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Purple wheat alleviates dyslipidaemia in rat model

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Abstract

To study the exact effect of purple wheat (PW) on dyslipidaemia in rats so as to lay the foundation of a dietary therapy for hyperlipidaemia. A total of 42 rats were randomly divided into two groups: normal control group (NC) consisting of 12 rats were fed with standard diet in the whole testing process, the other group rats were fed with high-fat diet to induce dyslipidaemia. The dyslipidaemic rats were averagely divided into three groups: dyslipidaemia control group (DC) with 60% common wheat flour, PW1 group with diet containing 60% purple wheat Jizimai1 flour, PW3 group with 60% purple wheat Jizimai3 flour. Six weeks later, the weight-gain-ratio of the rats in PW1 and PW3 groups were lower as compared to the rats in DC and NC groups. Further, we found that the levels of triglyceride, total cholesterol and low-density lipoprotein in the PW1 and PW3 groups were significantly reduced to almost that of normal levels. Finally, the results of hematoxylin and eosin staining showed that intaking of Jizimai1 and Jizimai3 could repair hepatocyte steatosis and kidney injury due to dyslipidaemia. Purple wheat diet therapy reduced lipid metabolism disorders and the liver tissue and renal injury in hyperlipidaemic rats.

Keywords: purple wheat; hyperlipidaemia; body weight; liver; kidney.

Practical Application: Purple wheat plays an important role in alleviating lipid metabolism disorders and reducing hepatocyte steatosis and renal injury.

1 Introduction

Cardiovascular and cerebrovascular diseases are a major threat to human health. Hyperlipidaemia is a potential and independent risk factor (De Moura et al., 2009; Hong et al., 2012; Navar-Boggan et al., 2015) for various cardiovascular and cerebrovascular diseases including atherosclerosis and coronary heart disease. In order to decrease the morbidity and mortality rates of cardiovascular and cerebrovascular diseases, it is important to prevent hyperlipidaemia (Bolton-Smith et al., 1991), for which dietary therapy and good lifestyle are important (Rosenthal, 2000; Jeon et al., 2001; Jenkins et al., 2003; Jacobson et al., 2015; Yu et al., 2015; Gao et al., 2017). Therefore, the prevention of cardiovascular and cerebrovascular diseases by consumption of foods with lipid-lowering activity has become a hot topic in the field of nutrition science. There are many reports (Vahouny, 1982; Hassona, 1993; Kahlon & Chow, 1997; Lecumberri et al., 2007) about the effect of dietary fibre on blood lipid. A study (Zhang et al., 2016) concluded that dietary fibre composed of wheat, corn, grains and beans can significantly reduce the levels of triglyceride (TG), total cholesterol (TC) and low-density lipoprotein (LDL) in hyperlipidaemic rats. Generally, dietary fibre from a mixture of grains and beans is more effective than a single dietary fibre of wheat or corn. Lundin et al. (2004) that whole wheat bread can significantly reduce the TC and TG levels in patients with colitis, Leinonen et al. (2000) demonstrated that whole purple wheat bread can effectively reduce the serum levels of TC and LDL in men. The most recent report (Liu, 2017) that black whole wheat flour can significantly reduce serum levels of TC, TG and LDL, and increase serum levels of the high-density lipoprotein (HDL) in hyperlipidaemic rats. These findings suggest that purple wheat can alleviate lipid metabolism disorders.

Purple wheat is nutritionally valuable (Li et al., 2002; Liu et al., 2002; Li et al., 2003; Jaafar et al., 2013; Lan et al., 2013), and it contains many microelements, amino acids, proteins, natural melanin and anthocyanins. Our previous report (Lan et al., 2013) demonstrated that the bread made from Jiziheiixoamai1 (also named Jizi439), a new purple wheat, could reduce the levels of postprandial blood glucose in diabetic patients, and different purple wheat varieties had different effects on the postprandial blood glucose levels. However, the role of purple wheat in reducing fat and the differences in lipid-lowering effects among different varieties of purple wheat remains unknown. To address these problems, we explored the effects of different purple wheat varieties on weight gain and lipid metabolism in hyperlipidaemic rats. We believe that this study will provide a theoretical basis for the prevention and treatment of diseases caused by a high-fat diet.

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†Equal contributors: Yaning Meng and Suque Lan led the implementation of the majority project, conducted majority of the data collection and analysis, and drafting the manuscript. Yaning Meng and Suque Lan contributed to the study equally.

2 Materials and methods

2.1 Sample

Two varieties of purple wheat (Jizimai1, also mamed Jizi439, and Jizimai3) were selected for the study, with 15% of the bran removed. Flour of the two varieties of purple wheat was obtained from the Institute of Cereal and Oil Crops, the Hebei Academy of Agricultural and Forestry Sciences (Shijiazhuang, China). Common wheat flour was purchased from the supermarket. The feed used in the experiment was produced by the experimental animal center of the Hebei Medical University (Shijiazhuang, China).

High-density lipoprotein cholesterol detection Kit (k075) and low-density lipoprotein cholesterol detection Kit (k076) were purchased from Changchun Huili Biotechnology Co., Ltd. (Changchun, China). A triglyceride detection kit (auz3592) and total cholesterol detection kit (auz3625) were purchased from Beckman Kurt Experimental System Co., Ltd. (Suzhou, China). Cholesterol and sodium cholate were purchased from Henan yipinshihua Co., Ltd. (Zhengzhou, China); yolk powder was purchased from Beijing Kaiyuan Feed Co., Ltd. (Beijing, China); and lard was obtained from Linyi Xincheng Jinluo Meat Products Group Co., Ltd. (Linyi, China).

2.2 Experimental animals

Forty-two male SPF (Specific Pathogen Free) grade SD (Sprague-Dawley), weighing 180-210 g, provided by Liaoning Changsheng Biotechnology Co., Ltd. (Benxi, China), license No.: scxk (Liao) 2015-0001, were used for the study. Forty-two rats were raised in separate cages in a controlled environment (22 °C ± 1 °C, 12-h light/12-h dark cycle). Rats were fed a common diet *ad libitum* and provided with filtered tap water for 7 days to acclimatize to their new environment.

2.3 Diets and experimental design

The study followed the national guidelines for care and use of animals, and all experimental procedures were approved by the Animal Ethics Committee at the second hospital of the Hebei Medical University (Shijiazhuang, China; Approval Number: 303-09-02-67). Forty-two rats were randomly divided into two groups. The normal control (NC) group consisted of 12 rats which were fed with a standard diet in the whole testing process. The model group consisted of 30 rats which were fed with a high-fat diet, after 4 weeks of feeding, blood was collected from the tail vein and serum levels of TC, TG, LDL and HDL were determined. Serum levels of TC, TG and LDL were significantly higher in the model group than in the control group ($p<0.05$). This indicated (Gao et al., 2017) that dyslipidaemia models were successfully established.

The thirty hyperlipidaemic rats were randomly divided into three groups, 10 rats in each group. The dyslipidaemia control (DC) group was fed with a diet containing 60% common wheat flour; the purple wheat 1 (PW1) group was fed with a diet containing 60% purple wheat Jizimai1 flour; and the purple wheat 3 (PW3) group was fed with a diet containing 60% purple wheat Jizimai3 flour. The composition of the experimental diets is presented in Table 1, and nutrition composition of different diet has been shown in Table 2. The rats were fed

Table 1. Composition of experimental diets (g/kg of mixture).

Ingredients	high-fat	standard	DC	PW1	PW3
	diet	diet	group	group	group
purple wheat flour	-	-	-	600	600
Common wheat flour	197	250	600	-	-
Corn flour	213	270	-	-	-
Soybean meal	166	210	130	130	130
Wheat bran	118	150	150	150	150
Fish meal	63	80	80	80	80
vitamins	10	15	15	15	15
Fish liver oil	12	15	15	15	15
Slat	10	10	10	10	10
cholesterol	10	-	-	-	-
sodium cholate	2	-	-	-	-
lard	100	-	-	-	-
yolk powder	100	-	-	-	-

Table 2. Nutrition compositions of the diets fed to rat.

Nutrient compositions	high-fat	standard	DC	PW1	PW3
	diet	diet	group	group	group
Total calorie(kcal)	399.25	311.45	301.20	304.70	307.60
Total protein(g/100g)	14.77	16.80	16.30	17.60	17.80
Total fat(g/100g)	16.19	5.70	4.00	4.00	4.00
Total carbohydrate (g/100g)	36.55	45.60	51.20	49.30	51.60
Total fiber(g/100g)	8.86	10.80	9.40	9.70	7.20
Total vitamins (g/100g)	1.00	1.50	1.50	1.50	1.50
Total minerals (mg)	1816.11	2281.70	2056.56	2359.99	2627.31

regularly and quantitatively every day for six weeks and their daily activities were observed. The bedding and drinking water were changed regularly and the weight of the rats was monitored once a week. At the end of the experiment, all rats were fasted overnight and euthanized using 4% fluothane in oxygen anaesthesia in an airtight container. Before euthanasia, blood was collected from the inner canthus vein at 3000 rmp for 15 min at 4 °C. The resulting supernatant was stored at -80 °C for biochemical analysis. Liver and kidney were excised, weighed, and stored at -80 °C. Flowchart of the experiment is presented in Figure 1.

2.4 Blood lipid estimation

The serum levels of TC, TG, HDL and LDL were measured according to the kit manufacturer's instructions.

2.5 Histopathological examination

For haematoxylin eosin (HE) staining, the liver and the kidneys of the rats were washed in cold 0.9% normal saline, and the weight of each organ was measured, the liver and the kidneys samples were fixed in 4% polyformaldehyde and embedded in paraffin. The organs were then sectioned and the sections were stained with haematoxylin and eosin, and observed under 10×and 40× magnification of a microscope.

2.6 Statistical analysis

Statistical analysis was performed using the Statistical Product and Service software, version 19.0 (SPSS, USA). The data have been expressed as mean ± SEM. Comparisons between two groups were performed using independent samples t-test. $p<0.05$ was considered statistically significant.

3 Results and discussion

3.1 Effect of purple wheat on the body weight of hyperlipidaemic rats

The weight of the rats was measured once a week. It was observed that the body weight of rats continuously increased up to six weeks; NC group rats had the highest body weight, followed by DC and PW1 group rats, and PW3 group rats had the lowest weight. In this study, we observed that the weight-gain-ratio of the PW1 and PW3 group rats was significantly lower than that of the DC group and the NC group rats (Figure 2), which was in agreement with previous reports (Liu, 2017). Furthermore, we found that the weight-gain-ratios of the PW3 group rats were significantly lower than that of the PW1 group rats in the sixth week ($p<0.05$), Therefore, purple wheat could lead to a reduction of weight gain in hyperlipidaemic rats, and different purple wheat varieties had different effects in reducing the weight gain.

3.2 Effect of purple wheat on blood lipid levels in hyperlipidaemic rats

This result shows that the serum levels of TG, TC and LDL in the PW1, PW3 and DC group rats significantly reduced after a dietary intervention as compared with those before a dietary intervention ($p<0.05$) (Table 3). As shown in Table 3, compared to the NC group, there was no significant difference in serum levels of TG, TC, HDL and LDL between the DC, PW1 and PW3 groups ($p>0.05$), reaching serum levels to that of normalcy. Our results were found to be consistent with those of a study conducted by Liu (Liu, 2017). We found serum levels of TC, TG and LDL decreased to varying levels in the PW1 and PW3 groups as compared with the DC group, although there was no significant difference. Liu (Liu, 2017) reported that Serum

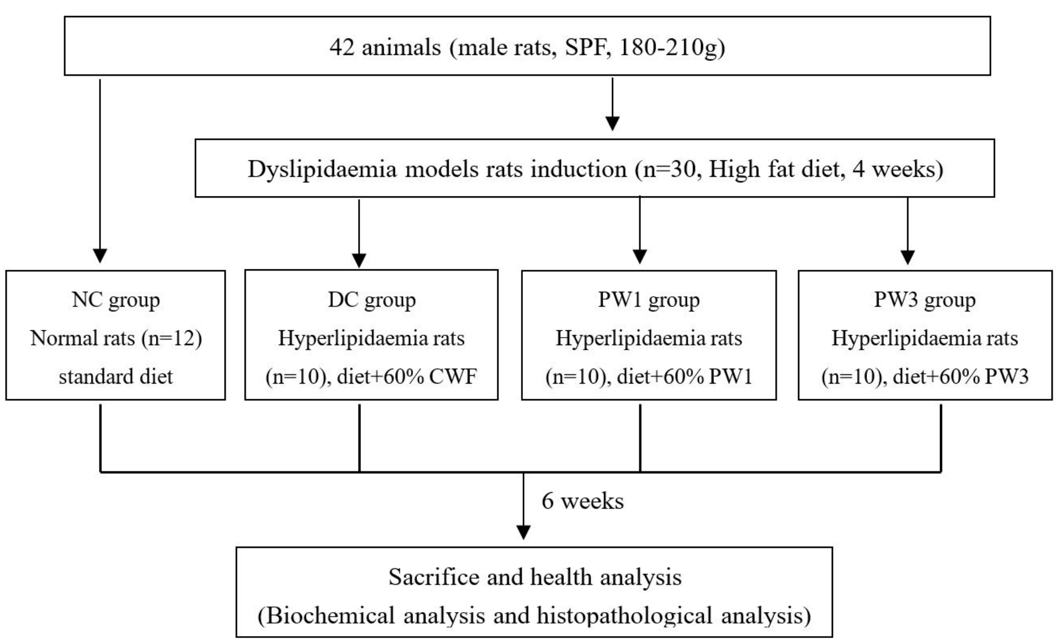

Figure 1. Flowchart of the experiment. SPF=Specific Pathogen Free; NC=normal control; DC=dyslipidaemia control; CWF=common wheat flour; PW1 =purple wheat Jizimai1 flour; PW3=purple wheat Jizimai3 flour.

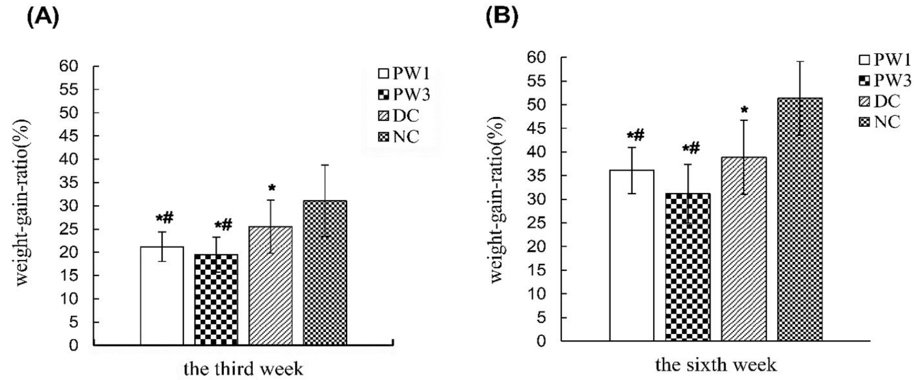

Figure 2. Weight-gain-ratio of rats in each group at different time periods of dietary intervention (A) Weight-gain-ratio of rats at the third week; (B) Weight-gain-ratio of rats at the sixth week. PW1 group: diet containing 60% purple wheat Jizimai1 flour; PW3 group: diet containing 60% purple wheat Jizimai3 flour; DC group: dyslipidaemia control group, diet containing 60% common wheat flour; NC: normal control, standard diet. Data have been expressed as mean ± S.D. * means *P*<0.05(vs NC group), # means *P*<0.05(vs DC group).

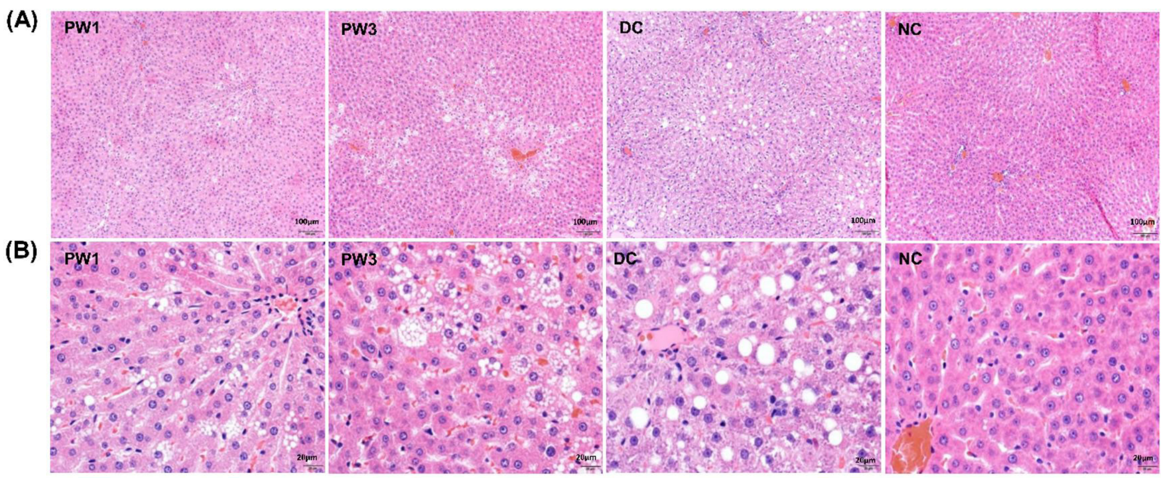

Figure 3. The liver of rats was examined with HE staining after six weeks of dietary intervention (A) 10x, scale bar=100μm; (B) 40x, scale bar=20μm. PW1 group: diet containing 60% purple wheat Jizimai1 flour; PW3 group: diet containing 60% purple wheat Jizimai3 flour; DC group: dyslipidaemia control group, diet containing 60% common wheat flour; NC: normal control, standard diet.

Table 3. The serum levels of TC、TG、HDL、LDL in different groups.

Time	Group	TG (mmol/L)	TC (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
Before dietary intervention	Model rats	1.21±0.46	3.16±0.42	0.93±0.16	2.18±0.29
dietary intervention for six weeks	PW1	0.66±0.25*	2.14±0.34*	0.45±0.06	1.40±0.30*
	PW3	0.54±0.20*	2.08±0.28*	0.41±0.14	1.43±0.21*
	DC	0.69±0.31*	2.33±0.44*	0.57±0.13	1.44±0.35*
Standard diet for six weeks	NC	0.71±0.12	2.07±0.26	0.43±0.15	1.35±0.42

Data are mean ±SDs, *means *p<0.05*.(compared with Before dietary intervention)

TC and TG significantly decreased, and HDL significantly elevated by the whole purple wheat. The results of this study are inconsistent with those. The reason may be the difference between different varieties of purple wheat, or purple wheat flour without 15% bran in the present study. It is generally known that dietary fiber is rich in bran, and many studies have reported that the dietary fiber of wheat can reduce the blood lipid level of rats with dyslipidaemia (Bakhsh & Chughtai, 1984; Zhang et al., 2015). Jing Wang et al. (2011) used wheat bran xylose as a new functional oligosaccharide instead of arabinose, and found that 5% wheat bran xylose oligosaccharide was beneficial to maintain the weight and lipid homeostasis (Wang et al., 2011). Therefore, whole flour of purple wheat Jizimai1 and Jizimai3 could be more effective in alleviating dyslipidaemia in rats.

3.3 Effect of purple wheat on the liver of hyperlipidaemic rats

The pathological changes in the liver of hyperlipidaemic rats were detected by the HE staining (Figure 3). As compared to the NC group, the DC group showed an unclear liver lobule structure, disordered arrangement of the hepatocyte cords, and disappearance of the cords (Figure 3, DC). Fatty degeneration was observed in almost all the hepatocytes of the DC group. Many

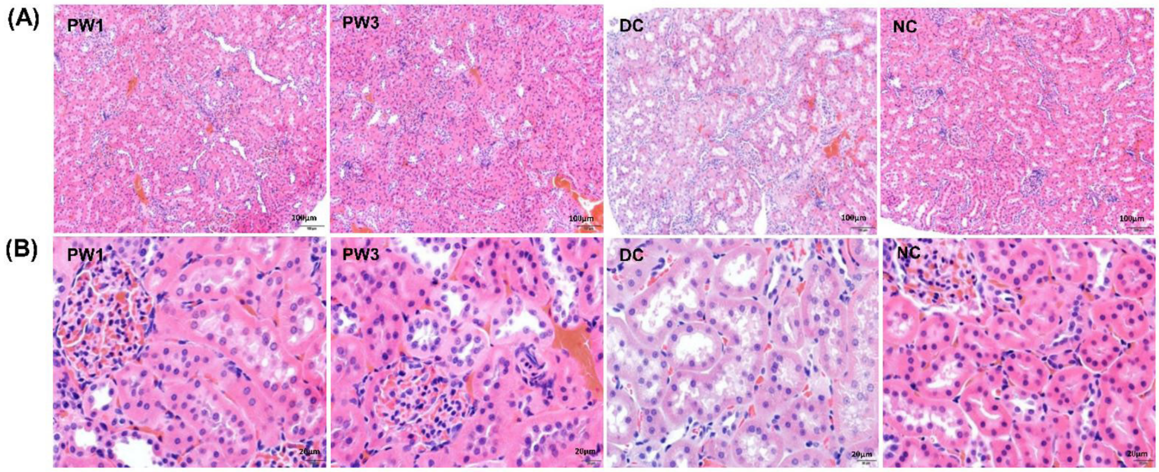

Figure 4. The kidneys of rats were examined with HE staining after six weeks of dietary intervention (A) 10x, scale bar=100μm; (B) 40x, scale bar=20μm. PW1 group: diet containing 60% purple wheat Jizimai1 flour; PW3 group: diet containing 60% purple wheat Jizimai3 flour; DC group: dyslipidaemia control group, diet containing 60% common wheat flour; NC: normal control, standard diet.

large-area fat vacuoles of different sizes could also be observed. Moreover, the size of hepatocytes was different, and some nuclei were observed to be squeezed and displaced.

The fat denaturation was lighter in PW1 (Figure 3, PW1) and PW3 groups (Figure 3, PW3) as compared to that in the DC group. In PW1 group, the arrangement of hepatocyte cords was more ordered than that in DC and PW3 groups, and the size of hepatocytes was more uniform than that in PW3 group. However, 30% of the hepatocytes in PW1 group showed steatosis, and a small number of nuclei were squeezed and displaced. In PW3 group, the arrangement of hepatocyte cords was disordered, fatty degeneration was observed in 60% of the hepatocytes, and a large number of fat vacuoles were observed in the liver tissue. Moreover, the size of hepatocytes in PW3 group was different, but they were more orderly than those in the DC group, and the phenomenon of a nuclear extrusion and displacement was lighter than that in the DC group. These results showed that purple wheat could attenuate the abnormal liver lipid metabolism and alleviate fatty degeneration in the liver.

3.4 Effects of purple wheat on the kidneys of hyperlipidaemic rats

HE staining was used to determine the pathological changes in the kidneys of rats (Figure 4). Compared to the NC group, the DC group showed a loose renal tissue structure (Figure 4, DC), disordered cell arrangement, and evident edema of the glomerular and renal tissue. Moreover, the DC group showed irregular renal tubular epithelial cells, displaced cell nuclei, atrophic cytoplasm in the proximal and distal convoluted tubules, broken cells in the lumen, widened lumen space, and evident renal tissue lesions.

Compared to the DC group, the adverse pathological changes in the kidneys of PW1 (Figure 4, PW1) and PW3 groups (Figure 4, PW3) were alleviated. In PW3 group, the renal tissue structure was uniform and orderly, the structure of tubulointerstitial area was clear, and the shape of renal tubuloepithelial cells was regular. Moreover, the size of the cells was consistent, the arrangement was orderly, and the nucleus was in the center of cells. The lumen space was normal and almost no significant pathological changes were seen in the kidneys. In PW1 group, the shape of renal tubular epithelial cells was slightly irregular, whereas all the other characteristics were normal. Therefore, we suggest that purple wheat plays an important role in alleviating the renal injury in hyperlipidaemic rats.

Hyperlipidaemia can lead to liver injury in rats, resulting in an abnormal lipid metabolism and accumulation of lipids in the liver (Calamita & Portincasa, 2007). The present study showed that after six weeks of dietary intervention, purple wheat reduced the fatty degeneration and alleviated the renal injury. In contrast, the DC group showed fatty degeneration in almost all hepatocytes and evident renal tissue lesions. Therefore, purple wheat flour is more effective than common wheat in protecting the rat liver and kidneys of hyperlipidaemic rats, both the varieties of purple wheat used in this study are rich in nutrients such as minerals, amino acids, proteins, and natural melanin (Li et al., 2002; Liu et al., 2002; Li et al., 2003). A large number of studies have shown that mineral elements can alleviate lipid metabolism disorders in hyperlipidaemic rats (Koo & Lee, 1988; Zaporowska & Wasilewski, 1992; Neggers et al., 2001; Wu et al.,2004; Wu et al., 2006; Huster et al., 2007), proper supplementation of zinc, selenium, copper, vanadium and magnesium can alleviate metabolic disorders in rats. Other studies have shown that anthocyanin can regulate lipid metabolism in hyperlipidaemic rats (Xia et al., 2006; Yang et al. 2011). This may be one of the reasons why purple wheat flour has a good lipid-lowering effect as compared to that by the common wheat flour. However, the mechanism by which purple wheat alleviates dyslipidaemia in rats remains unclear and needs further research.

4 Conclusion

(1) The weight-gain ratio of the PW1 and PW3 group rats decreased significantly after six weeks of dietary intervention, indicating that purple wheat could regulate the body weight of hyperlipidaemic rats, Jizimai3 being better than Jizimai1 in

reducing weight gain. (2) After six weeks of dietary intervention, the levels of serum TG, TC and LDL in hyperlipidaemic rats significantly reduced, suggesting that purple wheat could regulate the blood lipid levels of rats with abnormal lipid metabolism. (3) Moreover, purple wheat diet therapy reduced fatty degeneration of the liver tissue and alleviated lipid metabolism disorders and renal injury in hyperlipidaemic rats. Thus, purple wheat is more effective than the common wheat in alleviating liver and kidney injury in hyperlipidaemic rats.

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