Effect of Dates and Gahwa (Arabian Coffee) Supplementation on Lipids in Hypercholesterolemic Hamsters

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Abstract: The aim of present study to evaluate the effect of dates, gahwa and their combination on lipid metabolism in hypercholesterolemic hamsters. The increase intake of dates and gahwa (Arabian coffee) along with a high cholesterol diet in Saudi population as well as increased incidence of cardiovascular disease (CVD) has raised a concern about the effects of the Saudi diet on CVD risk. Golden Syrian hamsters were divided into six groups (six animals in each) as follows: 1) control (chow), 2) Dates-diet (50% date pulp with chow), 3) Dates-diet + gahwa (replaced with drinking water), 4) cholesterol-diet (1% cholesterol in chow), 5) dates-diet + 1% cholesterol, 6) Dates-diet + gahwa + 1% cholesterol. All the above dietary preparations were made every week and supplemented for 13 consecutive weeks. Plasma lipid profile including total cholesterol, triglycerides (TC), Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL) were estimated. Total cholesterol and TC were estimated in liver, heart and kidney tissues. The high cholesterol-diet caused significant increase in body and organs (liver and kidney) weights as compared to controls. Dates-diet, significantly reduced the body and liver weight that increased by the high cholesterol-diet. Plasma lipids were significantly elevated by high cholesterol-diet supplementation and this increase was significantly decreased by the dates-diet. However, hepatic TC levels further increased when dates were combined with high cholesterol-diet supplementation. Gahwa intake either with dates alone or with high cholesterol-diet was not induced any significant changes in lipid parameters. In conclusion, the dates lowering effects on body weights and plasma lipid profile shows its beneficial effects against atherosclerosis development in humans. Further investigations required for find out its potential constituents that affecting the CVD risk.

Key words: Dates, Arabian coffee, cholesterol, triglycerides, LDL, HDL, hamster

INTRODUCTION

Cardiovascular disease is a leading cause of global mortality, accounting for 17 million deaths annually (Smith et al., 2004); atherosclerosis, in particular, is the main contributor for the pathogenesis of myocardial infarction. Dietary cholesterol in excess promotes coronary plaque formation, which increases the susceptibility to myocardial ischemia and aggravates ischemic heart disease (Schwartz et al., 2001; Yaoota et al., 2005). Elevated levels of plasma LDL and triglycerides, accompanied by reduced HDL levels, is often associated with an increased risk of coronary heart disease (CHD) (Fki et al., 2005; Smith et al., 2004). Atherosclerosis represents a state of heightened oxidative stress, characterized by lipid and protein oxidation (Stocker and Keaney, 2004). Several studies indicate that LDL oxidation in an early event of process. Indeed, oxLDL is cytotoxic to a variety of vascular cells (Morel et al., 1983), induces the synthesis of monocyte chemotactic protein-1 (Rajavashisth et al., 1990), recruits inflammatory cells (Navab et al., 1991) and stimulate the production of autoantibodies (Salonen et al., 1992).

The date palms (Phoenix dactylifera L.) have been cultivated in the Middle East over at least 6000 years ago (Copley et al., 2001). Dates fruits have always played an important role in the economic and social lives of the people of these regions and they considered dates are a staple carbohydrate food (Al-Shahib and Marshall, 2003). They are also used in the production of local foods, beverages and spirits. Folk medicinal practices dates are considered as tonic and aphrodisiac. Recently, Al-Qarawi et al. (2005) reported its beneficial affect on experimentally induced gastric ulcers in rats. A first study, reported that date fruits has anti-tumor activity (Ishurd and Kennedy, 2005). It has proved for
antioxidant and anti-mutagenic properties (Vayali, 2002; Mansouri et al., 2005). Dry date fruits are used in Indian traditional medicine after birth as immunostimulants (Puri et al., 2000). Furthermore, Al-Shahib and Marshall, (2003) concluded that, in many ways, dates may be considered as an almost ideal food, providing a wide range of essential nutrients and potential health benefits.

The relation between coffee consumption and CVD has been extensively. Although many previous studies found no significant association between coffee and CHD (Kawachi et al., 1994; Willett et al., 1996), more recent results have been inconsistent. Case-control studies found a positive association between coffee consumption and risk of CHD (Tavani et al., 2001; Hammer et al., 2003), prospective cohort studies reported a lower risk among individuals with higher coffee consumption (Woodward and Tunstall-Pedoe, 1999; Kleemola et al., 2000). However, it has been reported that, boiled coffee enhances the serum cholesterol levels (Little et al., 1966; Bjelke, 1974). Scandinavian boiled coffee and Arabian coffee are prepared by boiling roasted ground coffee beans found to raise serum cholesterol levels in humans and in experimental animals (Zook et al., 1990; Urgert et al., 1995; Al-Khanhal et al., 1994). Dusseldorp et al. (1991) reported that, coffee boiling caused an increased extraction of hypercholesterolemic lipid factor from coffee, which powerfully raises the serum cholesterol level.

The increase intake of gahwa (Arabian coffee) with dates along with a high cholesterol diet in Saudi population as well as increased incidence of CHD (Lewis and Russel, 1985; Al-Shoshan, 1992) has raised a concern about the effects of the Saudi diet on CHD risk. The present study has designed to evaluate the effect of dates, gahwa and their combination on lipid metabolism in hypercholesterolemic hamsters.

MATERIALS AND METHODS

This study has undertaken in College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia during 2006.

Animals: Thirty-six male golden Syrian hamsters of same age weighing 60-70 g were received from Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. All the animals were maintained under controlled condition of temperature (24±1°C), humidity (50-55%) and light (12 h light/dark) and were provided with chow and water ad lib. The animals were kept for one week in similar conditions as incubation period and were divided in six groups by taking 6 hamsters in each group. The groups are as follows: 1) Control (chow, manufactured by Grain Silos and Flour Mills Organization, Riyadh, Saudi Arabia), 2) Dates-diet (50% date pulp with chow w/w), 3) Dates-diet with gahwa (drinking water was replaced with gahwa), 4) Cholesterol-diet (1% cholesterol in chow w/w), 5) Date + cholesterol (1% cholesterol in dates-diet) and 6) Date + gahwa + cholesterol (1% cholesterol in dates-diet and gahwa replaced with drinking water).

Diets: Dates-diet: Khalas (a variety of date) date pulp was mixed with hamster chow power (1:1, w/w), made into pellets and air-dried.

Cholesterol-diet: One percent (w/w) cholesterol (BDH Chemicals, Poole, England) was mixed with hamster chow power, made into pellets and air-dried.

Gahwa: The gahwa was prepared by boiling 30 g of medium roasted grounded Arabian coffee (obtained from local market) in 1 L of water for 20 min, 5 g of ground cardamom (Elettaria cardamomum Maton) was added to boiling coffee and continue for 5 more min. The gahwa was decanted to remove the sediments and used after cooled.

All the above dietary preparations were made every week and stored in refrigerator except gahwa. The treatments were continued for 13 weeks.

Biochemical analysis: At the end of the experimental period, animals were fasted for overnight and were anesthetized with ether. The blood samples were collected via cardiac puncture in EDTA containing tubes. The liver, heart and kidneys were excised and the blood was removed by rinsing in chilled normal saline. The tissues were pressed with blotting papers to remove water and weighed. The blood samples were centrifuged at 3000 rpm for 10 min and the plasma samples were stored at -20°C until analysis.

Plasma lipids including total cholesterol, triglycerides, LDL and HDL were estimated by using enzymatic kits (BioMerieux, France) on spectrophotometer (LKB Ultraspec II, Biochrom). The total lipids in tissues were extracted by the method of Folch et al. (1957) using a chloroform and methanol mixture at the ratio of 2:1 (v/v). The lipid fraction was dissolved in saline, total cholesterol and triglycerides were estimated by using enzymatic-kit (Spinreact, San Antonio).

Statistical analysis: The various parameters studied were subjected to statistical analysis with Student’s t-test.
RESULTS

The animals fed with dates-diet alone and with gahwa for 13 weeks, did not show any significant change in body weight increase or in organs weight. Cholesterol-diet supplementation caused significantly (p<0.05) increase in the body weights as compared to control group of animals. Liver and kidney weights were also increased significantly (p<0.001) and (p<0.01) in high cholesterol-diet fed hamsters as compared to controls, respectively. The dates-diet alone or with gahwa, significantly (p<0.05) decreased the body weight increased by high cholesterol-diet. Kidney weight also significantly (p<0.05) decreased by dates-diet supplementation as compared to high cholesterol-diet alone fed group (Table 1).

Plasma total cholesterol, triglycerides, HDL and LDL concentrations were not significantly altered either with dates-diet alone or with gahwa supplementation for 13 consecutive weeks. However, high cholesterol-diet supplementation showed significant increase in the levels of total cholesterol (p<0.001), triglycerides (p<0.001), HDL (p<0.05) and LDL (p<0.01) in plasma compared to controls. The dates-diet supplementation caused significant decrease in the concentrations of cholesterol (p<0.05), triglycerides (p<0.05) and LDL (p<0.05) in masters plasma as compared to high cholesterol-diet supplemented animals. However, drinking gahwa as with dates alone and or with high cholesterol-diet did not show any significant change in plasma lipid profile as compared to control or high cholesterol-diet alone supplemented animals, respectively (Table 2).

Dates-diet alone or with gahwa supplementation did not cause any significant alteration in the tissues (liver, heart and kidney) total cholesterol concentrations as compared to the control group of animals. High dietary cholesterol induced significant (p<0.01) increase in liver total cholesterol concentrations. The dates-diet supplementation caused further increase in total cholesterol concentrations of liver and kidney tissues but those were not statistically significant (p>0.05). However, heart total cholesterol levels were significantly (p<0.01) increased by dates-diet supplementation when it compared to high cholesterol-diet fed animals. Drinking gahwa either with dates or with hyperlipidemic diet caused no significant change in total cholesterol levels in liver, heart and kidney, respectively (Table 3).

Table 1: Effects of dates, gahwa (Arabic coffee) and cholesterol in diets alone or in combination with each other on body and organs weights (Mean±SE)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight increased (g)</th>
<th>Organ weights (g/100 g body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Chow)</td>
<td>38.78±2.26</td>
<td>2.58±0.23  0.35±0.03  0.65±0.04</td>
</tr>
<tr>
<td>Dates-diet</td>
<td>35.24±2.16</td>
<td>2.60±0.18  0.37±0.02  0.62±0.03</td>
</tr>
<tr>
<td>Dates-diet with gahwa</td>
<td>37.92±2.74</td>
<td>3.11±0.26  0.35±0.02  0.69±0.04</td>
</tr>
<tr>
<td>High cholesterol-diet</td>
<td>48.75±3.64*</td>
<td>5.69±0.52** 0.31±0.02  0.81±0.05*</td>
</tr>
<tr>
<td>Dates-diet + cholesterol</td>
<td>39.13±2.32*</td>
<td>5.47±0.28  0.38±0.03  0.68±0.03*</td>
</tr>
<tr>
<td>Dates-diet + cholesterol with gahwa</td>
<td>36.46±3.13*</td>
<td>6.09±0.49  0.38±0.03  0.69±0.05*</td>
</tr>
</tbody>
</table>

*p<0.05 and **p<0.001 Student's t-test, Groups 2, 3 and 4 were compared with group 1, Groups 5 and 6 were compared with group 4, Six animals were used in each group

Table 2: Effects of dates, gahwa (Arabic coffee) and cholesterol in diets alone or in combination with each other on plasma lipids including total cholesterol, TC, HDL and LDL concentrations (Mean±SE)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cholesterol (mg dl⁻¹)</th>
<th>Triglycerides (mg dl⁻¹)</th>
<th>HDL (mg dl⁻¹)</th>
<th>LDL (mg dl⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Chow)</td>
<td>109.70±11.83</td>
<td>95.90±10.26</td>
<td>60.20±5.71</td>
<td>16.90±4.45</td>
</tr>
<tr>
<td>Dates-diet</td>
<td>97.30±14.08</td>
<td>68.40±10.49</td>
<td>65.40±2.22</td>
<td>10.60±1.79</td>
</tr>
<tr>
<td>Dates-diet with gahwa</td>
<td>68.40±9.47</td>
<td>74.30±8.45</td>
<td>54.70±10.00</td>
<td>13.10±3.88</td>
</tr>
<tr>
<td>High cholesterol-diet</td>
<td>80.90±20.15***</td>
<td>210.90±3.80***</td>
<td>105.60±17.59*</td>
<td>61.50±9.47**</td>
</tr>
<tr>
<td>Dates-diet + cholesterol</td>
<td>240.80±22.25*</td>
<td>139.90±22.78*</td>
<td>126.80±37.56</td>
<td>35.20±8.33</td>
</tr>
<tr>
<td>Dates-diet + cholesterol with gahwa</td>
<td>272.80±29.84</td>
<td>231.10±27.16</td>
<td>126.80±37.56</td>
<td>35.20±8.33</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01 and ***p<0.001 Student's t-test, Groups 2, 3 and 4 were compared with group 1, Groups 5 and 6 were compared with group 4, Six animals were used in each group

Table 3: Effects of dates, gahwa (Arabic coffee) and cholesterol in diets alone or in combination with each other on tissues total cholesterol concentrations (Mean±SE)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver (mg/100mg)</th>
<th>Heart (mg/100mg)</th>
<th>Kidney (mg/100mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Chow)</td>
<td>85.32±12.04</td>
<td>17.79±0.65</td>
<td>92.47±23.72</td>
</tr>
<tr>
<td>Dates-diet</td>
<td>76.76±23.15</td>
<td>19.38±1.22</td>
<td>71.57±14.53</td>
</tr>
<tr>
<td>Dates-diet with gahwa</td>
<td>90.96±22.78</td>
<td>16.12±0.37</td>
<td>79.87±23.92</td>
</tr>
<tr>
<td>High cholesterol-diet</td>
<td>258.09±39.64***</td>
<td>22.47±1.39*</td>
<td>107.49±18.57</td>
</tr>
<tr>
<td>Dates-diet + cholesterol</td>
<td>276.04±44.78</td>
<td>28.21±0.77**</td>
<td>124.58±14.86</td>
</tr>
<tr>
<td>Dates-diet + cholesterol with gahwa</td>
<td>260.20±35.60</td>
<td>24.36±2.69</td>
<td>94.48±21.02</td>
</tr>
</tbody>
</table>

*p<0.05 and **p<0.01 Student's t-test, Groups 2, 3 and 4 were compared with group 1, Groups 5 and 6 were compared with group 4, Six animals were used in each group
Table 4: Effects of dates, gahwa (Arabic coffee) and cholesterol in diets alone or in combination with each other on tissues triglycerides levels (Mean±SE)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver (mg/100g)</th>
<th>Heart (mg/100g)</th>
<th>Kidney (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Chow)</td>
<td>102.0±10.33</td>
<td>25.2±3.14</td>
<td>122.5±10.98</td>
</tr>
<tr>
<td>Dates-diet</td>
<td>97.4±12.61</td>
<td>25.3±3.79</td>
<td>117.0±4.99</td>
</tr>
<tr>
<td>Dates-diet with gahwa</td>
<td>130.7±21.84</td>
<td>28.6±5.29</td>
<td>141.5±12.70</td>
</tr>
<tr>
<td>High cholesterol-diet</td>
<td>181.4±13.72**</td>
<td>41.2±4.02**</td>
<td>165.3±12.37*</td>
</tr>
<tr>
<td>Dates-diet + cholesterol</td>
<td>254.6±26.21*</td>
<td>42.8±2.82</td>
<td>154.4±10.86</td>
</tr>
<tr>
<td>Dates-diet + cholesterol with gahwa</td>
<td>243.8±17.76*</td>
<td>45.4±2.14</td>
<td>130.8±16.21</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01 and ***p<0.001 Student's t-test. Groups 2, 3 and 4 were compared with group 1, Groups 5 and 6 were compared with group 4. Six animals were used in each group.

Supplementation with high cholesterol-diet caused significant increase in liver (p<0.001), heart (p<0.01) and kidney (p<0.05) triglycerides levels compared to the control group of animals. Dates in high cholesterol-diet caused further significant increase in liver triglycerides concentrations. However, such increases in liver triglycerides were not seen in the group of animals supplemented with dates and gahwa along with high cholesterol-diet (Table 4).

**DISCUSSION**

The customary diet of a population is one of the most important factors determining susceptibility to heart disease, through its effects on lipid metabolism. The simplest and clearest measures of this dietary effect are the total cholesterol and triglyceride concentrations in serum and tissues (Shaper, 1987). Golden Syrian hamster is a small animal model that is frequently used for studying cholesterol metabolism and atherosclerosis (Martellino et al., 2006; Sohn et al., 1999). The rate of cholesterol synthesis in the hamsters can be easily altered in response to changes in cholesterol intake and the plasma lipoprotein to dietary changes makes the hamster more comparable to humans. Thus, the present study designed to investigate the effect of Saudi traditional serve (gahwa and dates) on the cholesterol metabolism in the hamsters fed a hypercholesterolemic diet.

It is well known that high dietary cholesterol increases the body weights (Vasu et al., 2005), total cholesterol, triglycerides and lipoprotein levels in blood and tissues (Valcheva-Kuzmanova et al., 2006; Al-Kanhal et al., 1994) and that elevated lipids levels constitute the major risk for atherosclerosis (Fki et al., 2005; Smith et al., 2004). The rise in plasma cholesterol caused by supplemented cholesterol also observed in the present study. This rise presumably could be due to the limited capacity of the liver to respond to changes to dietary cholesterol addition (Spady and Dietschy, 1985). Since the rate of sterol synthesis by the liver in hamsters has shown to be disproportionately low compared to rats, the liver of the hamster cannot rapidly adapt to dietary changes and thus alters the rate of LDL cholesterol transport (Spady et al., 1983). In the present study, plasma HDL concentrations were significantly increased in high cholesterol-diet fed hamsters as compared to controls. This rise may be due to alteration in composition of HDL which reduces the typical protein-rich HDL and an increase in larger more cholesterol-rich lipoprotein called HDLe. However, dates inclusion in high cholesterol-diet showed further elevation in plasma HDL levels but not statistically significant. It has properties both like HDL and LDL and gets enriched with cholesterol during high cholesterol feeding (Mahley et al., 1978).

In the present study, date consumption produced a hypolipidemic effect in hamsters but was not significant as compared to controls. A similar significant effect was reported in rats (Al-Orf, 1992) after feeding a date diet for 17 weeks. The insignificance of the present results could be due to shorter experimental duration. However, the results of the present study provide further evidence that date fed with a high cholesterol diet, significantly decreased the plasma cholesterol, triglycerides and LDL concentrations as compared to cholesterol diet alone fed animals. The significance of both LDL and HDL concentrations in affecting CHD risk is well established. Control clinical trials have shown that a 1% reduction in total and LDL-cholesterol concentrations resulted in almost 1.5% decreases in the incidence of CHD (American Academy of Pediatrics, 1992). The reduction in plasma triglycerides and cholesterol by the addition of date to the diet of hamsters is most likely mediated by its inhibition of absorption of dietary fat, cholesterol and bile acids similar to the hypolipidemic effect of green tea epicatechin (Chan et al., 1999). Recently date fruits are been proven as antioxidants, antimutagenic, anticancer, anti-gastric ulcer and as immunostimulants experimentally (Vayali, 2002; Al-Farsi et al., 2005; Al-Qarawi et al., 2005; Puri et al., 2000). Further studies required to evaluate the hypoglycemic effect of date fruits.

Coffee is a major source of caffeine, which stimulates fat oxidation in muscle (Spriet et al., 1992) and increases basal energy expenditure (Astrup et al., 1990). Coffee-boiling causes increase in extraction of hypercholesterolemic lipid factor from coffee, which powerfully raises the serum cholesterol level
(Dusseldorf et al., 1991; Zock et al., 1990; Urgert et al., 1995; Al-Kahhal et al., 1994; Al-Kahhal, 1996). In the present study, gahwa alone group was not taken, it was supplemented with date-diet and further it combined with date and high cholesterol-diet so that the exact effect of gahwa was not seen. However in both the groups no significant changes were seen as compared to controls and cholesterol-diet alone fed animals respectively. These results are in agreement with an earlier study performed in hypercholesterolemic diet supplemented rats (Al-Kahhal et al., 1994).

The date’s addition to the cholesterol-diet caused significant increase in liver total cholesterol and triglycerides levels as compared to group supplemented with cholesterol-diet alone. This increase mechanism is not clearly known. However, fructose feeding had shown to stimulate the hepatic triglyceride by promoting the esterification of circulating non-esterified fatty acids and by stimulating the de novo synthesis of fatty acids (Southgate, 1999; Park et al., 1992) and facilitating triglyceride production in hamsters (Kasim-Karakas et al., 1996).

In conclusion, dates supplementation showed hypolipidemic effect on hypercholesterolemic hamsters by reducing the plasma total cholesterol, triglycerides and LDL levels. Remarkably, plasma HDL levels increased in the hypercholesterolemic hamsters supplemented with dates. It has been reported that the lipid metabolism in hamsters is more similar to that of human beings. Therefore, these data suggest that date fruits have potential to control the risk of atherosclerosis development in humans. Further investigations required to know more about date’s hypolipidemic effects on lipid metabolism.

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