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Effects of crude flavonoids from tatary buckwheat on alloxan-induced oxidative stress in mice

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Abstract

The present study was undertaken to evaluate the effects of crude flavonoids from tatary buckwheat (FTB) on alloxan-induced oxidative stress in mice. After induction of diabetes, diabetic mice were randomly divided into four groups: One diabetic control group and three different doses of FTB (100, 200 and 400 mg/kg) treated groups, with non-diabetic mice used as the normal control group. The mice were intragastrically administered once daily for 28 days. Fasting blood glucose (FBG), serum insulin, superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and malondialdehyde (MDA) were measured. The results showed that FTB could significantly reduce FBG levels and MDA contents, and increase serum insulin levels, SOD, GPx and CAT activities. These data suggested that FTB possess hypoglycemic effects and could reduce alloxan-induced oxidative stress in mice. Therefore, FTB might be of use as an antidiabetic drug.

Introduction

Diabetes mellitus (DM) is a group of metabolic disorders with different underlying etiologies, characterized by hyperglycemia due to underutilization of glucose (Zhang et al., 2003). It is the third most life-threatening disease whose mortality is right after cancer and cardiovascular disease. Consequently, diabetes is rapidly emerging as a global health care problem that threatens to reach pandemic levels by 2030, and the total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million (Gong et al., 2009; Li et al., 2009; Chen et al., 2011). There is much evidence that oxidative stress is involved in the etiology of diabetes and its complications (King and Loeken, 2004; Lopes et al., 2008; Drel and Sybirna, 2010; Bîcu et al., 2010). Oxidative stress results when the rate of oxidant production exceeds the rate of oxidant scavenging. Hyperglycemia, a key clinical manifestation of diabetes mellitus, can increase oxidative stress due to increased mitochondrial production of the superoxide anion nonenzymatic glycation of proteins and glucose autoxidation (Aronson, 2008). Free fatty acids (FFA), which are elevated in diabetes and insulin resistance, may also contribute to the increased production of reactive oxygen species (ROS) due to increased mitochondrial uncoupling and ß-oxidation (Bikopoulos et al., 2008). In addition, hyperglycemia and FFA-induced oxidative stress lead to the activation of stress-sensitive signaling pathways. Several studies have shown that anti-oxidants can be useful in preventing or attenuating the adverse effects of chronic hyperglycemia (Sivakumar et al., 2010; Li et al., 2011).

Tartary buckwheat (*Fagopyrum tataricum Gaertn.*) is a dry fruit that belongs to the *Polygonaceae* family. It is grown and used in the mountainous regions of Southwest China (Sichuan), in northern India, Bhutan, and

Nepal (Fabjan et al., 2003). In the past decade, as a unique food medicine dual-use cereal crop, tartary buckwheat has become more popular among consumers for both its nutritional and medicinal values. Epidemiological studies have revealed that buckwheat can reduce the risk of chronic diseases. The beneficial effects of tartary buckwheat have been attributed to its high content of flavonoids and its average content is 9-300 times as much as that of common buckwheat (Wang et al., 2009). Flavonoids from tartary buckwheat including the predominant flavonol rutin and minor flavonols quercetin 3-O-rutinoside-3'-O-β-glucopyranoside, kaempferol 3-O-rutinoside and quercetin. It has been reported that crude flavonoids from tatary buckwheat (FTB) exhibit multiple pharmacological activities, such as antihypertensive, antioxidant, anti hypercholesterolemia and anticancer (Ren et al., 2001; Yu and Wang, 2007; Li et al., 2010; Guo et al., 2011). Our previous study has also shown that FTB have potent anti-oxidant activity and free radical-scavenging activities (Zhang et al., 2012). In addition, we have reported that FTB have hypoglycemic effects in diabetic mice (Li et al., 2012). The present study was undertaken to evaluate the effects of FTB on alloxan-induced oxidative stress in mice.

Materials and Methods

Plant material

The air-dried tartary buckwheat grains were purchased from Liangshan agricultural institution (Sichuan, China). It was ground in a blender (FW177, Taisite Instrument Co., Ltd., Tianjin, China) for 10 sec to produce powder with an approximate size of 1 mm. The powders of tartary buckwheat grains were stored at -20°C before experiment.

Chemicals and reagents

Authentic standard rutin (>97%) and alloxan were purchased from Sigma chemical Co. (USA). Glucose Analyzer and strips were purchased from Roche Diagnostic Co. (USA). The kits for the determination of superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and malondialdehyde (MDA) were purchased from Nanjing Jiancheng Bioengineering Co. (Jiangsu, China). Reagents for serum insulin were purchased from Beijing Beifang Pharmaceutical Co. (Beijing, China). All of the other chemicals and reagents were standard commercially available biochemical quality. Triple-distilled water was used throughout the study.

Preparations of crude flavonoids from tatary buckwheat

The powders of tartary buckwheat grains (1.0 g) were accurately weighed and placed in a sealed vessel by adding 40 mL of the ethanol-water (60:40, v/v) solvent, then the sealed vessel was placed into the microwave extraction system (WF-4000C, PreeKem Scientific Instruments Co., Ltd., Shanghai, China). The extraction temperature was 70°C , extraction time was 20 min and microwave power was 600W. After that, the extract was centrifuged at 3,000 rpm for 10 min to remove the insoluble and the supernatant was filtrated through 0.45 mm of filter membrane to obtain a clarified solution. The filtrate was collected and evaporated with a rotary evaporator at 40°C until the sediment was formed. After collected and vacuum-dried at 40°C, the sediment was rightly the solid-state product of crude flavonoids from tartary buckwheat (FTB).

Determination of flavonoids content

The flavonoids content of the FTB was measured using a modified colorimetric method 21 (Jia et al., 1999). To 1.0 mL diluted sample, 0.3 mL $NaNO_3$ solution (5%), 0.6 mL $AlCl_3$ solution (0.1 g/mL and 2.0 mL NaOH solution (1.0 mol/L) were added. The final volume was adjusted to 10.0 mL with deionised water. The absorption was measured at 507 nm against the same mixture, without the sample as a blank. The amount of the total flavonoids was expressed as rutin quivalents (mg rutin/g sample). The calibration curve (Y = 9.265 X-0.0113, where Y is absorbance value, X sample concentration) ranged 0.75-6.0 mg/mL (R2= 0.9984). The content of total flavonoids was 27.97 mg/g.

Animals

Male mice of original Kun-ming strain, approximately 18 to 22 g, were obtained the Experimental Animal Center of Sichuan University (Sichuan, China). The animals were housed in a room maintained at 22 to 25° C with relative air humidity of 50 to 70% controlled room under a 12 hours light-dark cycle, and basal diet and water were supplied *ad libitum*. Approval of this experiment was obtained from the Institutional Animal Ethics Committee of XiChang College (Sichuan, China).

Preparation of alloxan-induced diabetic mice

Male mice were adapted to diet for 1 week before the experiment began. After a 14 hours fasting, mice were induced with a single injection of 4% alloxan prepared freshly at a dose of 200 mg/kg body weight (Gong et al., 2009; Li et al., 2011). Diabetes was confirmed by the determination of tail vein blood glucose levels on the third day after administration of alloxan. Mice having blood glucose levels greater than 11.1 mmol/L were considered diabetic and used for the study.

Experiment design and biological analyses

After confirmation of the diabetic state, the diabetic mice were randomly divided into 4 groups of 12 mice, and 12 non-diabetic mice received distilled water as a normal control group. Normal control group (NC): Non -diabetic mice received distilled water (2 mL/kg);

Diabetic control group (DC): Diabetic mice received distilled water (2 mL/kg); Low-dose FTB treated group (LF): Diabetic mice received FTB solution (100 mg/kg); Middle-dose FTB treated group (MF): Diabetic mice received FTB solution (200 mg/kg); High-dose FTB treated group (HF): Diabetic mice received FTB solution (400 mg/kg). The mice were intragastrically administered once daily for 28 days. FTB solution was prepared through dissolving it in distilled water. Fasting blood glucose (FBG) levels were measured for once every week. Blood was collected from tip of the tail vein (starting from 9:00 am) after a 12 to 14 hour overnight fast.

On the last day of experiment, the mice were deprived of food overnight and sacrificed by cervical dislocation. Blood was collected in polystyrene tubes without the anticoagulant. Serum was immediately separated by centrifugation at 3,000 rpm at room temperature for 10 min. Samples were stored at -20°C for the assay of insulin levels. The kidney and liver tissue were carefully removed and homogenized in ice-cold 0.15 M Tris -KCl buffer (pH 7.4) to yield a 10% (w/v) homogenate. The latter was next subjected to high- speed centrifugation for 30 min at 4°C. The resulting supernatant was used as such for assaying GPx, SOD, CAT activities and MDA contents.

Statistical analysis

All the data were expressed as means ± SD. Statistical significance was calculated using one-way analysis of variance (ANOVA). Significance was accepted at the $p<0.05$.

Results

As shown in Figure 1, FBG levels in NC group maintained constant during 28 days and was significantly lower than diabetic groups (DC, LF, MF and HF groups) before experiment ($p<0.05$). After 14 days, FBG levels of LF, MF and HF groups were significantly decreased as compared with DC group in a dose-dependent manner ($p<0.05$). After 28 days, FBG levels of MF and HF groups were increased as compared with the NC group, but not significantly ($p>0.05$).

As shown in Figure 2, serum insulin levels of LF, MF and HF groups were significantly increased as compared with DC group ($p<0.05$), but LF and MF groups were still significantly decreased as compared with the NC group ($p<0.05$).

As shown in Figure 3, SOD, GPx and CAT activities in kidney of LF, MF and HF groups were significantly increased as compared with DC group ($p<0.05$), but SOD activities of LF and MF groups still significantly decreased as compared with the NC group ($p<0.05$).

As shown in Figure 4, SOD, GPx and CAT activities in liver of LF, MF and HF groups were significantly increased as compared with DC group ($p<0.05$), but SOD and GPx activities of LF group still significantly

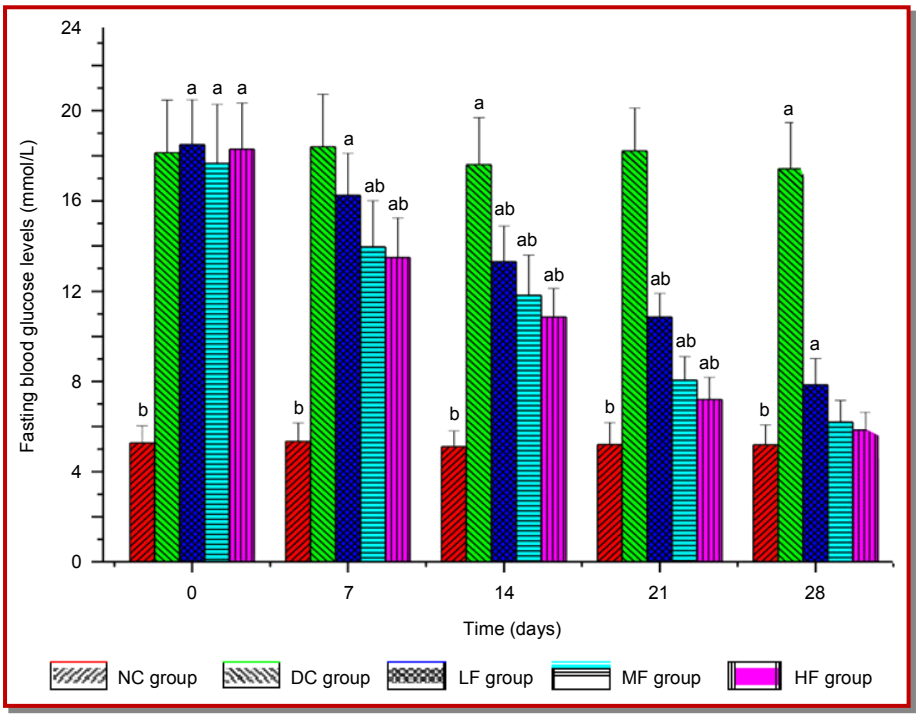

Figure 1: Effects of FTB on fasting blood glucose levels in mice. Data were presented as means ± SD. [a]$p<0.05$ as compared with the NC group. [b]$p<0.05$ as compared with the DC group

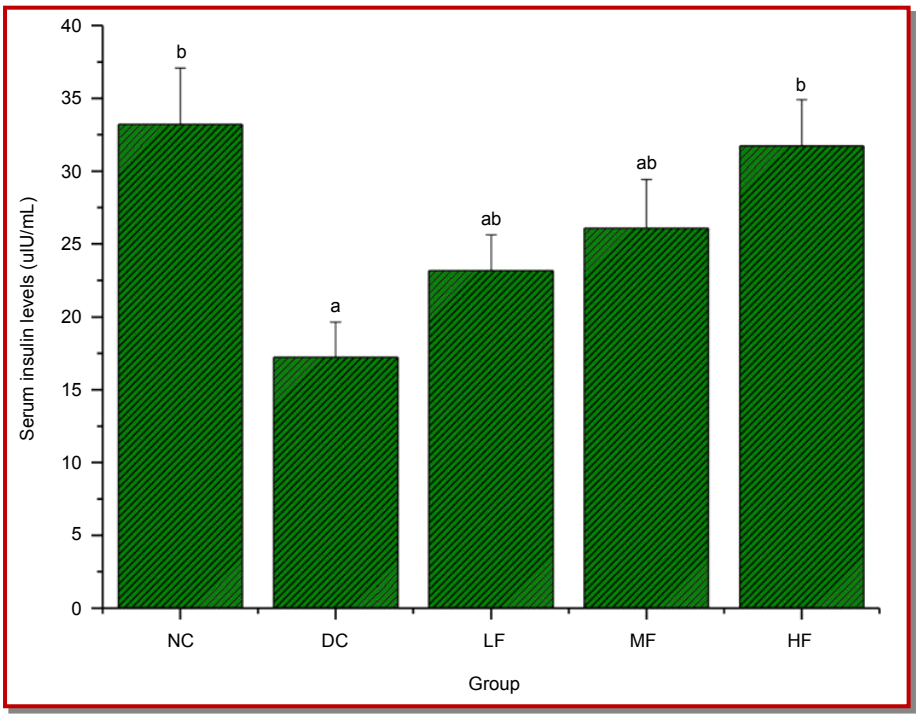

Figure 2: Effects of FTB on serum insulin levels in mice. Data were presented as means ± SD. [a]p<0.05 as compared with the NC group. [b]p<0.05 as compared with the DC group; NC means normal control; DC means diabetic control; Diabetic mice received low dose (LF) , middle dose (MF) and high dose (HF) of FTB

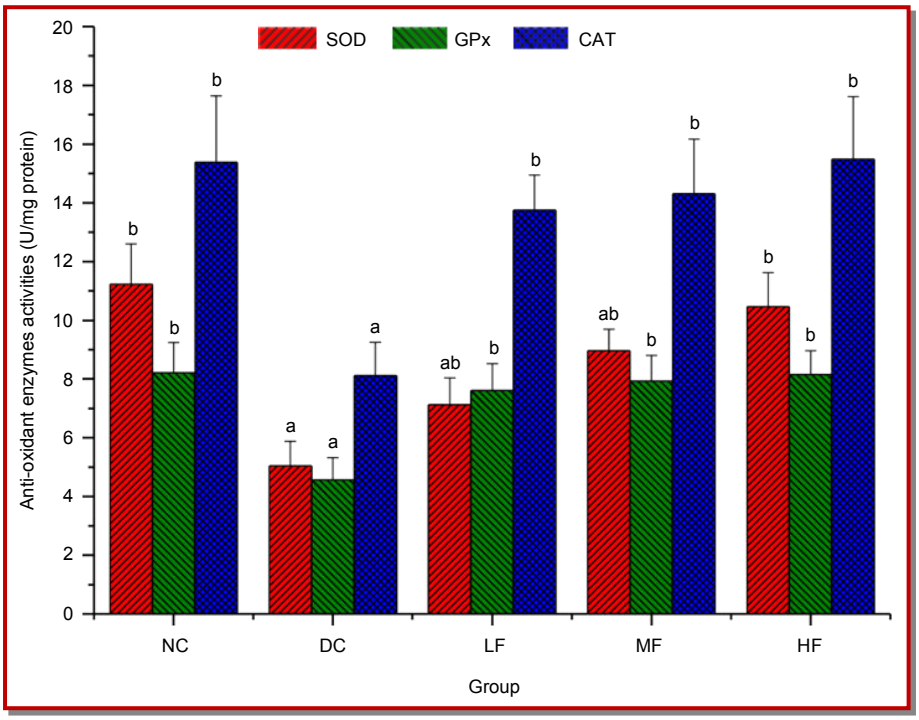

Figure 3: Effects of FTB on anti-oxidant enzymes activities in kidney in mice; NC means normal control; DC means diabetic control; Diabetic mice received low dose (LF) , middle dose (MF) and high dose (HF) of FTB

decreased as compared with the NC group (p<0.05).

As shown in Figure 5, MDA contents in kidney of LF, MF and HF groups were significantly decreased as compared with DC group (p<0.05), but LF group still significantly increased as compared with the NC group (p<0.05). MDA contents in liver of LF, MF and HF groups were significantly decreased as compared with

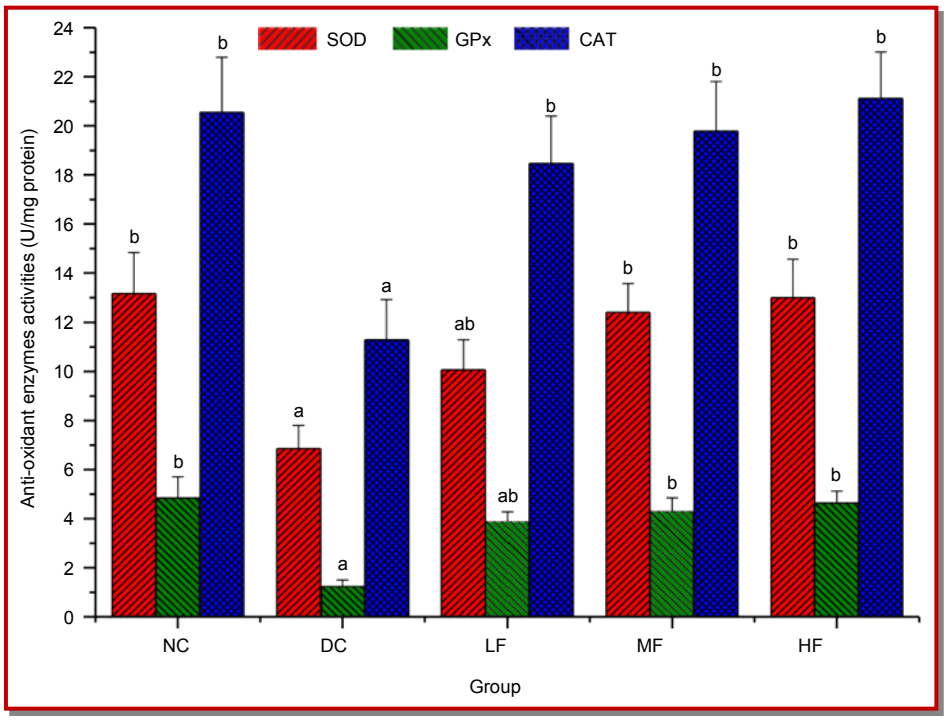

Figure 4: Effects of FTB on anti-oxidant enzymes activities in liver in mice; NC means normal control; DC means diabetic control; Diabetic mice received low dose (LF) , middle dose (MF) and high dose (HF) of FTB

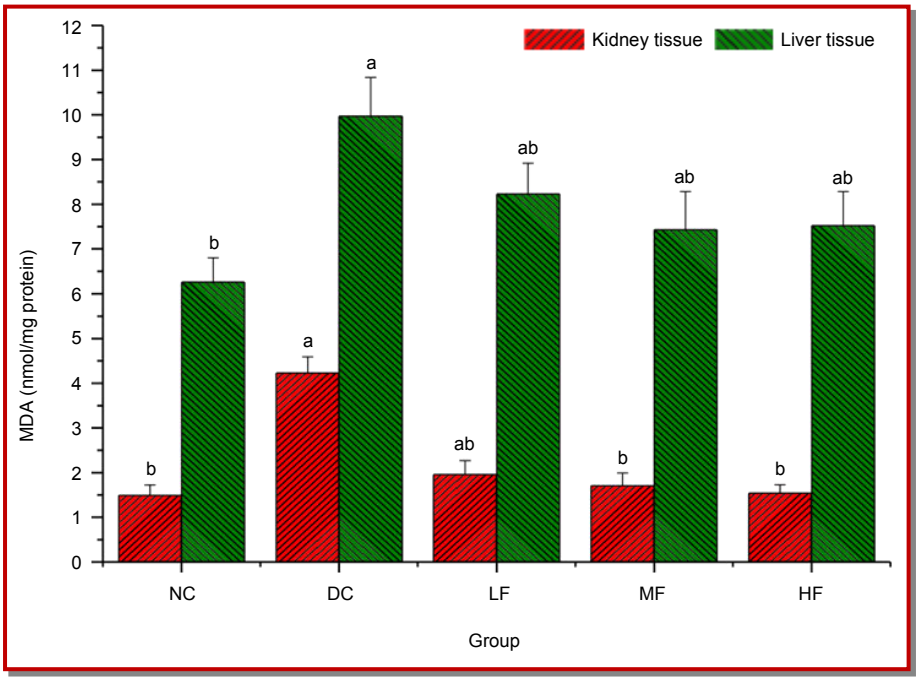

Figure 5: Effects of FTB on MDA contents in kidney and liver in mice; NC means normal control; DC means diabetic control; Diabetic mice received low dose (LF) , middle dose (MF) and high dose (HF) of FTB

DC group ($p<0.05$), but still significantly increased as compared with the NC group ($p<0.05$).

Discussion

Alloxan is a hydrophilic and chemically unstable pyrimidine derivative, which has been widely used to produce experimental diabetes in animals such as rabbits, rats, mice and dogs (Rohilla and Ali, 2012). Alloxan causes a massive reduction in insulin release, by the destruction of the β-cells of the islets of Langerhans and inducing hyperglycemia (Grover et al., 2000).

The present study showed that alloxan-induced diabetic mice presented obvious hyperglycemic symptoms, but FTB produced a significant drop in FBG levels in diabetic mice, which indicated that FTB possessed hypoglycemic effects, at the same time the 200 and 400 mg/kg dose could achieve ideal blood glucose levels.

Insulin is secreted by the β-cells of the pancreas directly into the portal circulation. Insulin suppresses hepatic glucose output by stimulating glycogen synthesis and inhibiting glycogenolysis and gluconeogenesis, thus decreasing the flow of gluconeogenic precursors and free fatty acids to the liver (Egan et al., 1991; Hordern et al., 2005). The present study showed that FTB had the ability to increase the serum insulin levels and the possible mechanism might be due to the renewal of β-cells in the pancreas or the recovery of partially destroyed β-cells. However, further detailed studies are required in order to prove this hypothesis.

Increased oxidative stress is a widely accepted participant in the development and progression of diabetes and its complications (Maritim et al., 2003). Several studies showed that diabetes is usually accompanied by increased production of free radicals or impaired antioxidant defenses, which may enhance membranes susceptibility to lipid peroxidation and lead to pancreatic β-cells dysfunction as well as other cellular organelles damage (Niedowicz and Daleke, 2005; Xue et al., 2009; Spadella et al., 2010). The increase in lipid peroxide levels is one of the most important contributing factors in the development of diabetes-related complications (Li et al., 2011). Thus, the ideal antidiabetic drug should combine both hypoglycaemic and antioxidative properties. The present study showed that FTB could significantly increase SOD, GPx and CAT activities, decrease MDA (a marker of lipid peroxidation) contents in kidney and liver in mice. These results suggested FTB had anti-oxidant properties and prevents lipid peroxidation in alloxan-induced diabetic mice. This meant that FTB had therapeutic preventative and protective effects in diabetes by decreasing oxidative stress.

Conclusion

The present investigation showed that FTB possessed hypoglycemic effects and could reduce alloxan-induced oxidative stress in mice. FTB may be of use as an antidiabetic drug, but there is a need for further research on long-term use in order to show its positive effects on diabetes and its complications.

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