Biochemical effects of feeding soft drink and ethanol

Arun Raj1, Praveen K V2, Sheeba Varghese2, J K Mukkadan2 & P K Joseph2*

1St. Xaviers Institute of Science and Technology, Peechanicadu, Angamaly 683 572, India
2Little Flower Institute of Medical Sciences and Research, Angamaly 683 572, India

Received 17 October 2008; revised 3 February 2009

This work was undertaken to study whether consumption of alcoholic beverage mixed with soft drinks could reduce the metabolic effect caused by ethanol. When 24 hr fasted rats were intragastrically fed rum (with 40% ethanol) diluted (1:1) with water, 3.0 ml (0.5 g ethanol) per 100 g body weight and sacrificed 12 hr later in fasting condition, exhibited higher levels of triacyl glycerol, glucose, total cholesterol, high density lipoprotein (HDL), aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) in serum, higher levels of total cholesterol, triacyl glycerol and thiobarbituric acid reactive substances (TBARS) in both liver and kidneys, and lower levels of serum albumin. When fasted rats were fed 3.0 ml soft drink (0.31 mg caffeine), they showed increased levels of triacyl glycerol, glucose, ALT and ALP in the serum, TBARS in liver and kidneys, triacyl glycerol and total cholesterol in kidneys and lower levels of serum albumin. Soft drink feeding did not reduce serum total cholesterol but reduced HDL levels. Also soft drink did not alter liver lipids. When a mixture of 1.5 ml diluted rum (0.25 g ethanol) and 1.5 ml soft drink (0.154 mg caffeine) were fed to the fasted rats, the serum parameters increased similar to rats fed rum only except that total cholesterol and HDL cholesterol were unaltered. TBARS in kidneys and liver were also increased but triacyl glycerol levels were not altered. Thus feeding ethanol with soft drink does not reduce the metabolic effects of ethanol but it will prevent ethanol induced serum HDL cholesterol rise.

Keywords: Rum, Serum, Soft drinks, Tissue changes

Consumption of soft drinks can lead to numerous health problems like diabetes mellitus, tooth decay, osteoporosis, heart disease, neurological disorders etc1-8. Amato et al9 have demonstrated that in immature rats plasma pH and ionized calcium were reduced after consumption of soft drinks, but such effects were not observed in adult rats. They concluded that immature animals show more derangement of calcium and phosphate metabolism related to soft drinks. Kandiah and Kies10 observed that feeding of soft drinks packed in aluminium cans to rats resulted in much higher levels of aluminium in blood, liver and bone. Aluminium is a well accepted neurotoxin11.

Bone mineral density of those with daily soft drink intake was 3.7% lower than that of non-consumers12. Such results are reported in rats also13. Some soft drinks contain caffeine. Caffeine is known to increase free fatty acids and triacyl glycerol and is known to produce hyperlipidemia and increase in liver marker enzymes14-16. Many alcoholics have a tendency to mix alcoholic beverage with soft drinks and drink for better taste. There is a belief that soft drinks may counteract some of the harmful effects of ethanol. Hence, studies have been undertaken by feeding rum (with 40% ethanol) mixed with a popular soft drink to rats

Materials and Methods

Six bottles of a soft drink containing caffeine and carbonated water were collected from different parts of Ernakulam District, Kerala state. They were opened just before the experiment, transferred to beakers and kept for 30 min at room temperature to remove excess CO2. Rum was purchased from Beverages Corporation outlets. Rats were purchased from veterinary breeding centre, Mannoothy, Kerala. Male albino rats weighing 120-150 g and fasted for 24 hr were divided into 4 following groups:

Group 1—Control rats fed 3.0 ml normal saline, intragastrically per 100 g body weight.

Group 2—Ethanol fed rats. Rats were fed intragastrically 3.0 ml/100 g body weight, rum diluted (1:1 v/v) with water equivalent to 0.5 g ethanol.
Group 3—Soft drink fed rats. They were fed intragastrically 3.0 ml (0.31 mg caffeine) soft drink/100 g body weight.

Group 4—Ethanol and soft drink fed rats. They were fed a mixture of 1.5 ml (0.25 g ethanol) diluted rum and 1.5ml (0.154 mg caffeine) soft drink/100 g body weight.

All the rats were fasted again for 12 hr after feeding and then sacrificed. Blood was collected and transferred to centrifuge tube and allowed to clot. After 5 min it was centrifuged. Glucose was estimated from 0.1 ml serum within 10 min. Tubes were kept at room temperature for 2 hr and centrifuged again. Other parameters were estimated from the serum thus obtained. Glucose, aspartate amino transferase (AST; EC 2.6.1.1) alanine amino transferase (ALT; EC 2.6.1.2), alkaline phosphatase (ALP EC 3.1.3.1), triacyl glycerol, HDL cholesterol and total cholesterol, in the serum were estimated using the enzymes kits supplied by Agappe Diagnostics, Thana, Maharastra. Serum albumin was estimated by the dye binding method using the kits supplied by the same company.

Kidney and liver (0.25 g each) were transferred soon after sacrifice into 9 volumes of 5% trichloroacetic acid (TCA). They were later homogenized and thiobarbituric acid reactive substances (TBARS) were estimated.

Results

Ethanol fed rats of group 2 (Table 1) exhibited significantly higher levels of glucose, triacyl glycerol, total cholesterol, HDL cholesterol, AST, ALT and ALP ($P<0.01$) and lower levels of albumin in the serum ($P<0.05$) compared to saline fed control rats of group 1. Ethanol fed rats also exhibited (Table 2) higher levels of TBARS, total cholesterol and triacyl glycerol compared to the control rats ($P<0.01$) in both liver and kidneys.

Soft drink fed rats of group 3 also exhibited higher levels of glucose triacyl glycerol, ALT and ALP and lower levels of HDL cholesterol and albumin in the serum ($P<0.01$) compared to group 1 control rats. TBARS in both liver and kidneys were increased by soft drink feeding ($P<0.01$) compared to group 1 rats (Table 2). Kidney total cholesterol ($P<0.01$) and triacyl glycerol ($P<0.05$) were moderately raised. Unlike the ethanol fed rats (group 2) the soft drink fed rats (group 3) did not alter liver lipids and serum cholesterol compared to control rats.

Table 1—Effect of alcohol and soft drink consumption on serum parameters in rats

<table>
<thead>
<tr>
<th>Serum parameters</th>
<th>Group 1 (normal rats)</th>
<th>Group 2 (alcohol fed rats)</th>
<th>Group 3 (soft drink fed rats)</th>
<th>Group 4 (alcohol + soft drink fed rats)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum glucose (mg/dL)</td>
<td>78.67 ± 3.92</td>
<td>118.47 ± 3.27*</td>
<td>111.66 ± 4.40*</td>
<td>126.18 ± 4.11*</td>
</tr>
<tr>
<td>Serum triacyl glycerol (mg/dl)</td>
<td>83.40 ± 3.54</td>
<td>124.40 ± 4.40*</td>
<td>96.94 ± 4.8*</td>
<td>119.32 ± 4.8*</td>
</tr>
<tr>
<td>Serum total cholesterol (mg/dl)</td>
<td>74.95 ± 3.6</td>
<td>131.46 ± 5.52*</td>
<td>77.22 ± 4.5</td>
<td>74.58 ± 4.5</td>
</tr>
<tr>
<td>Serum HDL cholesterol (mg/dl)</td>
<td>50.68 ± 3.98</td>
<td>82.03 ± 5.03*</td>
<td>40.9 ± 4.5</td>
<td>56.69 ± 4.6</td>
</tr>
<tr>
<td>Serum AST (IU/L)</td>
<td>27.31 ± 4.39</td>
<td>41.06 ± 5.03*</td>
<td>33.47 ± 3.87</td>
<td>36.63 ± 4.43*</td>
</tr>
<tr>
<td>Serum ALT (IU/L)</td>
<td>8.86 ± 2.7</td>
<td>16.47 ± 3.39*</td>
<td>18.47 ± 2.4</td>
<td>21.21 ± 2.38*</td>
</tr>
<tr>
<td>Serum ALP (KA units/100 ml of serum)</td>
<td>51.35 ± 5.29</td>
<td>73.03 ± 5.65*</td>
<td>75.73 ± 3.92</td>
<td>79.21 ± 6.46*</td>
</tr>
<tr>
<td>Serum albumin (g/dL)</td>
<td>0.29 ± 0.24</td>
<td>0.187 ± 0.42**</td>
<td>0.179 ± 0.33</td>
<td>0.194 ± 0.60*</td>
</tr>
</tbody>
</table>

Group 2, 3 and 4 are compared with Group 1

$P$ values; *$P<0.01$; **$P<0.05$
Table 2—Effect of alcohol and soft drink consumption on tissue parameters in rats

[Values are mean ± SD of 6 animals per group]

<table>
<thead>
<tr>
<th>Tissue parameters</th>
<th>Liver TBARS (µmoles/100 g of wet tissue)</th>
<th>Liver triacyl glycerol (mg/g of wet tissue)</th>
<th>Liver total cholesterol (mg/g of wet tissue)</th>
<th>Kidney TBARS (µmoles/100 g of wet tissue)</th>
<th>Kidney triacyl glycerol (mg/g of wet tissue)</th>
<th>Kidney total cholesterol (mg/g of wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (normal rats)</td>
<td>5.77 ± 0.55</td>
<td>3.88 ± 0.50</td>
<td>0.62 ± 0.18</td>
<td>9.65 ± 0.65</td>
<td>0.90 ± 0.12</td>
<td>0.46 ± 0.07</td>
</tr>
<tr>
<td>Group 2 (alcohol fed rats)</td>
<td>10.68 ± 0.38*</td>
<td>6.86 ± 0.72*</td>
<td>1.16 ± 0.25*</td>
<td>11.57 ± 0.92*</td>
<td>2.07 ± 0.46*</td>
<td>1.05 ± 0.09*</td>
</tr>
<tr>
<td>Group 3 (soft drink fed rats)</td>
<td>9.67 ± 1.11*</td>
<td>4.45 ± 0.50</td>
<td>0.91 ± 0.15</td>
<td>11.59 ± 0.85*</td>
<td>1.32 ± 0.28*</td>
<td>0.74 ± 0.14*</td>
</tr>
<tr>
<td>Group 4 (alcohol + soft drink fed rats)</td>
<td>12.10 ± 0.95*</td>
<td>3.45 ± 0.40</td>
<td>0.83 ± 0.16*</td>
<td>11.78 ± 1.16*</td>
<td>0.92 ± 0.16</td>
<td>0.46 ± 0.10</td>
</tr>
</tbody>
</table>

Group 2, 3 and 4 are compared with Group 1

*P values; *<0.01; **<0.05

Fig. 1—Cross section of liver. (a): normal rats; (b): ethanol (0.5 g/100 g body wt) fed rats; (c): soft drink (0.31 mg caffeine/100 g body wt) fed rats; (d): ethanol (0.25 g) + soft drink containing 0.154 mg caffeine/100 g body wt fed rats × 400.
Rats of group 4 which were fed a mixture of soft drink and rum also exhibited changes in serum levels of glucose, triacyl glycerol, AST, ALT, ALP and albumin and liver kidneys levels of TBARS ($P<0.01$) (Tables 1, 2) compared to group 1 control rats, similar to the levels of these parameters in ethanol fed rats of group 2. But group 4 rats did not show change in serum total cholesterol, HDL cholesterol or triacyl glycerol in liver or kidneys compared to group 1 rats.

Macroscopic examination of liver and kidneys of rats fed a mixture of ethanol and soft drink (group 4) appeared inflamed and showed yellow coloured nodules on the surface. Microscopic study revealed that liver cells of rats fed alcohol and soft drink together showed inflammation (Fig. 1). The kidney cells also showed damage in the form of inflamed glomerular cells (Fig. 2)

Discussion

Ethanol—induced changes in the serum liver and kidneys were similar to the results of earlier workers. Soft drink fed rats also exhibited similar rise in serum triacyl glycerol but not in the liver lipids. This drink contain caffeine. Caffeine is known to stimulate adrenal glands increasing gluconeogenesis in the liver and lipolysis in adipose tissue causing rise in free fatty acids in the serum and futile recycling of triacyl glycerol, which could be responsible for the rise of glucose and triacyl glycerol in the serum.

Ethanol is known to increase hepatic synthesis of both triacyl glycerol and cholesterol. Probably soft drink has no such effects as evidenced by the absence of hypercholesterolemia in the serum and absence of increase in triacyl glycerol and cholesterol in the liver. But soft drink fed rats showed lesser levels of HDL cholesterol, albumin and higher levels of ALT and ALP in the serum indicating that soft drink could be compromising liver function.

Group 4 rats fed a mixture of soft drink and ethanol exhibited rise in liver marker enzymes ALT and ALP,
lower levels of serum albumin showing reduced liver function. Both liver and kidney TBARS were raised in group 4 rats. Though triacyl glycerol was not raised in the liver but the liver and kidney tissues showed histopathological changes. Many alcoholics believe that rum mixed with soft drink will prevent the cirrhosis. But drinking rum mixed with soft drink appears to damage both liver and kidneys. Ethanol induced rise in HDL cholesterol (group 2) was absent in rats fed ethanol mixed with soft drink (group 4). Thus mixing of ethanol with caffeine containing soft drink is more damaging to the system than consuming rum alone.

References