Effects of Okinawan Sugar Cane Rind on Serum and Liver Cholesterol and Triglyceride Levels in the Rat

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Summary Okinawan sugar cane rind was fed to Wistar strain rats to examine its effects on the serum and liver cholesterol (Chol) and triglyceride (TG). At the same time, the effects of sugar cane rind on the fecal excretion of neutral sterols of the rats were examined.

There were no significant differences found in the food intakes and the liver weight between the rats fed with sugar cane rind and other groups. The addition of 1% Chol to the diet caused a significant increase in body weight gain but the supplementation of sugar cane rind (2%) showed an effect on weight control of rats.

The serum Chol and TG levels of the rats given sugar cane rind were lowered significantly. However, the lipid levels in the liver were almost the same when compared with the control groups.

The amount of feces excreted by the rats fed with sugar cane rind was about 37% more than that of the control group, and the fecal excretion of neutral sterols was significantly higher.

Key Words sugar cane rind, cholesterol, triglyceride, fecal excretion, neutral sterols

Since Portman et al. (1), and Grant and Fahrenbach (2) reported the experimental results that a high correlation between dietary sugar intake and blood lipid concentration had been found, many studies have been undertaken (3–6).

In man, it has been demonstrated that increasing dietary intake of simple sugars (especially sucrose) results in increased serum lipid levels, most noticeably those of the Chol and TG concentration. Keys et al. (7) observed that serum Chol levels were significantly higher, by 10%, in men fed simple sugars than when

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complex carbohydrates were ingested. Kaufmann et al. (8) in their work with hyperlipidemic patients, noted that in all cases patients fed sucrose or glucose diets had marked increases in the serum TG levels with serum Chol levels following in like fashion.

The main composition of black (raw) sugar is sucrose (84–90%) and then 7% water, 3% reducing sugar and a small amount of minerals, cellulose, organic acids, protein, starch, lipids and pigments are contained (9). In our previous report (10), we indicated that the rats fed black sugar as carbohydrate sources showed significantly lower serum Chol and TG levels than when white (refined) sugar was fed. The experimental results obtained by feeding corn starch and sugars (black and white) and sugars supplemented with tocopherol (11) also indicated that black sugar lowered serum Chol and TG levels but the lowering factor in black sugar was found to be other than tocopherol.

The purpose of the present study was to assess the effect of sugar cane rind on serum and liver lipid levels.

MATERIALS AND METHODS

Materials. The sugar cane (Kind, NCO-310; Brix, 21%) used was obtained from the Experimental Farm attached to the Agriculture Department, University of the Ryukyus. The rind materials were collected in January, the harvesting season, by scratching the surface of a cane with a straight razor.

Animals. Male Wistar rats (Nikon Rat Co., Saitama) weighing about 200 g were randomly divided into 4 experimental groups, 5 rats in each group, and caged individually. Experimental diets and water were provided ad libitum for 14 days, and the rats were sacrificed by decapitation after fasting overnight (for 16 hr). The blood was centrifuged at 8,000 rpm for 30 min to obtain serum. Liver was excised from each animal for the determination of liver lipid concentration. In addition, feces of the last three days of experimental periods of each animal were collected for sterol analysis.

Diets. The percentage composition of experimental diets are shown in Table 1.

Lipid analysis. Serum and liver lipids were extracted according to Folch et al. (12) using chloroform : methanol (2:1) as solvent. The methods used for determining each lipid concentration are as follows: Chol, Sperry and Webb method (13); TG, Fletcher method (14); and phospholipid (PL), Gomori method (15).

Sterol analysis. Feces collected were freeze-dry-powdered and sieved (42 mesh). Lipids were extracted from 0.5 g of each fecal sample according to Eneroth et al. (16) with chloroform : methanol (1:1) and 0.5 ml of each extract was used for sampling. The solvent was vaporized under a stream of nitrogen and the residual lipid was refluxed for 2 hr with 10 ml of ethanol, 1.2 ml of 2 N potassium hydroxide solution and with 5α-cholestane as an internal marker. Then 10 ml of water was added and after mixing well, unsaponifiable matter was extracted with petroleum
ether. The extract solvent was vaporized and trimethyl silylated with dry-pyridine: hexamethyldisilazane : trimethylchlorosilane (9 : 3 : 1) solution, and analyzed by gas-liquid chromatography. Chromatographic separation was performed on a Hitachi 163 type equipped with a hydrogen flame ionization detector. A glass column (3mm x 2m) packed with 3% OV-17 on Gas Chrome Q (60–80 mesh) was run at 280°C with nitrogen flow rate of 40 ml/min and injection temperature was kept at 300°C.

RESULTS

Growth and food consumption

As indicated in Table 2, the body weight gain of the rats fed lard + Chol (group II) was the highest and those fed Chol-free diet (group I) was the lowest, showing statistically significant differences from three other experimental groups.

The effect of sugar cane rind (group IV) on the body weight change was observed and it was significantly lower when compared with group II. The food consumption, feed conversion and liver weight of each experimental group was roughly similar, but feed conversion of group I was higher than that of the three other groups.

Serum lipid concentrations

The concentrations of serum Chol, PL and TG are shown in Table 3. The total Chol level of the rats fed corn oil (group III) was the lowest and followed by sugar cane rind diet (group IV), Chol-free diet (group I) and lard + Chol diet (group II) in increasing order.
Table 2. Effect of sugar cane rind on body weight gain, food consumption and liver weight.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Diets</th>
<th>Initial weight (g)</th>
<th>Weight gain (g/14 days)</th>
<th>Diet eaten per day (g)</th>
<th>Feed conversion (g)</th>
<th>Liver wt./100 g body wt. (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5% lard</td>
<td>206 ± 4*</td>
<td>62 ± 3</td>
<td>21 ± 2.6</td>
<td>4.7 ± 0.4</td>
<td>3.0 ± 0.4</td>
</tr>
<tr>
<td>II</td>
<td>5% lard + 1% Chol</td>
<td>210 ± 4</td>
<td>77 ± 2*</td>
<td>23 ± 1.7</td>
<td>4.2 ± 0.3</td>
<td>3.3 ± 0.4</td>
</tr>
<tr>
<td>III</td>
<td>5% corn oil + 1% Chol</td>
<td>211 ± 4</td>
<td>71 ± 3^b,d</td>
<td>21 ± 1.7</td>
<td>4.1 ± 0.3</td>
<td>3.3 ± 0.4</td>
</tr>
<tr>
<td>IV</td>
<td>5% lard + 1% Chol + 2% sugar cane rind</td>
<td>207 ± 4</td>
<td>67 ± 2^b,c</td>
<td>21 ± 1.5</td>
<td>4.4 ± 0.3</td>
<td>3.4 ± 0.5</td>
</tr>
</tbody>
</table>

* Mean ± SD. ^ Significantly different from group I (p < 0.005). ^ Significantly different from group I (p < 0.05). ^ Significantly different from group II (p < 0.01). ^ Significantly different from group II (p < 0.05).

Table 3. Effect of sugar cane rind on serum lipids.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Diets</th>
<th>Cholesterol</th>
<th>Phospholipid (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total (mg/dl)</td>
<td>Free (mg/dl)</td>
<td>Ester (%)</td>
</tr>
<tr>
<td>I</td>
<td>5% lard</td>
<td>89 ± 10.0*</td>
<td>21 ± 2.9</td>
<td>76 ± 2.8*</td>
</tr>
<tr>
<td>II</td>
<td>5% lard + 1% Chol</td>
<td>107 ± 14.0</td>
<td>28 ± 5.5</td>
<td>74 ± 1.1*</td>
</tr>
<tr>
<td>III</td>
<td>5% corn oil + 1% Chol</td>
<td>73 ± 5.0*</td>
<td>27 ± 2.5</td>
<td>63 ± 2.2^b</td>
</tr>
<tr>
<td>IV</td>
<td>5% lard + 1% Chol + 2% sugar cane rind</td>
<td>82 ± 4.8^b,c</td>
<td>23 ± 1.4</td>
<td>72 ± 3.3^d</td>
</tr>
</tbody>
</table>

* Mean ± SD. ^ Significantly different from group II (p < 0.05). ^ Significantly different from group II (p = 0.005). ^ Significantly different from group III (p < 0.05). ^ Significantly different from group III (p < 0.01). ^ Significantly different from group III (p < 0.005).
Table 4. Effect of sugar cane rind on liver lipids.

<table>
<thead>
<tr>
<th>Diets</th>
<th>Total Cholesterol (mg/g liver)</th>
<th>Free Cholesterol (mg/g liver)</th>
<th>Ester Cholesterol (mg/g liver)</th>
<th>Triglyceride (mg/g liver)</th>
<th>Phospholipid (mg/g liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% lard</td>
<td>5% lard +1% Chol</td>
<td>10% lard + 1% Chol</td>
<td>15% lard + 1% Chol + 2% sugar cane rind</td>
<td>4 ± 1.3 *</td>
<td>3 ± 1.1</td>
</tr>
<tr>
<td>2% lard</td>
<td>5% lard +1% Chol</td>
<td>10% lard + 1% Chol</td>
<td>15% lard + 1% Chol + 2% sugar cane rind</td>
<td>8 ± 5.8 *</td>
<td>3 ± 1.6</td>
</tr>
<tr>
<td>3% lard</td>
<td>5% lard +1% Chol</td>
<td>10% lard + 1% Chol</td>
<td>15% lard + 1% Chol + 2% sugar cane rind</td>
<td>8 ± 4.2 *</td>
<td>8 ± 4.2</td>
</tr>
<tr>
<td>4% lard</td>
<td>5% lard +1% Chol</td>
<td>10% lard + 1% Chol</td>
<td>15% lard + 1% Chol + 2% sugar cane rind</td>
<td>3 ± 9.5</td>
<td>3 ± 9.5</td>
</tr>
<tr>
<td>5% lard</td>
<td>5% lard +1% Chol</td>
<td>10% lard + 1% Chol</td>
<td>15% lard + 1% Chol + 2% sugar cane rind</td>
<td>3 ± 6.7</td>
<td>3 ± 6.7</td>
</tr>
</tbody>
</table>

*Mean ± SD. **Significantly different from group I (p < 0.005).

Table 5. Gas chromatographic analysis of sterol in feces.

<table>
<thead>
<tr>
<th>Diets</th>
<th>Intake (mg/g)</th>
<th>Cholesterol (mg/day)</th>
<th>Corosanol (mg/day)</th>
<th>Total sterol (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% lard</td>
<td>1.6 ± 0.8</td>
<td>6 ± 2</td>
<td>236 ± 10</td>
<td>15 ± 3</td>
</tr>
<tr>
<td>2% lard</td>
<td>1.7 ± 1.2</td>
<td>1.7 ± 1.2</td>
<td>300 ± 11</td>
<td>300 ± 11</td>
</tr>
<tr>
<td>3% lard</td>
<td>2.0 ± 1.2</td>
<td>2.0 ± 1.2</td>
<td>300 ± 11</td>
<td>300 ± 11</td>
</tr>
<tr>
<td>4% lard</td>
<td>2.4 ± 0.6</td>
<td>2.4 ± 0.6</td>
<td>234 ± 10</td>
<td>234 ± 10</td>
</tr>
</tbody>
</table>

*Mean ± SD. **Significantly different from group I (p < 0.005).

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The total Chol level of group IV was significantly lower than that of group II, indicating the lowering effect of sugar cane rind supplementation.

In regard to free-Chol level, group I was the lowest (21 mg/dl) and group II showed the highest (28 mg/dl) level, but there were no significant differences between groups.

The percentage of esterified Chol on the corn oil diet (group III) was the lowest, showing significant differences from the three other experimental groups. The PL level of Chol-free diet (group I) was the highest; however, there were no significant differences from the three other groups.

With regard to TG level, group II was the highest and groups III, I and IV followed in descending order. The lowering effect on TG level by supplementing sugar cane rind was also observed.

Liver lipid concentration
The concentrations of Chol, PL and TG in the liver are shown in Table 4. The total Chol level of the Chol-free diet (group I) was significantly lower than the three other groups, but free-Chol levels of all experimental groups were exactly the same. Therefore, a similar pattern was observed in the percentage of esterified Chol level. There were no significant differences observed in PL and TG levels among experimental groups.

Excretion of neutral sterols
The excretion of neutral sterols determined by gas chromatographic analysis is shown in Table 5.

The amount of feces excreted by the rats fed sugar cane rind (group IV) was 21–50% more than those of the three other groups.

With regard to the sterol intake, the Chol-free diet (group I) was the lowest as a matter of course, and by the addition of 1% Chol to the diet in the three other groups it noticeably increased. However, the corn oil diet (group III) was significantly higher than groups II and IV. The excretion of coprostanol and total sterol by group IV was significantly higher than that of group II.

DISCUSSION
The addition of 1% Chol to the diet caused an increase in weight gain. However, the results of the present study demonstrated that sugar cane rind has an effect on weight control of rats, showing a significant difference between groups II and IV.

Kaunitz and Johnson (17), and Gran and Nicolaysen (18) reported that feeding vegetable oil, which contains a high percentage of polyunsaturated fatty acids decreases serum Chol in rats. In our present experiment, rats fed corn oil (containing over 50% linoleic acid) instead of lard (group III) resulted in showing a significantly lower serum Chol level. On the other hand, those rats fed animal fat

lard, showed elevated serum Chol and TG levels when 1% Chol was added (group II); but when Chol was not added (group I) serum Chol and TG levels were almost the same as group III. Bronsgeest-Schoute et al. (19) indicated that there is a very variable response in the human population toward dietary Chol. In general, healthy subjects (human) on high Chol diets have lower synthetic rates than those on low Chol diets, but this is observed when the Chol intakes are kept within the normal range. In our present experiment, addition of 1% Chol to the diet which contains animal fat and sucrose (group II) resulted in high serum Chol and TG levels. These results agree with many previous studies.

Grundy (20) reported that the increased fecal excretion of sterols and bile acids by feeding vegetable oil that is high in polyunsaturated fatty acids is one factor that lowers the serum Chol level in rats.

In our present experiment, rats fed corn oil (group III) showed increased fecal excretion of sterols compared to those fed lard + Chol (group II). The lower serum Chol level and elevated fecal excretion of sterol by rats fed sugar cane rind has also been demonstrated.

Judging from the results of the present experiment, it could be concluded that the lowering effect of serum Chol level by feeding sugar cane rind to rats is likely to be due to the increased fecal excretion of sterols. It is also suggested that elements may exist which accelerate the excretion of fecal sterols. Further studies on the analysis of sugar cane rind materials should be carried out to determine the elements acting on serum lipid levels.

REFERENCES


