

## Original Article

# Preventive action of food seasoning spices mixture on fructose-induced lipid abnormalities

Ramu Suganthi MSc,<sup>1</sup> Shanmuganathan Rajamani MSc,<sup>1</sup> Mambakkam Katchapeswaran Ravichandran MSc,MPhil,PhD<sup>2</sup> and Carani Venkatraman Anuradha MSc, MPhil, PhD<sup>1</sup>

<sup>1</sup>Department of Biochemistry, Faculty of Science, Annamalai University, Annamalai Nagar, Tamil Nadu, India

<sup>2</sup>Department of Statistics, Faculty of Science, Annamalai University, Annamalai Nagar, Tamil Nadu, India

High fructose feeding in rats induces insulin resistance, hyperinsulinemia, hyperglycemia and dyslipidemia. The present study was undertaken to determine the hypolipidemic effect of food seasoning spices mixture on fructose-fed insulin resistant rats. Male Wistar rats received a daily diet containing either 60% fructose or 60% starch. They were administered with the spices mixture at three different doses (10mg, 30mg or 50mg/day/rat) orally 15 days later. At the end of 45 days of the experimental period fructose-fed rats displayed elevated plasma glucose and insulin levels and dyslipidemia which included elevated levels of cholesterol, triglycerides, free fatty acids, reduced high density lipoprotein cholesterol and increased very low density lipoprotein cholesterol. Alterations in tissue lipid levels were also observed. Simultaneous treatment with spices mixture along with fructose diet resulted in the normalization of plasma glucose and insulin levels and restoration of lipid levels in plasma and tissues. The insulin potentiating action of the active principles in these spices may contribute to the hypolipidemic effect of spices mixture in high fructose-fed rats.

**Key Words:** fructose, spices, cinnamon, cloves, fenugreek, cumin, cardamom, mace, garam masala, black pepper, ginger, nutmeg, insulin resistance, glucose, lipids

## Introduction

Rats fed high dosage of fructose in diet (60g/100g diet) form a useful model of the multi-metabolic syndrome or syndrome X, a clinical condition which involves a cluster of abnormalities such as insulin resistance, hyperinsulinemia, glucose intolerance, dyslipidemia and hypertension.<sup>1</sup> The insulin resistance in fructose-fed rats is associated with the defects in insulin signalling pathways.<sup>2</sup> The sites of fructose-induced insulin resistance are documented to be the liver, skeletal muscle<sup>3</sup> and adipose tissue.<sup>4</sup>

The dyslipidemia observed in high fructose-fed rats include elevated triglycerides (TG), free fatty acids (FFA) and lipoprotein abnormalities.<sup>5</sup> These alterations are secondary to the development of insulin resistance.<sup>1</sup> Spices mixture (SM) is a food-seasoning item commonly used in Indian homes and in several oriental countries. Spices have been used traditionally as flavouring agents. Most of the spices are reported to possess antioxidant, anti-inflammatory and antitumour properties.<sup>6</sup>

Recent studies have shown that the active constituents of some of these spices, in particular, cinnamon, bay leaf, clove and fenugreek seeds have insulin potentiating effects.<sup>7</sup> Agents that improve insulin action could also have beneficial effects on lipid metabolism. Based on the foregoing observations, the present study was undertaken to investigate the hypolipidemic effect of food seasoning SM in high fructose-fed rats, which form an acquired model of insulin resistance. The results obtained are compared with those obtained from untreated fructose-fed rats.

We have chosen to use the SM instead of individual spices owing to the wide spread use of SM commonly known as "Garam masala" in food preparation.

## Materials and methods

### Materials

The spices were purchased from the local market at Chidambaram. The authenticity of the samples was identified by Dr B Vembu, Professor, Department of Botany, Annamalai University. In our formulation we have chosen the following spices: 0.5g of black pepper (fruit of *Piper nigrum*), 1.0g of cumin seeds (seeds of *Cuminum cyminum*), 0.5g of cardamom (seeds of *Elettaria cardamomum*), 1.0g of cinnamon (bark of *Cinnamomum zeylanicum*), 1.0g of dry ginger (rhizome of *Zingiber officinale*), 0.5g of nutmeg (kernel of *Myristica fragrans*), 0.5g of mace (aril of *Myristica fragrans*), 0.5g of clove (flower bud of *Eugenia caryophyllatae*), 0.5g of Indian bay leaf (leaf of *Cinnamomum tamala*) and 1.0g of fenugreek seeds (seeds of *Trigonella foenum graecum*). The active constituents of spices used in preparation of SM are given in Table 1. These ingredients were ground into a smooth

**Correspondence address:** Dr.C.V. Anuradha Reader, Department of Biochemistry, Faculty of Science, Annamalai University, Annamalai Nagar-608 002, Tamil Nadu, India  
Fax: +91 04144 238343  
Email: cvaradha@hotmail.com  
Accepted 30th April 2005

powder in an electric grinder. A suspension was prepared in tap water. Preparations were made just before administration. Animals received these preparations through oral gavage.

#### Animals and experimental diet

Adult male Wistar albino rats weighing 150-170g were obtained from the Central Animal House, Rajah Muthiah Medical College, Annamalai University, Annamalai Nagar, India. They were housed in the animal room under controlled conditions on a 12h light - 12h dark cycle. They received a standard pellet diet (Karnataka State Agro Corporation, Agro Feeds Division, Bangalore, India) and water ad libitum. The animals used in the study were cared for according to the principles and guidelines of the Institutional Animal Ethics Committee and all procedures were cleared by the committee.

After a week of acclimatization, the animals were divided into two batches. One batch received a control diet containing starch as the source of carbohydrate while the other was fed with fructose-enriched diet for 15 days. The composition of the control and fructose diets is given in the Table 2. The diets were prepared fresh everyday. Treatment with SM was initiated on the 16<sup>th</sup> day and continued for the next 30 days. The following six experimental groups consisting of six rats each were maintained for a total experimental period of 45 days.

**Table 2.** Composition of diets (g/100g)

Ingredients	Control diet	High fructose diet
Corn starch	60	-
Fructose	-	60
Casein	20	20
Methionine	0.7	0.7
Groundnut oil	5	5
Wheat bran	10.6	10.6
Salt mixture <sup>†</sup>	3.5	3.5
Vitamin mixture <sup>‡</sup>	0.2	0.2

<sup>†</sup>The composition of mineral mix (g/kg)- MgSO<sub>4</sub>.7H<sub>2</sub>O, 30.5; NaCl, 65.2; KCl, 105.7; KH<sub>2</sub>PO<sub>4</sub>, 200.2; MgCO<sub>3</sub>, 3.65; Mg(OH)<sub>2</sub>. 3H<sub>2</sub>O, 38.8; FeC<sub>6</sub>H<sub>5</sub>O<sub>7</sub>. 5H<sub>2</sub>O, 40.0; CaCO<sub>3</sub>, 512.4; KI, 0.8; NaF, 0.9; CuSO<sub>4</sub>.5H<sub>2</sub>O, 1.4; MnSO<sub>4</sub>. 0.4 and CONH<sub>3</sub>. 0.05. <sup>‡</sup>One kg 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. 5mg; vitamin A acetate, 0.6g;  $\alpha$ -tocopherol acetate, 25g and choline chloride, 10g.

#### Experimental groups

##### Group - 1 (CON)

Control animals received control diet and tap water ad libitum for 45 days.

##### Group - 2 (FRU)

Fructose-fed rats received high fructose diet and tap water ad libitum for 45 days.

**Table 1.** The active constituents of spices used in the preparation of spices mixture

Spices	Botanical name	Principle active components
Dry ginger (rhizome)	<i>Zingiber officinale</i>	<i>d</i> -Camphene, $\beta$ -phellandrene, cineole, zingiberene, citral, gingerol, shogaol, zingerone
Cardamom (seeds)	<i>Elettaria cardamomum</i>	1,8-Cineol, $\alpha$ -teripinyl acetate, limonene, $\gamma$ -terpineol, $\alpha$ -pinene, $\beta$ -pinene, geraniol
Cinnamon (bark)	<i>Cinnamomum zeylanicum</i>	Cinnamic aldehyde, phellandrene, pinene, linalool, caryophyllene, eugenol
Clove (buds)	<i>Eugenia caryophyllatae</i>	Eugenol, esters, ketones, sesquiterpenes, alcohols
Cumin (seeds)	<i>Cuminum cyminum</i>	Cuminaldehyde, <i>p</i> -cymene, limonene, cuminyl ester
Black pepper (fruit)	<i>Piper nigrum</i>	Pipernol, chavicine, piperidine, piperetine, limonene
Bay leaf (leaf)	<i>Cinnamomum tamala</i>	<i>d</i> - $\alpha$ -Phellandrene, eugenol
Nutmeg (fruit)	<i>Myristica fragrans</i>	Myristicin, eugenol, <i>d</i> -pinene, <i>d</i> -camphene, isoeugenol, $\alpha$ -pinene, geraniol, safrole, myristic acid, dipentene, <i>p</i> -cymene
Fenugreek (seeds)	<i>Trigonella foenum graecum</i>	Vitexin, tricrin, naringenin, quercetin & tricrin-7-0-beta-D-glucopyranoside, trigonelline & coumarine
Mace (aril)	<i>Myristica fragrans</i>	Myristicin, eugenol, isoeugenol, <i>d</i> -camphene, <i>d</i> -pinene, dipentene, <i>p</i> -cymene, myristic acid, geraniol, safrole

**Table 3.** Initial and final body weights of control and experimental animals

Parameters	CON	FRU	FRU + SM1	FRU + SM2	FRU + SM3	CON + SM3
Initial weight (g)	153.33 ± 7.60	153.83 ± 3.92	156.50 ± 5.47	154.50 ± 3.61	152.66 ± 2.80	155.50 ± 6.22
Final weight (g)	198.50 ± 5.20	202.83 ± 7.49	202.83 ± 5.11	198.67 ± 7.92	195.00 ± 8.94	197.50 ± 5.24

Values are expressed as means ± SD, N = 6

#### Group – 3 (FRU+SM1)

Fructose-fed rats received high fructose diet and tap water ad libitum for 45 days. SM (10mg/day/rat) was administered from the 16<sup>th</sup> day of fructose feeding.

#### Group – 4 (FRU+SM2)

Fructose-fed rats received fructose diet and water ad libitum for 45 days. SM treatment (30mg/day/rat) was started from the 16<sup>th</sup> day of the experimental period.

#### Group – 5 (FRU+SM3)

Fructose-fed rats received fructose diet and water ad libitum for 45 days. SM (50mg/day/rat) was given from the 16<sup>th</sup> day of fructose feeding.

#### Group – 6 (CON+SM3)

Control animals received control diet and water ad libitum for 45 days. SM (50mg/day/rat) was initiated from the 16<sup>th</sup> day of the experimental period.

### Biochemical Analysis

At the end of 45 days the animals were sacrificed by cervical decapitation. Fasting blood samples were collected in heparinised tubes. Plasma was separated by centrifugation at 1000xg for 10 minutes. Glucose was estimated by Sasaki *et al.*<sup>8</sup> Plasma insulin was determined by ELISA method using UBI MAGIWEL kit obtained from California, USA and was expressed in  $\mu$ U/ml. Homeostasis model assessment (HOMA) was used as an index to measure the degree of insulin resistance, and was calculated by the formula: [insulin ( $\mu$ U/ml) x glucose (mmol/L)/22.5].<sup>9</sup> The extraction of plasma lipids was carried out according to the procedure of Folch *et al.*<sup>10</sup> Total cholesterol was estimated by Zlatki *et al.*,<sup>11</sup> phospholipids by Zilversmit and Davis,<sup>12</sup> free fatty acids by Falholt *et al.*,<sup>13</sup> and triglycerides by Foster and Dunn.<sup>14</sup> Cholesterol in the lipoprotein fractions was also determined. HDL-cholesterol (HDL-C) was analysed in the supernatant obtained after precipitation of plasma with phosphotungstic acid/Mg<sup>2+</sup>. LDL – cholesterol (LDL-C) and VLDL-cholesterol (VLDL-C) were calculated as follows:

$$\begin{aligned} \text{VLDL-C} &= \text{Triglycerides} / 5 \\ \text{LDL-C} &= \text{Total cholesterol} - (\text{HDL-C} + \text{VLDL-C}) \end{aligned}$$

### Statistical Analysis

Values are expressed as mean ± SD. Data within the groups were analysed using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). A value of  $P < 0.05$  was considered statistically significant.

## Results

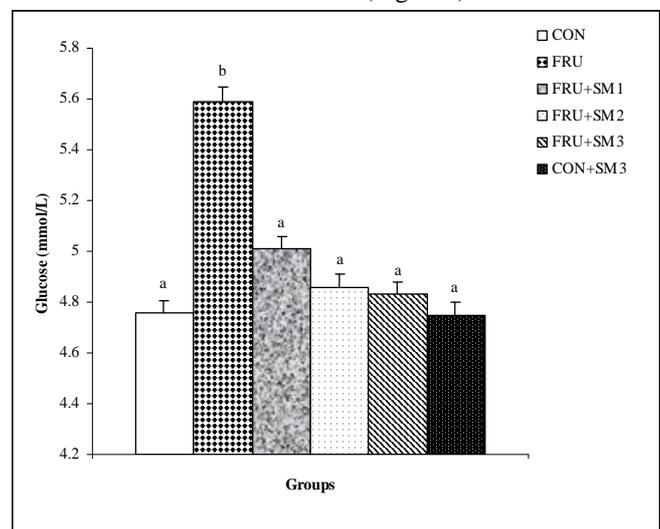
### Effect of SM on body weight

The initial and final body weights of the rats during the experimental period of 45 days are given in Table 3. The

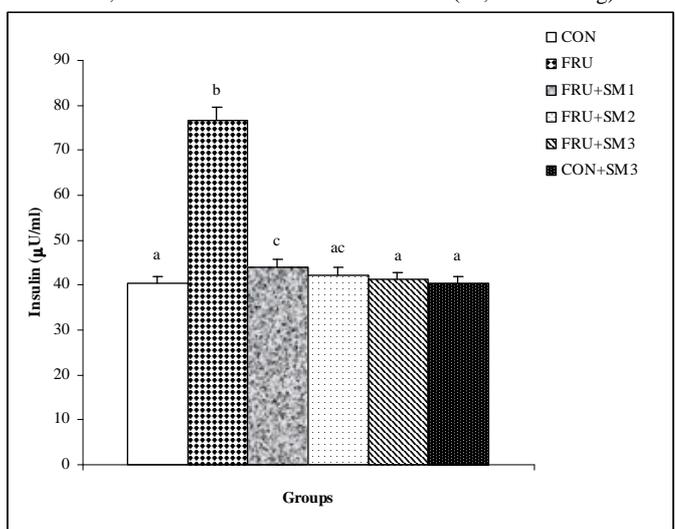
body weights of the animals increased progressively during the experimental period. There was a trend for the fructose animals to gain more weight than other rats. However the weight gain was not significantly different as compared with those of normal rats. Administration of SM did not significantly alter the final body weights of animals.

### Effect of SM on plasma insulin and glucose levels

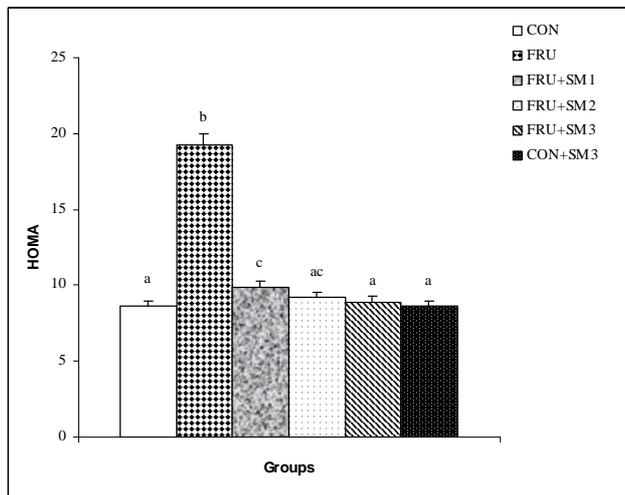
Fructose-fed rats showed significantly higher glucose and insulin levels in plasma and HOMA values as compared to control rats. On administration of SM the levels were decreased and were near-normal (Fig. 1-3).



**Figure 1.** Fasting plasma glucose concentration in control and experimental rats. Values are means ± SD of six animals. Values not sharing common superscript differ significant from each other at ( $P < 0.05$ ), ANOVA followed by DMRT. CON- Control; FRU- Fructose; SM- Spices mixture 1,2 and 3 refer to concentration of SM (10,30 and 50mg).



**Figure 2.** Fasting plasma insulin concentration in control and experimental rats. Values are means ± SD of six animals. Values not sharing common superscript differ significant from each other at ( $P < 0.05$ ), ANOVA followed by DMRT. CON- Control; FRU- Fructose; SM- Spices mixture 1,2 and 3 refer to concentration of SM (10,30 and 50mg).



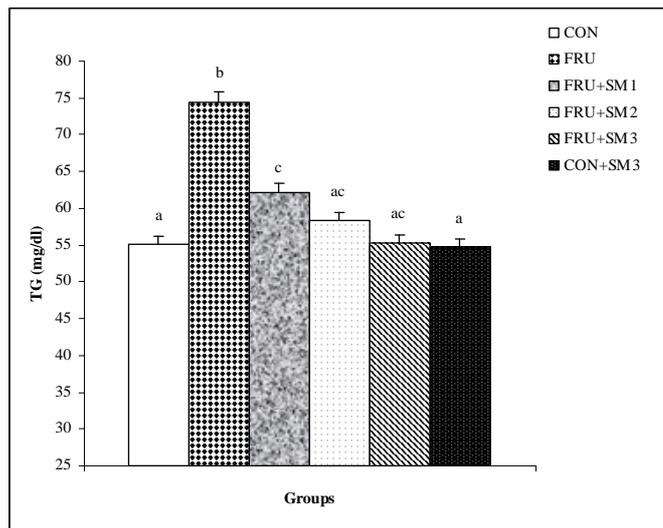
**Figure 3.** HOMA values in control and experimental rats. Values are means ± SD of six animals. Values not sharing common superscript differ significant from each other at ( $P < 0.05$ ), ANOVA followed by DMRT. CON- Control; FRU- Fructose; SM- Spices mixture 1,2 and 3 refer to concentration of SM (10,30 and 50mg). HOMA- Homeostatic model assessment (Insulin ( $\mu$  U/ml) x glucose (mmol/L) /22.5)

**Effect of SM on plasma lipids**

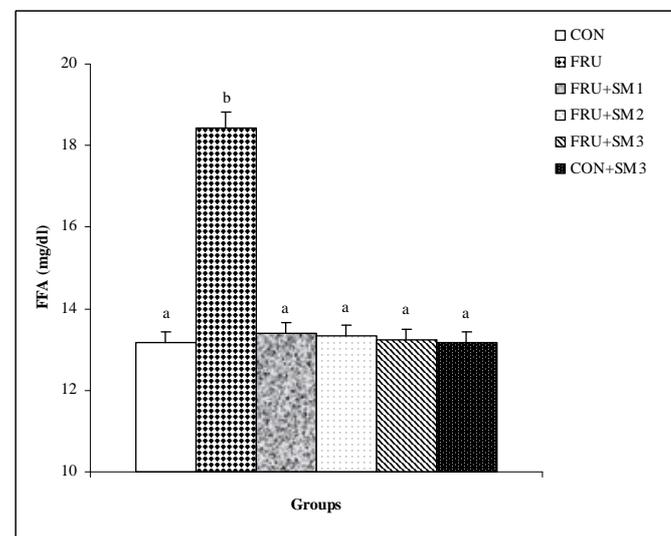
The concentration of plasma lipids in control and experimental animals are given in Figures 4,5,6 and 7. Fructose-fed rats had elevated levels of cholesterol in plasma as compared to control rats. Triglycerides are significantly elevated ( $P < 0.05$ ) in the FRU rats as compared to CON rats while FRU+SM rats showed a significant reduction ( $P < 0.05$ ) in cholesterol and TG levels as compared to FRU rats. FFA concentration was also elevated in FRU as compared to CON and was near normal after SM treatment. Phospholipid concentrations were elevated in the fructose groups as compared to control rats. SM supplementation significantly reduced the phospholipid level in fructose-fed rats.

**Effect of SM on cholesterol in lipoprotein fractions**

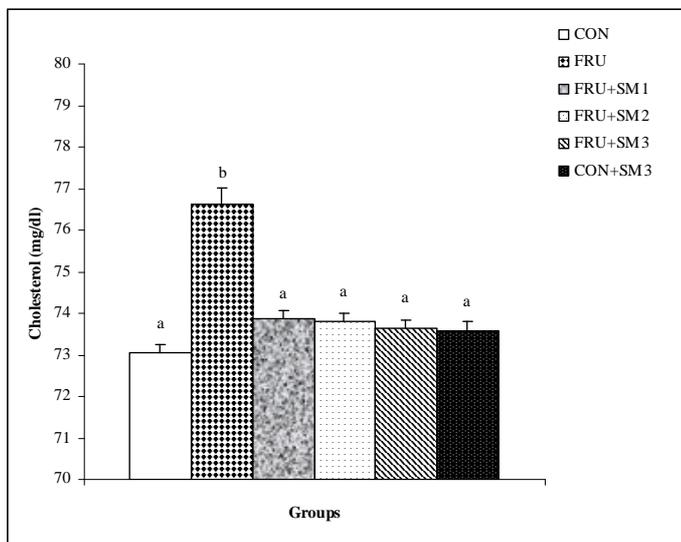
Concentration of plasma total cholesterol and that in lipoprotein fractions is given in Table 4. Significant increases in VLDL-C and LDL-C concentrations and a



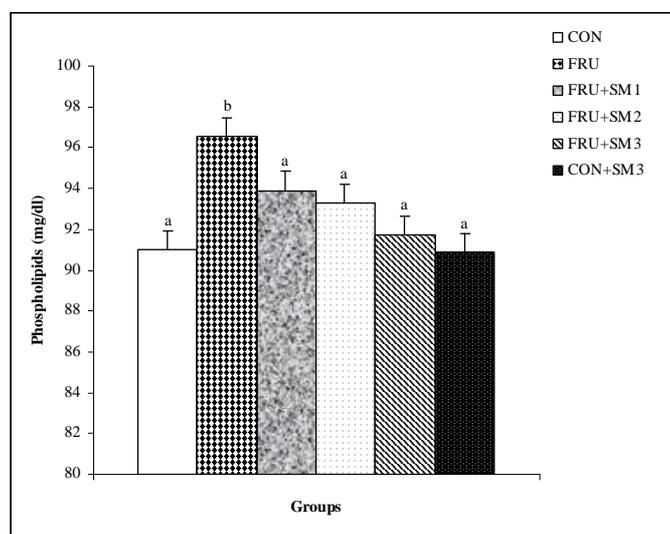
**Figure 5.** Concentration of TG (Triglycerides) in plasma of control and experimental rats. Values are means ± SD of six animals. Values not sharing common superscript differ significant from each other at ( $P < 0.05$ ), ANOVA followed by DMRT. CON- Control; FRU- Fructose; SM-Spices mixture 1,2 and 3 refer to concentration of SM (10,30 and 50mg).



**Figure 6.** Concentration of FFA (Free fatty acids) in plasma of control and experimental rats. Values are means ± SD of six animals. Values not sharing common superscript differ significant from each other at ( $P < 0.05$ ), ANOVA followed by DMRT. CON- Control; FRU- Fructose; SM- Spices mixture 1,2 and 3 refer to concentration of SM (10,30 and 50mg).



**Figure 4.** Concentration of cholesterol in plasma of control and experimental rats. Values are means ± SD of six animals. Values not sharing common superscript differ significant from each other at ( $P < 0.05$ ), ANOVA followed by DMRT. CON- Control; FRU- Fructose; SM- Spices mixture 1,2 and 3 refer to concentration of SM (10,30 and 50mg).



**Figure 7.** Concentration of phospholipids in plasma of control and experimental rats. Values are means ± SD of six animals. Values not sharing common superscript differ significant from each other at ( $P < 0.05$ ), ANOVA followed by DMRT. CON- Control; FRU- Fructose; SM- Spices mixture 1,2 and 3 refer to concentration of SM (10,30 and 50mg).

**Table 4.** Distribution of cholesterol in the lipoprotein fractions of control and experimental animals (values expressed as mg/dl)

Parameters	CON	FRU	FRU + SM1	FRU + SM2	FRU + SM3	CON + SM3
Total cholesterol	73.06 ± 2.25 <sup>a</sup>	76.63 ± 2.48 <sup>b</sup>	73.86 ± 1.37 <sup>a</sup>	73.79 ± 2.29 <sup>a</sup>	73.64 ± 1.29 <sup>a</sup>	73.59 ± 2.57 <sup>a</sup>
HDL-cholesterol	35.53 ± 2.44 <sup>a</sup>	30.55 ± 1.54 <sup>b</sup>	32.54 ± 1.87 <sup>c</sup>	35.22 ± 1.35 <sup>a</sup>	35.47 ± 1.87 <sup>a</sup>	35.78 ± 1.77 <sup>a</sup>
LDL-cholesterol	27.24 ± 2.48 <sup>ac</sup>	33.81 ± 1.42 <sup>b</sup>	28.98 ± 1.61 <sup>c</sup>	27.71 ± 2.28 <sup>ac</sup>	27.52 ± 0.62 <sup>ac</sup>	25.45 ± 2.18 <sup>a</sup>
VLDL-cholesterol	11.28 ± 0.75 <sup>a</sup>	13.19 ± 0.38 <sup>b</sup>	12.38 ± 0.25 <sup>c</sup>	11.68 ± 0.75 <sup>a</sup>	11.15 ± 0.61 <sup>a</sup>	10.95 ± 0.55 <sup>a</sup>

Values are expressed as means ± SD, n = 6 Values not sharing common superscript letter differ significantly from each other at  $P < 0.05$  (DMRT)

**Table 5.** Concentrations of lipids in liver of control and experimental animals. Values are expressed as mg/g wet

Parameters	CON	FRU	FRU + SM1	FRU + SM2	FRU + SM3	CON + SM3
Cholesterol	4.73 ± 0.14 <sup>a</sup>	5.66 ± 0.41 <sup>b</sup>	4.83 ± 0.35 <sup>a</sup>	4.78 ± 0.36 <sup>a</sup>	4.75 ± 0.15 <sup>a</sup>	4.73 ± 0.28 <sup>a</sup>
Triglycerides	5.13 ± 0.22 <sup>ac</sup>	5.92 ± 0.35 <sup>b</sup>	5.43 ± 0.08 <sup>c</sup>	5.23 ± 0.17 <sup>ac</sup>	5.16 ± 0.06 <sup>ac</sup>	5.13 ± 0.34 <sup>a</sup>
Free fatty acids	4.33 ± 0.25 <sup>a</sup>	10.44 ± 0.71 <sup>b</sup>	4.61 ± 0.27 <sup>a</sup>	4.55 ± 0.43 <sup>a</sup>	4.41 ± 0.20 <sup>a</sup>	4.35 ± 0.19 <sup>a</sup>
Phospholipid	18.47 ± 1.49 <sup>a</sup>	15.13 ± 1.22 <sup>b</sup>	17.42 ± 0.75 <sup>a</sup>	17.85 ± 0.84 <sup>a</sup>	18.03 ± 0.60 <sup>a</sup>	18.30 ± 1.77 <sup>a</sup>

Values are expressed as means ± SD, n = 6 Values not sharing common superscript letter differ significantly from each other at  $P < 0.05$  (DMRT)

decrease in HDL-C were observed in fructose-fed rats. In SM supplemented fructose-fed rats LDL-C and VLDL-C were lower while HDL-C was higher as compared to fructose-fed rats.

#### Effect of SM on liver lipids

Concentration of lipids in liver of control and experimental animals are given in Table 5. Cholesterol concentrations were elevated in FRU group as compared to CON rats. TG and FFA concentrations were significantly increased ( $P < 0.05$ ) in fructose-fed rats as compared to normal rats. SM supplementation to the fructose-fed rats resulted in significant decrease ( $P < 0.05$ ) in TG, cholesterol and FFA as compared to fructose-fed rats. The concentrations were restored to near normal values. The phospholipid concentration was significantly decreased ( $P < 0.05$ ) in fructose-fed rats as compared to that of control rats. SM administration at all three doses to fructose-fed rats brought the concentrations of lipid constituents in liver to near normal. The levels remained unaltered in control rats treated with SM.

SM was effective in controlling hyperglycemia and hyperlipidemia at all three doses tested. A dose-dependent effect was observed in certain parameters namely glucose, insulin, phospholipids and TG in plasma and cholesterol, FFA and TG in liver. In rest of the parameters the dose-dependent effect was not observed.

#### Discussion

Many studies have reported that fructose administration can have profound the effects on plasma and tissue lipids levels.<sup>15,16</sup> Michaelis *et al.*,<sup>15</sup> described an increase in total liver lipids in rats when glucose was isocalorically substituted by either sucrose or fructose. This effect was attributed to the induction of various lipogenic enzymes in liver by fructose.

Fructose feeding may lead to hypertriglyceridemia by increasing the formation of glycerol-3-phosphate, a precursor 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. Plasma lipoprotein lipase activity was reported to be lower in high fructose-fed rats.<sup>17</sup> The elevated TG concentration may be associated with impaired insulin action. Bieger *et al.*,<sup>18</sup> have shown that an increase in blood TG concentration can reduce the number of insulin receptors thereby reducing insulin sensitivity. A causative link between elevated circulating TG and impaired insulin action was observed in fructose-fed rats by Thorburn *et al.*<sup>16</sup>

Insulin has a regulatory effect of on FFA metabolism. A defect in the ability of insulin to regulate the FFA metabolism could contribute to increased FFA levels in fructose - fed rats. Elevated concentration of plasma FFA may play a key role in the pathogenesis of type 2 diabetes, by impairing peripheral glucose utilization and by promoting hepatic glucose overproduction.<sup>19</sup>

Cellular and membrane phospholipids are the major targets of damaging free radicals and therefore depletion of phospholipids in liver of high fructose-fed rats could attributed to oxidative stress.<sup>20</sup> Studies from our laboratory have shown that fructose facilitates oxidative damage in tissues.<sup>21</sup> Further a positive correlation between the levels of lipid peroxidation products and insulin resistance has been documented.<sup>22</sup>

Botanical products that show antihyperglycemic effect have a positive influence on lipid metabolism. This is due to the inter-relationship between metabolism of glucose and lipid and to the regulatory influences of insulin on both the metabolic pathways. Some spices especially fenugreek seeds,<sup>23,24</sup> clove,<sup>25</sup> cumin seeds,<sup>26</sup> cinnamon<sup>27</sup> and bay leaf<sup>7</sup> have been found to have both hypolipidemic and hypoglycemic effects.

Increase in blood glucose level associated with hyperinsulinemia in fructose-fed rats suggests impaired insulin action. This is supported by high HOMA values. Administration of SM lowered blood glucose and insulin levels and also reduced HOMA values. Administering SM to insulin resistant rats normalized the levels of lipids in

plasma and liver in the present study. The active principles may act through the insulin potentiating action.

Some of the spices like cinnamon, clove and bay leaf have been reported to have insulin-potentiating effect *in vitro*.<sup>28</sup> Cinnamon potentiates the action of insulin on carbohydrate metabolism more than three fold.<sup>28</sup> In the last decade, *in vitro* studies related that the cinnamon extract mimics the effect of insulin, which potentiates insulin action in isolated adipocytes.<sup>28,29</sup> Cinnamon extract (CE) administration to high fructose diet-fed rats prevented the development of insulin resistance possibly by enhancing insulin signaling pathway in skeletal muscle.<sup>30</sup> The active principle in cinnamon was identified to be methyl hydroxy chalcone polymers (MHCP). Further, Jarvill-Taylor *et al.*,<sup>31</sup> proposed that MHCP is an effective insulin mimetic which activates the pathways leading to glucose utilization in cells.

The hypoglycemic effect of fenugreek is thought to be largely due to its high content of soluble fiber, which acts to decrease the rate of gastric emptying by delaying the absorption of glucose from small intestine.<sup>23</sup> Also, fiber in general (except for cellulose), enhance fecal excretion of bile acid and cholesterol, which could explain in part the hypocholesterolemic properties of fenugreek seeds.<sup>24</sup> Hydroxy isoleucine which represent 80% of the free amino acid in fenugreek seeds may possess insulin stimulating properties.<sup>32</sup> Administration of fenugreek seeds to alloxan-induced diabetic rats produced significant fall in various serum lipids, especially total cholesterol in diabetic rats.<sup>33</sup>

Cinnamon significantly reduced the fasting blood glucose, TG, cholesterol and LDL-C, but did not alter the mean fasting HDL-C level in type 2 diabetic individuals.<sup>27</sup> The hypolipidemic effect of nutmeg in rabbits<sup>34</sup> and of cumin seeds in alloxan-induced diabetic rats have been reported.<sup>26</sup> Treatment of ginger juice to diabetic rats significantly reduced the serum cholesterol, TG and blood pressure in streptozotocin diabetic rats and also significantly reduced the insulin level in blood.<sup>35</sup> Platel *et al.*,<sup>36</sup> reported that the SM containing piperine, ginger and cumin favourably enhanced the activity of pancreatic lipase and stimulated secretory rate of bile acids. Hypolipidemic effect of clove in rats fed high fat diet has been reported.<sup>25</sup> These effects of spices could be responsible for the observed positive influence of SM on lipid metabolism.

Our findings indicate that administration of SM attenuates hyperglycemia and hyperinsulinemia and improves plasma and liver lipid concentrations in insulin resistant rats. Insulin resistance is a contributing factor for type 2 diabetes and is also shown to be a precursor for endothelial dysfunction, hypertension and dyslipidemia ultimately leading to cardiovascular disease.<sup>37</sup> Increased plasma lipid concentration represents a risk factor for CHD. In fact high fructose feeding is reported to induce atherosclerotic changes in the aorta<sup>38</sup> and hypertension<sup>16</sup> in animals. Lowering of plasma lipid levels through diet or drug therapy is associated with a decrease in the risk of vascular disease.

The results of our study are significant considering the high prevalence of insulin resistance in the general population and the increased fructose intake as high fructose

corn syrup. Three doses of spices (10, 30, 50mg/ day/rat) were administered. A clear dose response was not observed in the study. The lack of dose response indicates that the spices are effective at the low dose itself. The lower dose (10mg/day/rat) used in the study corresponds to the human consumption of 4-5g spices mixture/day. It is likely that life style changes especially modern food habits involving high refined sugar low starch and high fat content with low intake of traditional herbs, spices and other plant products could be implicated in the increased incidence of insulin resistance in India. Increasing the consumption of spices could serve as an effective support therapy in the prevention and management of insulin resistance. More research on the effect of spices on the other components of syndrome X and on the insulin-sensitizing effects of active components are certainly needed.

## References

1. Reaven GM. Role of insulin-resistance in human disease. *Diabetes* 1988; 37: 1595-1607.
2. Ueno M, Bezerra RMN, Silva MS, Tavares DQ, Carralho CR, Saad MT. A high-fructose diet induce changes in PP185 phosphorylation in muscle and liver of rats. *Braz J Med Biol Res* 2000; 33: 1421-1427.
3. Bezerra RNM, Ueno M, Silva MS, Tavares DQ, Caravvalho CR, Saad MJA. High fructose diet affects the early steps of insulin action in muscle and liver of rats. *J Nutr* 2000; 130: 1531-1535.
4. Vrana A, Fabry P, Slabochava Z, Kazdova L. Effect of dietary fructose on free fatty acid release from adipose tissue and serum free fatty acid concentration in the rat. *Nutr Metab* 1974; 17: 74-83.
5. Dai S, McNeill, JH. Fructose-induced hypertension in rats is concentration and duration-dependent. *J Pharmacol Toxicol Method* 1995; 33: 101-107.
6. Surh YJ. Anti-tumour promoting potential of selected spice ingredients with antioxidative and anti-inflammatory activities: a short review. *Food Chem Toxicol* 2002; 40: 1091-1097.
7. Khan A, Safdar M. Role of Diet, Nutrients, Spices and Natural products in Diabetes mellitus. *Pak J Nutr* 2003; 2: 1-12.
8. Sasaki T, Matsui S, Sonae A. Effect of acetic acid concentration on the colour reaction in the O-toluidine-boric acid method for blood glucose estimation. *Rinshokagaku* 1972; 1: 346-353.
9. Pickavance LC, Tadayyon M, Widdowson PS, Buckingham RE, Wilding JP. Therapeutic index for rosiglitazone in dietary obese rats. Separation of efficacy and haemodilution. *Br J Pharmacol* 1999; 128: 1570-1576.
10. Folch J, Loer M, Stanley GSH. A simple method for the isolation purification of total lipids from animal tissue. *J Biol Chem* 1951; 126: 497-509.
11. Zlatki A, Zak B, Boyle AJ. A new method for the direct determination of serum cholesterol. *J Lab Clin Med* 1953; 45: 486.
12. Zilversmit DB, Davis AK. Microdetermination of plasma phospholipids by trichloroacetic acid precipitation. *J Lab Clin Invest* 1950; 35: 155.
13. Falholt K, Falholt W, Lund B. An easy colorimetric method for routine determination of free fatty acids in plasma. *Clin Chim Acta* 1973; 46: 105.

14. Foster CS, Dunn O. Stable reagents for determination of serum triglycerides by a colorimetric Hantzsch condensation method. *Clin Chem* 1973; 19: 338-340.
15. Michaelis DC, Nace CS, Szepesi B. Demonstration of a specific metabolic effect on dietary disaccharides in the rat. *J Nutr* 1975; 105: 1186-1191.
16. Thorburn AW, Storlein LH, Jenkins AB, Khouri S, Kraegen EW. Fructose induced in vitro insulin resistance and elevated plasma triglycerides levels in rats. *Am J Clin Nutr* 1989; 49: 1155-1163.
17. Anitha Nandhini AT, Balakrishnan SD, Anuradha CV. Taurine improves lipid profile in rats fed a high fructose diet. *Nutr Res* 2002; 22: 343-354.
18. Bieger WP, Michel G, Barwich D, Wirth A. Diminished insulin receptors on monocytes and erythrocytes in hypertriglyceridemia. *Metab* 1984; 33: 982-987.
19. Boden G, Jadalyn F, Kian Y. Effect of fat on insulin stimulated carbohydrate metabolism in normal men. *J Clin Invest* 1991; 88: 960-966.
20. Slatter DA, Bolton CH, Bailey N. The importance of lipid derived malondialdehyde in diabetes mellitus. *Diabetologia* 2000; 43: 550-557.
21. Thirunavukkarasu V, Nandhini AAT, Anuradha CV. Lipoic acid restores antioxidant system in tissues of hyperinsulinaemic rats. *Indian J Med Res* 2003; 118: 134-140.
22. Thirunavukkarasu V, Anuradha CV. Influence of  $\alpha$ -lipoic acid on lipid peroxidation and antioxidant defence system in blood of insulin resistant rats. *Diab Obes Metab* 2004; 6: 200-207.
23. Madar Z, Abel R, Samish S, Arad J. Glucose-lowering effect of fenugreek in non-insulin dependent diabetics. *Eur J Clin Nutr* 1988; 42: 51-54.
24. Valette G, Sauvaire Y, Baccou JC, Ribes G. Hypocholesterolaemic effect of fenugreek seeds in dogs. *Atherosclerosis* 1984; 50: 105-111.
25. Shyamala MP, Venkumar MR, Latha MS. Antioxidant potential of the *Syzygium aromaticum* (Gaertn.) Linn. (Cloves) in rats fed with high fat diet. *Indian J Pharmacol* 2003; 35: 99-103.
26. Dhandapani S, Subramanian VR, Rajagopal S, Namasivayam N. Hypolipidemic effect of *Cuminum cyminum* L. on alloxan – induced diabetic rats. *Pharmacol Res* 2002; 46: 251-255.
27. Khan A, Safdar M, Khan MMA, Khattak KN, Anderson RA. Cinnamon improves glucose and lipids of people with type 2 diabetes. *Diabetes Care* 2003; 26: 3215-3218.
28. Khan A, Bryden NA, Polanski MM, Anderson RA. Insulin potentiating factor and chromium content of selected foods and spices. *Bio Trace Element Res* 1990; 24: 183-188.
29. Broadhurst CL, Polansky MM, Anderson RA. Insulin like biological activity of culinary and medicinal plant aqueous extracts in vitro. *J Agric Food Chem* 2000; 48: 849-852.
30. Qin B, Nagasaki M, Ren M, Bajotta G, Oshida Y, Sato Y. Cinnamon extract prevents the insulin resistance induced by high-fructose diet. *Horm Metab Res* 2004; 36 (2): 119-125.
31. Jarvill-Taylor KJ, Anderson RA, Graves DJ. A hydroxyl-chalcone derived from cinnamon functions as a mimetic for insulin in 3T3-L1 adipocytes. *J Am Coll Nutr* 2001; 20: 327-336.
32. Sauvaire Y, Petit P, Broca C, Manteghetti M, Baissac Y, Alvarez JF, Gross R, Roye M, Leconte A, Gomis R, Ribes G. 4-hydroxy isoleucine: a novel amino acid potentiator of insulin secretion. *Diabetes* 1998; 47: 206-210.
33. Khosla P, Gupta DD, Napal RK. Effect of *Trigonella foenum graecum* on serum lipids in normal and diabetic rats. *Indian J Pharmacol* 1995; 27: 89-93.
34. Ram A, Lauria P, Gupta R, Sharma VN. Hypolipidaemic effect of *Myristica fragrans* fruit extract in rabbits. *J Ethnopharmacol* 1996; 55: 49-53.
35. Akhiani SP, Vishwakarma SL, Goyal RK. Anti-diabetic activity of *Zingiber officinale* in streptozotocin type I diabetic rats. *J Pharm Pharmacol* 2004; 56: 101-105.
36. Platel K, Rao A, Saraswathi G, Srinivasan K. Digestive stimulant action of three Indian spice mixes in experimental rats. *Nahrung* 2002; 46: 394-398.
37. Hollenbeck CB. Dietary fructose effects on lipoprotein metabolism and risk for coronary artery disease. *Am J Clin Nutr* 1993; 58: 800s-809s.
38. Dai S, McNeill JH. Effect of fructose loading in streptozotocin diabetic and non-diabetic rats. *Can J Physiol Pharmacol* 1992; 70: 1583-1589.

## Preventive action of food seasoning spices mixture on fructose-induced lipid abnormalities

### 食品调料混合香辛料对果糖诱变脂质异常的预防作用

Ramu Suganthi MSc<sup>1</sup>, Shanmuganathan Rajamani MSc<sup>1</sup>, Mambakkam Katchapeswaran Ravichandran MSc, MPhil, PhD<sup>2</sup> and Carani Venkatraman Anuradha MSc, MPhil, PhD<sup>1</sup>

*1. Department of Biochemistry, Faculty of Science, Annamalai University, Annamalai Nagar, Tamil Nadu, India*

*2. Department of Statistics, Faculty of Science, Annamalai University, Annamalai Nagar, Tamil Nadu, India*

给老鼠喂食大量的果糖将导致胰岛素抵抗、高胰岛素血症、高血糖症和血脂代谢紊乱。本实验用62861只果糖喂食胰岛素抵抗的大鼠来测定食品调料混合香辛料对其的降血脂作用。雄的Wistar每天喂食含60%果糖或60%淀粉，15天后，用三种不同剂量的混合香辛料通过嘴喂食这些大鼠（每天每只大鼠10mg, 30mg或50mg）。实验时间45天后，用果糖喂食的大鼠的血浆葡萄糖浓度升高，糖尿病血脂异常甘油三酯及自由脂肪酸浓度均升高，高密度脂蛋白胆固醇降低，极低密度脂蛋白胆固醇升高。同样也可发现组织脂质的变化。同时用混合香辛料处理和果糖饮食一起的大鼠血浆葡萄糖和胰岛素水平都正常，血浆和组织中的脂质水平也恢复正常。这些香辛料的胰岛素加强作用的积极原理将有助于这些混合香辛料对高剂量果糖喂食大鼠的降血脂作用。

关键词：果糖饮食 香辛料 胰岛素抵抗 葡萄糖 脂质