (117 %) with significantly decreased HDL-C (12.4%) in comparison with C-group. These alterations in lipid profile resulted increased atherogenic index in group-F animals (84.2 %) compared to C-group. *O.sanctum* administration for 60 days restored the plasma lipid profile to normal values in group F + OSAE. A significant decrease in plasma TC (35.7%), LDL-C (50.2 %), VLDL-C (49%) and a significant increase in HDL-C (12 %) with a significant decrease in atherogenic index (56 %) in F+ OSAE-group compared to F-group.

The abnormal high concentration of serum lipids in diabetes is mainly due to the increase in the mobilization of free fatty acids from the peripheral depots, since insulin inhibits
Table 18: Effect of O. sanctum treatment on plasma lipid profile in four groups of animals

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Days</th>
<th>C</th>
<th>F</th>
<th>F + OS</th>
<th>C + OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>Initial</td>
<td>64.24±3.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67.58±3.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>66.16±4.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65.17±2.67&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>71.87±3.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>117.18±6.19&lt;sup&gt;d&lt;/sup&gt;</td>
<td>75.25±3.76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>61.19±4.22&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>Initial</td>
<td>68.89±3.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69.23±4.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.86±5.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>73.96±3.60&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>75.26±4.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>155.52±8.80&lt;sup&gt;d&lt;/sup&gt;</td>
<td>79.46±8.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>67.76±2.74&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>Initial</td>
<td>32.69±1.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.33±2.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.92±1.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.24±0.78&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>28.41±1.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.89±1.81&lt;sup&gt;d&lt;/sup&gt;</td>
<td>27.91±1.55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36.74±0.89&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>Initial</td>
<td>12.88±0.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.02±0.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.75±0.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.97±0.72&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>14.23±0.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.28±1.71&lt;sup&gt;d&lt;/sup&gt;</td>
<td>15.07±1.65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.73±0.54&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>VLDL-cholesterol (mg/dl)</td>
<td>Initial</td>
<td>16.63±3.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.18±3.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.45±2.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.91±2.85&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>27.15±2.93&lt;sup&gt;b&lt;/sup&gt;</td>
<td>58.95±4.36&lt;sup&gt;e&lt;/sup&gt;</td>
<td>30.03±2.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.74±3.82&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Atherogenic index</td>
<td>Initial</td>
<td>1.97±0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.09±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.07±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.09±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>2.53±0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.71±0.20&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.07±0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.67±0.18&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., (n=8 animals). Values with different superscripts within the row are significantly different from initial values of controls at P<0.05 (Duncan’s multiple range test).
The hormone sensitive lipase. On the other hand, glucagon, catecholamines and other hormones enhance lipolysis. The marked hyperlipidemia that characterizes the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots (Al-shamaony et al., 1994).

There are numerous studies in which dietary fructose has been shown to induce hyperlipidemia in rodents. Numerous mechanisms have been suggested to explain this phenomenon. These include enhanced hepatic lipogenesis, overproduction of VLDL, and impairment in their peripheral catabolism (Busserolles et al., 2002). As previously discussed, the two monosaccharides- glucose and fructose are metabolized differently. Hellerstein, (1996) showed that there is little de novo lipogenesis from glucose under eucaloric conditions in humans. In contrast, Schwarz et al, (2000) reported 3- to 15-fold increases in fractional de novo lipogenesis from fructose above fasting concentrations in obese and lean subjects and nearly 30 % of circulating triacylglycerol palmitate after fructose ingestion resulted from de novo lipogenesis derived from fructose.

In the past, fructose was considered to be beneficial in the dietary management of diabetes mellitus and insulin resistance because fructose ingestion results in smaller postprandial glycemic and insulin excursions than do glucose and complex carbohydrates (Glinsmann and Bowman, 1993). In contrast with low doses of fructose, when much larger amounts of fructose are consumed, fructose continues to enter the glycolytic pathway distal to phosphofructokinase, and hepatic triacylglycerol production is facilitated. Fructose can provide carbon atoms for both the glycerol and the acyl portions of acylglycerol molecules (Mayes, 1993). Thus, unlike glucose metabolism, in which the uptake of glucose is negatively regulated at the level of phosphofructokinase, high concentration of fructose, can serve as a relatively unregulated source of acetyl-CoA. Indeed, studies in human subjects have shown that fructose ingestion results in markedly increased rates of de novo lipogenesis (Schwarz et al., 1993, 1994) whereas de novo lipogenesis does not increase in response to eucaloric glucose ingestion (Hellerstein et al., 1996). Thus, fructose is more lipogenic than is glucose, an effect that might
be exacerbated in subjects with existing hyperlipidemia (Jeppesen et al., 1995) or insulin resistance or type-2 diabetes (Abraha et al., 1998).

In the present study dyslipidemia observed in fructose fed rats was characterized by elevating plasma TG, TC, LDL-C, and VLDL-C with decreased HDL-C. This pattern strongly correlates with cardiovascular risk.

There is overwhelming evidence that fructose increases plasma TG. Many studies, both of animals and humans, have reported increases in TG after consumption of diets with fructose compared with complex carbohydrates and other sugars. In humans, some groups are more susceptible than others to hypertriglyceridemia that follows fructose consumption. Postmenopausal women, hyperinsulinemic men, and type-2 diabetes are more likely to become hypertriglyceridemic after fructose consumption than normoinsulinemic and non-diabetic men and young women (Reiser, 1985). So, fructose has been discouraged for use in diabetic patients on the basis of its supposed effects on plasma triglycerol and there is concern about a relation between fasting and non-fasting triglycerols and cardiovascular diseases (Bansal et al., 2007; Nordestgaard et al., 2007; Sarwar et al., 2007).

Hypertriglyceridemia may be secondary to increases in the very low-density lipoprotein-triglyceride (VLDL-TG) secretion rate since elevations in plasma triacylglycerol levels have been correlated with rises in this rate (Zavaroni et al., 1982). Previous studies have shown that the VLDL-TG secretion rate in the liver of rats fed a high-sucrose or high-fructose diet was higher than that of controls (Frayn, 1993; Bjorntorp, 1994). Because insulin resistance and reduced insulin binding have been reported in hypertriacylglycerolemic persons (Bieger et al., 1984), this may be one mechanism by which fructose diets promote insulin resistance. Administration of benfluorex and bezafibrate, hypolipidemic agents, reversed the insulin resistance induced by fructose feeding in rats. The improvement was associated with the normalization of triacylglycerol concentrations (Storlien et al., 1993; Matsui et al., 1997). Further insulin resistance may contribute to hypertriglyceridemia by reducing the inhibitory effect of insulin on VLDL-TG secretion.
Although effects of fructose-containing diets on plasma TG are often substantial, the reported effects on plasma cholesterol in response to fructose in the diet are not clear-cut. The increase in the synthesis of VLDL, which contains 10-20% cholesterol, to transport TG produced in the liver may be a cause of the increase in plasma cholesterol after dietary fructose consumption reported in a few studies. Another mechanism by which fructose could increase plasma cholesterol is by interaction between fructose and copper. Animal (Failla and Seidel, 1988) and human (Reiser et al., 1985) studies report that diets containing fructose aggravate copper deficiency, which results in hypercholesterolemia and may be related to coronary disease (Klevay, 1975). The hypercholesterolemia of copper deficiency is not well understood, but may result from reduced lecithin: cholesterol acyl transferase and lipoprotein lipase activities found in copper deficient animals.

Elevated non-esterified fatty acids (NEFA) concentrations are one of the metabolic consequences of a chronic positive energy balance and increased body adiposity (Ji Equier and Tappy, 1999). The exposure to increased concentrations of NEFA may reduce insulin sensitivity by increasing the intramyocellular lipid content (Virkamaki et al., 2001). Increased portal delivery of NEFA, particularly from visceral adipose tissue, could also lead to impaired carbohydrate metabolism, because elevated portal NEFA concentrations increases hepatic glucose production (Rebrin et al., 1995; Steil 1998). In addition, over time increased NEFA concentrations may have a deleterious effect on cell function (Bergman and Ader, 2000).

In the present study *O.sanctum* administration improved the lipid profile by lowering TC, TG, LDL-C and VLDL-C and increasing HDL-C resulting in decreased atherogenic index in both STZ diabetic and fructose fed rats.

Various phytochemicals reported in *O.sanctum* like flavonoids, polyphenols and alkaloids may be responsible for its hypolipidemic activity. Eshrat Halim et al. (2001) reported that *O.sanctum* corrected the abnormal lipid profile seen in fructose fed rats as reflected by the decrease in serum TC and LDL-C.
TRANSMINASES

The data presented in the Table 11 reveal the activities of hepatic and renal transaminases in the four experimental groups. Fructose-induced insulin resistant rats (group-F) showed a significant increase in the activities of glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT) both in the liver (10.6%, 13.5%) and kidney (18.2%, 17.2%) compared to group-C. Treatment with OSAE for 60 days resulted in the prevention of fructose diet induced rise in the activities of GPT and GOT in liver and kidney in group-F+OSAE. No significant variation is observed in the activities of transaminases both in the liver and kidney between group-C and C+OSAE.

Measurement of enzyme activities of serum transaminases is of clinical and toxicological importance as changes in their activities are indicative of tissue damage by toxicants or in disease conditions whereas tissue transaminases particularly liver transaminases are useful to assess the liver functions. Both GOT and GPT are located in the cytoplasm and mitochondria of liver cells and also in cells of the heart, skeletal muscle, kidney and brain (Ringer and Dabich, 1979). According to Klaassen and Watkins (1999), the activities of GPT outside the liver are low and therefore this enzyme is considered more specific for hepatocellular damage.

The observed elevation of transaminase activities in liver and kidney of group-F rats as an indication of increased protein degradation and amino acid catabolism under hyperglycemic conditions thus providing precursors for gluconeogenesis. In addition, enhanced nonenzymic glycation and/or oxidation of proteins under hyperglycemic conditions may decrease the half life of proteins, thus contributing to the enhanced protein degradation (Vlassara and Palace, 2002). Liver is the major site of nitrogen metabolism in the body. In times of dietary surplus, the potentially toxic nitrogen of amino acids are eliminated via transamination, deamination and urea formation; the carbon skeletons are generally conserved as carbohydrate, via gluconeogenesis or as fatty acid via fatty acid synthesis pathways. Moreover, GOT and GPT activities act as indicators of liver function.

Liver, an insulin dependent tissue, plays a pivotal role in glucose and lipid homeostasis and it severely affected during diabetes. Risk of chronic liver disease is higher in diabetics.
TABLE 11: Effect of OSAE treatment on tissue transaminases in four groups of animals.

<table>
<thead>
<tr>
<th>TISSUES</th>
<th>TRANSAMINASES (µg of pyruvate formed/min/mg protein)</th>
<th>C</th>
<th>F</th>
<th>F+ OSAE</th>
<th>C+ OSAE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>GPT</td>
<td>2.83±0.08</td>
<td>3.13±0.03</td>
<td>2.91±0.04</td>
<td>2.82±0.05a</td>
</tr>
<tr>
<td></td>
<td>GOT</td>
<td>2.22±0.02</td>
<td>2.52±0.08</td>
<td>2.33±0.11</td>
<td>2.26±0.06a</td>
</tr>
<tr>
<td>Kidney</td>
<td>GPT</td>
<td>0.77±0.06a</td>
<td>0.91±0.04b</td>
<td>0.81±0.04a</td>
<td>0.78±0.07a</td>
</tr>
<tr>
<td></td>
<td>GOT</td>
<td>1.62±0.05a</td>
<td>1.90±0.07b</td>
<td>1.71±0.07a</td>
<td>1.60±0.05a</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M

Values with different superscripts within the row are significantly different at P<0.05 (Duncan's multiple range test).
Figure 18: Percent recovery from fructose diet induced alterations in hepatic and renal GPT and GOT activities by OSAE treatment.
Animals must metabolize proteins to amino acids, at the expense of muscle tissue, when blood sugar is low. The preference of liver transaminases for oxaloacetate or alpha-ketoglutarate plays a key role in funneling nitrogen from amino acid metabolism to aspartate and glutamate for conversion to urea for excretion of nitrogen. Similarly, in muscle the use of pyruvate for transamination gives alanine, which is carried by the blood stream to the liver (the overall reaction being termed "glucose-alanine cycle"). Tissue transaminase activities are increased in situations associated with enhanced gluconeogenesis. Higher ALT concentrations were cross-sectionally associated with obesity and whole-body and hepatic insulin resistance and prospectively associated with a decline in hepatic insulin sensitivity and the development of type-2 diabetes (Barbora et al., 2002). ALT was significantly higher in patients with metabolic syndrome and associated with lower adiponectin levels. As the number of features of metabolic syndrome increased, ALT increased and adiponectin decreased independent of glycemic control. Hypoadiponectinemia and metabolic syndrome may be linked with liver injury in patients with liver injury in patients with type 2 diabetes (Hickman et al., 2007).

It is the general assumption that herbal preparations would have fewer side effects, but chronic consumption of large amounts and/or prolonged consumption of traditional remedies must always be taken with caution. No deviation in the activities of tissue transaminases of group-C+OSAE from group-C clearly indicates the non-toxic nature of OSAE even in chronic treatments. Instead, the restoration of transaminase activities of liver and kidney of group-F+OSAE to their respective normal values compared to group-F further strengthens the hepatoprotective and nephroprotective effects of OSAE against fructose diet induced alterations.

CARBOHYDRATE METABOLIC STUDIES

FRUCTOSE METABOLISM

The consequence of the digestion of sucrose and other fructose containing foods such as honey, fruits, and some vegetables, is absorption and transport of fructose by the intestinal epithelium into the hepatic portal vein. Therefore, all fructose absorbed flows through the liver initially. Unlike glucose, fructose is not an insulin secretagogue. On the other hand, in contrast
to glucose, fructose in not metabolized in insulin sensitive tissues (peripheral tissue). Fructose is selectively taken up and almost completely metabolized by hepatocytes in the liver (the only other cells capable of metabolizing fructose under normal conditions are spermatozoa. Very little fructose escapes from the liver, and it is not metabolized to any great extent in the small intestine which lacks the fructose phosphorylating enzyme fructokinase.

Uptake of fructose in liver and skeletal muscle is through insulin independent facilitative glucose transporters i.e. GLUT2 in liver and GLUT5 in muscle. Because of the presence of an active hepatic enzyme system for metabolizing fructose, fructose readily passes into the liver. In humans it was shown that the liver metabolized at least 50 % of the fructose injected intravenously (Mendeloff and Weichselbaum, 1953). As a consequence of the high rate of extraction of fructose by the liver, correspondingly low concentrations of fructose are found in the systemic blood vessels after meals containing fructose or sucrose are consumed (Crossley and Macdonald, 1970). Some 20 % of fructose administered intravenously is taken up by the kidney (Bj Orkman and Felig, 1982) and considerably smaller fraction would be available for adipose tissue (Froesch and Ginsberg, 1962) and skeletal muscle (Bergstrom and Hultman, 1967). Recent studies revealed that GLUT5 is specifically localized in the plasma membrane of human skeletal muscle, and fractionation studies using human adipocytes or skeletal muscle demonstrated that it is not further recruited to the plasma membrane in response to insulin stimulation (Shepherd et al., 1992). A previous study showed that when fructose is infused into exercising subjects to maintain a concentration of 5.5 mmol/L, which is above physiological concentrations and above the glucose concentration, there is considerably more fructose oxidation by exercising and resting muscles (Ahlborg and Bj Orkman, 1990).

The metabolism of fructose appears to be different in different tissues depending on the tissue distribution of the various enzymes involved in fructose metabolism (Herman and Zakim, 1968).

Fructose is rapidly phosphorylated by ATP in the liver to form fructose-1-phosphate. Fructose-1-phosphate is split by liver aldolase (aldolase B) into glyceraldehyde and dihydroxyacetone phosphate, a member of the glycolysis sequence of intermediates.
Glyceraldehyde so formed is phosphorylated by triose kinase to form glyceraldehyde-3-phosphate, another intermediate of the glycolytic pathway. Thus, the pathways of glucose and fructose metabolism in the liver converge at the triose phosphate stage of metabolism and from this point on their metabolism are qualitatively similar (Mayes, 1993). The two trioses formed by the cleavage of fructose-1-phosphate can each follow three paths 1) Dihydroxyacetone phosphate can be isomerized to glyceraldehyde-3-phosphate and continue through the glycolytic pathway to ultimately yield pyruvate, which is converted to either lactic acid under anaerobic conditions or enters the citric acid cycle as acetyl-CoA under aerobic conditions. The acetyl-CoA can then either produce energy via the TCA chain or be used as the substrate for fatty acid synthesis. 2) Dihydroxyacetone phosphate may be reduced to glycerol-3-phosphate and provide the glycerol backbone of synthesized triacylglycerols, phospholipids, and other lipids. 3) Dihydroxyacetone phosphate may also be condensed with glyceraldehydes-3-phosphate by aldolase to form fructose-1,6-diphosphate, and ultimately glucose or glycogen (Hallfrisch, 1990).

In muscle and adipose tissue hexokinase converts fructose to fructose-6-phosphate (F-6-P) which enters into the glycolysis (Strickland and Ellis, 1975). The fructokinase content of muscle and adipose tissue seems to be quite low if present at all.

As a part of our objective to find the variation in the activities of key fructose catabolising enzyme i.e., fructokinase under fructose fed condition and the effect of OSAE administration on this enzyme activity, fructokinase activity was assayed in liver of the four experimental groups.

**FRUCTOKINASE**

The phosphorylation of fructose to form fructose-1-phosphate by ATP, in liver is catalyzed by the enzyme fructokinase. This enzyme is highly specific for fructose, because it is a ketohexokinase, and fructose is the only ketohexose of physiological significance in the diet. Fructokinase is virtually confined to the liver (Cori et al., 1951). Although fructokinase is negligible in tissues other than liver, some activity is present in kidney and intestine of several species including humans (Heinz et al., 1975).
TABLE 14: Effect of OSAE treatment on the hepatic fructokinase activity in four groups of animals.

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>C</th>
<th>F</th>
<th>F + OSAE</th>
<th>C + OSAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructokinase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(μmol NADPH oxidized/min/mg protein)</td>
<td>5.136 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.643 ± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.807 ± 0.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.339 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M (n=8)
Values with different superscripts within the row are significantly different at P<0.05 (Duncan's multiple range test).
The data presented in Table 14 represent the activity of fructokinase in the liver of the four experimental groups. No significant variation in the activity of hepatic fructokinase activity was observed among groups -C and C+OSAE. However, significantly increased hepatic fructokinase activity was observed both in group-F (68.0%) and group-F+OSAE (13.7%) compared to group-C. Administration of OSAE resulted in a significant decrease in hepatic fructokinase activity in group-F+OSAE compared to group-F indicating partial protection by OSAE.

Glucose is a general substrate for all body tissues while fructose has to be processed in the liver first and its intake represents a carbohydrate load targeted on the liver. A further aspect favoring the hepatic fructose metabolism is the high activity of fructokinase relative to that of glucose phosphorylating enzymes. This is a surprising mammalian characteristic feature since glucose is the major hexose from exogenous and endogenous sources available for energy production. Yet the maximal capacity of fructokinase exceeds that of hexokinase and glucokinase combined by a factor of 3 to 5 in different species including humans (Heinz et al., 1968). The high capacity of this enzyme ensures the channeling of the majority of fructose into hepatic metabolism.

Further, it was demonstrated that prolonged feeding is necessary for meaningful induction of enzyme, for example fructokinase activity rises gradually up to 2 fold during 4 weeks on a 50 % sucrose diet (Fukuda et al., 1983). Fructokinase induction is preventable by actinomycin both in liver and intestines confirming the transcriptable mode of substrate regulation of its synthesis (Grand et al., 1974). Pathological conditions also affect fructokinase activity. STZ diabetes in rats causes a decrease while insulin treatment restores the activity. Sucrose feeding of diabetic rats has a fructokinase inducing effect even in the absence of insulin (Shafrir and Overvi, 1984). The substantial inducibility of fructokinase shows that the mammalian liver is capable of adaptation to fluctuations in dietary fructose content by expanding its capacity for fructose utilization.
GLUCOSE METABOLISM

The concept of over and underutilization of glucose by peripheral tissues during diabetes and insulin resistance plays a pivotal role during the disarray of glucose metabolism leading to elevated systemic glucose. In order to understand reasons for the fructose diet induced hyperglycemia and in an attempt to gain an insight into the underlying biochemical mechanism of antihyperglycemic effect of OSAE, the hepatic and muscle glycogen content and the key enzymes of carbohydrate metabolism i.e. glycolysis, HMP shunt, gluconeogenesis, glycogenolysis were assayed in the four experimental groups.

Liver is the first major tissue to have an opportunity to remove glucose from the portal vein blood. Uptake of glucose in liver cells occur independent of insulin by means of GLUT2, a low affinity, high capacity glucose transporter. When blood glucose is high, the liver removes glucose for the glucose-consuming processes of glycogenesis and glycolysis. When blood glucose is low, the liver supplies the blood with glucose by the glucose-producing processes of glycogenolysis and gluconeogenesis. The liver is also the first organ exposed to the blood flowing from the pancreas and therefore “senses” the highest concentrations of hormones (glucagon and insulin) released from this endocrine tissue. These important hormonal regulators of blood glucose levels affect key enzyme-catalyzed steps in the liver.

Muscle and heart cells readily utilize glucose. Insulin stimulates transport of glucose into these cells by way of GLUT4. Binding of insulin to its receptor on the plasma membrane initiates a signaling cascade that promotes translocation and fusion of GLUT4 containing vesicles with the plasma membrane thereby placing GLUT4 in a position where it can facilitate glucose transport. Glucose taken into muscle and heart cells can be utilized by glycolysis and TCA cycle to provide ATP. Muscle is capable of synthesizing significant quantities of glycogen, an important fuel that the cells of these tissues store for later consumption.

HEPATIC AND SKELETAL MUSCLE GLYCOGEN

Glycogen is the principal polysaccharide that functions as the secondary long term energy storage in animal cells. It is made primarily by the liver and the muscle, but can also be
made by glycogenesis within the brain and stomach. Liver glycogen functions as a glucose reserve for the maintenance of blood glucose concentrations. The function of muscle glycogen is to act as readily available source of glucose units to glycolysis within the muscle. The concentration of tissue glycogen also depends upon the rate id its formation (glycogenesis) and degradation (glycogenolysis).

The glycogen content of liver and skeletal muscle of four experimental groups is represented in Table 12. Group-F showed significantly enhanced glycogen content both in liver (48.6%) and muscle (5.8%) when compared to group-C. Control rats treated with OSAE (group-C+OSAE) showed no significant variation in the glycogen content of liver and muscle as compared to group-C. However, co-administration of OSAE along with fructose diet for 60 days (group-F+OSAE) resulted in a significant decrease in hepatic glycogen (22.3%) and muscle glycogen content (4.17%) when compared to group-F. Thus OSAE treatment restored the glycogen content of muscle to normal values in group-F+OSAE; however, the glycogen content of liver is still significantly higher than group-C.

Glycogen is an important fuel reserve for several reasons. The controlled breakdown of glycogen and release of glucose, increase the amount of glucose that is available between meals. Hence glycogen serves as a buffer to maintain blood glucose levels. Although the metabolism of fructose and glucose share many of the same intermediate structures, they have very different metabolic fates in human metabolism. Fructose is metabolized almost completely in the liver in humans, and is directed toward replenishment of liver glycogen and TG synthesis, while much of dietary glucose passes through the liver where it is metabolized in skeletal muscle to CO₂, H₂O and ATP, and to fat cells where it is metabolized primarily to glycerol phosphate for TG synthesis as well as energy production (McGrane, 2006). The products of fructose metabolism are liver glycogen and de novo lipogenesis of fatty acids and eventual synthesis of endogenous TG can be divided into two main phases: The first phase is the synthesis of the trioses, dihydroxyacetone and glyceraldehyde; the second phase is the subsequent metabolism of these trioses either in the gluconeogenic pathway for glycogen replenishment and/or the complete metabolism in the fructolytic pathway to pyruvate, which
enters the Krebs cycle, and converted to citrate and subsequently directed toward "de novo" synthesis of the free fatty acid palmitate (McGrane, 2006).

The synthesis of glycogen in the liver following a fructose-containing meal proceeds from gluconeogenic precursors. Fructose is initially converted to DHAP and glyceraldehyde by fructokinase and aldolase B. The resultant glyceraldehyde then undergoes phosphorylation to glyceraldehyde-3-phosphate. Increased concentrations of DHAP and glyceraldehyde-3-phosphate in the liver drive the gluconeogenic pathway toward glucose-6-phosphate, glucose-1-phosphate and glycogen formation. It appears that fructose is a better substrate for glycogen synthesis than glucose and that glycogen replenishment takes precedence over triglyceride formation (Parniak, 1988). Once liver glycogen is replenished, the intermediates of fructose metabolism are primarily directed toward TG synthesis.

Studies of Forster (1974) demonstrated greater deposition of glycogen in animals infused with fructose than those infused with glucose. Insulin plays a direct role in the control of liver glycogen metabolism by the regulation of the glycogenesis. It controls this process by regulating the conversion of a glucose-6-phosphate-dependent form into a glucose-6-phosphate-independent form of glycogen synthase (Tan and Nuttall, 1976; Khandelwal et al., 1977). Basal amounts of insulin inhibit glycogenolysis by about 60%. Glycogen synthesis is impaired in diabetes either because of glycogen synthase inhibition or impaired hepatic glucose uptake and glucose phosphorylation. Because fructose phosphorylation does not require hexokinase it may overcome the glycogen synthesis in diabetes. Studies of Cuidad et al. (1988) demonstrated enhanced glycogen synthesis in isolated hepatocytes both from diabetic and normal rats when incubated in solutions containing fructose compared to glucose, due to stimulation of glycogen synthesizing enzymes.

Our results of enhanced glycogen content in fructose fed rats are in accordance with earlier studies (Youn et al., 1987; Murakami et al., 1997). However a few contradictory observations of decreased hepatic glycogen content in fructose fed rats also appeared in literature (Rajasekar and Anuradha, 2007).
When compared with glucose, the hexose most predominantly utilized by skeletal muscle is fructose. It appears to be more readily incorporated into glycogen. After an intravenous infusion, fructose has been demonstrated to disappear from the bloodstream more rapidly than glucose leading to a greater production of lactate (Zierath et al., 1995). In man, skeletal muscle glycogen content increased in response to fructose infusion (Nillson and Hultman, 1974). The observed increase in muscle glycogen has been reported to occur despite alterations in circulatory levels of glucose, and offers indirect evidence that a portion of circulating fructose is converted directly into muscle glycogen. Despite these findings, no change in diaphragm muscle glycogen content (Vrana et al., 1978) and decreased soleus muscle glycogen content (Thorburn et al., 1989) were observed.

Administration of OSAE to control rats (group-C+OSAE) showed no influence on glycogen content of liver and muscle whereas OSAE administration to fructose fed rats (group-F+OSAE) resulted in partial restoration of hepatic glycogen and complete restoration of glycogen content of skeletal muscle from fructose diet induced enhancement to near normal values.

GLYCOGEN PHOSPHORYLASE

Glycogen phosphorylase is one of the phosphorylase enzymes (EC 2.4.1.1). Glycogen phosphorylase catalyzes the rate limiting step in the degradation of glycogen in animals by releasing glucose-1-phosphate from the terminal alpha-1,4-glycosidic bond. The regulation of glycogenesis and gluconeogenesis in the liver is central to the maintenance of blood glucose level. Glycogen metabolism is majorly affected by several hormones. Insulin induces the synthesis of glycogen. Glucagon and epinephrine in contrast trigger the breakdown of glycogen.

In order to elucidate the mechanism for glycogen over accumulation in liver under fructose fed insulin resistant conditions, glycogen phosphorylase, the key regulatory enzyme of glycogen degradation, was assayed. Data on the activity of hepatic glycogen phosphorylase in four groups of experimental animals are represented in Table 12. When compared to group-C, the activity of this enzyme decreased significantly (27%) in group-F. Administration of OSAE along with fructose diet (group-F+OSAE) resulted in a significant increase (26.3%) in hepatic
TABLE 12: Effect of OSAE treatment on glycogen and activity of glycogen phosphorylase in four groups of animals.

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>TISSUE</th>
<th>C</th>
<th>F</th>
<th>F + OSAE</th>
<th>C + OSAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen</td>
<td>Liver</td>
<td>18.4 ± 0.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.35 ± 1.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.24 ± 1.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>19.44 ± 0.82&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>2.72 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.88 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.76 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.74 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glycogen phosphorylase</td>
<td>Liver</td>
<td>13.81 ± 0.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.07 ± 0.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.72 ± 0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.51 ± 0.36&lt;sup&gt;a&lt;/sup&gt;</td>
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</tbody>
</table>

Values are mean ± S.E.M
Values with different superscripts within the row are significantly different at $P<0.05$ (Duncan's multiple range test).
Figure 20: Percent change in hepatic and muscle glycogen content, and the activity of hepatic glycogen phosphorylase of control (C), Fructose fed (F), Fructose treated (F+OSAE) and control treated (C+OSAE) rats.

Figure 21: Percent recovery from fructose diet induced alterations in hepatic and muscle glycogen content and the activity of hepatic glycogen phosphorylase by OSAE treatment.
glycogen phosphorylase activity when compared to group-F and reached normal values. However, OSAE administered control rats (group-C+OSAE) showed no deviation in their hepatic glycogen phosphorylase activity from group-C.

Glycogen synthesis and breakdown is controlled by a complex series of reactions involving covalent modification by protein phosphorylation and dephosphorylation. Briefly, regulation centers around two rate-controlling enzymes—glycogen synthase and glycogen phosphorylase. The active form of glycogen synthase (synthase a) is the dephosphoenzyme whereas the inactive synthase b is phosphorylated. On the other hand, active glycogen phosphorylase a is the phosphoenzyme, whereas the inactive b form is dephosphorylated. Protein kinases carry out phosphorylations and protein phosphatases carry out dephosphorylations of these enzymes. Both processes are controlled by hormonal and allosteric modifiers (Ercan et al., 1996; Klinov and Kurganov, 2001).

A study of the literature reveals a disparity in results on whether fructose promotes liver glycogen deposition, with the balance of studies in vivo in the fed condition indicating that fructose is a better promoter of glycogenesis than glucose (Van den Berghe, 1986). The net deposition of glycogen appears to result from both activation of glycogen synthase (Hue et al., 1973; Whitton and Hems, 1975) and inhibition of glycogen phosphorylase (Hue et al., 1973; Thurston et al., 1974) which are brought about by several mechanisms. Phosphorylase a is inhibited by fructose-1-phosphate (Kaufmann and Froesch, 1973; Van den Berghe, 1973; Thurston et al., 1974), which accumulates after administration of fructose. Also, glucose-6-phosphate increases in concentration and activates glycogen synthase and inhibits phosphorylase (Hers et al., 1974).

α-D-glucose is a weak inhibitor of glycogen phosphorylase b and acts as a physiological regulator of hepatic glycogen metabolism. Glucose binds to phosphorylase at the catalytic site and results in a conformational change that stabilizes the inactive state of the enzyme promoting the action of protein phosphatase 1 and stimulating glycogen synthase. It has been suggested that, in the liver, glucose analogues with greater affinity for glycogen phosphorylase may be a more effective regulatory agent (Kimberly et al., 1994). Thus the hyperglycemia
observed under fructose fed condition may be responsible for inactivation of glycogen phosphorylase and activation of glycogen synthase. The elevated glucose phosphate pool results in a “push” of carbon mass toward glycogen. Glycogen synthase activated by glucose, increases flux to glycogen. However, this increased glycogen synthetic flux would be of little significance for glycogen deposition, if fructose does not inhibit glycogen phosphorylase and retains glycogen. Thus, according to the model, inhibition of phosphorylase by fructose is the crucial element by which this gluconeogenic compound increases glycogen accumulation (Youn et al., 1986). It has been demonstrated that fructose intake increases hepatic glycogen (Sonne and Galbo, 1986). Inhibition of glycogen phosphorylase by fructose 1-phosphate produced from fructose is suggested to be responsible for enhancing net glycogen store in liver (Youn et al., 1987). Fructose 1-phosphate can increase translocation of glucokinase to the cytosol and glucose phosphorylation and can inhibit glycogen phosphorylase (Niewoehner and Nuttall, 1986; Niewoehner et al., 1987; Davies et al., 1990). Thus, this intermediate may play an instrumental role in the regulation of glycogen accumulation when fructose delivery is increased.

The elevated hepatic glycogen content of group-F rats can be explained by the observed in the activity of glycogen phosphorylase and enhanced production of fructose-1-phosphate by increased activity of fructokinase observed in group-F animals. Further, inhibition of glycogen phosphorylase and activation of glycogen synthase by increased concentration of glucose-6-phosphate may also be possible in fructose fed rats because of enhanced operation of gluconeogenesis (Table 15). Thus conversion of fructose to liver glycogen increased because of enzyme adaptation. Partial correction of enhanced glycogen content by fructose feed in group-F+OSAE can be explained by the observed significant enhancement in glycogen phosphorylase activity and indirectly by decreased fructokinase activity, decreased gluconeogenesis (Figure 20) and also by its antihyperglycemic activity.

GLYCOLYTIC ENZYMES

Glycolysis is an almost universal central pathway of glucose catabolism, the pathway with the largest flux of carbon in most cells. The glycolytic breakdown of glucose is the main
**TABLE 13:** Effect of OSAE treatment on the activities of key glycolytic enzymes in four groups of animals.

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>TISSUE</th>
<th>C</th>
<th>F</th>
<th>F+ OSAE</th>
<th>C+ OSAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexokinase (μmol G6P formed/min/mg protein)</td>
<td>Liver</td>
<td>5.87 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.41 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.64 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.09 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>7.30 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.30 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.16 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.53 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Phosphofructo kinase (μmol F-1,6-bis phosphate formed/min/mg protein)</td>
<td>Liver</td>
<td>6.88 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.70 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.46 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.88 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>7.88 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.70 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.46 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.87 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pyruvate kinase (μmol NADH oxidized/min/mg protein)</td>
<td>Liver</td>
<td>2.24 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.27 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.48 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.27 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>11.46 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.47 ± 0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.86 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.44 ± 0.17&lt;sup&gt;a&lt;/sup&gt;</td>
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</table>

Values are mean ± S.E.M.

Values with different superscripts within the row are significantly different at P<0.05 (Duncan's multiple range test).
source of metabolic energy with the caveat that regulation of flux through glycolysis is
dependent on the tissue under consideration and, nutritional and hormonal state of the tissue.
Of the ten steps in glycolytic pathway, three are essentially irreversible. These are Hexokinase
(HK) reaction in which glucose glucose is phosphorylated to glucose-6-phosphate,
prophofructokinase (PFK) reaction, in which fructose-6-phosphate is phosphorylated to
fructose-1,6-bisphosphate and pyruvate kinase (PK) in which transfer of phosphate from
phosphoenolpyruvate to ADP takes place. Activities of HK, PFK and PK have shown to be very
sensitive markers of the glycolytic pathway (Murphy and Anderson, 1974). Measuring these
enzyme activities represents a method to assess the peripheral utilization of glucose.

The data on activities of key glycolytic enzymes i.e., HK, PFK and PK of liver and skeletal
muscle and HK of adipose tissues are presented in Table 13. Fructose fed rats (group-F) showed
significantly decreased activities of HK and PFK in liver (24.8% and 17.15% respectively) and
skeletal muscle (13.7% and 15% respectively) when compared to corresponding values of
group-C. Similarly HK activity of adipose tissue of group-F is significantly lower (30.6%) than
group-C. Unlike HK and PFK, the activity of PK was significantly enhanced both in the liver (46%)
and muscle (17.5%) of group-F animals compared to corresponding values of group-C animals.
Administration of OSAE along with fructose diet for 60 days prevented the fructose diet
induced alterations in the activities of HK, PFK and PK of liver, muscle and adipose tissues and
restored these values to normal. However, OSAE administration to control rats showed no
alterations in the activities of these three key glycolytic enzymes in liver, muscle and adipose
tissues when compared to control rats.

In the present study, group-F rats showed significantly decreased activities of HK and
PFK (two among three regulatory enzymes of glycolysis) indicating decreased operation of
glycolysis both in the insulin dependent tissue (skeletal muscle and adipose tissue) and insulin
independent tissue (liver). This is an indication of impaired utilization of glucose in group-F. The
decreased utilization of glucose, as found in the whole organism when placed on a high-sucrose
or fructose diet, was also reported in studies of individual tissues (Bender and Thadini, 1970;
Tuovinen and Bender, 1975).
Figure 22: Percent recovery from fructose diet induced alterations in the activities of hepatic and muscle glycolytic enzymes, and hepatic fructokinase by OSAE treatment.

Figure 23: Percent recovery from fructose diet induced alterations in hepatic and renal gluconeogenic enzyme activities by OSAE treatment.
Studies of Weber and Convery (1966) indicated that insulin administration restored the decreased glycolytic enzyme activities of diabetic animals. Insulin upregulates the transcription of glucokinase, PFK, and PK genes (Howard, 1995). The development of insulin resistance in fructose fed rats as also reported in the present study is well documented in the literature. Defects in post-receptor events in insulin signaling (Bezerra et al., 2000) and in enzymes involved in glucose metabolism (Blakely et al., 1981) were reported. The hyperglycemia prevailing in fructose fed conditions may lead to increased uptake of glucose into hepatocytes through GLUT2, an insulin independent glucose transporter. Besides, decreased operation of glycolysis is also responsible for the enhancement in the cellular glucose concentration in the liver. High intracellular glucose exerts toxic effects on structure and function of organs and induces insulin resistance, a phenomenon referred to as glucose toxicity was observed in diabetic rats (Kahn et al., 1991).

In the span of glycolytic reactions from glyceraldehyde-3-phosphate to pyruvate and lactate, the rate-controlling step is catalyzed by pyruvate kinase. In contrast to HK and PFK, PK activity significantly increased in liver of fructose fed rats which can be explained by the hepatic fructose metabolism. Rapid uptake of fructose by liver and its entry in to the glycolysis at the triose phosphate level after bypassing the PFK regulatory steps takes place. As a result of the loading of the initial pathways of fructose metabolism there is a tendency for intermediates of glycolysis to increase in concentration resulting in an increased flux through the pathway (Sahebjami and Scalettar, 1971) as evidenced by enhanced PK activity in fructose fed rats. Pyruvate kinase is normally under feed-forward control because of allosteric activation by fructose-1,6-bisphosphate. Although this metabolite may double in concentration when fructose is added to hepatocytes (Van den Berghe, 1986), of more significance are the large increases in fructose-1-phosphate concentrations which extend a similar but more enhanced activation of PK (Eggleston and Woods, 1970).

Even though low concentrations of fructokinase are reported in muscle and adipose tissue, tissue HK converts fructose to fructose-6-phosphate which enters the glycolysis (Katzen et al., 1965) because of the decreased HK activity observed in fructose fed rats resulting in less
production of fructose-6-phosphate the substrate of PFK. Thus fructose fed rats also showed decreased PFK activity indicating decreased entry of fructose into glycolysis. The decreased activity of the first two key enzymes of glycolysis also indicates decreased oxidation of glucose in the muscle of fructose fed rats. Muscle tissue shows decreased activity to metabolize glucose and increased ability to oxidize fatty acids after animals have been fed high fructose diets (Vrana et al., 1978).

Co-administration of OSAE along with fructose diet prevented the fructose feed induced decrease and increase operations of glycolysis pathway before and after triose phosphate level respectively in both liver and muscle tissues. Impaired insulin sensitivity with compensatory hyperinsulinemia and hyperglycemia are common pathogenic factors observed in the fructose fed rats. Under these conditions, skeletal muscle represents the main site of insulin resistance as it is one of the major sites for glucose consumption. The current results of glycolytic enzyme activities in four experimental groups clearly represent the beneficial effect of OSAE in rectifying insulin resistance not only in muscle and adipose tissues but also in liver.

GLUCONEOGENESIS

Glucogenesis is a metabolic pathway that results in the generation of glucose from non carbohydrate carbon substrates such as lactate, glycerol and glucogenic amino acids. It is one of the two main mechanisms the body uses to keep blood glucose levels from dropping too low (hypoglycemia). The other means of maintaining blood glucose level is through the degradation of glycogen. Both phenomena are inhibited by insulin and enhanced by a deficiency of insulin action (Friedmann et al., 1967). Increased hepatic glucose production is a major component of insulin deficiency and insulin resistance induced hyperglycemia. Glucogenesis is also a target therapy for type II diabetes, such as metformin, which inhibit glucose formation and stimulate glucose uptake by cells (Hundal et al., 1992).

In order to assess the role of glucogenesis in fructose diet induced hyperglycemia and to study the antihyperglycemic efficacy of OSAE, the key gluconeogenic enzymes i.e, fructose-1,6-bisphosphatase (F1,6Pase) and glucose-6-phosphatase (G6Pase) were assessed in
TABLE 15: Effect of OSAE treatment on the activities of key gluconeogenic enzymes in four groups of animals.

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>TISSUE</th>
<th>C</th>
<th>F</th>
<th>F + OSAE</th>
<th>C + OSAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructose-1,6-bis phosphatase</td>
<td>Liver</td>
<td>3.08 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.17 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.58 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.83 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(µmol F6P formed/min/mg protein)</td>
<td>Kidney</td>
<td>3.14 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.23 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.64 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.89 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glucose-6-phosphatase</td>
<td>Liver</td>
<td>16.45 ± 0.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.48 ± 0.80&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.77 ± 0.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.28 ± 0.61&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(nmol Pi liberated/min/mg protein)</td>
<td>Kidney</td>
<td>13.27 ± 0.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.59 ± 0.91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.66 ± 0.63&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.05 ± 0.76&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M
Values with different superscripts within the row are significantly different at P<0.05 (Duncan's multiple range test).
Group F showed significantly enhanced activities of F1,6Pase and G6Pase in both liver and kidney when compared with group C. The per cent increase in hepatic F1,6Pase and G6Pase activities of group F compared to group C is 100.3 and 36.6% respectively. The per cent increase in renal F1,6Pase and G6Pase activities of group F is 98.4 and 32.5% respectively when compared with group C. Co-administration of OSAE along with fructose diet prevented the increase in the activities of F1,6Pase and G6Pase both in the liver and kidney. However, OSAE treatment for control rats resulted in no significant variation in the activities of F1,6Pase and G6Pase, both in the liver and kidney of group C+OSAE as compared with group C.

Insulin regulates hepatic glucose production and gene expression of several proteins at transcription level including regulatory enzymes of gluconeogenesis (phosphoenolpyruvate carboxykinase and G6Pase) (Brien et al., 1995; Argaud et al., 1996; Gabbay et al., 1996). In the presence of insulin resistance insulin may also fail to regulate the transcription of these genes. In adult rat liver, phosphoenolpyruvate carboxykinase (PEPCK) gene transcription is upregulated by glucagon, glucocorticoids, and epinephrine and downregulated by insulin which exerts a dominant effect (Lucas and Granner, 1992). Activities of liver microsomal G6Pase have been reported to be up-regulated in both fasting and diabetic states (Ashmore et al., 1954; Segal and Washko, 1959), and administration of insulin to diabetic rats resulted in a reduction in the enzyme activity (Nordlie and Arion, 1965). Recent studies suggested that adipose hormones may be involved in the control of glucose metabolism in liver. Indeed the suppression of hepatic glucose production by insulin is enhanced by leptin and adiponectin (Fruhbeck and Salvador, 2000; Berg et al., 2001; Combs et al., 2001) but impaired by resistin (Rajala et al., 2003).

The glycolysis and gluconeogenic pathways make use of many common intermediates and enzymes but are controlled by several non-equilibrium enzymes unique to each pathway (Murray et al., 1993). Two pivotal reactions, for which the activities are reciprocal and coordinated, dominate these pathways. These are catalyzed by PFK in glycolysis and F1,6Pase in gluconeogenesis. Their activities are both induced by hormones and also by allosteric modifiers.
often acting on the key regulatory molecule-fructose-2,6-bisphosphate, which activates PFK and inhibits F1,6Pase (Hers and Schaftingen, 1982).

The liver can convert fructose into glucose, but this occurs by a pathway not involving sorbitol. The pathway probably involves the formation of fructose-1-phosphate followed by cleavage to D-glyceraldehyde and DHAP. D-glyceraldehyde is converted to glyceraldehyde-3-P which reacts with DHAP to form fructose-1,6-bisphosphate and successively fructose-6-phosphate, glucose-6-phosphate, and finally glucose (Ballard, 1965).

Increased activity of F1,6Pase and G6Pase in group-F animals indicates enhanced operation of gluconeogenesis in liver and kidney under insulin resistant condition. This may be attributed to the enhanced availability of gluconeogenic precursors.

Fructose is a highly lipogenic nutrient because its metabolism produces the precursors for lipogenesis and TG synthesis i.e., acetyl-CoA and glycerol (Mayes, 1993). Fructose infusions in humans resulted in dangerous increase in blood lactic acid, especially in patients with preexisting acidotic conditions such as anoxia, diabetes, postoperative stress, or uremia (Hallfrisch, 1990). Many studies revealed that more lactate is formed from fructose than from glucose (Sahebjami and Scalettar, 1971). The increased lactate production occurs because fructokinase activity is increased, the rate limiting step of glycolysis (PFK) is bypassed and PK activity is stimulated by activity of fructose-1-phosphate. Thus, fructose metabolism favours the formation of gluconeogenic substrates like glycerol, acetyl-CoA, pyruvate and lactate. In addition, enhanced protein degradation, reflected by increased tissue transaminases in fructose fed rats (Table 11), also provides gluconeogenic amino acid pool.

Basing on our observations, the antihyperglycemic effect of OSAE appears to be at least in part, due to increased glucose utilization by liver and muscle, and decreased glucose production by depression of key gluconeogenic enzymes i.e., F1,6Pase and G6Pase. Increased activity of these enzymes in group-F animals indicates enhanced operation of gluconeogenesis in liver and kidney under insulin resistant condition. This may be attributed to the enhanced availability of gluconeogenic precursors. Administration of OSAE for 60 days along with fructose feed prevented the enhanced gluconeogenesis by fructose diet. Activities of enzymes of
gluconeogenesis and insulin sensitivity are reciprocally related. Activities of gluconeogenic enzymes are inhibited by enhancing insulin sensitivity (Brazilai et al., 1999).

Barzilai et al. (1999) reported that suppression of PEPCK is associated with enhanced hepatic insulin sensitivity after removal of visceral fat in rats. They also demonstrated that lowering visceral fat by caloric restriction or leptin administration dramatically improved hepatic insulin sensitivity independent of whole body fat mass (Barzilai et al., 1997, 1998).

Thus OSAE treatment restored the enzyme activities to normal level. This can be explained by the enhanced insulin sensitivity by OSAE treatment in group-F+OSAE which can be attributed to its hypolipidemic activity reflected by preventing lipid accumulation in liver and muscle as well as by maintaining normal plasma lipid profile thus preventing fructose induced abnormalities in metabolism reflected by decreased visceral fat in group-F+OSAE and hence correcting the endocrine functions of the adipose tissue.

**HMP SHUNT (Glucose-6-phosphate dehydrogenase)**

HMP shunt is an alternate route for the metabolism of glucose. Liver is one of the tissue, is having active operation of the pathway. Glucose-6-phosphate dehydrogenase (G6PDH), the first and rate limiting step of pentose phosphate pathway, has long been regarded as major enzyme enzyme generating reducing power in the form of NADPH. The NADPH is used for reductive pathways, such as fatty acid biosynthesis, detoxification of drugs by monoxygenases and glutathione defense system against injury by reactive oxygen species. Thus HMP pathway provides reducing equivalents for the support of lipogenesis when lipogenesis is very active, as in experimental animals fed on sugar rich diet. Alterations in G6PDH activity can significantly alter oxidative stress induced cell death (Beutler, 1992; Vulliamy et al., 1992). It plays an important role in cell death by regulating intracellular redox levels. Glucose-6-phosphate dehydrogenase gene can be considered to be a constitutively expressed “housekeeping gene” in many tissues. There are several other tissues (liver, adipose, lung and proliferating cells) wherein modulation of cellular G6PDH activity represents an important component of the integrated response to external stimuli (hormones, growth factors, nutrients, and oxidative stress) (Rudack et al., 1971; Berdanier and Shubeck, 1979).
The data on activity of hepatic and adipose tissue G6PDH of four experimental groups is represented in Table 16. Chronic fructose feeding has shown differential response regarding G6PDH in liver and adipose tissue. G6PDH activity was significantly enhanced in hepatic tissue (115.6%) with no significant alterations in adipose tissue of group-F rats compared to group-C. Administration of OSAE along with fructose diet resulted in significantly decreased hepatic G6PDH activity compared to group-F. Group-F+OSAE showed only 40.32% increase in the G6PDH activity when compared with group-C. Thus OSAE treatment restored G6PDH activity in the liver of fructose fed rats to near normal values. However, OSAE administration to normal rats does not cause any changes in the hepatic and adipose tissue G6PDH activity compared to group-C.

It has been known for many years that the enzyme profile of the liver cell can be drastically changed by a great variety of dietary, hormonal, and pharmacologic stimuli (Tepperman and Tepperman, 1964). Of the NADP-linked liver enzymes, the hexose monophosphate shunt dehydrogenases and malic enzyme (ME) are among those which exhibit the largest variation from one physiological circumstance to another (Tepperman and Tepperman, 1964). The activities of liver G6PDH and 6-phosphogluconate dehydrogenase are regulated by dietary stimuli since they are elevated in fasting and refeeding (Young et al., 1964), intermittent starvation and refeeding (Leveille, 1966), after feeding diets high in glucose (Fitch and Chaikoff, 1960) or in fructose (Fitch and Chaikoff, 1960; Chevalier et al., 1972). They are also lowered by feeding diets high in fats (Leveille and Hanson, 1966) or in refeeding, after a period of starvation, with diets low in protein (Jomain and Hanson, 1969).

In addition, G6PDH has long been considered as a member of the family of lipogenic enzymes which also includes fatty acid synthase, acetyl-CoA carboxylase, and malic enzyme. This group of enzymes is induced in liver during episodes of dietary carbohydrate excess and is responsible for fatty acid synthesis, a process generally regulated by insulin (Katsurada et al., 1989). The elevated activity seems to be dependent upon the presence of insulin, glucocorticoids, and carbohydrate in the diet (Miksicek and Towle, 1982) whereas insulin is generally considered to be the predominant signal responsible for upregulation of expression of
TABLE 16: Effect of OSAE treatment on the activity of glucose-6-phosphate dehydrogenase in four groups of animals.

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>TISSUE</th>
<th>C</th>
<th>F</th>
<th>F+ OSAE</th>
<th>C+ OSAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose-6-phosphate dehydrogenase</td>
<td>Liver</td>
<td>1.86 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.01 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.61 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.95 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M (n=8)
Values with different superscripts within the row are significantly different at P<0.05 (Duncan's multiple range test).
the lipogenic enzymes including G6PDH. Other studies have shown that the glucocorticoids play an important role (Bouillon and Berdanier, 1980).

A significantly enhanced activity of hepatic G6PDH observed in fructose fed rats is in accordance with earlier reports (Moser and Berdanier, 1974; Fiebig et al., 1998). The liver is an organ in which glucose regulated gene expression has already been investigated. When glucose flux increases in the hepatocytes, flux through the pentose phosphate pathway also increases. In addition, pressure overload induces G6PDH, the enzyme catalyzing the flux generating step in oxidative pentose phosphate pathway (Zimmer, 1996). The induction of G6PDH in different tissue by a variety of non-hormonal agents suggests that the G6PDH gene may be a sentinel for oxidant stress and may be capable of responding rapidly to the need for NADPH for maintenance of the cellular redox state.

Dietary carbohydrate also influences the level of expression. It was suggested that a metabolite of the sugars is involved in upregulation of hepatic G6PDH expression (Fukuda et al., 1992). However, since both glucose and sucrose can elicit insulin secretion, it has been difficult to sort out secondary effects of the carbohydrates versus primary effects on gene expression in animals. Other studies demonstrated that fatty acids in the diet can repress induction of G6PDH activity, mRNA abundance (Tomlinson et al., 1988), and G6PDH gene transcription (Iritani, 1992) following fasting/refeeding. Several studies have explored the possible role of carbohydrate on G6PDH expression, since earlier studies in animals and hepatocytes in culture suggested that nutrient influenced G6PDH activity (Kelley and Kletzien, 1984; Salati et al., 1988). However, the reports demonstrate a lack of consensus; some groups indicate that glucose does not influence expression while others suggest that it does. A study by Fukuda et al. (1992) showed that glucose can enhance insulin and glucocorticoid-induced expression of all the lipogenic enzymes in addition to G6PDH, although the influence of the sugar in the absence of the hormones was not addressed. Thus, one is not sure if the sugar effect is directly linked to enhancement of gene expression or indirectly linked through an enhancement of hormonal signaling. Fukuda et al. (1992) also showed that fructose could replace glucose and that glycerol was also effective suggesting that a metabolite or glycolytic intermediate is mediating the effect.
of carbohydrate on G6PDH expression. Thus in the present study the hyperglycemia along with hyperinsulinemia in fructose fed rats might have mediated the G6PDH expression. In addition, fructose as well as its metabolite-glycerol and fructose induced oxidative stress has given additive effect in enhancing the G6PDH expression.

Restoration of enhanced G6PDH activity of liver by fructose feeding to normal values by OSAE administration indicates reduced availability of NADPH for lipogenesis even under fructose fed condition. Thus, improved insulin sensitivity by OSAE treatment might have resulted in normalizing the enhanced operation of HMP shunt by fructose diet.

INTESTINAL DISACCHARIDASES

The digestive enzymes of small intestinal mucosa play an important role in the overall digestion process. Complex polysaccharides must be digested by the entire digestive enzymes including α-glucosidase and intestinal and intestinal brush border disaccharidases. The traditional view of the interaction of nutritional substances and intestinal enzymes is that the enzymes act to digest and metabolize the foodstuffs that one eats. More recent knowledge has demonstrated that intestinal enzyme activity will vary according to the diet; i.e., the concept of adaptation to the diet ingested. This should not be surprising since adaptation to the environment is a fundamental principle of many biological systems.

Fructose feeding alters intestinal mucosal function in a variety of ways including the enhancement of both active transport processes and the activity of brush border hydrolases. Chronic diabetes enhances glucose transport by non specific increase in intestinal mass (Olsen and Korsme, 1975; Ferraris et al., 1993). Release of glucose and transport across the intestinal brush border membrane down to the blood stream has attracted much attention recently as potential targets to control postprandial hyperglycemia. Since both postprandial hyperglycemia and hyperinsulinemia were improved by treatment with a disaccharidase inhibitor in patients with NIDDM, the possibility that this drug might improve insulin resistance was suggested (Friedman et al., 1991; Shinozaki et al., 1996). Therefore, a disaccharidase inhibitor could be recommended to prescribe to obese and insulin resistance patients even without metabolic derangement in glucose homeostasis. So the present study was extended to understand the
TABLE 17: Effect of OSAE treatment on the activities of intestinal disaccharidases in four groups of animals.

<table>
<thead>
<tr>
<th>DISACCHARIDASE (nmol disaccharide hydrolyzed/min/mg protein)</th>
<th>C</th>
<th>F</th>
<th>F + OSAE</th>
<th>C + OSAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maltase</td>
<td>1.49 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.90 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.45 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.41 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sucrase</td>
<td>2.75 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.17 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.88 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.68 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lactase</td>
<td>2.17 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.45 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.22 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.11 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M (n=8)
Values with different superscripts within the row are significantly different at P<0.05 (Duncan’s multiple range test).
efficacy of OSAE administration in regulating the intestinal disaccharidases activity under insulin resistant condition.

Previous studies demonstrated the adaptable nature of intestinal disaccharidase activity in rats (Deren et al., 1967). Specific dietary sugars can alter enzyme activity in the small intestine of man in a specific fashion. Sucrose and fructose are able to regulate sucrase and maltase activity (Rosensweig and Herman, 1968). Yasutake et al. (1995) reported that dietary sucrose enhanced both sucrase-isomaltase mRNA and sodium/D-glucose transporter-1 (SGLT1) mRNA levels in the rat jejunum within 12 h. Similarly, Miyamoto et al. (1993) demonstrated that the mRNA levels of jejunal hexose transporters (SGLT1, GLUT5, and GLUT2) were elevated by feeding fructose diet to rats for 5 days.

The data presented in Table 17 reveal the activities of intestinal disaccharidases of four experimental groups. Fructose fed rats showed significantly enhanced activities of disaccharidases i.e., maltase (27.5%), sucrase (15.3%) and lactase (12.9%) when compared to group-C. Administration of OSAE along with fructose diet for 60 days showed significantly lower activities of maltase (23.7%), sucrase (9.15%) and lactase (9.15%) when compared with group-F. However it caused no significant alteration in the activities of intestinal disaccharidases in group-C+OSAE from group-C. Thus, OSAE administration prevented abnormal increase in the activities of disaccharidases induced by high fructose feeding.

The increased activities of intestinal disaccharidases (maltase, sucrase and lactase) observed in present study are in agreement with the earlier reports in fructose feed induced insulin resistant rats (Tominaga et al., 1997; Kishi et al., 1999).

Insulin deficiency or insulin resistance stimulates the functional activity of brush border membrane of the intestinal absorptive cell. For example, experimental diabetes has been reported to increase the enzymatic activity of many brush border hydrolases including the disaccharidases (Caspary et al., 1972) and stimulates a number of transport systems which are believed to reside in this membrane (Caspary, 1973; Hopfer, 1975). In diabetic patients and animals, in particular in rats with STZ induced IDDM, abnormal increases in the activities of sucrase and isomaltase were observed in the small intestine (Olsen and Korsmo, 1977;
Hoffmann and Chang, 1992). Furthermore, it has been reported that the postprandial metabolic profile is improved by the administration of an inhibitor of the sucrase and isomaltase in the experimental IDDM rats (Matsuo et al., 1992) suggesting that the abnormal increases in these enzyme activities exert a harmful influence on the metabolic imbalance under diabetic conditions (Porteous et al., 1979). Diabetes mellitus in man has also been associated with increased intestinal disaccharidase activity (Cerda et al., 1972; Tandon et al., 1975) and enhanced glucose absorption (Vinnik et al., 1965). Although, in man negative studies have also been reported (Genel et al., 1971; Arvanitakis and Olsen, 1974), perhaps because of differing severity of the disease and effects of treatment. According to Olsen and Korsmo (1977) enhanced sucrase-isomaltase enzyme activity of STZ diabetes is the consequence of an increase in concentration of sucrase-isomaltase protein due to decreased rate of its degradation.

Hormone secretion by the gut and the pattern of response after feeding may be abnormal in diabetes and might be regulatory for disaccharidases. The intestinal effects are prevented or markedly diminished by insulin therapy (Olsen and Rosenberg, 1970) suggesting that they are in fact the result of insulin deficiency or insulin resistance.

The α-glucosidase inhibitors are a new class of antihyperglycemic drugs that have a unique effect on the glycemic profile. Their major action is to lower postprandial plasma glucose levels by inhibiting the breakdown of complex carbohydrates within the intestine resulting in delayed absorption of glucose with the small bowel and a consequent reduction in postprandial glucose levels. The α-glucosidase inhibitors not only delay carbohydrate absorption but they also alter the gastrointestinal hormonal axis. These drugs decrease postprandial secretion of gastric inhibitory polypeptide (potentiates insulin secretion under hyperglycemic condition) and increases postprandial levels of glucagon-like peptide which may play a role in the regulation of insulin secretion (Sunil and Sadekar, 1999). Therefore, α-glucosidase inhibitors such as acarbose, voglibose and miglitol are widely used either alone or in combination with insulin secretagogues in patient with type-2 diabetes (Saito et al., 1998; Standl et al., 1999). Acarbose is the first commercially available α-glucosidase inhibitor and inhibits both amylase and membrane bound α-glucosidase with approximately equal affinity. It
Figure 24: Intestinal disaccharidases of Control (C), Fructose fed (F), Fructose treated (F+OSAE) and Control treated (C+OSAE) rats.

Figure 25: Percent recovery from fructose diet induced alterations in hepatic and cardiac total cholesterol, triglycerides, free fatty acids, phospholipids and hepatic total lipids by OSAE treatment.
has a potent inhibitory effect on sucrase but weak effect on the maltase and no effect on β-glucosidase such as lactase. Thus lactose is digested normally (Chait and Brunzell, 1996). Acarbose does not interfere with the sodium dependent glucose transporter thereby not affecting absorption of glucose. Friedman et al. (1991) reported that the decreased levels of GLUT4 of fatty Zucker rats are normalized by the treatment with a disaccharidase inhibitor, acarbose. Since GLUT4 level is the rate-limiting step of insulin stimulated glucose disposal, normalization of GLUT4 level might considerably improve in vivo insulin resistance of fatty Zucker rats.

Voglibose, a N-substituted derivative of valiolamine isolated from the fermentation broth of Streptomyces hygroscopicus, is a potent and structurally novel inhibitor of the intestinal disaccharidases. It had a potent inhibitory effect on maltase but with a short inhibitory duration (Kameda et al., 1984; Goto et al., 1995; Taira et al., 2000; Koyama et al., 2000). Shinozaki et al. (1996) found an improvement of in vivo sensitivity to insulin in non-diabetic hyperinsulinemic patients by administration of a disaccharidase inhibitor AO-128 (voglibose).

In this category, a majority of recent studies report the potential of antidiabetic medicinal plants on inhibition of carbohydrate hydrolyzing enzymes, α-amylase and α-glucosidase and manipulation of intestinal glucose transporters. A wealth of literature has emerged now showing the potential effect of phytochemicals in inhibiting α-amylase (Kim, 2000) and α-glucosidase (Watanabe et al., 1997). Watanabe et al. (1997) also reported the potential effect of phytochemicals in inhibiting α-glucosidase activity resulting in lowering of in vivo postprandial hyperglycemia.

In the present study the enhanced activities of intestinal disaccharidases of insulin resistant rats were prevented by the OSAE treatment. Therefore the improvement of glycemic control by the delayed absorption of monosaccharides with a disaccharidase inhibitor is likely responsible for the observed improvement of insulin resistance in group-F+OSAE rats. Thus, antidiabetic property of OSAE may also contribute due to its intestinal disaccharidase inhibitory activity.
STUDIES ON LIPID METABOLISM

Metabolic syndrome is associated with insulin resistance and abdominal obesity; therefore, it has a great significance both in carbohydrate metabolism and lipid metabolism. High fructose diets induce well characterized metabolic dysfunction i.e., dyslipidemia, typically resulting in a rapid elevation of serum triglycerides with a corresponding increase in blood pressure within two weeks. Animals maintained on high-fructose diet for longer periods of time develop elevated FFAs and hyperinsulinemia at the expense of glycemic control. Further, this dietary manipulation also results in enhanced lipids in the muscle which was found to be closely correlated to the degree of insulin resistance (Oakes et al., 1997; Jucker et al., 1998).

Fructose is considered as a highly lipogenic nutrient. The role of fructose in lipid metabolism was suggested long ago by Higgins (1916) who from measurements of the respiratory quotient arrived at a conclusion that fructose has a greater tendency for conversion into lipids than glucose. Fructose has both immediate and long-term effects on lipid metabolism. Short-term or acute effects are those that occur as a result of fructose metabolism by existing enzyme capacity, whereas long-term effects result mainly from enzyme adaptation to diets containing high concentrations of fructose.

Liver plays an important role in carbohydrate and lipid metabolism and gets severely affected metabolically during insulin resistance. Liver is involved in lipid metabolism through uptake, oxidation and metabolic conversion of FFAs, synthesis of cholesterol and phospholipid secretion of plasma lipoproteins. Because of the importance of the liver in fructose uptake from the blood, many of its effects on lipid metabolism are found in this organ.

In this metabolic model, compounds that lower circulating lipid levels, increase insulin sensitivity, or inhibit TNF-α production reduce serum TGs and improve blood pressure (Inoue et al., 1995; Mangaloglu et al., 2002). In the present study as the plant extract showed antihypertriglyceridemic activity in fructose fed animals, it may also have beneficial effect in correcting the altered lipid metabolism reported under high fructose fed condition. As a part of it, we assayed tissue lipid profile of liver and heart and, the activities of fatty acid synthetase.
and malic enzyme in both liver and adipose tissues, and lipoprotein lipase in adipose tissue of four experimental groups.

HEPATIC AND CARDIAC LIPIDS

The data presented in the Table 18a reveals the hepatic and cardiac tissue total lipids, cholesterol, TGs, phospholipids and FFAs of the four experimental groups. All fractions of tissue lipids except phospholipids enhanced significantly in group-F compared to group-C. In comparison to group-C, the per cent increase in hepatic and cardiac cholesterol, TG and FFA are 26.8, 48.2 and 67.4%, and 46.3, 34.3, and 45.7% respectively in group-F. Group-F also showed significantly enhanced total lipid content of liver (34.04%) when compared with group-C. Group-F showed no significant variation in the hepatic phospholipid concentration when compared with group-C; however, cardiac phospholipids of these animals are significantly lower (22.4%) than group-C. Thus insulin resistance rat model showed higher lipid accumulation in both liver and heart tissues. Co-administration of OSAE along with fructose diet for 60 days prevented fructose diet induced abnormalities in tissue lipids. However, group-C+OSAE showed no deviation in tissue lipid fractions when compared to group-C.

The observed abnormalities in tissue lipids by fructose feeding are in accordance with the previous reports (Rajasekar et al., 2005). The accumulation of TGs and other fractions of lipids in liver and cardiac tissue of fructose fed rats could occur due to increased lipogenesis, decreased clearance or reduced fatty acid oxidation. Kelley et al. (2004) hypothesized that high fructose fed animals exhibit altered lipid metabolism due to hepatic stress as a result of the burden of fructose metabolism. Additionally, fructose bypasses two regulatory steps of glycolysis; glucokinase and PFK, thus potentially providing unregulated accumulation of glycolytic intermediates, DHAP and pyruvate. DHAP is in equilibrium with glycerol-3-phosphate, cosubstrate for esterification of long-chain acyl-CoA in the synthesis of TGs and phospholipids. TG is the major precursor and determinant of VLDLs secreted by the liver which constitutes the bulk of endogenously derived plasma TG (Havel et al., 1962). Fructose also generates pyruvate, which, besides forming lactate, enters the mitochondrion to form acetyl-CoA as a result of pyruvate dehydrogenase (PDH) activity. Fructose causes increase in PDH activity by increasing
<table>
<thead>
<tr>
<th>TISSUE</th>
<th>C</th>
<th>F</th>
<th>C+OSAE</th>
<th>F+OSAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>2.98 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.78 ± 0.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.94 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.24 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Heart</td>
<td>1.97 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.93 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.14 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.13 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
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<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>C</th>
<th>F</th>
<th>C+OSAE</th>
<th>F+OSAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol (mg/g tissue)</td>
<td>5.58 ± 0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.27 ± 0.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.54 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.94 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Triglycerides (mg/g tissue)</td>
<td>3.66 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.39 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.02 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.80 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Free Fatty Acids (mg/g tissue)</td>
<td>0.83 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.38 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.38 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.38 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Phospholipids (mg/g tissue)</td>
<td>25.48 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.79 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.22 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.22 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total LIPIDS (mg/g tissue)</td>
<td>25.48 ± 1.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.79 ± 1.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.22 ± 1.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.22 ± 1.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M (n=8)
pyruvate concentrations. Acetyl-CoA is the major carbon source for lipogenesis. Acetyl-CoA is converted to long-chain fatty acid via the important cytosolic intermediate malonyl-CoA.

By these pathways, fructose provides carbon atoms for both the glycerol and the acyl portions of the acylglycerol molecule.

Rajasekar et al. (2005) reported increased activity of HMG-CoA reductase in fructose fed rats which signify an increase in cholesterol synthesis. The addition of fructose to cultured rat hepatocytes increases HMG-CoA reductase approximately 3-fold (Spence et al., 1985). The observed accumulation of cholesterol in liver and heart of fructose fed rats in the present study can be linked to this. The increase in FFA could be one mechanism by which fructose could produce insulin resistance. Increased delivery of FFAs to the muscle interferes with glucose utilization through the principles of Randle cycle (Randle, 1998). This can attenuate insulin signaling and exacerbate insulin resistance. Conversely, diminished insulin-stimulated glucose disposal could lead to impaired FFA reesterification and thereby to higher circulating FFA concentrations.

The dyslipidemia observed in insulin resistance is thought to be initiated by the resistance of fat storing cells. The inability to store TG results in its mobilization to plasma and also to non-fat storing tissues such as liver. Over accumulation of TG in the liver and muscle produces excess of metabolites such as fatty acids, ceramides and diacyl glycerol. These may enter deleterious non-oxidative pathways and induce a state of lipotoxicity (Unger, 2002). Further, these metabolites induce a cascade of serine/tyrosine phosphorylation reactions that diminish the glucose transport activity and other events that desensitize insulin receptor signaling (Shulman, 2000).

Marked increase in the lipid concentration in the liver during fructose fed (Rajasekar et al., 2005) and diabetic conditions (Agheli et al., 1998) resulting into fatty liver. The liver, once fatty, is insulin resistant and overproduces both glucose and VLDL leading to hyperglycemia, hypertriglyceridemia, and a low HDL-C concentration (Wasastjerna et al., 1972). Liver fat is significantly and linearly correlated with all components of the metabolic syndrome independent of obesity. Individuals with a fatty liver are more likely to have excess intra
abdominal fat and inflammatory changes in adipose tissue. In humans, exogenous insulin therapy (70 IU/d for 7 months) decreases liver fat significantly suggesting that hyperinsulinemia may be a consequence rather than cause of liver fat (Juurinen et al., 2007). Insulin normally inhibits the production of VLDL from the liver. Once fatty, this action of insulin is impaired whereas VLDL clearance remains unchanged. The overproduction of VLDL results in hypertriglyceridemia and a lowering of HDL-C (Nikkila and Taskinen, 1978).

It is conceivable that if the muscular fat accumulates directly within the cytosol, that is, in the intramyocellular compartment, this lipid and its metabolism could contribute to the development of insulin resistance and hence to the pathogenesis of type-2 diabetes (Jacob et al., 1999). Thus the present study revealed enhanced lipid accumulation in both liver and heart tissues under fructose fed condition and this may be responsible for decreased insulin sensitivity in these tissues. Coadministration of OSAE along with fructose diet has prevented fructose diet induced accumulation of tissue lipids representing the beneficiary effect of this plant in enhancing the insulin sensitivity.

**FATTY ACID SYNTHETASE, MALIC ENZYME AND LIPOPROTEIN LIPASE**

Fatty acid synthetase (FAS) plays a central role in de novo lipogenesis in animals by catalyzing all the reactions in conversion of acetyl-CoA and malonyl-CoA to palmitate (Wakil et al., 1983). Malic enzyme (ME) catalyze the oxidative decarboxylation of malate to pyruvate and CO₂, with the concomitant reduction of the cofactor NAD(P)⁺ to NAD(P)H, which is utilized in reductive lipogenesis. Malic enzymes have been found in most living organisms from E.coli to man. Lipoprotein lipase (LPL) is an enzyme responsible for the hydrolysis of triacylglycerols from plasma lipoproteins, mainly chylomicrons and very low-density lipoproteins and its activity is influenced by nutritional and hormonal status and by environmental conditions (Kraemer et al., 1998; Merkel et al., 2002). Adipose tissue LPL initiates the entry of lipoprotein packaged fatty acids into adipose tissue for storage (Appel and Fried, 1992). Research carried out over the past two decades have not only established a central role for LPL in the overall lipid metabolism and transport but have also identified additional, non catalytic functions of the enzyme. Furthermore, abnormalities in LPL function have been found to be associated with a number of
TABLE. 19: Effect of OSAE treatment on the activities of key lipid metabolic enzymes in four groups of animals.

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>TISSUE</th>
<th>C</th>
<th>F</th>
<th>F + OSAE</th>
<th>C + OSAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipoprotein lipase (μmol PNP released/min/mg protein)</td>
<td>Adipose</td>
<td>14.42 ± 0.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.47 ± 0.76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.95 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.25 ± 0.63&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fatty acid synthetase (μmol NADPH utilized/min/mg protein)</td>
<td>Liver</td>
<td>0.62 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.86 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.66 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.60 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Adipose</td>
<td>5.79 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.19 ± 0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.37 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.98 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Malic enzyme (μmol NADPH oxidized/min/mg protein)</td>
<td>Liver</td>
<td>7.10 ± 0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.50 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.96 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.19 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Adipose</td>
<td>40.40 ± 1.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.83 ± 1.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36.38 ± 1.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.45 ± 1.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. (n=8)
Values with different superscripts within the row are significantly different at P<0.05 (Duncan's multiple range test).
pathological conditions, including atherosclerosis, obesity, Alzheimer’s disease and dyslipidemia associated with metabolic syndrome (Mead et al., 2002).

The data on activity of hepatic and adipose tissue FAS and ME, and adipose tissue LPL of four experimental groups are furnished in the Table 19. Group-F showed significantly enhanced activities of hepatic FAS (38.7%) and ME (33.8%) when compared to group-C. Administration of OSAE to fructose fed rats (group-F+OSAE) resulted in significant decrease in hepatic FAS (23.2%) and ME (16.2%) activities when compared to group-F and reached normal values. There is no statistical significance in the activities of these enzymes between groups-C+OSAE and C. Group-F showed significantly decreased activity of adipose tissue FAS (27.6%) and ME (28.6%) when compared to group-C. Administration of OSAE to fructose fed rats (group-F+OSAE) resulted in a significant increase in adipose tissue FAS (28.2%) and ME (26.2%) activities when compared to group-F and restored to normal values. The enzyme activities of group-C+OSAE did not deviate from group-C. A significantly decreased activity (34.3%) of adipose tissue LPL was observed in fructose fed rats (group-F) when compared to group-C. Group-F+OSAE showed a significantly enhanced adipose tissue LPL activity (57.8%) when compared to group-F. However, administration of OSAE for 60 days showed no significant variation in this enzyme activity in group-C+OSAE as compared to group-C.

*In vitro* studies as well as the enzyme data from both liver and adipose tissue suggest that a diet containing a high percentage of fructose causes a shift in the site of lipid synthesis from adipose tissue to liver (Chevalier et al., 1972). Thus, while fatty acid synthesis in adipose tissue of fructose fed animals decreased, fatty acid synthesis from fructose in liver increased. Since the rate of lipogenesis from acetate was not significantly affected by dietary carbohydrate, the adaptive step probably lies in the conversion of glucose and fructose to acetyl CoA and not in the conversion of acetate to fatty acids (Chevalier et al., 1972). Since the adipose tissue lacks fructokinase (Adelman et al., 1968) and glucokinase (Katzen and Shimke, 1965), both glucose and fructose must by phosphorylated by hexokinase. This enzyme was shown to be adaptive (Chevalier et al., 1972) and may be responsible for the reduction in fatty acid synthesis in adipose tissue of the fructose-fed animal.
Thus in the present study the enhanced tissue lipids in fructose fed rats can be explained by enhanced hepatic lipogenesis as reflected by the increased activities of FAS, ME and G6PDH in the liver along with decreased activity of adipose tissue LPL, under fructose fed condition.

The activities of adipose tissue FAS, ME, G6PDH and HK suggest that fructose feeding reduces the capacity of the adipose tissue to synthesize fatty acids. The depressed lipogenesis from both glucose and fructose in the fructose-fed animals as well as the reduction in the activities of ME, citrate cleavage enzyme and HK was already reported by Chevalier et al. (1972). The activity of the NADPH-producing enzymes has previously been shown to parallel adipose tissue fatty acid synthesis activity (Leveille, 1966). The activity of ME, particularly high in lipogenic tissues, is elevated in conditions favoring fatty acid synthesis. The role of ME in lipogenesis has recently received renewed attention in view of the calculations of Flatt and Ball (1964) and of Landau et al. (1965) that the NADPH generated by the pentose phosphate pathway is only about 50% of that needed for fatty acid synthesis and that the remainder could be derived from NADP-linked ME. The liver also appears to adapt to dietary fructose. Fatty acid synthesis from fructose was more rapid in the liver of the fructose-fed animal than in that of the glucose-fed animal. The activity of hepatic ME increased by fructose feeding. Activity of this enzyme has been correlated with the rate of fatty acid synthesis in liver (Tepperman and Tepperman, 1964).

The beneficiary role of OSAE against fructose feed induced lipid accumulation in tissues is justified by preventing the enhanced lipogenesis under fructose fed conditions by keeping the enhanced lipogenic enzymes of liver and decreased activity of these enzymes in adipose tissue to the normal level. Thus OSAE treatment has given protection against fructose feed induced insulin resistance in target tissues by preventing lipid accumulation. Similar type of protection was reported by *Tinospora cordifolia aqueous* stem extract treatment in fructose fed rats (SS Reddy et al., 2006). The antihyperlipidemic action of this plant against fructose diet induced hyperlipidemia observed in the present study may also due to correction of altered lipid metabolism observed in liver and adipose tissue. The lipid lowering activity of OSAE was also
Figure 26: Percent recovery from fructose diet induced alterations in hepatic and adipose tissue lipid metabolic enzyme activities by OSAE treatment.

Figure 27: Percent recovery from fructose diet induced alterations in liver, pancreas and heart aldose reductase and sorbitol dehydrogenase activities by OSAE treatment.
revealed in STZ induced diabetic rats by Eshrat Halim et al. (2007) and cholesterol fed rabbits (Swetha Guptha, 2005). There are evidences that plant extracts can effectively prevent fructose diet induced alterations in lipid profile. For example, Cinnamon barks extract (Kannappan et al., 2006), *Tinospora cordifolia* aqueous stem extract (SS Reddy et al., 2006) prevented high fructose diet induced alterations in the plasma cholesterol, TGs, FFAs and phospholipids.

**IN VIVO ANTIOXIDANT STUDIES ON OSAE**

**OXIDATIVE STRESS**

Oxidative stress occurs when there is an imbalance between free radical reactions and the scavenging capacity of antioxidative defense mechanism of the organism (Sies, 1991). Oxidative stress and associated tissue damage represent a common end-point of chronic diseases such as arteriosclerosis, rheumatoid arthritis and diabetes (Hiramatsu and Arimori, 1988; Baynes and Thrope, 1999). If cellular antioxidants do not remove free radicals, radicals attack and damage proteins, lipids and nucleic acids. The oxidized or nitrosylated products of free radical attack have decreased biological activity leading to loss of energy metabolism, cell signaling, transport and other major functions. These altered products are also targeted for degradation further decreasing cellular functions. Accumulation of such injury ultimately leads the cell to die through necrotic or apoptotic mechanisms. Hence, the comparative evaluation of intra and extracellular oxidizing and antioxidant/reducing responses are important tools in the determination of an oxidative stress.

ROS are generated by the electron transport chain in mitochondrial respiration and are thus increased in conditions associated with enhanced oxidation of energy substrate such as glucose and FFAs. Furthermore, ROS is produced by NADPH oxidase, which is activated by various cytokines. The state of insulin resistance is accompanied by increases in the levels of blood glucose, FFAs, and adipocytokines and is thus regarded as a state of increased exposure to ROS (Evans et al., 2002, 2003).

When glucose and FFA increase, they cause oxidative stress along with activation of stress-sensitive signaling pathways (Evans et al., 2003, 2005). Activation of these pathways, in
turn, worsens both insulin action and secretion leading to overt NIDDM. Insulin resistance leads to abnormal glucose and lipid metabolism with an increase in reactive aldehydes. These aldehydes react with the sulfhydryl and amino groups of proteins to form advanced glycation end products, adversely affecting body proteins, including antioxidant enzymes. This leads to oxidative stress. Advanced glycation end products and ROS perpetuate a pro-oxidant state (Vasdev et al., 2006).

Increases in circulating FFA and hyperglycaemia, chief characteristics of NIDDM, can both lead to leakage of \( \cdot \text{O}_2 \) from the mitochondrial respiration process and activation of NADPH oxidase, a membrane-bound enzyme (Evans et al., 2002; Nisoli et al., 2007). NADPH oxidase, a major source of \( \cdot \text{O}_2 \) generation, is found in a variety of cells, including adipocytes, vascular smooth muscle cells, endothelial cells, fibroblasts and monocytes/macrophages (Griendling et al., 2000).

ROS can damage cellular DNA, membranes, lipids, and proteins, and drive inflammatory gene expression that inhibits metabolic pathways induced by insulin, leading to insulin resistance (Nisoli et al., 2007).

Experimentally, ROS is implicated in the development of obesity-induced insulin resistance. Systemic markers of oxidative stress are increased with adiposity because of dysregulation of adipocytes (Keaney et al., 2003). Increased adiposity in target organs is associated with an accumulation of macrophages, which are a major source of TNF-\( \alpha \) (Weisberg et al., 2003; Desruisseaux et al., 2007). Localised production of ROS by NADPH oxidase in adipose tissue increases oxidative stress in remote tissues, causing dysregulation of adipocytes, increasing secretion of TNF\( \alpha \), plasminogen-activating inhibitor-1 (PAI-1), and MCP-1, resistin, and leptin; and, decreased secretion of adiponectin, leading to a worsening of insulin resistance (Trayhurn and Wood, 2005).

Manipulation of mediators of oxidative stress can lead to disease modification both short- and long-term (Warnholtz et al., 1999). Thus, treatment aimed at reducing the degree of oxidative stress and activation of oxidative stress signaling pathways would appear to warrant
The direct measurement of free radicals, particularly in vivo is extremely difficult. Usually, the products of radical damage in the cell-viz., lipids, proteins and DNA are considered good markers of oxidative stress (Piconi et al., 2003). In order to understand the extent of oxidative stress in metabolic syndrome and to evaluate the protective role of OSAE against metabolic syndrome induced oxidative stress, markers of this process like lipid peroxidation, protein oxidation and antioxidant status were assessed in tissues of four experimental groups.

**LIPID PEROXIDATION AND PROTEIN OXIDATION**

Polyunsaturated fatty acids are particularly vulnerable to free radical attack leading to lipid peroxidation (LPO) and it has probably the most extensively investigated process induced by free radicals. The measurement of LPO is a convenient method to monitor oxidative damage. Lipid peroxides are derived from the oxidation of poly unsaturated fatty acids of membranes and are capable of further LPO by a free radical chain reaction (Das et al., 2002). Malondialdehyde (MDA) is a breakdown product of peroxidation of long chain fatty acids which accumulates when LPO increases (Ceconi et al., 1992). MDA levels in liver may also be used to investigate the oxidative damage of proteins and lipoproteins which is a possible pathogenic mechanism for liver injury (Kojic et al., 1998). These peroxide products are cytotoxic and highly reactive, leading to free-radical damage to proteins and DNA. Skeletal muscle and liver of obese insulin-resistant subjects contain a higher amount of intramyocellular lipids and, more importantly, these lipids showed a higher degree of LPO (Golay et al., 2003). Protein oxidation is defined as the covalent modification of a protein induced either directly by reactive ROS or indirectly by reactions with secondary byproducts of oxidative stress (Sermin et al., 2007).

The extent of LPO and protein oxidation in liver, pancreas and heart of four experimental groups are summarized in the Table 22. The extent of LPO in all experimental groups indicates that the intensity of LPO is highest in liver followed by heart and pancreas (L > H > P). Group-F showed significantly enhanced LPO in liver (95.35%), pancreas (121.3%) and heart (69.74%) when compared to corresponding values of group-C (Figure). Thus under
fructose fed condition the per cent increase in LPO is more in pancreas than liver and heart. Administration of OSAE along with fructose feed for 60 days prevented the increased tissue LPO observed in fructose fed conditions (Figure 29). However, administration of OSAE for normal rats showed no deviation in tissue LPO levels from corresponding values of group-C.

Group-F showed a significant increase in the level of protein oxidation in liver (55.56 %), pancreas (12.9 %) and heart (12.5 %) compared to group-C. The altered protein oxidation levels are reinstated to normal values by OSAE supplementation in group-F+OSAE (Figure 29). However, OSAE treated control group i.e. C+OSAE showed no deviation in the extent of protein oxidation in these three tissues compared to group-C.

Increased LPO impairs membrane functions by decreasing membrane fluidity and changing the activity of membrane-bound enzymes and receptors (Chen et al., 2002). Oxidative stress could also participate in the progression of insulin resistance since the incubation of adipocytes in the presence of H2O2 decreases the sensitivity of cells to insulin and glucose transport (Hansen et al., 1999). Kocic et al. (2007) reported that positive correlation was found between the MDA level and index of insulin sensitivity.

Insulin resistance is also associated with increased LPO and free radical formation and increased formation of TBARS (thiobarbituric acid reactive substances) is associated with insulin perturbations. LPO is one of the characteristic features of metabolic syndrome. Oxidative stress induces a variety of cellular damage directly or indirectly through LPO of reactive aldehydes, such as 4-hydroxynonenal (HNE) (Schaur, 2003; Yang et al., 2003). LPO and therefore aldehydes represent markers of oxidative injury and may be more deleterious than the initial product, ROS, because they diffuse within the cell and thus propagate their noxious action. HNE is the major unsaturated aldehyde end product and the most toxic one, produced during oxidative stress in relatively large amounts (Benedetti et al., 1979). At high concentrations, HNE exerts cytotoxic, mutagenic, and genotoxic activities and is largely responsible for cytopathological effects observed during oxidative stress (Esterbauer et al., 1991).

Previous studies strongly suggest that oxidative stress occurs in rats fed a high fructose diet (Srividhya and Anuradha, 2002). Detrimental effects of fructose are enhanced when
Antioxidant defenses are decreased or when free radical production is increased (Rayssiguier et al., 1981, 1993). High fructose diet has prooxidant effects (Busserolles et al., 2002). Both enhanced oxidative damage to cellular constituents and diminished antioxidative capacity have been reported in fructose fed-rats (Faure et al., 1997; Busserolles et al., 2002).

High fructose diets may have a hypertriglyceridemic and prooxidant effect, and fructose fed rats have shown less protection from LPO. Moreover, the susceptibility of tissues to oxidative stress may depend on alterations in lipid composition. Enhanced lipid accumulation observed in the tissues of fructose fed rats may also contribute to increase LPO in these animals. Further heightened catabolism of fructose would result in energy depletion in cells making them more susceptible to peroxidation (Fields et al., 1992). Besides hyperglycemia, hypertriglyceridemia and hyperinsulinemia along with lipid over load in non-adipose tissues by fructose feeding can be related to increased lipid peroxide levels found in these rats.

Sandrine et al. (2005) showed that insulin resistance induced by high fructose diet in rats was associated with oxidative stress. Thus enhanced protein oxidation under fructose fed conditions may be one of the factors for development of insulin resistance. Recently the role of oxidative stress in the development of atherosclerosis in the insulin resistance syndrome has been evoked. Major components of insulin resistance syndrome (IRS) (insulin resistance, hypertension, and dyslipidemia) generate oxidative stress in response to an overproduction of superoxide anion by the activation of NADPH oxidase (Lee, 2001).

The antioxidants like vitamin E, vitamin C (Cinar et al., 2001; Kedziora-kornatowska et al., 2003), α-lipoic acid (Kocak et al., 2000; Thirunavukkarasu et al., 2004) and L-carnitine (Rajasekar and Anuradha, 2007) received much attention for their potential role in controlling oxidative stress in fructose fed insulin resistant conditions. In addition to the many antioxidants examined, a number of commonly used drugs have shown promising antioxidant activity in addition to their primary pharmacological activity. These drugs include thiazolidinediones, metformin and HMG-CoA reductase inhibitors (statins), and inhibitors of the rennin-angiotensin system.
TABLE 22: Effect of OSAE on Tissue lipid peroxidation, protein oxidation and reduced glutathione levels in four groups of animals

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tissue</th>
<th>C</th>
<th>F</th>
<th>F+ OSAE</th>
<th>C + OSAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid peroxidation (nmol MDA formed/min/mg protein)</td>
<td>Liver</td>
<td>23.02±1.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>44.97±3.40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.42±2.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.59±1.66&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Pancreas</td>
<td>10.69±0.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.66±0.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.380±0.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.19±0.32&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>11.37 ±0.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.30±0.55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.65±0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.12±0.31&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GSH (µg/mg protein)</td>
<td>Liver</td>
<td>6.24±0.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.31±0.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.76±0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.35±0.25&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Pancreas</td>
<td>4.12±0.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.31±0.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.58±0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.21±0.33&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>5.53±0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.25±0.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.42±0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.63±0.34&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Protein oxidation (µmol protein carbonyls/mg protein)</td>
<td>Liver</td>
<td>1.62±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.52±0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.62±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.52±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Pancreas</td>
<td>2.85±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.22±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.92±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.77±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>3.04±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.42±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.11±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.99±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., (n=8 animals). Values with different superscripts within the row are significantly different at P<0.05 (Duncan's multiple range test).
Figure 28: Percent recovery from fructose induced diet alterations in the activities of hepatic, pancreatic and cardiac glutathione independent antioxidant enzymes by OSAE treatment.

Figure 29: Percent recovery from fructose diet induced alterations in Lipid peroxidation, GSH and Protein oxidation by OSAE treatment.
Administration of OSAE for 60 days to fructose fed rats alleviated the lipid accumulation in the heart and liver tissues. This may depend upon its TG lowering and insulin sensitivity effects. Further, its antihyperglycemic effect could bring a favorable metabolic environment avoiding the prooxidant conditions with reduced oxidative stress in OSAE treated fructose fed rats. This protection against oxidative stress by OSAE is further reflected by controlled LPO and protein oxidation in group-F+OSAE.

*Ocimum sanctum* leaf extract showed strong protective effects against reactive oxygen species (Umadevi *et al.*, 2000). The ethanolic extract of OS leaves was found to prevent noise induced oxidative stress in discrete organs of the brain (Samson *et al.*, 2007). The anti stressor activity of essential oil from leaves and seeds of OS in rats exposed to restrained stress has been reported (Sen *et al.*, 1994). Protective effect of OSAE against LPO in different oxidative stress models have been reported (Geetha and Vasudevan, 2004). The antioxidants interrupt the free radical chain of oxidation by donating hydrogen from phenols hydroxyl groups, thereby forming stable free radicals, which do not initiate or propagate further oxidation of lipids. Therefore, it can be assumed that OS may be acting on similar lines.

It may be presumed that ursolic acid, which is one of the key constituents of OS leaf extract, is responsible for inhibition of lipid peroxidation (Balanehru and Nagarajan., 1992). Phenolic compounds like eugenol, apigenin and other flavonoids present in OS have excellent antioxidant activity (Anita *et al.*, 2009). The flavonoids which were extracted from OS have been shown significant antioxidant activity both invivo and invitro (Umadevi *et al.*, 2000). Presence of flavonoids in OS may be held responsible for its attenuating activity because flavonoids have been reported as potentially useful exogenous agents in protecting the organs and tissues of the body against free radical induced damage (Tabassum *et al.*, 2009).

**POLYOL PATHWAY**

Polyol pathway also known as sorbitol-aldose reductase pathway consists of two enzymes. The first enzyme, aldose reductase (AR), reduces glucose to sorbitol with the aid of its co-factor NADPH, and the second enzyme, sorbitol dehydrogenase (SDH), with its co-factor NAD⁺, converts sorbitol to fructose. Activation of polyol pathway results in a decrease of
reduced NADP⁺ and oxidized NAD⁺; these are necessary cofactors in redox reactions throughout the body. The decreased concentration of these cofactors leads to decreased synthesis of reduced glutathione, nitric oxide, myoinositol and taurine. Sorbitol may also glycate nitrogens on proteins such as collagen and the products of these glycations are referred to as advanced glycation end products (AGE). AGEs are thought to cause disease in the human body, one effect of which is mediated by receptor for advanced glycation end products (RAGE) and the ensuring inflammatory responses induced. In animal models, treatment with AR inhibitors was effective in preventing the development of various diabetic complications including cataract, neuropathy, and nephropathy (Oates and Mylari, 1999).

Most cells require the action of insulin for glucose to gain entry into the cell, the cells of the retina, kidney and nervous tissues are insulin independent, so glucose moves freely across the cell membrane, regardless of the action of insulin. The cells will use glucose for energy as normal and any glucose not used for energy will enter the polyol pathway. When blood glucose is normal, this interchange causes no problems, as aldose reductase has a low affinity for glucose at normal concentrations. In a hyperglycemic state, the affinity of AR for glucose rises, causing much sorbitol to accumulate, and using much more NADPH, leaving less NADPH for other processes of cellular metabolism (Brownlee, 2001).

The activities of AR and SDH in liver, pancreas and heart tissues of four experimental groups are given in the Table 20. Aldose reductase enzyme activity was highest in liver, than heart followed by pancreas. A significant increase in the activity of AR and SDH was observed in liver (12.0 and 16.2%), heart (16.5 and 9.0%) and pancreas (30.1 and 8.4%) of group-F compared to group-C. Administration of OSAE along with fructose diet prevented the increase in the activities of both AR and SDH in the three tissues. Activities of these two enzymes in group-C+OSAE did not deviate from that of group-C. Thus prevention of enhanced polyol pathway enzyme activities by OSAE supplementation in fructose fed animals (group-F+OSAE) avoided the deleterious alterations due to enhanced operation of polyol pathway towards oxidative stress.
TABLE 20: Effect of OSAE treatment on the activities of aldose reductase and sorbitol dehydrogenase enzymes in four groups of animals.

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>TISSUE</th>
<th>C</th>
<th>F</th>
<th>F + OSAE</th>
<th>C + OSAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldose reductase (µmol NADPH oxidized/min/mg protein)</td>
<td>Liver</td>
<td>3.11 ± 0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.48 ± 0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.19 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.97 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Pancreas</td>
<td>1.36 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.77 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.50 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.29 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>1.58 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.84 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.64 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.52 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sorbitol dehydrogenase (µmol NADH oxidized/min/mg protein)</td>
<td>Liver</td>
<td>4.68 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.44 ± 0.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.85 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.62 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Pancreas</td>
<td>3.21 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.48 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.30 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.12 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>3.35 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.65 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.52 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.30 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M (n=8)

Values with different superscripts within the row are significantly different at P<0.05 (Duncan's multiple range test).
The present study clearly indicates enhanced operation of polyol pathway in fructose fed rats, which may be correlated to the hyperglycemia observed in these rats, which may be correlated to the hyperglycemia observed in these rats. In hyperglycemic state, the affinity of AR for glucose rises, causing much sorbitol to accumulate. The sorbitol cannot cross cell membranes, and when it accumulates, it produces osmotic stress on cells by drawing water in.

There are three potential mechanisms for the polyol pathway to contribute to oxidative stress.

1. AR activity depletes its co-factor NADPH which is also required for glutathione reductase to regenerate GSH. Under hyperglycemic condition, as much as 30% of the glucose is channeled into the polyol pathway, causing a substantial depletion of NADPH and consequently a significant decrease in the GSH level. Thus, during hyperglycemia, AR activity diminishes the cellular antioxidant capacity (Cheng and Gonzalez, 1986).

2. Oxidation of sorbitol to fructose by SDH causes oxidative stress because its co-factor NAD\(^+\) is converted to NADH in the process, and NADH is the substrate for NADH oxidase to generate ROS (Morre et al., 2000). Oxidation of sorbitol by NAD\(^+\) increases the cytosolic NADH: NAD\(^+\) ratio, which tends to inhibit glyceraldehyde phosphate dehydrogenase activity. This can lead to increased levels of triose phosphates, methylglyoxal, and diacylglycerol. This chain of events is also associated with consumption of NAD\(^+\) by activated poly (ADP-ribose) polymerase. This in turn was associated with intracellular AGE formation and activation of PKC, the hexosamine pathway and NF-kB (Du et al., 2003).

3. The polyol pathway converts glucose to fructose. Because fructose and its metabolites, fructose-3-phosphate and 3-deoxyglucosone are more potent non-enzymatic glycation agents than glucose, the flux of glucose through the polyol pathway would increase advance glycation end products (AGE) formation. AGES, as well as binding of AGE to their receptors, are known to cause oxidative stress (Chung et al., 2003).

Restoration of enhanced polyol pathway enzymes to normal levels by OSAE supplementation along with fructose diet in group-F+OSAE avoided the deleterious alterations.
due to enhanced operation of polyol pathway towards oxidative stress and this effect of OSAE may be attributed to its antihyperglycemic activity. Reports are available for polyol pathway inhibiting activity of many plant extracts and phytochemicals. Aqueous extracts of Ocimum sanctum, Withania somnifera, Curcuma longa, Azadirachta indica are having AR inhibiting activity (Halder et al., 2003). Flavonoid treatment inhibits the polyol pathway in experimental diabetes (Vertommen et al., 1994). Turmeric and curcumin supplementation was also reduced the oxidative stress encountered by the diabetic rats. This was demonstrated by the lower levels of TBARS, which may have been due to the decreased influx of glucose into the polyol pathway (Suryanarayana et al., 2005). Results from our study revealed another beneficial property of OS by decreasing the polyol pathway towards preventing the oxidative stress induced in metabolic syndrome.

ANTIOXIDANT SYSTEM

Antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols (Sies, 1997). Cells must contain the levels of antioxidants, often defined as antioxidant potential, through dietary uptake or denovo synthesis. Excess production of free radicals can deplete the intra cellular antioxidants resulting in oxidative stress.

The body has developed several endogenous antioxidant systems to deal with the production of ROS. These systems can be divided into enzymatic and non-enzymatic groups. Enzymatic antioxidant defenses include GSH independent SOD and CAT and GSH dependent GPx, GR and GST. The non-enzymatic antioxidants include the lipid-soluble vitamins, vitamin E and vitamin A or provitamin A (β-carotene), and the water-soluble vitamin C and GSH. Vitamin E has been described as the major chain-breaking antioxidant in humans (Packer, 1992). Because of its lipid solubility, vitamin E is located within cell membranes, where it interrupts LPO and may play a role in modulating intracellular signalling pathways.
There are mainly four routes for action of antioxidants:

1. Chain breaking reactions, e.g. α-tocopherol which acts in lipid phase to trap ROS.

2. Reducing the concentration of ROS, e.g. glutathione.

3. Scavenging initiating radicals e.g. superoxide dismutase which acts in aqueous phase to trap superoxide free radicals.

4. Chelating the transition metal catalysts: A group of compounds serves as antioxidant function by sequestering of transition metals that are well-established pro-oxidants. In this way, transferrin, lactoferrin, and ferritin function to keep iron induced oxidant stress in check and ceruloplasmin and albumin as copper sequestrants.

The development of oxidative stress, an imbalance between free radical production reactions and scavenging capacity of antioxidative defense mechanism of the organisms, has been shown to play an important role in mediating insulin resistance (Paolisso and Giugliano, 1996; Ceriello, 2000). Through in vitro studies and in animal models, it has been found that antioxidants improve insulin sensitivity (Rudich et al., 1999; Maddux et al., 2001). Several clinical trials, have also demonstrated that treatment with vitamin E, vitamin C, or glutathione improves insulin sensitivity in insulin-resistant individuals and/or patients with type-2 diabetes (Evans and Goldfine, 2000; Jacob et al., 2000). So the present study was further extended to study the antioxidant status in four experimental groups.

GLUTATHIONE

Glutathione (GSH) is present in all mammalian tissues, provides reducing capacity for several reactions and plays an important role in detoxification of hydrogen peroxide and free radicals (Meister and Anderson, 1983). It has been established in several different animal models, as well as humans, that a decrease in GSH concentration may be associated with aging and pathogenesis of many diseases (Gambhir et al., 1997). The GSH is an important constituent of cell and considered to be a vital component and redox buffer in the cell. It
provides protection to cell non-enzymatically, by acting as a free radical scavenger, a nucleophilic shield and as a reductant (Masella et al., 2005).

While all cells in the human body are capable of synthesizing GSH, majority of it is synthesized in the liver, and approximately 40% is secreted in the bile. The biological role of GSH in bile is believed to be defence against dietary xenobiotics and LPO in the lumen of the gut and protection of the intestinal epithelium from oxygen radical attack (Aw, 1994).

During ROS over production, intracellular GSH is oxidized to disulfide form GSSG, which is then reconverted to GSH by GR. The GSH/GSSG ratio also defines the so-called GSH redox state which plays an important role in cellular activation, gene expression, mRNA stability, protein folding, metabolic regulation, and cell protection against oxidative damage (Cappel and Gilbert, 1988; Tran et al., 2004). Thus GSH is an important antioxidant that functions directly in elimination of toxic peroxides and aldehydes and indirectly in maintaining vitamin C and vitamin E and SH dependent enzymes in their reduced and functional forms. Resistance of many cells against oxidative stress is associated with high intracellular levels of GSH (Meister, 1991; Estrela et al., 1995).

Depleted plasma GSH and tissue GSH was well documented in type-1 diabetic animal models (Venkateswaran and Pari, 2003; Pari and Latha, 2004), NIDDM patients (Seghrouchni et al., 2002) and fructose diet-induced insulin resistant rats (Rajasekar et al., 2005). It has recently been shown that GSH improves insulin sensitivity in insulin-resistant individuals and/or patients with type-2 diabetes (Evans and Goldfine, 2000; Zancan and Sola-Penna, 2005). Hence, the measurement of cellular GSH provides the information about GSH associated scavenging system against free radicals induced LPO in the metabolic disease conditions and aging.

The reduced glutathione of liver, pancreas and heart of four experimental groups are summarized in the Table 22. A significant decrease in the tissue GSH content was observed in liver (46.96%), pancreas (31.33%) and heart (20.26%) of group-F compared to group-C (Figure 29). Administration of OSAE along with fructose diet (group-F+OSAE) prevented fructose diet induced depletion of GSH in all the three tissues. Group-F+OSAE showed significantly higher GSH values in liver (74%), pancreas (38.37%) and heart (27.5%) when
compared with group-F, while there is no significant difference when compared with group-C. However, OSAE treated control rats (group-C+OSAE) showed a slight but not significant increase in the GSH content of liver (1.76%), pancreas (2.18%) and heart (1.8%) when compared with corresponding values of control rats.

The significantly decreased GSH content in various tissues of group-F compared to group-C may be due to increased utilization of GSH or decreased synthesis because of enhanced oxidative stress. The decrease in GSH level may be due to increased utilization of GSH by the liver cells or due to oxidative stress. Earlier studies also revealed the decreased concentration of GSH in fructose fed insulin resistant rats. Thus significant decrease in GSH content of liver, pancreas and heart of fructose fed rats reflected the enhanced oxidative stress in the form of increased LPO and protein oxidation. Girard et al. (2005) suggesting that the decreased GSH concentration plays a role in development of hyperglycemia induced complications. It has been proposed that pathways of glucose metabolism are involved in the control of myocardial GSH (Mak et al., 1996; Bersin and Stacpoole, 1997; Squires et al., 2003). In the case of diabetes, the well-documented decreases in myocardial insulin signaling and glucose utilization (Rodrigues et al., 1995; Huang et al., 2003) are likely factors contributing to alterations in GSH status. Accordingly, insulin replacement therapy in type-1 diabetic models maintains normal cardiac GSH levels (Yadav et al., 1997; Xu et al., 2002). Recent studies of Shumin et al. (2007) also provide functional evidence for a link between glucose metabolism and cell GSH. Thus the significant decrease in glucose utilization in fructose fed rats might have resulted in decreased GSH content in the tissue of fructose fed animals.

The enhanced GSH content of liver, pancreas and heart of OSAE treated fructose fed rats indicate that OSAE treatment has either increased the biosynthesis of GSH or decreased the utilization of GSH due to decreased oxidative stress or both. Previous studies indicate that GSH may also be important in blood pressure and glucose homeostasis, consistent with the involvement of free radicals in both essential hypertension and diabetes mellitus (Sagar et al., 1992; Paolisso et al., 1992, 1993 and 1994; Moran et al., 1993). Changes in the GSH/GSSG ratio affect the β-cell response to glucose and improve insulin action (Ceriello et al., 1991). According
to Paolisso et al. (1992) intravenous infusion of GSH in type-2 diabetic patients improved insulin secretion and glucose tolerance during oral glucose tolerance tests. Maintenance of ample concentrations of antioxidants seems to be necessary for efficient insulin action. Efficient expression of insulin receptor gene requires certain transcription factors that are activated by GSH (Araki et al., 1991). Vitamin E also has a beneficial effect on insulin action as its supplementation could restore the GSH concentration in fructose fed rats and improve the physical state of plasma membrane and insulin action in NIDDM patients (Paolisso et al., 1993). Thus prevention of GSH depletion seen in fructose fed rats by OSAE supplementation may also be responsible for the enhanced insulin sensitivity observed in group-F+OSAE.

GSH DEPENDENT ANTIOXIDANT ENZYMES

Glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-S-transferase (GST) are considered as GSH dependent antioxidant enzymes.

Glutathione peroxidase (EC 1.11.1.9) is a selenoenzyme with peroxidase activity whose main biological role is to protect the living cell from oxidative damage. The biochemical function of GPx is to reduce lipid hydro peroxides to their corresponding alcohols and to reduce hydrogen peroxide to water. GPx has a complementary catalytic activity with catalase. The Km value for \( H_2O_2 \) of GPx (0.25 mmol/l) is lower than that of CAT (25 mmol/l) providing a preferential pathway for the degradation of even low concentration of \( H_2O_2 \) present in intact cell (Wohaieb and Godin., 1987). GPx catalyses the reduction of peroxides with GSH to form GSSG and the reduction product of \( H_2O_2 \). This enzyme is specific for its hydrogen donor GSH, and non-specific for the hydro peroxides ranging from \( H_2O_2 \) to organic peroxides (Freeman and Crapo., 1982) thus offering a major defense role in cells against peroxidative damage of complex biochemical compounds such as lipids and nucleic acids (Meister and Anderson, 1983). This detoxifying action of GPx against \( H_2O_2 \) protects cell membrane against oxidative damage (Jacob, 1995).

Glutathione reductase (GR) (EC 1.8.1.7) reduces oxidized glutathione (GSSG) to reduced glutathione (GSH), which is an important cellular antioxidant. For every mole of oxidized glutathione, one mole of NADPH is required to reduce GSSG to GSH. So, for every GSSG
and NADPH, two molecules are gained, which can again act as antioxidants scavenging reactive oxygen species in the cell (Meister, 1998).

Members of the GST (EC 2.5.1.18) family of enzymes are best known for their role in the detoxification of various exogenous compounds. These enzymes catalyze the nucleophilic attack of the thiol group of GSH, at an electrophilic site of the second substrate. This reaction most frequently results in the covalent linkage of GSH to the second substrate yielding a GSH conjugate, which is generally less toxic than the parent compound (Gerald and Patrick, 1993). GSTs may also bind toxins and function as transport proteins, and, therefore, an early term for GSTs was “ligandin” (Litwack et al., 1971). It is a multifunctional protein found in many tissues showing a broad specificity for organic hydroperoxides but not for H₂O₂ (Bruce et al., 1982). Thus it plays an important role in detoxification of xenobiotic compounds thereby protecting the cells from peroxidative damage (Deneke and Fanburge, 1989).

The activities of GSH dependent antioxidant enzymes: GR, GPx and GST in liver, pancreas and heart of four experimental groups are summarized in the Table 23. Group-F showed significantly decreased activities of these enzymes in liver, pancreas and heart when compared with group-C (Figure 30). Compared to control rats, the activities of GPx, GR and GST decreased in liver (18.7, 28.7 and 18.05%), pancreas (10.5, 33.1 and 35.6%) and heart (22.3, 27.5 and 31.8%) of fructose fed rats. Administration of OSAE along with fructose diet (group-F+OSAE) completely prevented fructose diet induced decline in the activities of these three enzymes in liver, pancreas and heart (Figure). However, OSAE administration to control rats (group-C+OSAE) for 60 days caused no alteration in the activities of these GSH dependent antioxidant enzymes.

Decreased activity of GPx and GST in group-F could be directly explained by the low content of GSH found in these rats which is a substrate and cofactor of GPx and GST (Domingues et al., 1998). GSH, the most important antioxidant metabolite, plays an important role in maintaining good levels of GPx activity. Flohe (1971) reported that the kinetics of GPx are in the first order in respect to GSH. GPx is a relatively stable enzyme, but
TABLE 23: Effect of OSAE treatment on glutathione dependent enzyme activities in four groups of animals

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tissue</th>
<th>C</th>
<th>F</th>
<th>F+ OSAE</th>
<th>C+ OSAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione reductase</td>
<td>Liver</td>
<td>38.29 ± 0.84a</td>
<td>27.30 ± 0.76b</td>
<td>36.22 ± 0.93a</td>
<td>39.43 ± 0.92a</td>
</tr>
<tr>
<td>(umol NADPH oxidized/min/mg protein)</td>
<td>Pancreas</td>
<td>22.46 ± 0.11a</td>
<td>15.04 ± 0.11b</td>
<td>21.61 ± 0.12a</td>
<td>24.62 ± 0.14a</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>19.47 ± 0.08a</td>
<td>14.11 ± 0.09b</td>
<td>18.62 ± 0.04a</td>
<td>20.21 ± 0.06a</td>
</tr>
<tr>
<td>Glutathione peroxidase (ug GSH consumed/min/mg protein)</td>
<td>Liver</td>
<td>7.68 ± 0.24a</td>
<td>6.24 ± 0.28b</td>
<td>7.79 ± 0.21a</td>
<td>7.84 ± 0.17a</td>
</tr>
<tr>
<td></td>
<td>Pancreas</td>
<td>11.78 ± 0.08a</td>
<td>10.54 ± 0.12b</td>
<td>11.24 ± 0.09a</td>
<td>11.82 ± 0.07a</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>14.86 ± 0.42a</td>
<td>11.54 ± 0.33b</td>
<td>14.93 ± 0.55a</td>
<td>15.04 ± 0.08a</td>
</tr>
<tr>
<td>Glutathione-S-transferase (umol CDNB-GSH conjugate formed/min/mg protein)</td>
<td>Liver</td>
<td>720.87 ± 12.56a</td>
<td>590.75 ± 16.54b</td>
<td>702.5 ± 18.65a</td>
<td>724.25 ± 12.78</td>
</tr>
<tr>
<td></td>
<td>Pancreas</td>
<td>85.25 ± 1.25a</td>
<td>54.75 ± 1.11b</td>
<td>78.37 ± 2.25a</td>
<td>85.5 ± 1.25a</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>99.75 ± 1.45a</td>
<td>68.00 ± 2.02b</td>
<td>92.37 ± 1.11a</td>
<td>102.62 ± 2.34a</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., (n=8 animals). Values with different superscripts within the row are significantly different at P<0.05 (Duncan's multiple range test).
Figure 30: Percent recovery from fructose diet induced alterations in the activities of GSH dependent antioxidant enzymes by OSAE treatment.
it may be inactivated under conditions of severe oxidative stress (Condell and Tappet, 1983). The low activity of GPx causes accumulation of \( \text{H}_2\text{O}_2 \) in group-F rats. The depletion in the activities of GST and GPx may result in the involvement of deleterious oxidative changes due to accumulation of toxic products. Thus the decreased activities of these GSH related antioxidant enzymes of group-F may be responsible for elevated LPO and protein oxidation observed in this group of rats.

The observed decrease in GR activity in fructose fed rats may be due to the decreased availability of NADPH by enhanced operation of polyol pathway (Peterson et al., 1990) as well as due to the inactivation of the enzyme by non-enzymatic glycation (Arai et al., 1987). The decreased activity of GR may lead to decreased regeneration of GSH from GSSG. Maintenance of ample concentrations of antioxidants seems to be necessary for efficient insulin action, for example, expression of insulin receptor gene requires certain transcription factors whose activity is modulated by GSH (Araki et al., 1991). The enhanced GR activity in the tissues of F+OSAE group compared to group-F reveals the protective effect of OSAE against oxidative damage by keeping normal GSH levels in the tissues which is further reflected by enhanced activity of GPx and GST in group F+OSAE rats.

SUPEROXIDE DISMUTASE AND CATALASE

Superoxide dismutase (SOD) and catalase (CAT) are considered as GSH independent antioxidant enzymes. SOD and CAT are widely distributed in all animal cells.

Superoxide dismutases (EC 1.15.1.1) are a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. As such, they are an important antioxidant defense in nearly all cells exposed to oxygen. SOD and CAT are widely distributed in all animal cells. SOD appears in three forms: (1) Cu-Zn SOD in the cytoplasm (2) Mn-SOD in the mitochondrion (3) Cu-SOD, a third extracellular SOD. SOD is considered fundamental in the process of eliminating ROS by reducing (adding an electron to) superoxide to form \( \text{H}_2\text{O}_2 \). Catalase and the glutathione peroxidase are responsible for reducing \( \text{H}_2\text{O}_2 \) to \( \text{H}_2\text{O} \).
<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>TISSUE</th>
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<th>F + OSAE</th>
<th>C + OSAE</th>
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<tr>
<td></td>
<td>Liver</td>
<td>46.30 ± 1.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.34 ± 0.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47.61 ± 1.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45.79 ± 1.02&lt;sup&gt;a&lt;/sup&gt;</td>
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<td></td>
<td>Pancreas</td>
<td>25.36 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.84 ± 0.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.93 ± 0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.10 ± 0.33&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Heart</td>
<td>23.04 ± 0.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.77 ± 0.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23.14 ± 0.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.20 ± 0.66&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Superoxide dismutase (U/mg protein)</td>
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<tr>
<td>Catalase (mmol H&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt; consumed/min/mg protein)</td>
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<tr>
<td>Liver</td>
<td>67.76 ± 1.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57.89 ± 2.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>66.80 ± 1.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67.83 ± 1.63</td>
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<tr>
<td>Pancreas</td>
<td>15.21 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.08 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.35 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.73 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>54.6 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.26 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>54.19 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.16 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M
Values with different superscripts within the row are significantly different at P<0.05 (Duncan’s multiple range test).
The respective enzymes that interact with superoxide and H$_2$O$_2$ are tightly regulated through a feedback system. Excessive superoxide inhibits GPx and CAT to modulate the equation from H$_2$O$_2$ to H$_2$O. Likewise, increased H$_2$O$_2$ slowly inactivates CuZn-SOD. Meanwhile, CAT and GPx, by reducing H$_2$O$_2$, conserve SOD. SOD, by reducing superoxide, conserves CAT and GPx. Through this feedback system, steady low levels of SOD, GPx, and CAT, as well as superoxide and H$_2$O$_2$ are maintained, which keeps the entire system in a fully functioning state (Fridovich, 1995). SOD also exhibits antioxidant activity by reducing O$_2^-$ that would otherwise lead to the reduction of Fe$^{3+}$ to Fe$^{2+}$ thereby promoting OH formation. When CAT activity is insufficient to metabolize the H$_2$O$_2$ produced by SOD, will increase the tissue oxidant activity. Hence, it was found that the antioxidant enzymes function as a tightly balanced system and any disruption of this system would lead to promotion of oxidation.

Recent studies demonstrated that a hyperglycemia-induced process of overproduction of O$_2^*$ by the mitochondrial electron-transport chain appears to be the initial and key event in the activation of all other pathways involved in the pathogenesis of diabetes-related complications (Brownlee, 2001). These include increased polyol pathway flux, increased formation of AGEs, activation of protein kinase C and nuclear factor κB. An increased expression of inducible nitric oxide synthase (iNOS), accompanied by increased generation of NO, and an over activity of the reduced form of NADP which, in turn, over generates O$_2^*$ (Garcia Soriano et al., 2001; Guzik et al., 2002). So maintenance of intracellular level of SOD enzyme is necessary to prevent free radical toxicity. Mc Cord and Fridovich (1969) proved that this enzyme is required to sustain life in aerobic condition. Oxygen free radical (O$_2^*$) is known to inactivate CAT which is involved in the detoxification of H$_2$O$_2$. Wohaieb and Godin (1987) suggested that the reactive oxygen free radicals could inactivate and reduce the hepatic SOD and CAT activities.

Catalse (EC 1.11.1.6), present in the peroxisome of nearly all aerobic cells, serves to protect the cell from the toxic effects of H$_2$O$_2$ by catalyzing its decomposition into molecular oxygen and water without the production of free radicals. H$_2$O$_2$ is considered a key metabolite because of its relative stability, its diffusion and its involvement in cell signaling cascade (Khan
and Wilson., 1995; Sujuki et al., 1997). Studies of Corrochr et al. (1986) demonstrated that the administration of CAT resulted in protection against H$_2$O$_2$ mediated LPO.

The activities of SOD and CAT of liver, pancreas and heart of four experimental groups are summarized in the Table 21. Group-F showed significantly decreased activities of SOD and CAT in three tissues compared to group-C (Figure 28). The per cent decrease in the SOD activity in liver, pancreas and heart tissues of group-F are 12.87, 33.6 and 31.55% respectively compared to group-C. Respective per cent decrease in the CAT activity of group-F in liver; pancreas and heart are 14.54, 40.3 and 20.7% compared to group-C. The per cent alterations in SOD and CAT activities are prominent in pancreatic tissue compared to liver and heart in fructose fed rats. Administration of OSAE for 60 days prevented fructose diet induced decline in the activities of SOD and CAT in these three tissues (Figure). Further OSAE administration to control group i.e., group-C+OSAE showed no alterations in these enzyme activities in liver, pancreas and heart when compared with group-C.

In metabolic syndrome the autooxidatin of glucose results in formation of H$_2$O$_2$ which inactivates SOD. Therefore, the accumulation of H$_2$O$_2$ may be one of the explanations for decreased activity of SOD in group-F rats. Copper and zinc depletion may results in reduced activity of Cu/Zn dependent enzymes like SOD. Earlier studies also indicated the decreased activities of these SOD and CAT in fructose fed insulin resistant rats (Anuradha and Selvam, 1993; Joyeux-Faure et al., 2006). Earlier studies also revealed that decreased Cu and Zn in fructose fed animals may contribute to the observed decrease in SOD activity in group-F animals (Joyeux-Faure et al., 2006).

The results on the SOD and CAT activity clearly show that TCAE treatment resulted in enhanced scavenging activity which could exert a beneficial effect against pathologic alterations caused by the presence of O$_2^-$ and H$_2$O$_2$. Restoration of SOD activity in group-F+TCAE reveals an efficient defense against the first line of oxidative stress i.e., O$_2^-$ radicals which are known to inactivate CAT. Benhamou et al. (1998) demonstrated that over expression of CAT in human islets had given protection against oxidative stress induced by xanthine oxidase-hypoxanthine. Xu et al. (1999) demonstrated that over expression of CAT in mouse islet cells had given
protection against H2O2 induced oxidative stress which resulted in normal insulin secretion and reduced the diabetogenic effect of STZ in vivo.

Therefore, as reported, the levels of all biomarkers of oxidative stress are modified in experimental animals and humans with insulin resistance which suggests that insulin resistance is associated with an over generation of free radicals. Importantly, further support is provided by a study showing that hyperglycemia-induced insulin resistance can be prevented by antioxidant treatment. Several trials have shown that improving glycemic control does not necessarily improve accompanying oxidative stress (Seghrouchni et al., 2002). One might infer from such observations that specific therapy directed towards oxidative stress is obvious. Moreover, the large evidence for oxidative stress in insulin resistance and diabetes has logically prompted the use of antioxidants like a self-evident treatment (Marfella et al., 1995; Ting et al., 1996).

The results from the present study indicate that fructose fed insulin resistant rats have increased oxidative stress and a compromised antioxidant defense system in the liver, pancreas and heart. This increase in oxidative stress could be completely prevented by OSAE coadministration along with fructose diet. Further, OSAE exerts a protective effect against LPO and protein oxidation in liver, pancreas and heart by scavenging ROS and elevating the activities of antioxidant enzymes in insulin resistant condition.

OS has been used for thousands of years in Ayurveda for its diverse healing properties. OS is considered to be an adaptogen, balancing different processes in the body and helpful for adapting to stress. Marked by its strong aroma and stringent taste, it is regarded in Ayurveda as a kind of elixir of life and believed to promote longevity (Puri and Harbans singh., 2002).

It appears that apart from acting on carbohydrate metabolic targets compounds present in medicinal plants alone or in combination, possess a variety of beneficial activities and have the potential to impart therapeutic effect holistically in complicated disorders like insulin resistance and diabetes.
Various natural products have long been used in traditional medical systems for treating diabetes (Shapiro and Gong, 2002). Most of them contain a wide range of antioxidants with a potent scavenging activity for ROS. A similar type of protection against oxidative stress by enhancing the antioxidant enzymes was also observed in diabetic rats treated with other plants such as Salacia oblonga (Krishnakumar et al., 1999), Tinospora cordifolia (Stanely et al., 1999), Anoectochilus formosanus (Shih et al., 2002), Morinda officinalis (Soon and Tan, 2002), Morus indica (Andallu and Varadacharyulu, 2003), Ficus carica (Perez et al., 2003), Coccinia indica (Venkateswaran and Pari, 2003), Gymnema montanum (Ananthan et al., 2004) and Eugenia jambolana (Ravi et al., 2004).

Many phytochemicals are reported to enhance antioxidant enzymes by inducing gene expression of these enzymes. Induction of the hepatic GSH antioxidant system by chemopreventive agents was reported in several studies (Mahn et al., 2005; Yeh and Yen, 2006). Several phytochemicals were reported to act against the deleterious effects of oxidative stress such as anthraquinones in aloe vegetables (Malterud et al., 1993), total saponins from Pinax ginseng (Yukozawa et al., 1996), polyphenols (Tiwari, 2001) and flavonoids from Sideritis raeseri (Gabrieli et al., 2005).

Some of the phytochemicals (alkaloids and flavonoids) present in the OSAE either alone or in combination might have also exerted similar effects in the present study. However, further studies are needed to prove the assumptions on the exact mechanism of action. Therefore, it may be assumed that these products or isolated natural compounds could play a very important role in adjuvant therapy for insulin resistance.

The results from the present study suggest that OSAE has antioxidant potential against oxidative stress along with antihyperglycemic and hypolipidemic potential. These results may lend further support to mount evidence to show that OSAE contains compounds which, if taken in sufficient quantities, could conceivably be beneficial in prevention and/or management of insulin resistance.
TRACE ELEMENTAL ANALYSIS OF OSAE

In the present study, estimation of trace elements by X-ray fluorescence (XRF) analysis was performed to determine the content of elements in this plant. Results show that the OSAE contains appreciable amounts of the elements K, Sn, Cl, Fe, Ca, Cr, Mn, Cu, and Zn. (Table 24). Metabolic syndrome is a disease of metabolic disorders in which elements may play important role in the management. The various elements present in these medicinal plants have either direct or indirect role in the control and management of metabolic syndrome since this disease is associated with marked alterations in the concentrations of trace elements. Regulation of trace elemental concentrations has therefore been proposed as a potential preventive and treatment strategy for this disease (Jayasri et al., 2008).

POTASSIUM

OSAE contained 7.592 % of potassium. A potassium-depleted diet was found to lead to insulin resistance at post-receptor sites, a resistance that was reversed when potassium was resupplied (Norbiato et al., 1984). Currently, no information is available on potassium supplementation under other circumstances; however, this mineral appears to have a close association with insulin resistance and merits future investigation.

CHROMIUM

OSAE contained 0.017% chromium. Chromium deficiency has been associated with glucose intolerance and insulin resistance in patients on long term parenteral nutrition. The active form of chromium is an oligopeptide called chromomodulin which enhances the tyrosine-kinase activity of the occupied insulin receptor. The chromium ion is transported in the bloodstream on transferring (Fernandez-Real et al., 2002). Animal experiments have shown that a deficiency in chromium can result in insulin resistance (Striffler et al., 1995; 1999). Evidence also suggests that diet induced insulin resistance in experimental animals can be improved by chromium (Striffler et al., 1998). In humans, there also seems to be an association between insulin resistance and chromium status (Fulop et al., 1987). Evidence also suggests that individuals consuming diets with the lowest amounts of chromium tend to have

Anderson et al. (1991) reported a chromium-induced improvement in both glucose tolerance and circulating insulin among non-diabetic individuals with moderate post-glucose challenge hyperglycemia. The experimental group received 200 mcg chromium picolinate daily. Observed changes in glucose and insulin levels following the intervention period were suggestive of increased tissue sensitivity to insulin.

ZINC

Zinc concentration in the OSAE is 0.014%. Preliminary evidence suggests a relationship between zinc deficiency and the response to insulin. In human subjects, information contained in an abstract of a Japanese research article implied a clinical correlation between low zinc levels and insulin resistance (Chen et al., 1991). The prevalence of several of the diseases or metabolic dysfunctions associated with insulin resistance is also much more common among individuals consuming low zinc diets (Singh et al., 1998).

MANGANESE

XRF analysis of OSAE indicated the presence of 0.054% of Mn. Manganese is an important cofactor in the key enzymes of glucose metabolism. A deficiency of Mn resulted in diabetes in guinea pigs. It also resulted in the frequent birth of offspring who develop pancreatic abnormalities or no pancreas at all. Diabetics have been shown to have only one-half the manganese of normal individuals. Nicoloff et al. (2004) suggested that decreased concentrations of manganese are associated with the development of micro vascular complications in diabetic children.
Table 24: Elemental analysis of OSAE BY X-Ray Flourescence

<table>
<thead>
<tr>
<th>Element</th>
<th>OSAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Si</td>
<td>0</td>
</tr>
<tr>
<td>S</td>
<td>0.009</td>
</tr>
<tr>
<td>Cl</td>
<td>4.565</td>
</tr>
<tr>
<td>K</td>
<td>7.592</td>
</tr>
<tr>
<td>Ca</td>
<td>0.312</td>
</tr>
<tr>
<td>Ti</td>
<td>0</td>
</tr>
<tr>
<td>Cr</td>
<td>0.017</td>
</tr>
<tr>
<td>Mn</td>
<td>0.054</td>
</tr>
<tr>
<td>Fe</td>
<td>0.126</td>
</tr>
<tr>
<td>Ni</td>
<td>0.021</td>
</tr>
<tr>
<td>Cu</td>
<td>0.050</td>
</tr>
<tr>
<td>Zn</td>
<td>0.014</td>
</tr>
<tr>
<td>Se</td>
<td>0.014</td>
</tr>
<tr>
<td>Br</td>
<td>0.108</td>
</tr>
<tr>
<td>Sr</td>
<td>0.041</td>
</tr>
<tr>
<td>Zr</td>
<td>0.012</td>
</tr>
<tr>
<td>Mo</td>
<td>0.024</td>
</tr>
<tr>
<td>Pd</td>
<td>0.151</td>
</tr>
<tr>
<td>Sn</td>
<td>4.892</td>
</tr>
<tr>
<td>I</td>
<td>0</td>
</tr>
<tr>
<td>Ba</td>
<td>0.213</td>
</tr>
</tbody>
</table>

AE=Ocimum sanctum aqueous extract
figures are in weight % (i.e. x% means that out of the 100 gm of sample x gm is that particular element)
SELENIUM

XRF analysis of OSAE indicated the presence of 0.014% of Se. Selenium compounds exert their biological effects either directly or by being incorporated into enzymes and other bio-active proteins. The main inorganic dietary form of selenium is sodium selenite (Na\textsubscript{2}SeO\textsubscript{3}). In the organic forms selenomethionine and selenocysteine, a selenium atom is present in the position occupied by a sulfur atom in the amino acids methionine and cysteine. Selenium compounds exert their biological effects either directly or by being incorporated into enzymes and other bio-active proteins. The main inorganic dietary form of selenium is sodium selenite (Na\textsubscript{2}SeO\textsubscript{3}). In the organic forms selenomethionine and selenocysteine, a selenium atom is present in the position occupied by a sulfur atom in the amino acids methionine and cysteine. Se-methylselenocysteine is selenocysteine modified by the replacement of the hydrogen atom with a methyl group on the selenium atom. The glutathione and thioredoxin antioxidant systems function to regenerate other antioxidants while themselves providing the primary antioxidant defense in the water phase of the body. Glutathione peroxidase and thioredoxin reductase are two natural antioxidant enzymes that contain selenium and depend upon selenium activity for their antioxidant functionality. Both the glutathione and thioredoxin systems enhance their own antioxidant activity by inducing the production of other natural antioxidant enzymes, including superoxide dismutase [Hansen et al., 2004]. Both selenite and selenomethionine supplementation have been shown to significantly reduce oxidative DNA damage due to ultraviolet radiation.

HISTOPATHOLOGICAL STUDIES

The histopathological studies of the liver, heart, kidney, pancreas, small intestine and adipose tissue were conducted to know the alterations under fructose fed conditions and to study the protection/harmful effects of OS administration in metabolic syndrome rat model and normal rats.

LIVER

Liver is the central player in the whole body homeostasis by its ability to metabolize glucose and fatty acids. When energy intake is abundant, mammals preferentially burn carbohydrates to generate ATP and surplus glucose after replenishing glycogen stores is converted to fatty acids (lipogenesis) for use in the synthesis and storage of triglycerides in white adipose tissue. Although white adipose tissue functions essentially as a limitless reservoir
to accumulate triglycerides, the liver is also able to store significant quantity of lipids in conditions associated with prolonged excess energy consumption or impaired fatty acid metabolism manifesting as steatosis (fatty liver syndrome). The liver is the major target in the fructose fed rats since fructose uptake is very high by the tissue and long term fructose enriched diet leads to fatty liver and treatments that reduce or prevent hepatic lipid accumulation improves insulin sensitivity in the liver.

In the present study, the examination of liver revealed marked macroscopic and microscopic lesions in group-F rats. Grossly, the livers of group-F rats were enlarged in size, pale in colour, shiny in appearance and soft in consistency. Liver of group-C and group-C+OSAE rats were grossly normal and group-F+OSAE rats showed recovery from the features of group-F rats to group-C rats. Further weight of the liver, glycogen and fat content (TL, TG and TC) were higher in fructose fed rats compared to normal rats. Further, the biochemical studies revealed altered carbohydrate metabolism favoring lipid biosynthesis with enhanced activities of lipogenic enzymes in liver of group-F rats.

Histological observations of liver sections from group-F rats showed degenerative changes in the liver with severe congestion of central vein, hemorrhages in sinusoidal spaces, granular appearance of hepatocytes with cloudy swelling (hazy nucleus) with fatty infiltration with many ruptured hepatocytes (Fig 31c). These findings are supported by earlier workers (Baynes, 1999). Histological observations of group-C and group-C+OS rat livers showed normal hepatocytes architecture with normal central vein, prominent nucleus and normal hepatocytes (Fig 31a & 31b). Whereas, group-F+OS showed regeneration from hepatotoxic observations under fructose fed condition (Fig 31d). In group-F, severe hepato toxicity was evidenced by profound necrosis with fatty changes in the liver. Fatty changes in the liver means enlargement of hepatocytes containing fat droplets. This slows down blood flow throughout the hepatic sinusoid and may result in the development of portal hypertension. Non alcoholic fatty liver disease (NAFLD), a hepatic manifestation of MS is the most common explanation for the elevated hepatic transaminase activities. Fructose diet may have a role in the pathogenesis of NAFLD. Fructose is lipogenic and stimulates higher triglyceride secretion from the liver than
**Figure 31a:** Sections of the liver of group-C rats showing normal hepatic architecture.

**Figure 31b:** Sections of the liver of group-C+OSAE rats showing normal hepatic architecture.

**Figure 31c:** Sections of the liver of group-F rats showing degenerative changes and wending sinusoids

**Figure 31d:** Sections of the liver of group-F+OSAE rats showing reduced sinusoids and regenerative changes.

(CVH- Central vein with mild hemorrhage; H- Hepatocytes).
equimolar amount of glucose. The hepatic metabolism of fructose favors de novo lipogenesis and ATP depletion. A potential mechanism by which fructose may cause liver injury also exists. The metabolism of fructose is distinct from glucose. Before converging with glycolytic pathway, initial fructose metabolism involves phosphorylation of fructose to fructose-1-phosphate by fructokinase using the substrate ATP. Unlike glucokinase, the phosphorylation of fructose by fructokinase is specific for fructose and is not rate limiting. The enhanced activity of fructokinase in the liver of fructose fed rats results in hepatic ATP depletion (Havel, 2005). Indeed fructose has been shown to cause ATP depletion in humans (Marchesini et al., 2008). In rats, fructose administration increases hepatic lipid peroxidation and activation of inflammatory pathways (Kelley et al., 2004) and promotes hepatic necroinflammation. Thus in the present study the enhanced fructokinase activity and increased fat content of the liver, enhanced lipid peroxidation and protein oxidation with decreased antioxidant capacity in the liver of F-group rats might have ultimately resulted in the hepatic necro inflammation reflected by enhanced ALT, AST activities and histological observations. There was significant association seen between hepatic inflammation, fibrosis and features of MS. Both inflammation and fibrosis correlates significantly with serum insulin and HOMA for insulin resistance and hypertriglyceridemia. Liver is an aerobic organ which generates ROS that induce oxidative tissue damage. The enhanced oxidative stress observed in the liver of fructose fed rats may be an important pathological mediator for the tissue damage.

Nevertheless, there is an ongoing debate that reduction of these triglycerides and reactive free radicals by antioxidant molecules may improve liver function and histology. Vitamin C and vitamin E have been found to be both protective and therapeutic effects on NAFLD in an animal model (Harrison et al., 2003) which is linked to antioxidant function of these vitamins. Many phytochemicals like flavonoids and Phenolic compounds widely distributed in plants have been reported to exert multiple biological activities including antioxidant and free radical scavenging abilities. In our study, group-F+OS rats showed reduced plasma and hepatic triglycerides and enhanced antioxidant capacity compared to group-F might have accompanied by significant protection against fructose induced hepatotoxicity.
Our biochemical studies were also revealed the antioxidant property of OSAE. It may be presumed that urosolic acid, which is one of the key constituents of OS leaf extract, is responsible for inhibition of lipid peroxidation (Balanehru and Nagaraju, 1991). Membrane stabilizing property of OS has been shown to be responsible for its hepatoprotective action (Sen et al., 1988). Thus though the exact mechanism of hepatoprotective action of OS is not yet known, its antioxidant activity as revealed in some earlier studies seems to be the most important mode of its hepatoprotective action. In addition hepatoprotective activity of OS was also reported in carbon tetrachloride (Girish et al., 2009) and paracetamol (Chattopadhyay et al., 1992) induced liver damage.

HEART

High fructose diets have been used to induce cardiovascular symptoms such as hypertension, hypertriglyceridemia, lipid abnormalities; increased collagen deposition in the heart associated with increased oxidant concentration and decreased antioxidant defenses. Since fructose is more lipogenic than glucose, fructose consumption is able to produce greater elevation of triglycerides which in turn increases intramyocellular triglyceride content in the skeletal and cardiac muscle causing insulin resistance. Insulin resistance is an important risk factor for the development of hypertension, atherosclerotic heart disease, left ventricular hypertrophy and atherosclerotic heart failure. In addition, fructose-induced hyperuricemia results in endothelial dysfunction and insulin resistance and might be a causal mechanism of the metabolic syndrome.

The present study also revealed the biochemical changes which are the risk factors for heart tissue damage in group-F rats. Although, these studies have defined the biochemical changes, the structural and histological changes especially the cardiovascular systems have not been thoroughly addressed. In order to characterize the structural changes in the heart muscle of fructose fed rats and to assess the cardio protective role of OS, histological studies are conducted in four experimental groups.

Macroscopically, the heart from group-F animals is larger with greater relative weight (mg/100 g body wt.) compared to group-C. In contrast to fructose fed rats, group-C+OSAE and
Figure 32a: Sections of the heart of group-C rats showing normal architecture of heart.

Figure 32b: Sections of the heart of group-C+OSAE rats showing normal architecture of heart.

Figure 32c: Sections studies of the heart of group-F rats showing degenerated myocardial fibers and blood clumps.
F- Fibrocytes; C- Capillary; NMC-Nucleus of heart muscle cell

Figure 32d: Sections of the heart of group-F+OSAE showing normal heart architecture and normal myocardial fibers.
group-F+OSAE animals did not show significant variation in the size and weight of heart when compared to group-C. Microscopic histology of heart tissue revealed that group-C and group-C+OSAE rats show normal architecture of myocardium with organized pattern of cardiomyocytes (Figure 32a & 32b). Contrastingly, histological evaluation of group-F rats demonstrated marked edema, confluent areas of myonecrosis, degenerative myofibers, fat deposition and inflammation compared to group-C animals (Figure 32c). Whereas, sections of heart of group-F+OSAE revealed marked improvement especially with regard to the degree of myonecrosis and preserved the normal myocardial cellular integrity and prevented the fat deposition (32d).

Insulin is a potent anabolic hormone and is essential for tissue development, growth, and maintenance of whole body glucose homeostasis. In the present study the biochemical investigations revealed disturbed blood glucose homeostasis (mild hyperglycemia) with hyperinsulinemia resulted in insulin resistance in fructose fed rats. Similar to our observations, studies of Jen-Ying Deng et al. (2007) found decreased GLUT4 and increased FATP1 levels in fructose fed animals, which indicated that cardiac glucose uptake was reduced, whereas fatty acid uptake might have been elevated. Transgenic over expression of FATP1 in the heart caused lipotoxic cardiomyopathy, suggesting that increases in fatty acid supply to the heart adversely affect cardiac contractile functions (Chiu et al., 2005). Recent findings have indicated that the perturbations in cardiac energy metabolism and insulin resistance are among the earliest diabetes-induced events in the myocardium, preceding both functional and pathological changes (Rodrigues and McNeill, 1992). Furthermore, studies have found myocardial insulin resistance in advance dilated cardiomyopathy limits both glucose uptake and oxidation and impairs the heart's ability to generate much needed adenosine triphosphate (Shah and Shannon, 2003).

Cardiac insulin signal not only regulates metabolic energy homeostasis but also generates signals for cardiac growth, programmed cell death, and programmed cell survival. During insulin resistance or diabetes, the heart rapidly modifies its energy metabolism, resulting in augmented fatty acid and decreased glucose consumption. Accumulating evidence suggests
that this alteration of cardiac metabolism plays an important role in the development of cardiomyopathy.

*Ocimum sanctum* containing potent antioxidants, flavonoids (orientin, vicenin) and phenolic compounds (eugenol, cirsilineol, apigenin). The ancient systems of medicine, including Ayurveda, Greek, Roman, Siddha and Unani, have mentioned its therapeutic applications in cardiovascular disorders, diabetes and asthma (Uma *et al.*, 2000). However, its potential as a cardio protective agent has not been extensively studied. Besides its antioxidant properties, OS interacts by various other mechanisms in a complex way to elicit its therapeutic effects.

Histopathological examination further confirmed its cardio protective effects. Most importantly, treatment with OS decreased myocardial necrosis, inflammation, and improved cardiac functions by different mechanisms. Decreased myocardial necrosis and augmentation of endogenous antioxidants, i.e. myocardial adaptation and restored antioxidant status, all contribute to its cardio protective effects. The additional mechanisms by which OS may reduce myocardial injury and potential clinical implications require further investigation. In addition cardio protective activity of OS was also reported in isoproterenol induced myocardial infarction in rats (Meenu Sharma *et al.*, 2001).

**KIDNEY**

Recent evidence indicates that MS is related to the development of renal disease and also a predictor of poor outcome in patients with chronic renal failure (Coresh *et al.*, 2003). In addition, recent studies in humans have shown that MS is associated with increased risk for a reduced GFR and microalbuminuria (Kasiske *et al.*, 1988). Interestingly it has been reported that patients with essential hypertension and MS have glomerular hypertension and increased albumin excretion. If we consider the animal and epidemiological data, it is reasonable to hypothesize that fructose consumption may be among the factors that contribute the epidemic of MS and consequently to the epidemic of chronic renal disease (Nakagawa *et al.*, 2006). Renal injury is also seen in other models of the MS including Zucker fatty diabetic rats and db/db mice (Ziyadeh *et al.*, 2000). Primarily, fructose was reported to cause glomerular hypertrophy and
Figure 33a: Sections of the kidney of group-C rats showing normal glomeruli and normal tubular epithelial cells.

Figure 33b: Sections of the heart of group-C+OSAE rats showing normal glomeruli and normal tubular epithelial cells.

Figure 33c: Sections of the kidney of group-F rats showing degenerated renal cells and bowmans Capsules.

Figure 33d: Sections of the kidney of group-F+OSAE showing normal renal cells and bowmans Capsule.

G- Glomeruli; EC- Epithelial cells; BC-Bowman’s capsule; DRT- Destructed renal tubules)
subtil renal damage (Boot-Handford and Heath, 1981). More recently, it was reported that consumption of fructose induces glomerular hypertension and sclerosis of the afferent arterioli (Sanchez-Lozada et al., 2007).

Grossly kidney from the group-F animals was markedly larger than those from group-C. Histologically the kidney from group-F rat showed degenerated renal cells (Figure 33c). Photomicrographs of renal section of group-C and C+OSAE rats showed normal architecture of kidney with normal glomeruli and tubular epithelial cells (Figure 33a & 33b). Whereas group-F+OSAE rats showed regenerative changes with normal epithelial cells and glomeruli (Figure 33d).

Similar to our observation studies, Rasch (1980) also revealed that kidney from fructose fed animals showed more glomerular necrosis, tubular dilatation, tubular atrophy, intestinal inflammation, myofibroblast tubular osteopontin and interstitial collagen. Several mechanisms explain how MS could contribute to the development of chronic kidney diseases. The MS is also associated with release of inflammatory cytokines and presence of endothelial dysfunction and oxidative stress (Wisse, 2004), all which could contribute to the development of glomerular sclerosis. Insulin resistance also may have direct role in the pathogenesis of renal injury, as a consequence of stimulating the sympathetic nervous system and the rennin angiotensin-aldosterone system (Sowers, 2004). In the present study, insulin levels were elevated, and it is known that treatment with troglitazone to correct hyperinsulinemia slows progression of kidney disease (Yoshida et al., 2001). Dyslipidemia, which is a feature of the metabolic syndrome, may induce toxic and inflammatory tubulointerstitial injury (Sun et al., 2002). Lipotoxicity is an attractive mechanism, inasmuch as the cholesterol derangements in fructose feeding are striking, and treatment with statins has been shown to slow the progression of kidney disease in the RK model (Kasiske et al., 1988). Hall et al. (2002) proposed that lipid deposition in the inner medulla increases intrarenal pressure, leading to decreased tubular flow, which results in increased sodium reabsorption in Henle loop, volume expansion, and the development of systemic hypertension. The main pathophysiologic mechanism by which uric acid causes these conditions involves an inhibition of endothelial nitric oxide bioavailability (Khosla et al., 2005), activation of the renin angiotensin system (Mazzali et al., 2001), and direct
actions on endothelial cells and vascular smooth muscle cells (Kang et al., 2005). The importance of these pathways is suggested by a recent prospective study in which lowering uric acid in individuals with hyperuricemia and renal dysfunction was associated with improved BP control and slower progression of renal disease (Siu et al., 2006).

The regenerative changes in the kidney are evident from the histomicrophotographs of F+OS group by showing normal pattern of architecture of kidney. The improvement of renal morphology and function in fructose fed rats after treatment with OSAE in the present investigation could be attributed to its antidiabetic action resulting in alleviation of altered metabolic status in animals. However, the excellent recovery of renal function expected with treatment of OSAE can be explained by the regenerative capability of the renal tubules (Kissane, 1985). Kidney sections of healthy rats treated with OSAE showed no pathological changes and were comparable to those of normal control rats.

**PANCREAS**

No macroscopic lesions are observed in the pancreas of group-F, group-F+OSAE and group-C+OSAE rats, when compared to group-C. Microscopic observations of pancreatic sections revealed variations among groups. Group-C and group-C+OSAE rats showed normal architecture of pancreas with lobular arrangement of acini with islets of langerhans (Figure 34a & 34b). Whereas, sections of pancreas from group-F rats showed hyperplasia of pancreatic cells with amyloid deposition and abnormally increased deposition of fat (Figure 34c). The sections of pancreas of group-F+OSAE rats showed regenerative changes almost, giving the appearance of normal pattern of architecture similar to group-C (Figure 34d).

Increasing evidence suggest that variations in insulin demand as a result of physiological and pathological states such as aging, pregnancy and obesity can lead to adoptive changes in the β-cells that include hyperplasia, hypertrophy and increased insulin synthesis and secretion. In several patients with T2DM, there is an initial peripheral resistance to insulin, which may be compensated some time by β-cell hyperplasia and/or hyper function (Lingwen Zhong et al., 2007). As long as pancreas is able to maintain a sufficient production of insulin, diabetes may not develop. However, upon failure of β-cell function, which may occur due to the cells own
Figure 34a: Sections of the pancreas of group-C rats showing normal architecture of pancreas.

Figure 34b: Sections of the pancreas of group C+OSAE showing normal architecture of pancreas.

Figure 34c: Sections of the pancreas of group-F rats showing degenerated islets and beta cells.

Figure 34d: Sections of the pancreas of group-F+OSAE showing regenerative changes.

IL: Islets of Langerhans; DIL: Destructed islets of Langerhans.
incapability to sustain high hormone production or to a continuously increasing peripheral need for insulin, diabetes will ensue.

Increased infiltration of fat in the pancreas has been associated with obesity, increased age, Cushing’s syndrome, cystic fibrosis, and lipomatous pseudo hypertrophy. In human obesity, the proportion of fat has been reported to be higher in the pancreas than in muscle and liver (Kovanlikaya, 2005). If this situation progresses, it not only causes abnormalities in glucose metabolism and gene expression but also induction of β-cell apoptosis (Unger and Orci, 2002).

Extreme fatty replacement of the exocrine pancreas is likely to be associated with a decrease in pancreatic function. Massive fatty replacement has been described in the entity of lipomatous pseudo hypertrophy of the pancreas. Interestingly, pancreatic islet cells are resistant to fatty infiltration (Walters, 1966). In addition, elevated insulin levels are known to generate oxidative stress and fibrogenesis (McCullough, 2006). Present study demonstrated the existence of mild hyperglycemia, hyperinsulinemia and enhanced oxidative stress in the pancreas of fructose fed rats. Similar types of observations are reported in fat diet fed rats (Yen et al., 2006). These changes would result in an inflammatory state of non alcoholic fatty pancreatic disease (NAFPD), which may lead to non alcoholic steatopancreatitis (NASP).

Regeneration of islet cells by dietary components and stimulated insulin secretion by different plant extracts have been reported. Treatments with antioxidants have been shown to reduce insulin resistance in rats (Song et al., 2005). Moreover, treatments of diabetic animals with probucol (lipid-lowering and antioxidant) (Kaul et al., 1995) or with vitamin E or with N-acetylcysteine (free radical scavenger and glutathione donar) have been shown to reduce oxidative stress and insulin resistance. It is plausible to speculate that antioxidants have a protective role against insulin resistance induced pancreatic damage.

In conclusion, this study has shown that OSAE administration prevented glucose intolerance in F+OSAE rats. In agreement with our study, beneficial effects of OS against to pancreatic damage have been reported by earlier workers. Hennan et al. (2006) were reported that Ocimum sanctum leaf extracts stimulate insulin secretion from perfused pancreas, isolated
islets and clonal pancreatic β-cells. Agrawal et al. (1996) have suggested that leaves of OS improved the B cell function and enhanced insulin secretion.

**SMALL INTESTINE**

Modern westernized diets and eating habits have changed, increasing the amounts of dietary fructose and fat, which are probably a nutritional basis for the obesity and type 2 diabetes pandemics. Intestinal adaptation to these diets may be an early event to the onset of metabolic disorders due to the rapid increase in sugar transport capacities and the alteration of insulin action in enterocytes.

Fructose is absorbed by a two step membrane-transport process involving the sodium-dependent glucose cotransporter (SGLT1) and the facilitative fructose transporter i.e., GLUT5 in the brush border membrane lining the lumen and GLUT2 in the basolateral membrane ensures sugar exit into the blood stream. The expression of GLUT5 and GLUT2 in the small intestine increases in rats or mice fed a diet high in fructose or perfused with increased fructose concentrations (Tobin et al., 2008). Recent studies have demonstrated that fructose induced hypertension is initiated by increased absorption of salt and fat in the intestine. Further, insulin resistance is reported to increase the intestinal sugar absorption as reflected by higher initial rise blood glucose after oral glucose load (Sharon Barone et al., 2009).

In the present studies of small intestine, in the four experimental groups revealed normal architecture of small intestine with normal intestinal villi in group-C (Figure 35a) and group-C+OSAE (Figure 35b). Whereas, group-F rats showed degenerative changes of intestinal mucosa (Figure 35c). In contrast to group-F, coadministration of OS in group-F+OSAE showed retained normal architecture of the small intestine as that of group-C, indicating protective effect of OS against to the degenerative changes observed in fructose fed rats (Figure 35d).

In adipocytes and muscle cells, insulin provokes a massive translocation of GLUT4 to the plasma membrane. In sharp contrast, insulin internalizes brush boarder membrane GLUT2 into intracellular pools. Insulin, by regulating GLUT2 and GLUT4 traffic in opposite direction, controls glucose homeostasis and limits glucose excursion in the course of digestion by increasing peripheral GLUT4-dependent glucose uptake and slowing down intestinal GLUT2-dependent
Figure 35a: Sections of the small intestine of group-C rats showing normal architecture of Small intestine.

Figure 35b: Sections of the small intestine of group C+OSAE rats showing normal architecture of Small intestine.

Figure 35c: Sections of the small intestine of group-F rats showing degenerated intestinal mucosa and damaged intestinal cells.

Figure 35d: Sections of the small intestine of group-F+OSAE rats showing regenerated intestinal mucosa and normal intestinal cells.

(V-Villi; KF-Kicking's folds; ST-Submucosal tissue; LPM-Lamina propria mucosae; IL-Intestinal lumen)
sugar delivery. Insulin sensitivity and glucose tolerance are important factors in the regulation of GLUT2 trafficking. In insulin resistant mice obtained by fructose rich or high fat diets, the distribution of GLUT2 was drastically altered, basolateral membrane GLUT2 was permanently high, and insulin was unable to come out GLUT2 internalization (Tobin et al., 2008).

The protective effective of OS in F+OSAE group by preventing fructose diet induced insulin resistance and by its antioxidant potential might have responsible for preventing fructose diet induced lesions in the small intestine.

**ADIPOSE TISSUE**

Adipose tissue plays an important role in regulating whole body homeostasis and obesity related insulin resistance. The hallmark of obesity is manifested by massive expansion of adipose tissue, attributable to excessive food (calories) consumption and sedentary life style in modern society. Recent studies indicate that inflammatory events in the adipose tissue may play a critical role in insulin resistance. Recent studies demonstrated that adipose tissue is an important source for providing inflammatory molecules in the obese state primarily due to accumulation of macrophages.

In the present study, macroscopic observation revealed that white adipose tissue was at the abdominal region was clearly visible in group-F rats. Whereas, clarity of the visceral adipose tissue was not observed in group-C and C+OSAE. Co administration of OSAE along with fructose diet (F+OSAE) attenuated the white adipose tissue in F+OSAE group indicating the anti inflammatory activity of OSAE. Histological observations of adipose tissue of four experimental groups are represented in Fig.

Group-C and C+OSAE rats showed normal architecture of adipose tissue (Figure 36a & 36b). Fructose fed rats showed enlarged adipocytes with degenerative changes (Fructose 36c). Whereas, group F+OSAE showed regenerative changes showing similar architecture of adipose tissue to that of group-C (Figure 36d). Thus fructose fed rats revealed inflammation of the adipose tissue.
Figure 36a: Sections of the adipose tissue of Group-C rats showing normal adipocytes.

Figure 36b: Sections of the adipose tissue of group C+OSAE showing normal adipocytes.

Figure 36c: Sections of the adipose tissue of Group-F rats showing degenerative changes in

Figure 36d: Sections of the adipose tissue of group F+OSAE rats showing normal adipocytes.

V-Vacuole; AC-Adipocyte)
Group-F rats showed a marked accumulation of visceral white adipose tissue compared to control rats. Biochemical studies supported the present observations by the decreased activity of lipoprotein lipase in the adipose tissue and increased insulin resistance in fructose fed rats. It has been shown that adipocyte death increases significantly in obese individuals and mice via mechanism of necrosis. The majority of macrophages in adipose tissue are localized around dead adipocytes to form crown like structure (Cinti et al., 2005). The macrophage content is depot dependent. Omental fat of obese humans contains more macrophages than subcutaneous fat. Omental fat macrophage infiltration correlates to fasting glucose, insulin and hepatic fibroinflammatory lesions in obese patients (Cancello et al., 2006). In addition, obese subjects with impaired glucose homeostasis have preferential omental adipose macrophage infiltration (Harman-Boehm et al., 2007). Thus the observed impairment in glucose homeostasis, hyperglycemia and hyperinsulinemia in group-F rats may be correlated to the inflammation of adipocytes and degenerative changes observed in adipocytes.

Weight loss in obese subjects either through low calorie diet or bariatric surgery have decreased expression of inflammatory factors and increased expression of anti-inflammatory molecules, accompanied with decreased macrophage number and improved metabolic profile (Cancello et al., 2005). The anti-inflammatory activity of OS reported by earlier workers (Singh et al., 1999) might have been attributed the abnormalities visceral adipose tissue of fructose fed rats. Development of new therapeutic agents targeting the repression of adipose inflammation may be useful approach to attenuate obesity associated metabolic disorders (Ping Jiao and Haiyan Xu., 2008).