

Note

Effects of Some Chinese Spices on Body Weights, Plasma Lipids, Lipid Peroxides, and Glucose, and Liver Lipids in Mice

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The purpose of this study was to investigate the long-term effects of 12 diets, containing small quantities (1%) of different Chinese spices, on plasma lipids, lipid peroxides, and glucose, and liver lipids in mice. The mice were fed with experimental diets containing one of 12 different spices and control diets for 90 day. There was a significant reduction in plasma triacylglycerol concentrations in mice in the star anise, clove and tsao-ko diet groups, and it was significantly higher in cassia diet group. The TBARS values were remarkably reduced in mice in the white pepper and tsao-ko diet groups, and it were significantly increased in the mice administered star anise and fennel. Plasma glucose concentrations were evidently lower in animals in the cassia and tsao-ko diet groups. In conclusion, some spices used in the preparation of Chinese food may have some positive effects on maintaining human health and preventing life-style diseases.

Keywords: chinese spices, lipids, glucose, plasma, liver, mice

Introduction

Spices are amongst the thousands of food flavoring substances used worldwide (Cadby, 2004). They have also been used for generations, as components of traditional medicines (Lai and Roy, 2004). From very early times spices are believed to have played an important role in Ayurvedic preparations. In traditional Chinese medicine, herbal remedies are classified into categories of low, intermediate and superior; spices are almost exclusively in the superior category.

There is now a growing body of scientific evidence to support the concept that spices can have medicinal properties and it is possible that spices not only alleviate symptoms, but also help prevent disease (Platel and Srinivasan, 2004; Srinivasan, 2005; Hirasu and Takemasa, 1998A, B; Rahman and Lowe, 2006; Westerterp-Plantenga *et al.*, 2006; Cichewicz and Thorpe, 1996; Ross *et al.*, 2001; Mousumi *et al.*, 2004; Chithra and Leelamma, 1997; Khan *et al.*, 1996). Spices are generally rich in ash and fiber and their protein content can be comparable with that of whole grains and mature dry legumes. The average content of calcium, magnesium, iron, sodium, and potassium are higher in spices from leaves (herbs) than in those spices obtained from other parts of plants. Spices from seeds have the highest phosphorus content (Murphy *et al.*,

1978). It has been shown that the commonly consumed spices stimulate digestive enzymes of the pancreas and small intestine, and affect the composition of bile and activate its secretion (Platel and Srinivasan, 2004; Srinivasan, 2005; Hirasu and Takemasa, 1998B). Various physiological activities of spices, particularly suppression of blood lipid levels and obesity (Rahman and Lowe, 2006; Westerterp-Plantenga *et al.*, 2006), and antibacterial activity are well documented (Hirasu and Takemasa, 1998C; Cichewicz and Thorpe, 1996; Ross *et al.*, 2001). However, it should be noted that as well as their beneficial effects spices can be a vehicle for various micro-organisms with the associated implications for consumers and for the shelf life of foods (Mousumi *et al.*, 2004).

Currently, the scientific information on whether spices can reduce risk factors for various life-related diseases such as heart disease and diabetes are well known (Lai and Roy, 2004; Rahman and Lowe, 2006; Westerterp-Plantenga *et al.*, 2006; Chithra and Leelamma, 1997; Khan *et al.*, 1996). Star anise (*Illicium verum* J.D. Hooker), cassia (*Cinnamomum cassia* Presl), prickly (*Zanthoxylum bungeanum* Maxim), fennel (*Foeniculum vulgare* P. Miller), clove (*Syzygium aromaticum* (L.) Merr. et Perry), tangerine (*Citrus reticulata* Blanco), white pepper (*Piper nigrum* L.), coriander (*Coriandrum sativum* L.), longan (*Euphoria longana* Lam), villosum (*Amomum villosum* Lour), tsao-ko (*Amomum tsao-ko* Crevest et Lemair) and bay leaf (*Laurus nobilis*) are among the most commonly used spices in Chinese food and drinks,

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and are also used in traditional Chinese medicine as a natural remedy for many ailments. However, there are few scientific data on the effects of the spice on the risk factor of life-related disease. The purpose of this study was to investigate the effect of these spices, ingested at a level normally used in Chinese food, on plasma lipid, and glucose contents and liver lipid content in mice. The possible effects of the spices on the physiological functions of animals are discussed.

Materials and Methods

Diet The compositions of the experimental diets were as follows: 47.8% cornstarch, 20% casein, 15% sucrose, 6% lard, 5% cellulose powder, 4% mineral mixture, 2% vitamin mixture and 0.2% L-methionine and 1% spice. The control diet contained cornstarch instead of spices. The lard was supplied from NOF Co., Ltd., Tokyo, Japan. Mineral and vitamin mixtures were purchased from Oriental Yeast Co., Ltd., Tokyo, Japan. The spices added for each dietary group was as follows: (1) control (no spice); (2) star anise (*Illicium verum* J.D. Hooker); (3) cassia (*Cinnamomum cassia* Presl); (4) prickly (*Zanthoxylum bungeanum* Maxim); (5) fennel (*Foeniculum vulgare* P. Miller); (6) clove (*Syzygium aromaticum* (L.) Merr. et Perry); (7) tangerine (*Citrus reticulata* Blanco); (8) white pepper (*Piper nigrum* L.); (9) coriander (*Coriandrum sativum* L.); (10) longan (*Euphoria longana* Lam); (11) villosum (*Amomum villosum* Lour); (12) tsao-ko (*Amomum tsao-ko* Crevost et Lemair); (13) bay leaf (*Laurus nobilis*). The percentage of dietary spices used in this study was based on the assumption that an individual eating Chinese food would consume 5 g of spice per day. The control and experimental diets were made once a month, and stored below 5°C.

Animals Male mice of the Crlj: CD-1(ICR) strain (4 weeks old) were obtained from Charles River Japan Inc. (Atsugi, Kanagawa, Japan). All animals were switched from a laboratory chow, MF (Oriental Yeast Co., Ltd., Tokyo, Japan) to the control and experimental diets at 11 weeks of age. Mice were randomly divided into 13 groups of 8 animals each and each group was fed on the control or an experimental diet for 90 days. The diets and water were given *ad libitum*. The feeding amount were controlled to same calorie through experimental period. Body weights were measured once a month. The animals were housed in suspended stainless-steel cages with wire mesh bottoms. The animal room was kept at $24 \pm 0.5^\circ\text{C}$ and the relative humidity at $65 \pm 5\%$. Room lighting consisted of 12-hour periods of light and dark. All mice were maintained according to the guidelines for experimental animals of the National Food Research Institute, Japan.

Preparation of Plasma Samples and Liver Homogenates At the end of the feeding trials, all mice were fasted for 20 hours before being anesthetized with diethyl ether. Blood was then collected from the inferior vena cava with a heparinized syringe and placed in ice-cold tubes. The plasma was separated by centrifugation at 900 g for 20 min at 4°C. After collecting the blood, the livers were removed and homogenized with 1/15 mol/L phosphate-

buffered saline (pH=7.4) using a Teflon-glass homogenizer. Plasma samples and liver homogenates were stored at -30°C until required for lipid and glucose analyses.

Lipid and Glucose Analyses Total cholesterol, triacylglycerol, and phospholipid concentrations in the plasma samples and liver homogenates were determined by Wako commercial analytical kit (Wako pure chemical industries, Ltd, Osaka, Japan). Plasma non-esterified fatty acid (NEFA) and glucose concentrations were measured by Wako commercial analytical kit. Plasma concentrations of thiobarbituric acid reactive substances (TBARS) were measured by the method of Yagi (Yagi, 1976).

Statistical Analyses All results were expressed as mean \pm SD. The statistical significance of differences in lipid components, glucose, and fatty acid percentages between the dietary groups were determined by one-way analysis of variance using the STATISTICA statistical program package (Stat-Soft Inc., Oklahoma, USA). When the F test was significant, comparisons between the dietary groups were done using the Duncan's multiple range tests at $p < 0.05$.

Results

Food Intake and Body Weight The average food intake (g/mouse/day) was as follows: control group, 4.31 ± 0.21 ; star anise group, 4.27 ± 0.31 ; cassia group, 4.30 ± 0.21 ; prickly group, 4.24 ± 0.25 ; fennel group, 4.28 ± 0.24 ; clove group, 4.34 ± 0.23 ; tangerine group 4.33 ± 0.21 ; white pepper group, 4.07 ± 0.36 ; coriander group, 4.09 ± 0.31 ; longan group, 4.25 ± 0.30 ; villosum group, 4.29 ± 0.28 ; tsao-ko group, 4.34 ± 0.26 ; and bay leaf group, 4.32 ± 0.21 . There were no significant differences in the average food intake between any of the experimental diet groups. The changes in body weights are shown in Table 1. The final mean body weights of the villosum and tsao-ko diet groups were significantly lower than that of the control group. To determine any suppression of body weight increase, by the dietary spices, the ratio of body weight gain (g) to the amount of food intake (g) was calculated in all groups after 90 days (Fig. 1). This ratio was lower in animals in the star anise, fennel and tsao-ko diet groups, and higher in the coriander diet group than that of the control group.

Blood Lipids, TBARS and glucose The plasma total cholesterol, triacylglycerol, phospholipid and NEFA concentrations of mice fed the control and experimental diets are shown in Fig. 2. The plasma total cholesterol concentration was significantly higher in the bay leaf diet group than in the control, star anise, cassia, prickly, fennel, clove, white pepper, coriander, villosum and tsao-ko diet groups. The tsao-ko and fennel diet groups had a significantly lower plasma total cholesterol concentrations than the cassia, tangerine, coriander, longan and bay leaf diet groups.

The plasma triacylglycerol concentration was significantly lower in the star anise, clove, tangerine and tsao-ko diet groups when compared with the control diet group; it was significantly higher in cassia diet group than in all other diet groups except the prickly diet group. The plasma phospholipid concentrations were significantly

Table 1. Body weight in mice fed the diet supplemented with the 1% of different Chinese spices (g).

Dietary group	Days			
	0	30	60	90
Control	44.5 ±2.57 ^a	45.03 ±2.69 ^{bc}	46.49 ±3.58 ^{bcde}	48.84 ±4.71 ^{bc}
Star anise	44.19 ±3.38 ^a	43.31 ±2.6 ^{ac}	44.99 ±3.28 ^{ae}	44.19 ±3.18 ^{ac}
Cassia	43.31 ±2.22 ^a	43.9 ±1.71 ^{acd}	46.65 ±2.16 ^{bcde}	47.95 ±3.41 ^{bc}
Prickly	43.69 ±2.14 ^a	46.34 ±2.8 ^{bd}	48.15 ±3.87 ^{be}	48.63 ±3.97 ^{bc}
Fennel	44.78 ±2.93 ^a	44.04 ±2.51 ^{acd}	45.04 ±2.95 ^{ae}	44.74 ±3.07 ^{acd}
Cloves	45 ±3.72 ^a	45.29 ±4.79 ^{bc}	45.56 ±4.94 ^{ae}	45.54 ±4.89 ^{acde}
Tangerine	43.94 ±2.61 ^a	45.71 ±1.78 ^{bc}	47.61 ±2.9 ^{bd}	49.1 ±4.64 ^{bc}
White pepper	44.75 ±2.66 ^a	43.13 ±2.17 ^{ac}	44.15 ±2.9 ^{ad}	45.14 ±4.49 ^{acde}
Coriander	44.75 ±3.38 ^a	46.63 ±3.01 ^{bd}	49.76 ±3.03 ^b	52.01 ±3.52 ^b
Longan	45.19 ±0.96 ^a	47.56 ±2.03 ^b	49.58 ±2.54 ^b	48.85 ±1.79 ^{bd}
Villosum	42.11 ±2.74 ^a	41.19 ±2.33 ^a	43.26 ±2.61 ^{ac}	42.99 ±3.25 ^a
Tsao-ko	42.72 ±1.77 ^a	42.77 ±2.37 ^{ac}	42.58 ±3.15 ^a	42.09 ±2.48 ^a
Bay leaf	45.25 ±3.44 ^a	47.17 ±3.48 ^b	48.54 ±4.82 ^{be}	49.89 ±4.88 ^{be}

Values are means ±SD. The statistical significance of differences between each dietary groups were determined at $P < 0.05$ by Duncan test. Different superscript letters represent differences between groups.

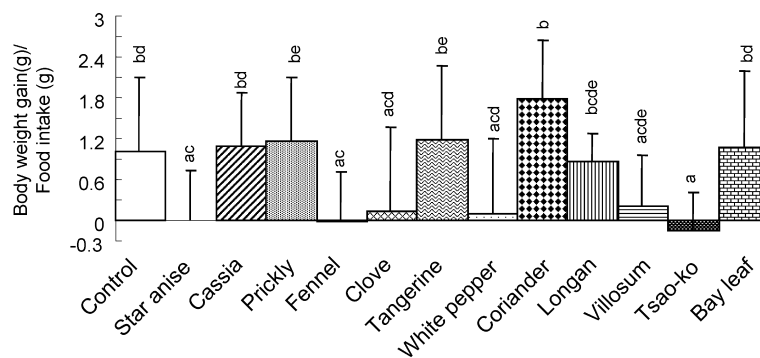


Fig. 1. The ratios of body weight gain/food consumption of mice fed the control and experimental diets for 90 days ($n = 8$ /group). Values are means ±SD. The statistical significance of differences between each dietary groups were determined at $P < 0.05$ by Duncan test. Different superscript letters represent differences between groups.

higher in the longan diet group than in the control, fennel, clove, tangerine, white pepper, villosum and tsao-ko diet groups. The NEFA concentrations were significantly increased in cassia diet group compared with all other diet groups except the prickly group. There was a significant reduction in NEFA concentrations in the star anise, clove, white pepper, tangerine and tsao-ko diet groups when compared with the control diet group.

The plasma TBARS and glucose concentrations of mice fed the control and experimental diets are shown in Fig. 3. The plasma TBARS concentrations of white pepper and tsao-ko diet groups were significantly lower than the control, star anise, prickly, fennel, clove, tangerine and bay leaf diet groups. In contrast, TBARS concentrations were significantly increased in the mice administered star anise, prickly, fennel, clove and bay leaf compared with

those fed the control diet. The plasma glucose concentration was significantly lower in the cassia and tsao-ko diet groups than the control, prickly, fennel, tangerine, coriander, longan, and bay leaf diet groups. However, the tangerine supplemented diet resulted in a significant increase in the plasma glucose concentrations compared the other diets, with the exceptions of those containing prickly, coriander or bay leaf.

Liver lipids There was a significant decrease in the total cholesterol content in the star anise and clove diet groups compared with the control diet group (Fig. 4). There was a significant reduction in liver triacylglycerol levels in the star anise, fennel, clove, villosum and tsao-ko diet groups compared with the control diet group. Only mice fed the coriander diet had a significantly higher liver triacylglycerol content than the control and all other

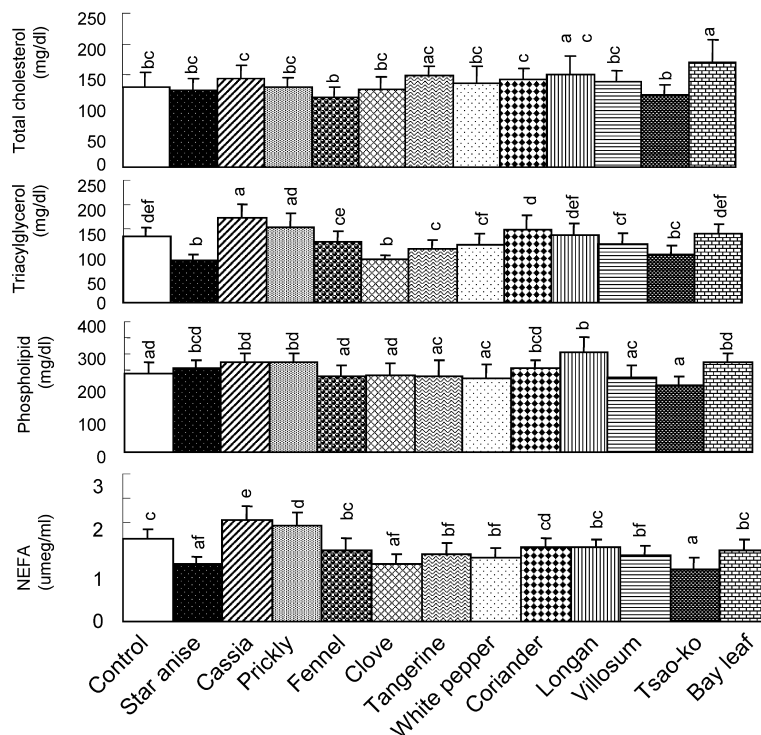


Fig. 2. Plasma total cholesterol, triacylglycerol phospholipid and NEFA concentrations of mice fed the control and experimental diets for 90 days (n=8/group). Values are means±SD. Values for each sample with different little letters in the same lipid were significantly different at $P<0.05$ by Duncan test.

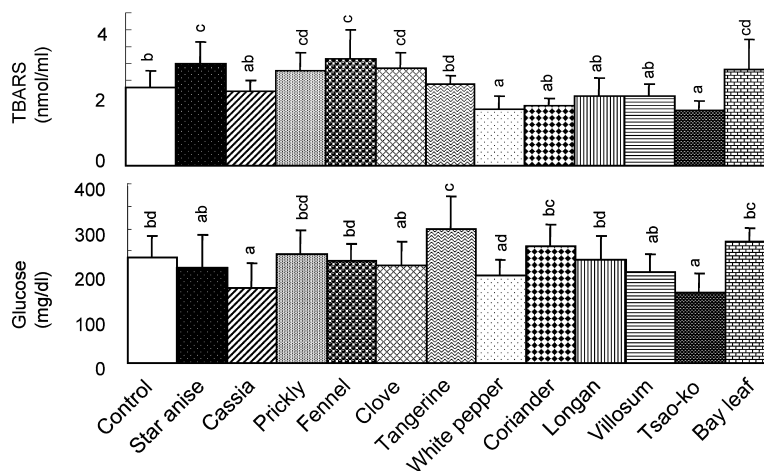


Fig. 3. Plasma TBARS and glucose concentrations of mice fed the control and experimental diets for 90 days (n=8/group). Values are means±SD. Values for each sample with different little letters in the same TBARS and glucose are significantly different at $P<0.05$ by Duncan test.

experimental diet groups. The phospholipid contents of liver from mice fed star anise and villosum diets were significantly higher than those from animals that were fed the control, cassia, tangerine, white pepper, coriander, longan and tsao-ko diets.

Discussion

In Western countries, excess adipose mass, or obesity, has reached epidemic proportions, resulting in metabolic syndrome or syndrome X, which is typified by type-2 diabetes, cardiovascular disease, hypertension and hyper-

lipidemia. It is becoming evident that metabolism of adipose tissue is important in the control of body fat content (Arner, 2000; Kopecky *et al.*, 2001). In the present study villosum and tsao-ko spices inhibited an increase in body weight in mice fed ad libitum despite of the same level of locomotion among animals on control and experimental diets (Table 1). When body weight was assessed in relation to food intake there was an increase in the control group but no change in the tsao-ko, star anise, fennel, clove and white pepper diet groups (Fig. 1). The reason for this perceived control of body weight is cur-

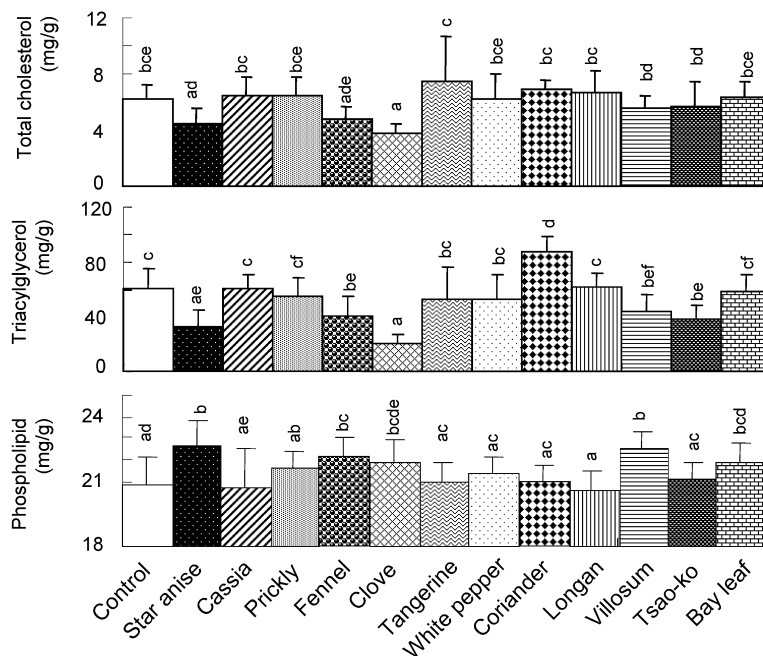


Fig. 4. Total cholesterol, triacylglycerol and phospholipid concentrations of liver in mice fed the control and experimental diets for 90 days. Values are means \pm SD. Values for each sample with different little letters in the same lipid were significantly different at $P < 0.05$ by Duncan test.

rently unknown and, there have been no other studies of a similar nature reported in the literature.

Literature reports on the influence of spices on lipid metabolism are limited but hypolipidemic and hypocholesterolemic activities of some spice have been demonstrated (Srinivasan, 2005). It has been suggested that certain spices may promote the activities of key enzymes in lipid metabolism (Chithra and Leelamma, 1997; Sambaiah and Srinivasan, 1991; Kuda *et al.*, 2004). Very few of the Chinese spices investigated in the present study have been used in other studies. Consistent with our results, a normal level of dietary cassia was not found to have a beneficial hypocholesterolemic influence in experimentally induced hypercholesterolemic rats (Malini *et al.*, 1999). In another study, the levels of total cholesterol and triacylglycerol decreased significantly in the tissues of the animals that had received coriander seeds (Chithra and Leelamma, 1997; Chithra and Leelamma, 1999). Our results showed no significant difference in the plasma or liver total cholesterol contents between the mice fed coriander diets (Fig. 2, Fig. 4). However, the liver triacylglycerol content was higher in mice fed a coriander seed diet than in animals fed control diets. Animals fed star anise, clove and tsao-ko also had a reduction in liver triacylglycerol, but to a lesser extent. Further, liver total cholesterol levels were markedly reduced in the star anise and clove groups compared with the control group (Fig. 4). A decrease in levels of this lipid are associated with inhibition of fat absorption (Han *et al.*, 2002; Chan *et al.*, 1999) and synthesis (Brusq *et al.*, 2006; Moriyama *et al.*, 2004), and enhancement of β -oxidation (Moriyama *et al.*, 2004; Huang *et al.*, 2006). Since we used a relatively low fat diet, this reduction in triacylglycerol is likely to be related to inhibition

of fat synthesis and/or enhancement of β -oxidation. These results suggest that ingestion of some spices may help prevent fat accumulation in the liver. In our study lower amounts of coriander seed was used than in the previous report which could account for the lack of influence on plasma and liver cholesterol concentrations (Chithra and Leelamma, 1997). From the present results it would appear that some spices could modulate plasma triacylglycerol levels. For example, in mice fed tsao-ko, star anise and clove there was decreased plasma triacylglycerol concentrations, whereas cassia fed mice had an increase in this concentration (Fig. 2). It has been reported that the piperine and coriander have a significant hypolipidemic action in rats fed a high fat diet with added cholesterol (Chithra and Leelamma, 1997; Kuda *et al.*, 2004). In our study there was no effective hypolipidemic action in the white pepper and coriander groups (Fig. 2), but again this may be due to the low spice concentration given to these mice. These spice levels were chosen to be consistent with those used in china human consumption.

Free fatty acid (FFA) levels are associated with the regulation of insulin secretion. In studies of hyperinsulinemic clamp, with portal glucose loading, elevated free fatty acids (FFA) impaired hepatic glucose uptake (HGU) as well as suppressing insulin-mediated endogenous glucose production (Nakahara *et al.*, 2004). Our results demonstrate that the administration of several spices was associated with a large reduction in plasma FFA (NEFA) concentrations, the order of potency was tsao-ko, star anise, clove, white pepper and tangerine (Fig. 2). These results suggest a beneficial effect of these spices, as impaired HGU that is associated with an elevated FFA may contribute to the development of insulin resistance in

obesity and type-2 diabetes (Nakahara *et al.*, 2004). However, it should be noted that not all spices appeared to have this effect and the concentrations of NEFA were significantly higher in the cassia and prickly diet groups than in the control diet group.

In previous studies decreases in plasma glucose concentrations were observed following an intake of spices (Malini *et al.*, 1999; Shapiro and Gong, 2002; Yadav *et al.*, 2002). This may have been a consequence of an increase in insulin secretion and a greater sensitivity to insulin (Broadhurst *et al.*, 2000). Cassia was also shown to have beneficial effects on blood glucose, as well as triacylglycerol, total cholesterol, HDL cholesterol, and LDL cholesterol levels (Khan *et al.*, 2003). It is evident from our results that the supplementation of diets with cassia and tsao-ko caused a significant hypo-glycemic action in mice. It has been demonstrated that the actions of some spices on glucose and insulin metabolism is due to stimulation of some key enzymes (Yadav *et al.*, 2002). Our observations could be due to a higher rate of glycolysis, possibly due to an increased activity of hexokinase and phosphoglucumutase.

The antioxidant properties of several spices were investigated in rats by measuring lipid peroxidation both in vivo and in vitro (Hirasa and Takemasa, 1998B; Reddy and Lokesh, 1992; Nakatani, 2000; Maheshwari *et al.*, 2006; Materska and Perucka, 2005). Antioxidant activity was most pronounced in the presence of clove followed by cassia, pepper, ginger, garlic, mint and onion (Shobana and Naidu, 2000). While cumin, capsicum and eugenol (clove) were found to be effective antioxidants, piperine (black pepper), zingerone (ginger), linalool (coriander) and cuminaldehyde (cumin) exhibited only a marginally inhibitory effect on lipid peroxidation (Reddy and Lokesh, 1994). In the present study, the antioxidant ability of the spices was evaluated by detection of TBARS. However, in contrast to other studies, there was no effect of clove on plasma TBARS concentration. On the other hand, white pepper (piperine) and tsao-ko groups significantly decreased the plasma TBARS concentration (Fig. 3). It has been suggested that spices may inhibit lipid peroxidation by quenching oxygen free radicals and by enhancing the activity of endogenous antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase and glutathione transferase.

In conclusion, in the present 12-week comparative study of spices added as a dietary supplementation (1%) to mice, tsao-ko reduced blood lipids and glucose concentrations and had some anti-peroxidative properties, star anise and clove decreased the level of blood or liver lipids and white pepper had an anti-peroxidative effect. Further detailed studies in humans and in non-insulin dependent diabetes mellitus animal models will be needed to help elucidate the mechanisms by which these spices exert their effects and any role in risk reduction for life-style diseases.

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