A Prospective Study of Biochemical Assay in Mice Fed Refined Sugar Diet and Unrefined Sugar Diet

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Abstract: The aim of our present study is to assess the basic biochemical parameters in mice fed palm jaggery diet and high fructose diet. 36 Female albino mice of age 21 days were randomly divided into six groups. The mice are fed Palm jaggery diet, High fructose diet, and control diet tap water ad libitum. Animals were maintained in the respective diet for 30 and 60 days. At the end of the experimental period, the animals were sacrificed and serum samples were separated. It was observed that mice fed high fructose diet shows significant elevated serum glucose and insulin levels, TBARS and also shows the features of dyslipidemia. Group 4 shows decreased total cholesterol level than compare to Group 6. On the basis of the results obtained in the present study, we conclude that Our findings indicate that mice fed high fructose diet shows biochemical features of metabolic syndrome or Polycystic ovary syndrome.

Keywords: female albino mice, high fructose diet, palm jaggery diet

I. Introduction

It is well-known and tragic fact that in the formulation of the great majority of today’s processed foods, the primary considerations of the manufacturer are taste, mouth-feel, appearance, shelf life and profit. Sadly, the all-important considerations, these being the nutritional value of the product, and the effect that the product will have upon the health of men, are not always given attention that they deserve. Added sugars have become a major part of the human diet since the commencement of modern methods of food processing [1].

Fructose is the sweetest tasting carbohydrate, found in many fruits and vegetables. In the past, dietary intake of fructose was used to be 16-20 grams per day, mainly from fresh fruits and vegetables. But in the last three decades, increased consumption of industrialized foods such as soft drinks, fruit juices, bakery products, canned fruits, jams, jellies and cookies, containing added sugars (sucrose, high fructose corn syrup, honey, molasses, and other syrups) has resulted in a significant increase in fructose intakes of 85-100 grams per day [1, 2]. Fructose is generally regarded as 1.73 times as sweet as sucrose [3, 4].

Advances in technology in the 1960s made possible the production of inexpensive high-fructose syrups from corn starch [5]. In the mid-1980s, 55% high-fructose syrup was adopted by the carbonated-beverage industry and became the predominant sweetener in soft drinks. Reports confirmed by animal and human clinical studies, indicating that the excessive fructose intakes induce adverse metabolic effects [6-11] however there is no direct evidence from epidemiological studies to clarify the association between current amounts of dietary fructose intake and the metabolic syndrome components. Metabolic syndrome, a worldwide epidemic health problem, is characterized by central obesity, hypertension, insulin resistance, and lipid profiles abnormalities [12].

Palm Jaggery is a traditional Indian sweetener made from the extract of Palm Trees and is believed to be a healthy substitute for sugar. The process of making jaggery from the plant sources, does not involve any chemical agents and hence all the natural mineral salts are retained without adding any preservatives of chemicals. Palm Jaggery is known to have various medicinal properties and other health benefits. It is a relatively unrefined sugar. While manufacturing processes in sugar utilizes chemicals such as sulphur dioxide, lime and other bleaching agents, but Palm Jaggery is prepared in a natural way, without removing minerals. Palm Jaggery is reported to have more nutritional and medicinal value than cane sugar. It is a healthy alternative to white sugar and is commonly known as ‘medicinal sugar’, because of its various health benefits.

In human studies, fructose consumption was associated with the development of hepatic and adipose tissue insulin resistance and dyslipidemia due to its ability to induce hepatic de novo lipogenesis [13]. Metabolic syndrome and its components are common in women with polycystic ovary syndrome. Insulin resistance, a feature of
metabolic syndrome is found to be the result of high intake of dietary fructose. There is growing interest in the metabolic abnormalities that occur in women with PCOS; these resemble the characteristic findings of the metabolic syndrome. Central obesity, hyperinsulinemia, insulin resistance, glucose abnormalities predictive of type 2 diabetes mellitus, dyslipidemia, and hypertension has been associated with PCOS.14

There is a scarcity of studies regarding high fructose diet and palm jaggery diet in animal models particularly in mice. In view of all the above, the present study was carried out to investigate the effect of high fructose diet (refined sugar diet), palm jaggery diet (unrefined sugar diet) on basic biochemical parameters in relation to polycystic ovary syndrome.

II. Materials And Methods

Experimental Animals
Female albino mice (Wistar strain) of three weeks old, weighing approximately 15-25 g were selected. The animals were maintained in the Central Animal House, Rajah Muthiah Medical College, Annamalai University. The animals were housed (3 per cage) in polypropylene cages (47×34×18 cm) lined with sterile paddy husk renewed every 24 h, with relative humidity (55%) in a 12 hour light/dark cycle at 25º ± 2º C. The animals were provided with control diet, experimental diet and water ad libitum. The experiment were carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and approved by the Institutional Animal Ethics Committee (IAEC), Annamalai University. (Approved number: 160/1999/CPCSEA/1072).

Experimental groups
The animals were divided into six groups. Each group consists of six animals.

- **Group 1** Control diet (normal diet) - 30 days: Animals fed normal control diet for 30 days.
- **Group 2** Control diet (normal diet) - 60 days: Animals fed normal control diet for 60 days.
- **Group 3** unrefined high sugar diet – 30 days: Animals fed Palm jaggery diet for 30 days.
- **Group 4** unrefined high sugar diet – 60 days: Animals fed Palm jaggery diet for 60 days.
- **Group 5** refined high sugar diet – 30 days: Animals fed High fructose diet for 30 days.
- **Group 6** refined high sugar diet – 60 days: Animals fed High fructose diet for 60 days.

Experimental Diets
Diets were formulated based on American institute of nutrition 93G (AIN-93G) to meet recommended nutrients levels for mice as showed in table 1. Fructose, casein, vitamin mix and mineral mix were purchased from SDFCL, Mumbai, NICE CHEMICALS Pvt. Ltd., Kerala, India. All other food ingredients were purchased from local market, Chidambaram. Diets were prepared fresh daily.

Sample collection
On the 30th and 60th day of experimental period, the mice were fasted overnight. Blood samples were collected by cardiac puncture after sacrificing the animals by cervical decapitation. Serum was separated by centrifugation at 3000 rpm for 15 min at 4º C and stored at -20º C until assayed. Assays were carried in serum for glucose, insulin, thiobarbituric acid reactive substances (TBARS) and Lipid profile.

Analytical procedures
Serum glucose concentrations were determined by Kricka L 2006 et al.16. Serum insulin was estimated by direct sandwich technique. Insulin levels were expressed as µIU/ml. Fasting glucose (mg/dl)-Insulin (µIU/ml) ratio was also calculated.17. Homeostatic model assessment (HOMA)18 as a measure of insulin resistance was calculated by the formula:

\[
\text{HOMA} = \frac{\text{Fasting insulin} \times \text{Fasting glucose}}{\text{n}}
\]

\[n = 405 \text{ (if fasting glucose value is mg/dl)}
\]

\[n = 22.5 \text{ (if fasting glucose value is mmol/L)}
\]

Serum levels of triglycerides (TG) and total cholesterol were determined by Nader et al R. 2006.19. TBARS in erythrocyte was quantified by the method of Donnan. 1950 et al. High - density lipoproteins was separated by using polyionic along with bivalent metal ion.21. LDL – cholesterol (LDL-C) and VLDL-cholesterol (VLDL-C) were calculated as follows:

\[
\text{VLDL-C} = \frac{\text{Triglycerides}}{5}
\]

\[
\text{LDL-C} = \frac{\text{Total cholesterol} - (\text{HDL-C} + \text{VLDL-C})}{5}
\]
Determination of erythrocyte thiobarbituric acid reactive substances (TBARS)

The levels of TBARS in erythrocyte were estimated by the method of Donnan (1950) [20]. The reaction mixture in a total volume of 1.7 ml containing 0.2 ml of erythrocytes and 1.5 ml of 10% TCA. The mixture was filtered through a Whatman No. 1 filter paper. 1.2 ml of TBA was added to 0.6 ml of filtrate. The mixture was heated in a boiling water bath for 15 min, cool and the colour developed was measured at 535 nm. Values were expressed as pmol/mg Hb.

III. Statistical Analysis

Statistical analysis was performed with SPSS (version 17.0). Results are expressed as mean ± SD and the statistical analysis of data was done using student’s ‘t’ test. Probability level less of 0.05 was considered statistically significant.

IV. Results

Effects on fasting Blood Glucose

Fasting glucose level of Group 6 (HFD-60 days duration), Group 5 (HFD-30) was significantly higher than Group 2 (control group-60 days duration) and group 1 (control group-30 days duration) respectively. Fasting glucose level of group 4 (PJD-60 days duration) was significantly higher than group 2 (CON-60 days duration).

Effects on fasting serum Insulin and IR (HOMA, QUICKI), G: I ratio

Fructose-fed mice (Group 5, 6) showed significantly higher glucose and insulin levels in serum and HOMA, QUICKI, G:1 ratio values as compared to control mice (Group 1, 2).

Effects on serum Lipid Profile

Serum Triglyceride (TG), total cholesterol and very low-density lipoprotein cholesterol (VLDL-C) concentrations were significantly increased in fructose-fed mice (Group 5, 6) as compared to control diet mice (Group 1, 2). Whereas high-density lipoprotein cholesterol (HDL-C) was lowered in fructose-fed mice (HFD 30 & 60). LDL was increased in group 5 (HFD 30) but it was statistically not significant. While TC level was reduced in both palm jaggery diet group (Group 3, 4) (table 2, figure 1).

Effects on erythrocyte TBARS (Thiobarbuturic acid reactive substances)

Erythrocyte MDA (Malondialdehyde) was significantly elevated in fructose fed mice of both groups (Group 5 and 6) than control group (Group 1 and 2). No significant changes were observed between palm jaggery group (3, 4) and control group 1, 2. While palm jaggery fed mice (Group 3) showed significant changes. No significant change was observed in Group 4.

V. Discussion

Our results show that fructose feeding in mice resulted in hyperglycemia and hyperinsulinemia suggests impaired insulin action. Serum insulin level is a crucial factor to control normal blood glucose level [22]. It was significantly higher (P < 0.05) in fructose fed mice compared to the control group. Chronic fructose consumption caused structural alterations in pancreatic β cells [23,24] to cause hyperinsulinaemia. Insulin resistance may occur due to a defect in insulin binding caused by decreased receptor number or affinity, or defects at the level of effector molecules such as glucose transporters and enzymes involved in glucose metabolism [25-27]. This is supported by high HOMA values in our study. The presence of insulin resistance is indicated by higher values of HOMA. These findings are in agreement with Rajasekar et al [28], Ramu Suganthi et al [29].

Our study shows, mean level of fasting glucose was elevated slightly in mice fed PJD (Group 4) when compared with respective control group (Group 2) but it was not statistically significant. Studies have shown that jaggery doesn’t raise blood glucose immediately [30]. Jaggery is unrefined sugar. It comes in blocks, bricks, cups or pastes. It contains up to 50% sucrose, up to 20% invert sugars, up to 20% moisture, and the remainder is made up of other insoluble matter such as wood ash, proteins and fibers [31]. The best thing about jaggery is that it is digested and absorbed gradually which in turn doesn’t raise blood sugar level immediately [32].

Total cholesterol, TG concentration in fructose fed mice (HFD) was significantly elevated. HDL-C was decreased whereas there was a significant increase in LDL-C and VLDL-C (Table: 2). these findings are consistent with other investigators [33, 34]. Serum VLDL and LDL concentrations were also significantly higher in mice fed the high-fructose diet as compared with the control mice. Both VLDL and LDL have a positive role in obesity and other related disorders [35, 36, 37]. HDL is considered to be a beneficial lipoprotein [38] and has a negative effect on...
hyperlipidemia, obesity. In our study, plasma HDL concentration was significantly lower in mice that received a high-fructose diet than the control mice.

Fructose feeding may lead to hypertriglyceridemia by increasing the formation of glycerol-3-phosphate, a pre-cursor of lipid synthesis. Hypertriglyceridemia may also arise due to defect in removal of VLDL from plasma or increased secretion of VLDL in the liver. Lipoprotein lipase is an important enzyme responsible for the hydrolysis of TG from chylomicrons and LDL. The increased TG concentration may be associated with impaired insulin action. Bieger et al., [30] have shown that an increase in blood TG concentration can reduce the number of insulin receptors thereby reducing insulin sensitivity. A causative link between increased circulating TG and impaired insulin action was observed in fructose-fed rats by Thorburn et al [34].

The high potassium content in jaggery is often associated with its weight loss benefits as potassium helps in reducing water retention in the body. Potassium helps build muscle, maintains the body’s electrolyte balance and improves metabolism-the conditions which support weight loss. This might be linked with reduced cholesterol level in palm jaggery fed mice [40].

Lipid peroxidation (LPO) is frequently investigated in biomedical research, and the assays for thiobarbituric acid-reactive substances (TBARSs) are more widely used than any other index of LPO in biological samples. Thiobarbituric acid reacts with LPO aldehydes, such as malondialdehyde (MDA).

Erythrocyte Malondialdehyde (MDA) levels were measured as thiobarbituric acid reactive substances (TBARS), which serves as an index of extent of lipid peroxidation. In the present study the lipid peroxidation product i.e. MDA levels have been increased significantly in erythrocytes of fructose fed mice compared to controls group mice. Rise in MDA could be due to increased generation of reactive oxygen species (ROS) due to the excessive oxidative damage generated in fructose fed mice. These oxygen species in turn can oxidize many other important biomolecules including membrane lipids. Similar reports of elevated MDA levels have been reported by Balasubramanian Vanithadevi et al [41].

ROS production could be enhanced during fructose feeding by well-described mechanisms like auto oxidation and glycation due to hyperglycemia [37-42]. In addition, hyperinsulinemia, depletion of ATP due to increased catabolism of fructose, increased aldehyde formation and reduced generation of reducing equivalents could be the other contributing mechanisms [43].

The increase in TBARS (MDA) level in fructose fed mice indicates enhanced peroxidation leading to a failure of the antioxidant defense mechanism to prevent formation of excess free radicals [44]. It indicates increased release of lipid peroxides from Erythrocytes of fructose induced mice than control group. Mice fed Palm jaggery diet (Group 4) prevented significantly lipid peroxidation (LPO) either directly or through reduced glutathione (GSH) by scavenging the free radicals. These results indicate the potential electron donating ability of Jaggery. These findings are in agreement with results of earlier investigations [45]. Due to scarcity of literature regarding palm jaggery we could not able to explain our results in detailed manner.

VI. Conclusion

On the basis of the results obtained in the present study, we conclude that Our findings indicate that mice fed high fructose diet (Refined sugar diet) shows biochemical features of metabolic syndrome or Polycystic ovary syndrome. Our study suggest that intake of palm jaggery will be a good alternative to fructose to maintain the blood cholesterol in normal range. Based on our study, we suggests Unrefined, dehydrated sugar (Palm jaggery) is better than white or black sugar (Refined sugar). Further detailed analysis is necessary for unrefined sugar (PJD) diet to understand their actions clearly.

References

A Prospective Study of Biochemical Assay in Mice Fed Refined Sugar Diet and Unrefined Sugar Diet


[40] mywellness.in/is-jaggery-better-for-diabetic.


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Table 1 Composition of diets (g/100g)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>HFD</th>
<th>PJD</th>
<th>CONT</th>
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<tbody>
<tr>
<td>Corn starch</td>
<td>-</td>
<td>-</td>
<td>60</td>
</tr>
<tr>
<td>High fructose</td>
<td>60</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Palm jaggery</td>
<td>-</td>
<td>60</td>
<td>-</td>
</tr>
<tr>
<td>Casein (fat free)</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.7</td>
<td>0.7</td>
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</tr>
<tr>
<td>Groundnut oil</td>
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<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Unrefined sesame oil</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Refined sesame oil</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>10.6</td>
<td>10.6</td>
<td>10.6</td>
</tr>
<tr>
<td>Salt mixture♣</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Vitamin mixture*</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
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</table>

HFD - High fructose diet
PJD - Palm jaggery diet
CONT - Control diet

♣ The composition of mineral mix (g/kg) MgSO₄·7H₂O-30.5; NaCl -65.2; KCl - 105.7; KH₂PO₄-200.2; MgCO₃ - 3.65; Mg (OH)₂ - 40.0; CuSO₄-512.4; KI-0.8; NaF-0.9; CuSO₄·5H₂O-1.4; MnSO₄-0.4, and CONH₃-0.05.

* One kilogram of vitamin mix contained thiamine mononitrate, 3g; riboflavin, 3g; Pyridoxine HCl, 3.5g; nicotinamide, 15g; d-calcium pantothenate, 8g; folic acid, 1g; d-biotin, 0.1g; cyanocobalamin, 5 mg; Vitamin A acetate, 0.6g; α-tocopherol acetate, 25g, and choline chloride, 10g.

Table 2 Basal biochemical parameters in control and experimental diet groups of 60 days duration

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control</th>
<th>PJD</th>
<th>HFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Glucose</td>
<td>81.41±1.68</td>
<td>83.66±2.46</td>
<td>157.28±6.77*</td>
</tr>
<tr>
<td>Insulin</td>
<td>8.12±3.93</td>
<td>8.12±6.94</td>
<td>12.07±5.42*</td>
</tr>
<tr>
<td>G:I Ratio</td>
<td>10.02±0.19</td>
<td>10.3±0.35</td>
<td>13.02±0.55*</td>
</tr>
<tr>
<td>HOMA</td>
<td>1.63±3.93</td>
<td>1.67±0.04</td>
<td>4.69±0.20*</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.35±1.21</td>
<td>0.35±0.00</td>
<td>0.3±1.83*</td>
</tr>
<tr>
<td>TC</td>
<td>93.45±3.36</td>
<td>106.63±16.46</td>
<td>169.08±7.83*</td>
</tr>
<tr>
<td>TGL</td>
<td>137.98±5.38</td>
<td>138.73±2.58</td>
<td>223.1±14.59*</td>
</tr>
<tr>
<td>HDL</td>
<td>35.76±2.01</td>
<td>35.95±2.10</td>
<td>31.7±2.14*</td>
</tr>
<tr>
<td>LDL</td>
<td>30.08±2.35</td>
<td>46.09±17.23</td>
<td>92.68±6.50*</td>
</tr>
<tr>
<td>VLDL</td>
<td>27.59±1.07</td>
<td>27.74±0.51</td>
<td>44.62±2.91*</td>
</tr>
<tr>
<td>TBARS</td>
<td>2.08±4.26</td>
<td>2.22±0.25</td>
<td>3.72±0.52*</td>
</tr>
</tbody>
</table>

* P<0.05 compared to control

Figure 1: fasting glucose and lipid profile level in control and experimental diet groups of 30 days duration
Figure 2: Erythrocyte TBARS level in control and experimental diet groups of 30 days duration

Figure 3: HOMA and QUICKI values in control and experimental diet groups of 30 days duration

Figure 4: HOMA and QUICKI values in control and experimental diet groups of 30 days duration
Figure 5: G:I ratio values in control and experimental diet groups of 30 days duration

Figure 6: showing the process of intracardiac blood collection from control mice