

# Pre-germinated brown rice alleviates non-alcoholic fatty liver disease induced by high fructose and high fat intake in rat

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In past researches, we had been proved the action mechanism of pre-germinated brown rice (PGBR) to treat metabolic syndrome and diabetes mellitus. This study was to investigate the protective effect of PGBR in high fructose and high fat-induced non-alcoholic fatty liver disease (NAFLD) in rodents. WKY rats were divided into: Control group was fed normal drinking water and diet; FLD group was fed 10% high-fructose-water (HFW) and high-fat-diet (HFD); PGBR group was given HFW, and HFD mixed PGBR. After four weeks, the body, hepatic and cardiac weight gains of FLD group had significant increases than that of Control group. The enhanced blood pressure and heart rate, hypertriglyceridemia, hyperuricemia, and higher liver function index (GPT levels) were observed; meanwhile, the IL-6 and TNF- $\alpha$  levels of serum, and TG level of liver were also elevated in FLD group. The related protein expressions of lipid synthesis, inflammation, cardiac fibrosis, and hypertrophy were deteriorated by HFW/HFD. However, in treatment group, PGBR decreased all above influenced parameters, additionally GOT; and related protein expressions. PGBR treated HFW/HFD-induced NAFLD and cardiac complications might be via improving lipid homeostasis, and inhibiting inflammation. Together, PGBR could be used as a healthy food for controlling NAFLD and its' cardiac dysfunction.

**Key Words:** PGBR, NAFLD, cardiac fibrosis and hypertrophy, inflammation

Most adults and children ingest too many sugary foods and drinks daily. Sucrose, the disaccharide composed of equal portions of glucose and fructose, is widely used in many snacks and meals, and the amount of sucrose intake is increased yearly.<sup>(1)</sup> Fructose, a monosaccharide, as for its sweetness, palatability, and taste enhancement, is also heavily applied in many foods, especially in beverage.<sup>(2)</sup> Nowadays, the excess sugar consumption leads to the increasing epidemics of obesity, metabolic syndrome, diabetes mellitus (DM), non-alcoholic fatty liver disease (NAFLD), and cardiovascular risks in worldwide.

It is worth noting that high fat diet (HFD) contributes to NAFLD, when fructose is combined with HFD, much more severe NAFLD may occur.<sup>(3)</sup> In addition, excessive fructose ingestion is reported to be associated with the rising prevalence of NAFLD, prompting damage of many tissues and organs.<sup>(4)</sup> The metabolism of fructose is different from that of glucose. Fructose is transported into cell by glucose transporter 5 (GLUT5), not insulin-sensitive pathway, and metabolized in liver

via fructolysis. The major metabolites of fructose include glucose, lactate, free fatty acid, triglyceride (TG), uric acid, and methylglyoxal.<sup>(2)</sup> Those metabolites are considered as risk factors with leading to NAFLD, and potential to disturb hepatic tissues and functions directly.<sup>(5)</sup>

Unnecessary absorption and the metabolites of fructose trigger inflammation response and oxidative stress, and then affect the insulin signaling pathway in insulin target tissues.<sup>(2)</sup> Subsequently, irregular insulin secretion and sensitivity obstruct the glucose and lipids catabolism to induce diabetes and hyperlipidemia.<sup>(2,6,7)</sup> High fructose intake is proved to enhance the expression of nuclear factor- $\kappa$ B (NF- $\kappa$ B),<sup>(7,8)</sup> in which plays a key role to regulate inflammation and cell proliferation. NF- $\kappa$ B accelerates more serious inflammation to decline endothelial nitric oxide formation, to decrease vasorelaxation and then to lead to hypertension.<sup>(7-9)</sup> NF- $\kappa$ B also induces growth factors, such as transforming growth factor (TGF) and matrix metalloproteinase (MMP) to aggravate myocardium proliferation and then to cause cardiomyopathy.<sup>(10)</sup>

According to our previous researches, pre-germinated brown rice (PGBR) contains rich dietary fiber and antioxidants that improves insulin activity, the glucose and lipids metabolism, lipids absorption, and inflammation. We have proved PGBR can treat metabolic syndrome, DM and their cardiovascular complications.<sup>(11-13)</sup> To date, there are few studies to investigate the effects of PGBR on NAFLD. In this study, Wistar-Kyoto (WKY) rats were fed 10% high fructose water (HFW) and HFD to induce NAFLD, or fed HFW and HFD, in which the carboxyhydrate of HFD were replaced by PGBR for treatment. We evaluated the effects and elucidated the action mechanisms of PGBR to ameliorate NAFLD and its' cardiac complication.

## Materials and Methods

**Animals.** All animal procedures were complied with the standards of animal welfare in laboratory. This study was approved by the Animal Care and Use Committee of Kaohsiung Veterans General Hospital (approval number: VGHKS-2020-A028). The WKY rats were purchased from National Laboratory Animal Breeding and Research Center (Taipei, Taiwan). After an acclimatization period, the six-week-old rats were randomly

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divided into three groups. One group fed water and normal chow (Control group,  $n = 8$ ) (cat No. 5001, contained 13.6 cal% fat, 57.5 cal% carbohydrates, 28.9 cal% protein and dietary fiber 5.3 g/100 g, Test Diet, Richmond, VA). One group fed 10% HFWD and HFD (cat No. 58G9, contained 60 cal% fat, 21.4 cal% carbohydrates, 18.6 cal% protein and dietary fiber 5.5 g/100 g; Test Diet, Richmond, VA) for 4 weeks to induce NAFLD (FLD group,  $n = 8$ ). Other group fed HFWD, and HFD which the carbohydrates were replaced with PGBR (Asia RICE Biotech, Inc, Taiwan), for 4 weeks (PGBR group,  $n = 8$ ),<sup>(11,12)</sup> and the dietary fiber in this PGBR chow was 5.8 g/100 g. The body weight, blood pressure, and heart rate were recorded every week, and the cardiac and hepatic weight were recorded after rat sacrifice. And the heart and liver were collected, and stored these tissues in protein-lysis buffer solution at  $-80^{\circ}\text{C}$  until analysis. NAFLD induction method was modified from previously described.<sup>(4)</sup>

**Measurement of biochemical parameters.** At the end of the study, blood samples were collected to measure blood glucose, glycosylated haemoglobin (HbA1c), TG, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), Non-HDL, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), creatinine, and uric acid which were performed using HITACHI Clinical Analyzer 7070; and insulin, interleukin-6 (IL-6), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and hepatic TG levels were performed using ELISA kits (Merckodia, Uppsala, Sweden; and R&D Systems, Minneapolis, MN).

**Western blot analysis.** The homogenized tissues were centrifuged at  $12,500 \times g$  for 30 min and the supernatants were stored at  $-70^{\circ}\text{C}$  until further analysis. Aliquots of tissue homogenates and protein quantification were used for the Western blot performed with a Bio-Rad protein assay reagent (Bio-Rad Laboratories Taiwan, Ltd., Taipei, Taiwan). The homogenates were probed for proteins of fructose metabolism: fructokinase, aldolase; lipid synthesis proteins: peroxisome proliferator-activated receptor- $\alpha$  (PPAR- $\alpha$ ), PPAR- $\gamma$ , sterol regulatory element-binding protein 1 (SREBP1), acetyl-CoA carboxylase (ACC), stearoyl-CoA desaturase (SCD-1), fatty acid synthase (FAS), glycerol-3-phosphate dehydrogenase (G3PD), glycerol kinase; inflammatory signaling proteins: toll-like receptor 4 (TLR4), TNF- $\alpha$ , extracellular signal regulated kinase 1/2 (ERK1/2), c-Jun-amino-terminal kinase 1/2 (JNK1/2), NF- $\kappa$ B, cyclooxygenase (COX-2), inducible nitric oxide synthase (iNOS), NADPH oxidase (NOX2); free radical scavengers proteins: superoxidase dismutase (SOD), catalase; cardiac hypertrophy proteins: matrix metalloproteinase-9 (MMP9), collagen I,

transforming growth factor- $\beta$  (TGF- $\beta$ ), and connective tissue growth factor (CTGF). The relative expression of those proteins in each tissue was quantified by densitometric scanning of the Western blots using Image-Pro Plus Software (Media Cybernetics, Rockville, MD).

**Statistical analyses.** Statistical differences between groups were calculated by using one-way analysis and variance (ANOVA) and Student-Newman-Keuls test. That was carried out by using SigmaStat (ver. 2.03) (Systat Software, Point Richmond, CA). A probability level of less than 0.05 ( $p < 0.05$ ) was considered as significant. The graphs were drawn using SigmaPlot (ver. 10.0) (Systat Software).

## Results

**Effect of PGBR on HFWD/HFD induced weight gain of body, liver, and heart.** After 4 weeks, the FLD group had more weight increases in body, liver, and heart than Control group. Treatment with PGBR led to a clear reduction not only in body weight but also hepatic and cardiac weight gain, compared with FLD group (Table 1).

**Effect of PGBR on HFWD/HFD enhanced blood pressure and heart rate.** In FLD group, the systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure (MBP), and heart rate were increased after eating HFWD/HFD, compared those of Control group (Table 2). However, in PGBR group, the blood pressure and heart rate were ameliorated significantly than that of FLD group.

**Effect of PGBR on HFWD/HFD influenced biochemical parameters.** HFWD/HFD increased the levels of fasting blood glucose (FBG), insulin, TG, and GPT in WKY (Table 3), meanwhile, IL-6, TNF- $\alpha$ , and hepatic TG were also enhanced in FLD group (Table 3). The fatty liver disease was induced successfully in this animal model. However, in PGBR group, the concentrations of TG, GOT, GPT, uric acid, IL-6, TNF- $\alpha$ , and hepatic TG were reversed, except insulin. It was interesting that GOT levels were not changed obviously in Control and FLD groups, but decreased in PGBR group (Table 3). PGBR could decrease GOT and GPT, and ameliorate fatty liver disease, hyperuricemia and inflammation effectively. As for the level of HbA1c, there were no significant differences in three groups. The levels of FBG and insulin were increased by HFWD/HFD, but PGBR did not improve them effectively (Table 3).

**Effects of PGBR on HFWD/HFD induced lipid synthesis in liver.** Comparing with Control rats, the FLD group had abnormal fructose metabolic enzyme levels as fructokinase and

**Table 1.** Effects of PGBR on the weight of body, liver and heart in fatty liver disease rats

Feeding time (week)	Control group ( $n = 8$ )	FLD group ( $n = 8$ )	PGBR group ( $n = 8$ )
Body weight (g)			
0	320.2 $\pm$ 9.5	315.5 $\pm$ 12.2	318.3 $\pm$ 16.8
1	327.5 $\pm$ 12.2	337.2 $\pm$ 14.0	343.5 $\pm$ 20.3
2	337.5 $\pm$ 12.4	347.8 $\pm$ 16.6	353.2 $\pm$ 22.9
3	339.5 $\pm$ 12.7	356.3 $\pm$ 17.7	362.3 $\pm$ 24.6
4	342.5 $\pm$ 12.9	374.5 $\pm$ 13.7*	364.5 $\pm$ 11.3
Hepatic weight (g)			
4	13.1 $\pm$ 0.8	17.1 $\pm$ 1.1*	15.2 $\pm$ 1.1#
Cardiac weight (g)			
4	1.74 $\pm$ 0.17	2.10 $\pm$ 0.25*	1.65 $\pm$ 0.13#

Control: male WKY rats were fed with a regular water and diet. FLD: male WKY rats were fed with 10% of fructose water and high fat diet. PGBR: male WKY rats were fed with 10% fructose water, and high fat diet mixed with PGBR. \* $p < 0.05$  vs control, # $p < 0.05$  vs FLD.

**Table 2.** Effects of PGBR on the blood pressure and heart rate in fatty liver disease rats

Feeding time (week)	Control group (n = 8)	FLD group (n = 8)	PGBR group (n = 8)
1			
SBP (mmHg)	115.4 ± 8.4	107.8 ± 10.8	104.7 ± 8.9
DBP (mmHg)	83.2 ± 7.4	76.7 ± 10.0	72.0 ± 9.6
MBP (mmHg)	98.2 ± 8.0	92.3 ± 8.9	88.4 ± 8.2
HR (beat/min)	191.5 ± 29.8	204.3 ± 20.5	203.5 ± 30.9
2			
SBP (mmHg)	109.6 ± 14.1	108.3 ± 13.6	99.8 ± 13.7
DBP (mmHg)	73.4 ± 7.5	74.7 ± 5.7	71.1 ± 9.1
MBP (mmHg)	91.5 ± 8.6	91.5 ± 8.5	85.5 ± 10.7
HR (beat/min)	173.2 ± 23.7	230.5 ± 14.86*	174.5 ± 15.5#
3			
SBP (mmHg)	85.2 ± 5.3	136.4 ± 3.4*	122.3 ± 6.5#
DBP (mmHg)	65.1 ± 4.4	83.6 ± 5.4*	78.7 ± 4.2
MBP (mmHg)	75.2 ± 4.2	110.0 ± 4.0*	100.5 ± 4.4#
HR (beat/min)	156.2 ± 22.2	286.8 ± 30.0*	162.8 ± 18.5#
4			
SBP (mmHg)	93.7 ± 8.7	142.4 ± 10.1*	127.0 ± 7.8#
DBP (mmHg)	63.9 ± 5.0	85.0 ± 4.8*	83.1 ± 11.4
MBP (mmHg)	78.8 ± 4.8	118.7 ± 6.3*	101.0 ± 6.8#
HR (beat/min)	152.3 ± 27.7	266.3 ± 34.4*	202.8 ± 17.8#

Control: male WKY rats were fed with a regular water and diet. FLD: male WKY rats were fed with 10% of fructose water and high fat diet. PGBR: male WKY rats were fed with 10% fructose water, and high fat diet mixed with PGBR. SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; HR, heart rate. \* $p < 0.05$  vs control, # $p < 0.05$  vs FLD.

**Table 3.** Effects of PGBR on biochemical parameters in fatty liver disease rats

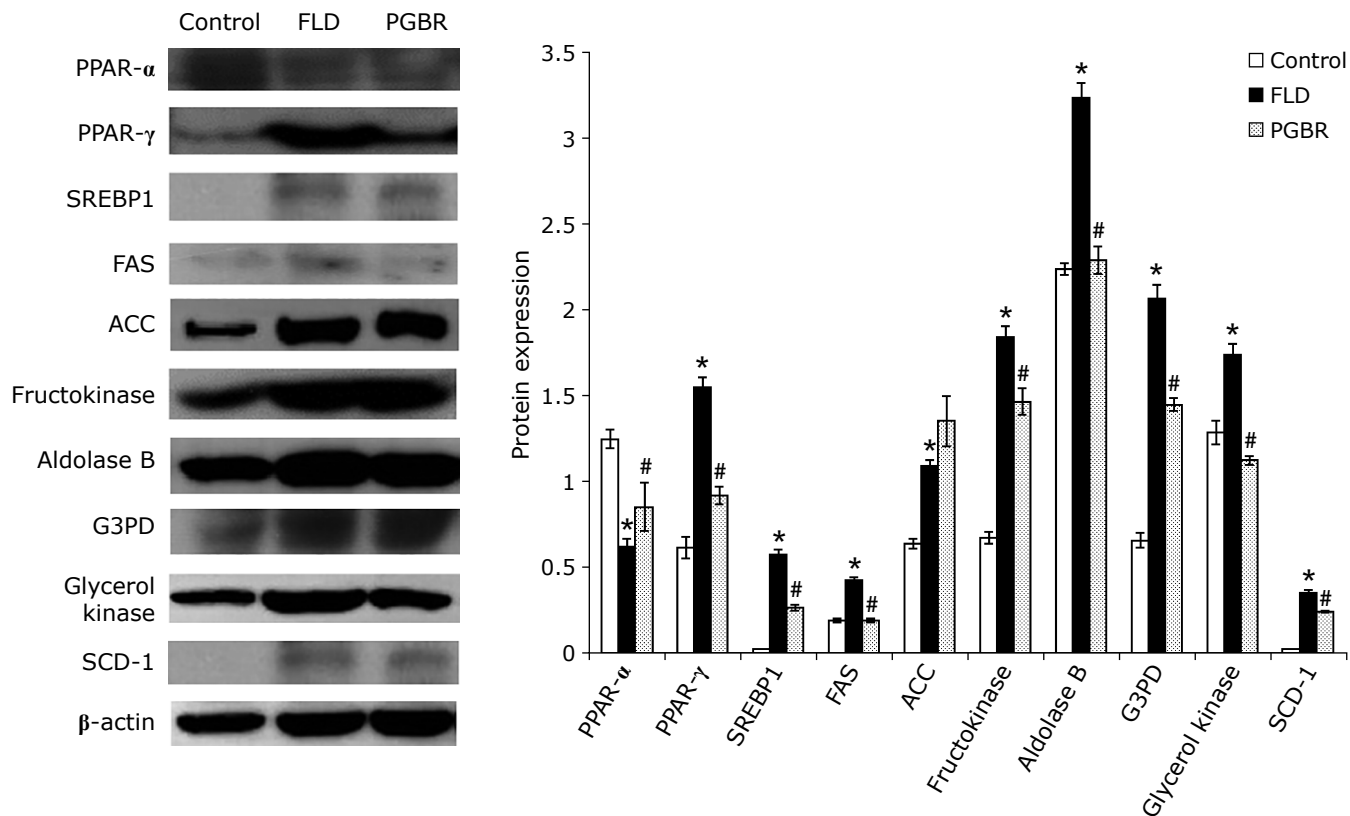
Items	Control group (n = 8)	FLD group (n = 8)	PGBR group (n = 8)
TG (mg/dl)	61.5 ± 6.2	142.5 ± 8.3*	124.3 ± 10.8#
TC (mg/dl)	91.2 ± 6.4	84.8 ± 6.3	84.5 ± 7.1
LDL-C (mg/dl)	5.2 ± 0.5	4.9 ± 0.7	4.7 ± 0.5
HDL-C (mg/dl)	25.6 ± 1.1	24.3 ± 1.2	24.8 ± 1.5
Non-HDL (mg/dl)	65.6 ± 5.4	60.6 ± 1.6	63.7 ± 7.2
AI	2.6 ± 0.1	2.5 ± 0.1	2.4 ± 0.5
FBG (mg/dl)	114.3 ± 12.8	242.2 ± 28.6*	252.1 ± 17.5
HbA1c (%)	4.3 ± 0.06	4.28 ± 0.07	4.33 ± 0.16
Insulin (ng/ml)	0.17 ± 0.05	40.41 ± 5.10*	42.33 ± 4.74
GOT (U/dl)	105.5 ± 19.6	114.7 ± 10.5	65.5 ± 6.9#
GPT (U/dl)	34.3 ± 3.8	44.8 ± 7.6*	34.3 ± 5.1#
Creatinine (mg/dl)	0.49 ± 0.05	0.55 ± 0.06	0.57 ± 0.04
Uric acid (mg/dl)	1.95 ± 0.36	2.56 ± 0.29*	1.83 ± 0.26#
IL-6 (pg/ml)	19.7 ± 2.1	274.5 ± 90.3*	47.0 ± 21.6#
TNF- $\alpha$ (pg/ml)	106.7 ± 19.2	137.4 ± 6.2*	78.2 ± 10.2#
Hepatic TG (nmol/g)	1,782.2 ± 275.1	2,850.7 ± 207.8*	2,031.4 ± 223.4#

Control: male WKY rats were fed with a regular water and diet. FLD: male WKY rats were fed with 10% of fructose water and high fat diet. PGBR: male WKY rats were fed with 10% fructose water, and high fat diet mixed with PGBR. AI, atherosclerosis index = [(TC - HDL)/HDL]. \* $p < 0.05$  vs control, # $p < 0.05$  vs FLD.

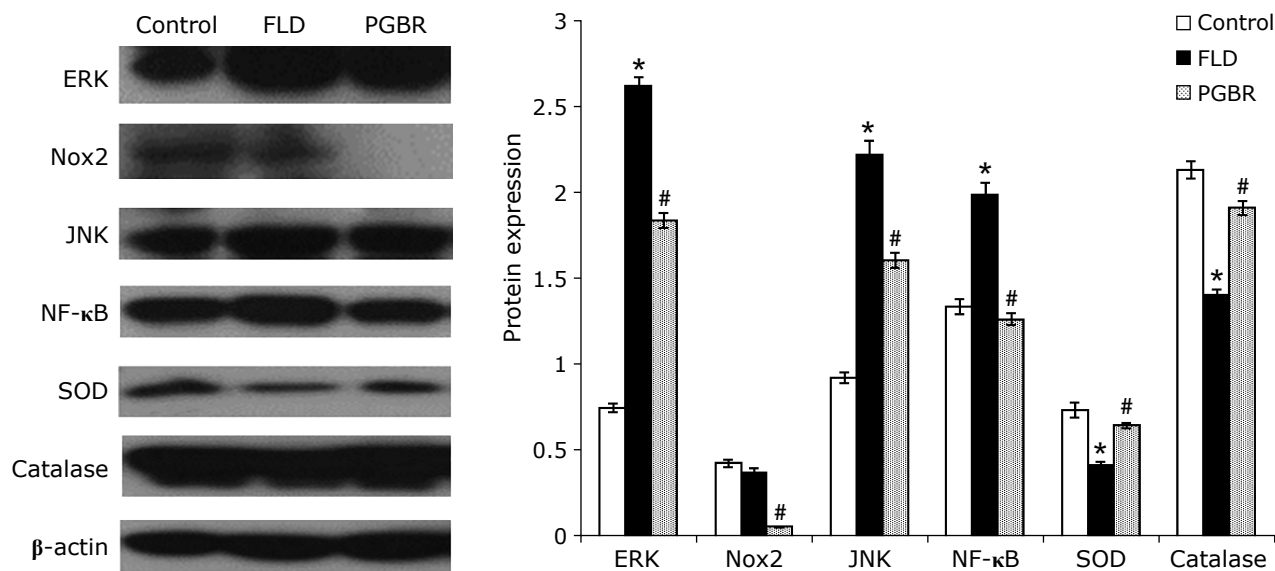
aldolase. Meanwhile, the regulated lipid synthesis protein levels (PPAR- $\gamma$ , SREBP-1, ACC, SCD-1, FAS, G3PD, and glycerol kinase) of FLD group had obvious enhancement than Control group did (Fig. 1). In FLD group, the regulated lipid metabolism protein PPAR- $\alpha$  level was decreased more than in Control group.

On the other hand, PGBR significantly decreased the protein levels of fructokinase and aldolase. It demonstrated that PGBR

might inhibit the metabolism of fructose in the liver. Simultaneously, PGBR also reversed lipid synthesis signaling PPAR- $\gamma$ , SREBP-1, SCD-1, FAS, G3PD, and glycerol kinase; and elevated lipid metabolism protein PPAR- $\alpha$  (Fig. 1). Combining the results of TG in blood and liver (Table 3), we believed that PGBR might reduce the lipid synthesis by eliminating the metabolism of fructose, especially TG synthesis.



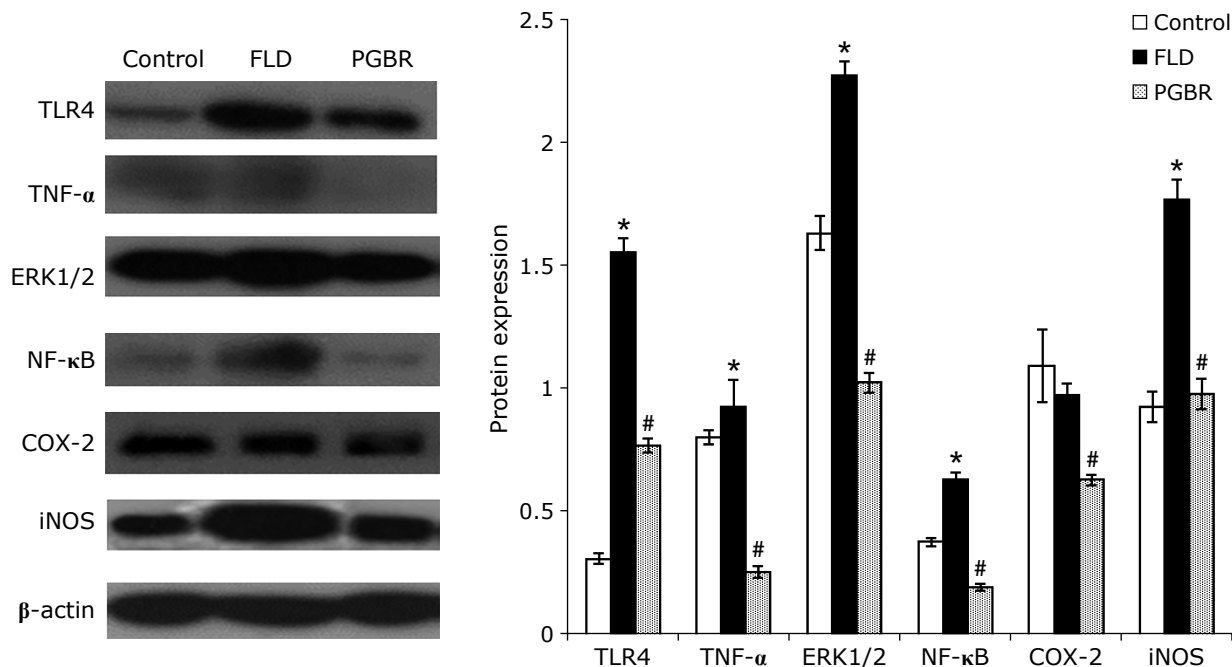
**Fig. 1.** The effects of PGBR on the fructose metabolism and lipid synthesis pathway in liver from NAFLD WKY. (A) Representative western blot images of protein bands. (B) Analysis of protein band density ratios after normalization against  $\beta$ -actin. Values are represented as means  $\pm$  SD ( $n = 8$ ). \* $p < 0.05$  (compared to Control group), # $p < 0.05$  (compared to FLD group).



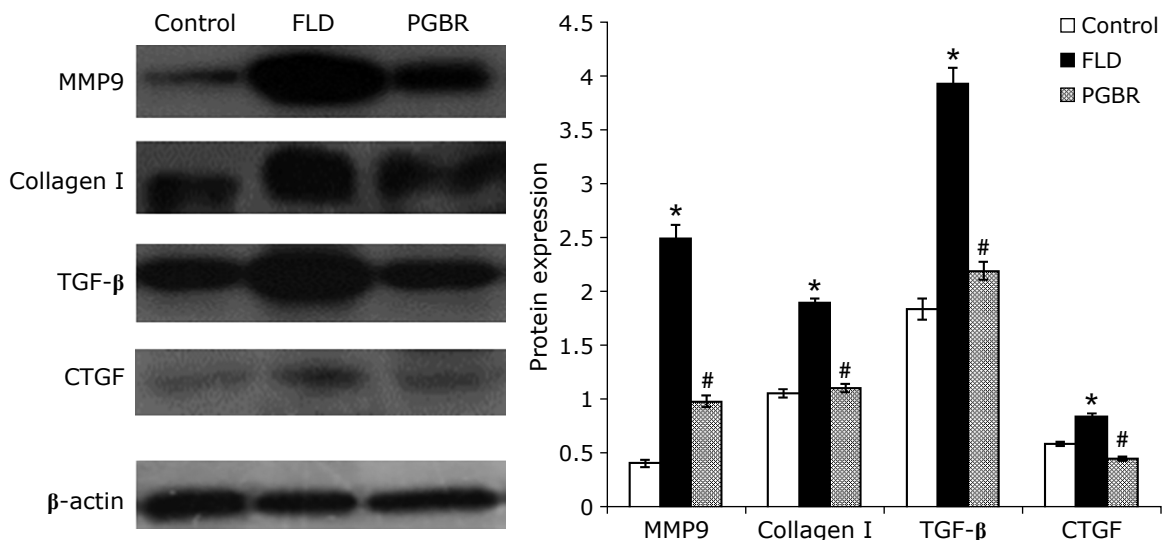
**Fig. 2.** The effects of PGBR on the inflammatory signaling and antioxidant enzymes in liver from NAFLD WKY. (A) Representative western blot images of protein bands. (B) Analysis of protein band density ratios after normalization against  $\beta$ -actin. Values are represented as means  $\pm$  SD ( $n = 8$ ). \* $p < 0.05$  (compared to Control group), # $p < 0.05$  (compared to FLD group).

**Effect of PGBR on HFW/HFD influenced inflammation and free radical scavengers in the liver.** We found the protein expressions of inflammatory signaling, ERK1/2, JNK1/2, and NF- $\kappa$ B in the liver between Control group and FLD group were

different. The levels of ERK1/2, JNK1/2, and NF- $\kappa$ B were increased in FLD group, compared with Control group (Fig. 2). Meanwhile, FLD group had lower expressions of SOD and catalase, in which were the proteins scavenged the free radical



**Fig. 3.** The effects of PGBR on the inflammatory signaling in heart from NAFLD WKY. (A) Representative western blot images of protein bands. (B) Analysis of protein band density ratios after normalization against  $\beta$ -actin. Values are represented as means  $\pm$  SD. ( $n = 8$ ). \* $p < 0.05$  (compared to Control group), # $p < 0.05$  (compared to FLD group).



**Fig. 4.** The effects of PGBR on aberrant cardiac hypertrophy and fibrosis markers in heart from NAFLD WKY. (A) Representative western blot images of protein bands. (B) Analysis of protein bands density ratio after normalization against  $\beta$ -actin. Values are represented as means  $\pm$  SD ( $n = 8$ ). \* $p < 0.05$  (compared to control group), # $p < 0.05$  (compared to DM group).

and reactive oxygen species (ROS). However, treating with PGBR not only decreased inflammatory and peroxidation (NOX2) signaling protein changes, but also reversed free radical scavenger protein variations. Combining the results of GPT, IL-6, and TNF- $\alpha$  in blood (Table 3), we believed that HFW/HFD increased abnormal liver index and inflammation were related to the deterioration of inflammatory and peroxidation signaling and the reduction of free radical scavengers. Then PGBR efficiently improved these risk factors (Fig. 2).

**Effect of PGBR on HFW/HFD induced inflammation and cardiac fibrosis and hypertrophy in the heart.** The FLD group had elevated levels of the inflammatory proteins: TLR4, TNF- $\alpha$ , ERK1/2, NF- $\kappa$ B, and iNOS in myocardium (Fig. 3). Followed cardiac fibrosis and hypertrophy related proteins: MMP9, collagen I, TGF- $\beta$ , and CTGF were also increased in FLD group (Fig. 4), compared to the Control group, confirming that HFW/HFD induced cardiac inflammation, fibrosis, and hypertrophy. PGBR clearly lowered the expression of the cardiac



inflammatory proteins, including COX-2, and the fibrosis and hypertrophy related proteins. From our results, we demonstrated that PGBR had anti-cardiac fibrosis and hypertrophy effects (Fig. 3 and 4).

## Discussion

Following our previous reports,<sup>(11–13)</sup> this study we further proved PGBR that possessed the effects of treating NAFLD and its' related complications in animal model induced by high fructose and high fat ingestion. PGBR decreased the metabolism of fructose, the synthesis of fatty acid and inhibited the inflammation in liver and heart, ameliorated the weight gains of liver and heart, higher blood pressure, and heart rate, liver index (GOT and GPT), cardiac fibrosis and hypertrophy.

Metabolic syndrome, such as obesity, hyperlipidemia and diabetes, is considered to be related with NAFLD.<sup>(14)</sup> If those risk factors are not improved, the hepatic steatosis, lesions, fibrosis, and cirrhosis, etc. pathologic changes will be gradually developed over time.<sup>(15)</sup> Much evidence indicates that NAFLD is a risk factor for the development of hypertension, cardiomyopathy, and coronary heart disease, which enhances cardiovascular morbidity and mortality.<sup>(16)</sup> We found that the HFW and HFD were given in WKY for 4 weeks, and NAFLD and cardiomyopathy occurred. Why the high fructose and high fat intake can cause NAFLD? Short term fructose intake encourages thermogenesis and metabolic rate.<sup>(17,18)</sup> In contrast, long-term fructose intake diminishes resting energy expenditure. Fructose intake stimulates *de novo* lipogenesis and blocks hepatic  $\beta$ -fatty acid oxidation in animals.<sup>(19)</sup> First, fructose is phosphorylated by fructokinase to form fructose-1-phosphate in the liver. Then fructose-1-phosphate is divided by hepatic aldolase to form dihydroxyacetone phosphate and glyceraldehyde. In addition, dihydroxyacetone phosphate is an intermediate metabolite in both the gluconeogenic and glycolytic pathway. The circulating glucose will be increased after gluconeogenesis.<sup>(20)</sup> Secondly, dihydroxyacetone phosphate is transformed to glycerol-3-phosphate by G3PD, and then glycerol phosphate combines fatty acid to TG. At the same time, ingestion of high fructose and high fat increases the glycerol levels that to convert to glycerol-3-phosphate by glycerol kinase, is also an important pathway to form TG.<sup>(20)</sup> Regardless of fructose or glucose provides pyruvate to transform malonyl-CoA by ACC. Then malonyl-CoA converts to fatty acyl-CoA that combines with glycerol and fatty acid to TG. In the results, we found the fructokinase, aldolase, G3PD, glycerol kinase and ACC were increased in FLD group. However, treatment with PGBR could decrease those over-expressed enzymes (Fig. 1), except ACC, and ameliorate hepatic weight (Table 1) and hypertriglyceridemia (Table 3). On the other hand, the metabolism of glucose or fructose by fructokinase requires the consummation of adenosine triphosphate (ATP), and then ATP is catabolized into adenosine monophosphate (AMP) and uric acid.<sup>(21)</sup> PGBR could diminish the levels of uric acid through inhibiting fructokinase and fructose metabolism (Table 3).

In fact, fructose also simultaneously influences other key enzymes that biosynthesize lipids, for example, SREBP-1, SCD-1, and FAS. As we are known, SREBP-1 is a transcriptional activator and regulates the cholesterol and fatty acid synthesis and metabolism.<sup>(22)</sup> SCD-1 is an endoplasmic reticulum enzyme that catalyzes the biosynthesis of monounsaturated fatty acids from saturated fatty acids that are either synthesized or derived from the diet.<sup>(22)</sup> FAS is a multi-enzyme protein that catalyzes fatty acid synthesis and participates in metabolism of energy.<sup>(23)</sup> We confirmed that SREBP-1, SCD-1, and FAS were enhanced by HFW/HFD. When we treated PGBR concurrently with HFW/HFD, those enzymes were reduced (Fig. 1). We suggested that PGBR could decrease the metabolism of fructose and the

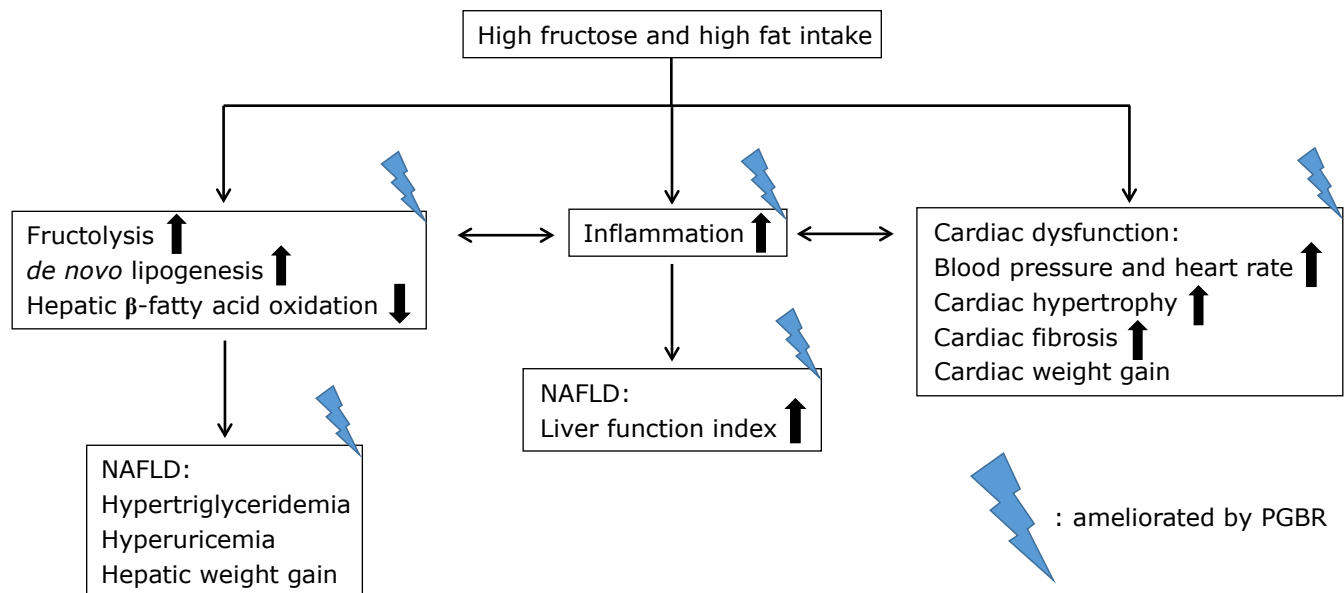
biosynthesis of lipids.

PPARs are transcription factors that modulate the expression of genes and enzymes involved in lipid metabolism, energy homeostasis and inflammation being altered by diet.<sup>(24)</sup> In addition, PPAR- $\alpha$  is the main regulator in lipid catabolism and lipoprotein metabolism. PPAR- $\alpha$  activation leads to  $\beta$ -oxidation as it allows the fatty acid to reach the mitochondrial matrix, and markedly decreased by HFW/HFD intake.<sup>(25)</sup> PPAR- $\gamma$  is related to adipogenesis and insulin-sensitizing effects through the diversion of fatty acids to adipose tissue storage. Patients with NAFLD exhibit abnormal high expression of PPAR- $\gamma$  in the liver, which coincides with overexpression of SREBP-1 and the consequent hepatic lipogenesis.<sup>(4)</sup> In the results, we also confirmed that PPAR- $\alpha$  was decreased, and that PPAR- $\gamma$  was induced by the ingestion of high fructose and high fat. In treating group, PGBR improved the aberrant PPAR- $\alpha$  and PPAR- $\gamma$  (Fig. 1). We believed other mechanism of PGBR's anti-NAFLD effect was to ameliorate the expression of PPARs. In brief, PGBR effectively reversed the enzymes of fructose metabolism and lipids biosynthesize, so the TG levels in blood and liver induced by HFW/HFD were decreased. We suggested that the fat accumulation was inhibited, in which the enhancement of weight gain in PGBR group was gentler than that of FLD group.

Persistence and chronic inflammation caused by metabolic syndrome, DM or NAFLD has been considered as a potent mediator to induce oxidative stress for the production of more ROS, reactive nitrogen species (RNS). Many reports demonstrate that mitogen-activated protein kinases (MAPKs) pathway plays the key role in it.<sup>(26,27)</sup> On the other hand, several protective systems in the body diminish the ROS and free radical formation. For instance, SOD and catalase are the enzymes which scavenge ROS and free radical and improve the inflammation.<sup>(27)</sup> In our results, we found that the expressions of ERK, JNK, and NF- $\kappa$ B were induced, at the same time, the expression of NOX-2, a superoxide generating enzyme, was not changed, but the expressions of SOD and catalase were reduced by HFW/HFD in the liver. Treatment of PGBR ameliorated these proteins significantly (Fig. 2). We suggested that was the reason why PGBR could improve liver index (GOT and GPT).

NAFLD not only causes a progressive inflammation, but also plays a risk factor for development of cardiac fibrosis, hypertrophy and cardiomyopathy.<sup>(16)</sup> TNF- $\alpha$  binding to its' receptor, TLR4, irritates MAPKs and NF- $\kappa$ B signaling pathway, and then induces the downstream protein, such as iNOS to generate more serious inflammation and ROS.<sup>(28)</sup> In the myocardium, MAPKs and NF- $\kappa$ B pathway stimulate MMPs, collagen TGF- $\beta$  and CTGF to induce cardiac fibrosis and hypertrophy.<sup>(29)</sup> In the results, the expressions of TNF- $\alpha$ , TLR4, ERK1/2, NF- $\kappa$ B, iNOS, MMPs, collagen, TGF- $\beta$ , and CTGF were significantly increased in the heart, meanwhile, the cardiac weight, blood pressure and heart rate were enhanced by NAFLD. After treatment with PGBR, those proteins, including COX-2, were obviously improved, and the cardiac dysfunctions were reversed evidently. PGBR could improve cardiovascular dysfunction induced by DM and NAFLD through inhibiting MAPKs and NF- $\kappa$ B pathway,<sup>(11–13)</sup> and then decreasing the related proteins of cardiac hypertrophy and fibrosis (Fig. 3 and 4).

In conclusion, high fructose and high fat intake could induce NAFLD and its' related cardiac complications in this short duration animal model. PGBR ameliorated hypertriglyceridemia, hyperuricemia, liver function index, hepatic weight gain; and improved blood pressure and heart rate enhancement, cardiac hypertrophy and fibrosis, cardiac weight gain. We suggested that was associated with PGBR amending the metabolism of fructose and the biosynthesis of lipids, expression of PPARs and inflammation (Fig. 5). Finally, PGBR is worthy to recommend as a healthy staple food controlling NAFLD.



**Fig. 5.** Hypothetical mechanisms of PGBR in high fructose/high fat intake-induced non-alcoholic fatty liver disease (NAFLD). The action mechanisms of PGBR ameliorating the metabolism of fructose and the biosynthesis of lipids, expression of PPARs and inflammation. Taken together, PGBR was able to ameliorate hypertriglyceridemia, hyperuricemia, liver function index, hepatic weight gain; and improved blood pressure and heart rate enhancement, cardiac hypertrophy and fibrosis, cardiac weight gain.

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## Conflict of Interest

No potential conflicts of interest were disclosed.

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