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Thai red curry paste lowers glucose, oxidative stress and insulin levels in type II diabetic rats

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Abstract: In order to evaluate whether daily intake of Thai red curry in an amount equivalent to that consumed in a normal Thai meal could decrease blood glucose and oxidative stress of type 2 diabetes mellitus (T2DM), six-weeks old male Sprague Dawley rats were fed a high-fat (HF) diet for 2 weeks and T2DM was induced with an injection of streptozotocin (25 mg/kg body weight). Rats then were fed a HF diet supplemented with 0.5% freeze dried Thai red curry paste (TRCP). After 3 weeks, beneficial effects were seen in the lowering of blood glucose level, liver enzyme activities and hyperinsulinemia. In addition, TRCP improved moderately antioxidant activity. These findings suggest that TRCP intake may ameliorate insulin sensitivity and oxidative stress in T2DM condition. These benefits might be attributed to the numerous bioactive compounds present in TRCP. Further studies on long term effects and optimal dosage will have to be conducted in the T2DM rat model before recommendations are made regarding the efficacy of TRCP as a dietary supplement for patients with T2DM.

Keywords: Thai red curry paste, Type 2 diabetic rat model, oxidative stress, blood glucose, Streptozotocin

Introduction

Patients with type 2 diabetes (T2DM) account for up to 95% of all diabetic patients worldwide (National Diabetic Information Clearing house, 2008). Overweight is one of risk factors becoming T2DM in addition to increasing age, family history of diabetes, previous history of gestational diabetes, physical inactivity, and certain ethnicities (National Diabetic Information Clearing house, 2008; American Diabetes Association, 2008). Previously, T2DM was named as adult onset diabetes but it is now increasingly diagnosed in children and adolescents with obesity (National Diabetic Information Clearing house, 2008; American Diabetes Association, 2008). At the pre-stage of T2DM, cells do not respond to insulin effectively, (insulin resistance), which is compensated by an increase in insulin production resulting in hyperinsulinemia, and eventually pancreatic beta cells become damaged and insulin production is decreased. As insulin level drops, cells cannot control glucose uptake properly, and this will result in a rise of blood glucose level (Feher *et al*., 1987; American Diabetes Association, 2008). High level of blood glucose (hyperglycemia) in diabetic patients can alter several metabolic pathways, especially that of glucose oxidation. This causes a higher production of free radicals, which can overwhelm endogenous antioxidant defenses resulting in a condition known as oxidative stress (Saxena *et al*., 1993), causing damage to cellular proteins, membrane lipids and nucleic eventually leading to cell death and thereby contributing to the development and progression of diabetes and its complications, such as dyslipidemia, and endothelia dysfunction (Leinonen and Rantalaiho, 1998; Maritim *et al*., 2003).

Food contains various antioxidant and bioactive compounds, which are believed to prevent T2DM and its complication caused by oxidative stress. Thai red curry paste (TRCP) is one of ingredients used traditional Thai curries. TRCP is not only made from chili (*Capsicum frutescens* L), but it also contains several ingredients, such as pepper (*Piper nigrum* Linn), shallot (*Allium ascalonicum* L.), garlic (*Allium sativum*), lemon grass (*Cymbopogon citratus Stapf*) and fermented shrimp paste. Each ingredient of TRCP provides its own bioactive compounds, such as vitamin C, vitamin E, carotenoids, flavonoids, which are generally known to have antioxidants properties TRCP also provides other specific bioactive compounds, such as capsaicinoids form red chili (Islam and Choi, 2008) , sulfur-containing amino acid (alliin) from garlic (Matsuura, 1997; Amagase, 2006), allyl propyl

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disulphide (APDS) from shallot (Leelarungrayub *et al*., 2006), 1-acetoxychavicol acetate from galangal (*Alpinia galanga*) (Shen, 2000), piperine from pepper (Pathak and Khandelwal, 2007), and fibrinolytic enzymes from fermented shrimp paste (Jeong, 2001). Those bioactive ingredients contained in TRCP have been shown to have ameliorating effects on hyperlipidemia and hyperglycemia. In this study the positive effects of TRCP were investigated in T2DM rat model.

Materials and Methods

Thai red curry paste preparation

TRCP consisting of 23% (w/w) of dried chili (*Capsicum frutescens* Linn.), 20% garlic (*Allium sativum* Linn.), 17% lemon grass (*Cymbopogon citratus* Stapf), 11% shallot (*Allium ascalonicum* Linn.), 6% of galanga (*Alpinia galanga*), 4% fermented shrimp paste, 3% of dried kaffir lime peel (*Citrus hystrix*) mixed dried pepper seed (*Piper nigrum* Linn.) and 16% sodium chloride was prepared by chopping all of the fresh ingredients obtained from local markets and homogenizing in a kitchen blender at 4°C. The mixture then was freeze-dried, ground and stored in air-tight aluminum bag until used for rat diet preparation. From an analysis of nutritive values (AOAC, 1990), freeze-dried TRCP contained 13.3% of protein, 3.5% fat, 51% carbohydrate, 29.2% crude fiber and 287.2 Kcal/100 g.

Animal and experimental design

All animals were maintained in accordance with the rules and regulations of the Animal Care Ethical Committee of Kobe University, Japan. Six-week-old male Sprague-Dawley rats weighing 180–200 g were obtained from SLC Japan (Shizuoka). Three rats were housed per stainless steel cage at ambient humidity and temperature in light-dark (12:12) controlled room. After 4 days of free access to chow diet (MR stock, standard feed, SLC Japan) for acclimation, rats received free access to drinking water and either normal diet (n = 6) or high fat diet (see below) (n = 12) for 2 weeks. Then, diabetes was induced by a single intraperitoneal injection of (streptocotocin) STZ (25 mg/kg body weight) dissolved in 2 ml of citrate buffer (pH 4.5) into overnight-fasted high fat diet group. Only citrate buffer (2 ml/kg BW) was injected into the normal diet rat group as vehicle. Two days after STZ injection, non-fasting glucose levels of blood collected from tail vein of all animals were determined using a portable gluco-meter (Accu-Check Active, Roche Diagnostics Ltd, Germany). Animals are considered diabetic when non-fasting blood glucose value is ≥ 300 mg/dL. Animals were allowed to consume water and their respective diet *ad libitum* for a further 3 weeks. During this period, the amount of food intake was measured daily. Body weight and blood glucose levels were monitored weekly. Normal control group (Control; n = 6) continued to feed on normal diet. Diabetic control group (DM; n = 6) continued to feed on the high fat diet. Diabetic animals treated with freeze dried TRCP (DM+TRCP; n = 6) were fed freeze dried TRCP mixed with high fat diet (405 mg/100 g diet).

Diet

Normal rat diet (in powder form) consisted 60% carbohydrate (C), 8.9% protein (P) and 4.11 % fat (F), with energy distribution of 66% C, 23% P and 11%. F. The high fat diet (purchased from Test Diet, USA) consisted of 27.8% C, 24.2% P and 34.7% F, energy distribution of 21% C, 19% and 60% F. TRCP supplement was equivalent to 2 servings/day (60 kg human weight to rat weight) of TRCP consumed in a normal Thai diet.

Intraperitoneal glucose tolerance test (IPGTT)

In the final week of experiment, IPGTT was performed as follows. Animals were fasted overnight prior to receiving an intraperitoneal injection of glucose (2 g/kg BW). Glucose concentrations subsequently were measured in blood collected from the tail vein at 0 (just before injection), 15, 30, 60, 90, 120 and 180 minutes after injection using a portable gluco-meter. The area under the curve (AUC) was calculated according to the trapezoid rule.

Biochemical analyses

At the end of the experimental period, blood pressure in tail artery of fasted rats was measured using Muromachi Non-Invasive Blood Pressure Monitor, Japan. After anesthesia with diethyl ether (Wako, Japan), blood (5 ml) was drawn from the abdominal aorta into centrifuge tube containing potassium-EDTA, sedimented at 3,000 *g* for 15 min, and immediately frozen at -80°C until analyzed. Liver was perfused with 0.9% NaCl through hepatic portal vein, then collected, weighed and kept at -80°C until analyzed. Pancreatic tissue was harvested and immediately preserved at -80°C for subsequent analysis. Blood glucose concentration was measured using a portable gluco-meter. Serum insulin concentration was determined using a rat insulin ELISA kit (U-E type, SHIBAYAKI, Japan) employing a multi-plate ELISA reader (Biorad-680, Biorad Ltd, Japan). Serum lipid profile, liver enzyme activities and creatinine were measured using an auto-analyzer (Hitachi 917; Roche Diagnostics).

Oxidative stress analysis

Malonaldehyde (MDA) contents in serum and pancreas tissues were determined using thiobarbituric acid reactive substance (TBARS) assay kit (Cayman, USA). Reactive oxygen species in serum was analysed by d-ROMs test (FRAS4, H&D srl, Italy).

Histopathological procedure

Pancreatic tissues were fixed in 10% neutral buffered formalin solution (Wako, Japan), embedded in paraffin and dehydrated with 99.5% alcohol (Wako, Japan). Sections were stained with haematoxylin and eosin for microscopic examination (Ross, 1989).

Statistical analysis

All data are presented as mean ± standard error of mean (SE). One- way analysis of variance with least significant difference post hoc test was used for statistical analysis. Significance is assigned for any p value ≤ 0.05.

Results

IPGTT

Three days before the end of the experimental period, rats were tested for glucose intolerance. There were no significant differences on area under the curve among control, DM and DM + TRCP groups (8196.25 ± 1531, 10496.25 ± 1865, and 10058.5 ± 2017 respectively (Figure 1). However, blood glucose level of DM + TRCP group was significantly lower than DM group ($p \leq 0.05$).

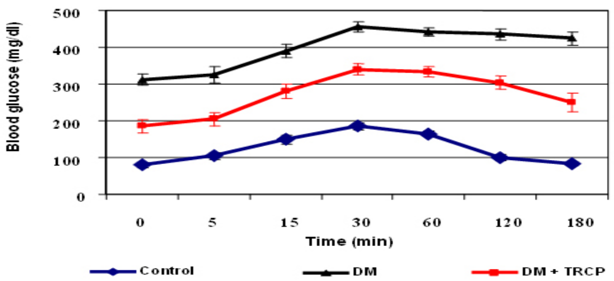


Figure 1. Effect of Thai red curry paste on intraperitoneal glucose tolerance test (all rats were fasted overnight prior to intraperitoneal injection of glucose (2 g/kg BW), glucose concentrations subsequently were measured from the tail vein at 0, 15, 30, 60, 90, 120 and 180 min). Results are presented as mean ± SE (n = 6).

Body weight and biochemical parameters

After 3 weeks on TRCP-supplemented diet, no significant differences of body weight among control, DM and DM + TRCP groups were observed (Table 1). Blood glucose levels of DM+TRCP group declined significantly ($p \leq 0.05$) compared to DM. Interestingly, insulin secretion in untreated DM group was statistically higher than in control group and DM+TRCP group. The triglyceride levels significantly increased in control DM and DM+TRCP groups compared to control. The DM+TRCP group showed a significantly lower AST, ALT and ALP levels than DM group and displays no difference in those of control, whereas DM group exhibited significant increase in these indicators ($p \leq 0.05$) compared to control group (Figure 2).

Table 1. Body weight and biochemical parameters

	Control	DM	DM+TRCP
Body weight before treatment (g)	282.2 ± 4.4	279.6 ± 6.7	281.3 ± 7.2
Body weight after treatment 1 wk (g)	308.0 ± 5.0	291.3 ± 11.7	308.3 ± 7.6
Body weight after treatment 2 wk (g)	341.8 ± 4.9	327.2 ± 16.0	343.6 ± 7.4
Body weight after treatment 3 wk (g)	369.6 ± 4.9	358.9 ± 16.4	368.3 ± 8.9
Blood Glucose before treatment	82.0 ± 6.0^{b}	402.3 ± 21.4^{a}	410.1 ± 28.6^{a}
Blood Glucose after treatment 1 wk	86.1 ± 4.6^{b}	389.2 ± 28.3^{a}	396.4 ± 26.4^{a}
Blood Glucose after treatment 2 wk	89.0 ± 5.5^{b}	378.2 ± 28.6^{a}	285.2 ± 45.8^{a}
Blood Glucose after treatment 3 wk	83.4 ± 2.3^{b}	394.1 ± 30.7^{a}	256.5 ± 22.4^{ab}
Blood Glucose fasting after treatment 3 wk	80.0 ± 5.2^{b}	312.2 ± 21.9^{a}	185.1 ± 27.7^{ab}
Blood pressure (SP mmHg)	121.3 ± 2.4	125.3 ± 3.7	123.3 ± 9.3
Insulin levels (pg/ml)	514.6 ± 151.5^{b}	1620.0 ± 313.0^{a}	1050.8 ±114.1^{ab}

Data are mean ± SE (n = 6), a : significant at $P \leq 0.05$ compared to Control

b : significant at $P \leq 0.05$ compared to DM

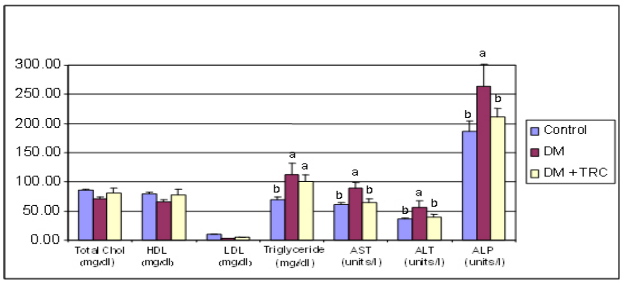


Figure 2. Effect of Thai red curry paste on the lipid profile and liver enzymes. Results are presented as mean ± SE (n = 6). (a: $p \leq 0.05$ compared to Control, b: $p \leq 0.05$ compare to DM).

Oxidative stress indicators

Serum MDA significantly increased in DM group to compare with control, while no significant differences of serum MDA between control and DM+TRCP groups (Table 2). The level of reactive oxygen species in DM+TRCP shows significantly lower than DM group ($p \leq 0.05$).

Histopathology of pancreas

Microscopic examinations of pancreatic histology in control group showed normal characteristics with exocrine portion containing several acini of regular shape of pyramidal acinar cells, while endocrine portion showed intact islets of langerhans (Figure 3). The islets itself was seen to contain a distribution of small dark purple and few pink granules (Figure 3A). On the other hand, DM pancreas had numerous amorphous acinar cells with amorphous small islets containing pale purple granules, suggesting cell necrosis (Figure 3B). The numbers of islets in DM group were difficult to observe (picture not shown). In DM+TRCP group, the exocrine portion appeared to have more regular pyramidal acinar cells than those of DM group. The islets in DM+TRCP group were intact with greater numbers of dark and pale granules, suggesting improved condition of the cells (Figure 3C).

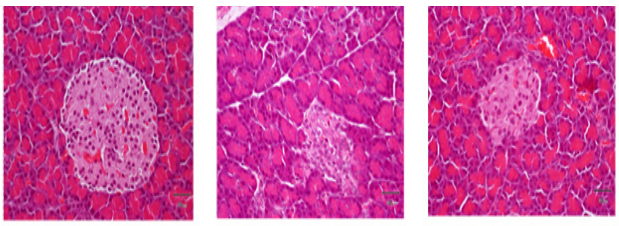
Table 2. Antioxidant indicators

	Control	DM	DM+TRCP
Serum MDA (μM)	12.7 ± 4.5 [b]	17.7 ± 1.8 [a]	14.0 ± 3.4
Pancreas MDA (μM/g of pancreas)	1036.5 ± 12.9 [b]	1395.9 ± 11.9 [a]	1281.3 ± 25.0 [a]
Reactive Oxygen Species (d-ROMs, UCarr) 1Ucarr=0.08% H_2O_2	131.5 ± 8.6 [b]	154.8 ± 4.73 [a]	144.2 ± 7.7 [ab]

Data are mean ±SE (n = 6). a: significant at $P \leq 0.05$ compared to Control,
b: significant at $P \leq 0.05$ compared to DM

dROMs : measured hydroperoxides (R-OOH), a class of reactive metabolites of oxygen

MDA : measured malondialdehyde (R-OOH), metabolites of lipid peroxidation



A. Control B. DM C. DM+TRCP

Figure 3. Effect of Thai red curry paste on histopathology of pancreas. Animals were treated as described in legend to Figure 1. (pancreatic samples were fixed in 10% neutral buffered formalin solution, embedded in paraffin, dehydrated with 99.5% alcohol, sectioned and stained with haematoxylin and eosin for light microscopy). Magnification ×40. Scale bar = 30 µm.

Discussion

Although many individual traditional plants have long been reported to be effective agents with hypoglycemic, hypolipidemic and antioxidative properties in diabetic rats, only some researchers are able to show the positive effects of specific plant on T2DM. For instance, Jalal and colleagues (Jalal *et al*., 2007) investigated the effectiveness of aqueous extracts of shallot and garlic (500 mg/ kg body weight/day) on fructose-induced insulin resistance and found that both significantly decreased fasting blood glucose in 8 weeks. Only shallot extract significantly improves intraperitoneal glucose tolerance. In 2008, Isam and Choi were the first to demonstrate the effectiveness of dietary dose of red chilli in a high fat/STZ rat model. They found that feeding of red chili for 4 weeks has no effect on fasting blood glucose and serum lipid concentrations, whereas serum insulin concentration is significantly ($p \leq 0.05$) increased in the 2% red chili group. However, excessive intake of whole red chilies can cause adverse effects to the stomach and intestine (Desai, 1973; Denis, 1976; Satyanarayana, 2006).

Most of T2DM patients also have insulin resistance, which is the major cause of hypertension, obesity, hyperglycemia, and decreased HDL and high triglyceride levels (Standl, 2005). In our study, consumption of 2 doses of TRCP, equivalent to 500 mg/kg body weight/day for 3 weeks continuously, decreased fasting blood glucose level compared to DM group. TRCP supplementation also restored liver function enzymes activities, from DM to normal levels. Hyperinsulinemia is a characteristic of type 2 diabetes mellitus (T2DM) and is believed to play a role in cellular inflammation (Ruge *et al*., 2009). Three weeks of TRCP consumption, in addition, showed a significance to lower the elevated insulin secretion, reactive oxygen species and serum MDA content. MDA is the product of lipid peroxidation and is generally used as an indicator of cellular oxidative stress (Yagi, 1998). TRCP contains several bioactive ingredients such as chili (*Capsicum frutescens* L), pepper (*Piper nigrum* Linn), shallot (*Allium ascalonicum* L.), garlic (*Allium sativum*) and lemon grass (*Cymbopogon citratus Stapf*). The ameliorating effects of T2DM rats would be the combined effects from these bioactive ingredients.

No any adverse effects of food plants within an amount of human consumption have been reported in health rats (Lim *et al*., 1997; Yoshioka *et al*., 1998; Ahuja *et al.,* 2006). Thus, it is suggested that 0.4% Thai red curry supplemented diet, approximately equivalent to the amounts of 2 serving that Thai people usually consume, could attenuate T2DM, possibly by attenuating glycemic, insulin and oxidative stress levels through synergistic effects of the various bioactive ingredients of TRCP.

Conclusions

This is the first report demonstrating that Thai red curry paste, composed of many ingredients, each of which has been reported to be able to ameliorate most of T2DM condition, is able to act similarly by reducing blood glucose and protecting against liver damage. In addition, Thai red curry paste tended to reduce hyperinsulinemia and oxidative stress to more desirable levels. However, further research is needed to support these findings, including studies to determine the effectiveness of long-term intake of Thai red curry paste and the optimal dietary amounts in type 2 diabetic patients.

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