БИОХИМИЯ И БИОТЕХНОЛОГИЯ

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Changqing Tong, Xinyao Li, Chengcheng Cai, Xuan Shi, Wei Li
College of Food Science and Engineering, Dalian Ocean University,
116023, Dalian, China

HEPATOPROTECTIVE AND LIPID-LOWERING EFFECT OF AN APPLE VINEGAR BEVERAGE WITH OYSTER POLYSACCHARIDES

The hepatoprotective and lipid-lowering effects of apple vinegar with oyster polysaccharides (OPAV) were investigated. The protective effect of OPAV on acute ethanol-induced liver injury and high-fat diet were investigated in BALB/c mice. Treatment with OPAV decreased serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), and malondialdehyde aldehyde (MDA) levels and increased superoxide dismutase (SOD) and alcohol dehydrogenase (ADH) activity in an acute ethanol-induced liver injury model. Treatment with OPAV decreased serum total cholesterol (TC) and triglycerides (TG), and liver tissue TC and TG levels, and it increased high-density lipoprotein cholesterol (HDL-C) and excrement TC and fat content in a high-fat diet model.

Key words: *apple vinegar, oyster polysaccharides, hepatoprotective, lipid-lowering effect.*

Чанцин Тун, Синьяо Ли, Чэнчэн Цай, Сюань Ши, Вэй Ли
Колледж пищевых наук и инженерии, Даляньский океанологический университет,
116023, Далянь, КНР

ГЕПАТОПРОТЕКТОРНОЕ И ГИПОЛИПИДЕМИЧЕСКОЕ ДЕЙСТВИЕ НАПИТКА ИЗ ЯБЛОЧНОГО УКСУСА С ПОЛИСАХАРИДАМИ УСТРИЦ

Были исследованы гепатопротекторное и гиполипидемическое действие яблочного уксуса с устричными полисахаридами. Защитный эффект при остром повреждении печени, вызванном этанолом, и диете с высоким содержанием жиров был исследован на белых мышах. Лечение с использованием яблочного уксуса с устричными полисахаридами снижало уровни аспартатаминотрансферазы, аланинаминотрансферазы и малонового диальдегида сыворотки крови, а также повышало активность супероксиддисмутазы и алкогольдегидрогеназы при остром поражении печени, вызванном этанолом. Лечение с помощью яблочного уксуса с устричными полисахаридами снижало общий уровень холестерина и триглицеридов в сыворотке крови и уровень холестерина и триглицеридов в тканях печени, а также повышало содержание холестерина липопротеинов высокой плотности (ЛПВП-Х), способствовало выведению холестерина и жира в модели диеты с высоким содержанием жиров.

Ключевые слова: *яблочный уксус, устричные полисахариды, гепатопротекторное и гиполипидемическое действие.*

Introduction

Excessive consumption of ethanol leads to fat accumulation, inflammation in the liver, cirrhosis and hepatocellular carcinoma [1]. Ethanol abuse is associated with serious health problems of the liver. High-fat food also can also potentially damage the liver and cause the accumulation of a large amount of fat in hepatocytes [2, 3]. Numerous studies have focused on identifying the effects of protective agents on ethanol-induced liver injury and lowering the hepatic lipid levels [4–14]. Therefore, therapies that prevent alcoholic liver disease may also be beneficial in the later stages of the disease.

Oysters are used as food products and have been used as traditional medicinal shellfish for a long time [15]. Oysters are rich in protein and polysaccharides. In recent years, the polysaccharides present in oysters have become the focus of intense interest because of their various bioactivities. A water-soluble polysaccharide (CGPS-1) with hepatoprotective effects was isolated from *Crassostrea gigas* [16]. Unfortunately, data regarding the use of CGPS-1 in food products are limited.

Vinegar is used as a condiment, and because of its physiological effects, it also has traditional medicinal uses, including promoting recovery from exhaustion [17], regulating blood glucose [18] and blood pressure [19], and exerting antioxidant activity [20]. Vinegar can be made from various sources. Fruit vinegar beverages are produced by fermenting fruits, including apples, grapes, strawberries, pears and hawthorn berries, as the main raw material [21–25]. Fruit vinegar beverages have often been considered to have physiological health benefits [26].

The effect of the blended foods or food components on biological systems is greater than or different from the corresponding effects of the individual food or food components [27]. This study establishes the possibility of formulating a 'liver-healthy' functional apple vinegar beverage using hepatoprotective CGPS-1. Blending apple vinegar with oyster polysaccharide CGPS-1 (OPAV) could enhance its physiological effects. In this report, we describe the potential hepatoprotective and lipid-lowering effects of OPAV. The hepatoprotective effect of OPAV was investigated using an ethanol-induced (acute) hepatocyte toxicity mouse model. The protective effect against ethanol-induced liver injury was assessed by measuring alanine aminotransferase (ALT), aspartate aminotransferase (AST), malondialdehyde (MDA), and trace glutathione (GSH) levels and superoxide dismutase (SOD) activity. The lipid-lowering effect of OPAV was investigated using a fatty liver mouse model and assessed by measuring low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), triglycerides (TG) and fat content and by evaluating histopathological changes.

Materials and methods

Materials

Apple vinegar was purchased from Henan Zhengxin Tianyuan Food Co., Ltd. (Xindeng, China). Oyster polysaccharides (CGPS-1) were isolated and separated according to the method described by Shi et al. (17). Tiopronin was purchased from Henan Xinyi Pharmaceutical Co., Ltd. (Xinxiang, China). Simvastatin was purchased from Harbin Pharmaceutical Group Sanjing Pharmaceutical Co., Ltd. (Suihua, China). Assay kits for alcohol dehydrogenase (ADH), ALT, AST, MDA, SOD, LDL-C, HDL-C, TC and TG were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The male Kunming mice were purchased from Dalian Medical University Animal Center (Dalian, China).

Preparation of OPAV

To prepare the OPAV, the 1 % yeast (W/V) was added into the oyster polysaccharides solution and cultured at 30 °C for 30 min. After culturing, the apple vinegar beverage was added to the fermentation solution and centrifuged at 4000 × g for 15 min. The resulting supernatant was the OPAV. The general OPAV composition used in this study is given in Table 1. The OPAV components consisted of water, carbohydrate, sodium, total acid, malic acid, citric acid and lactic acid were 948.65, 47.00, 0.20, 3.28, 0.56, 0.29 and 0.02 g/kg, respectively.

Animals and treatment protocol

Six- to eight-week-old BALB/c male mice were purchased from the Laboratory Animal Center of Dalian Medical University (China) and maintained in a well-ventilated room with free access to sterile standard mouse chow and water. The mice were housed under standard husbandry conditions (22 ± 2 °C, 60-70 % relative humidity and 12-h light-dark cycle). The animals were handled according to the rules and regulations of the Institutional Animal Ethics Committee of Dalian Ocean University, China. The high-fat diet consisted of 77.6 % standard laboratory diet, 10 % egg yolk, 10 % pig fat, 2 % cholesterol and 0.4 % sodium cholate and was prepared by our laboratory.

Table 1

General composition of the apple vinegar beverage (g /kg)

Component	Amount
Water	948,65
Carbohydrate	47,00
Lipid	0
Protein	0
Sodium	0,20
Total acid	3,28
Malic acid	0,56
Citric acid	0,29
Lactic acid	0,02

The intoxication time, potential hepatoprotective effect and lipid-lowering effect of OPAV were evaluated using the following three treatment protocols:

a) BALB/c male mice were randomly divided into five groups (ten animals per group). After fasting for 24 h, the mice were gavaged with 52 % aqueous ethanol solution (10, 12.5, 15, 17.5 and 20 mL/kg body weight [b.w.] oral dose [p.o.]). The intoxicated time is defined as the disappearance time of the righting reflex.

b) BALB/c male mice were randomly divided into six groups of twelve animals each: (1) Group 1, treated with saline as a negative control; (2) Group 2, treated with saline and given 52 % (v/v) ethanol [12.5 mL/kg b.w. p.o. for 6 days]; (3) Group 3, treated with tiopronin (50 mg/kg b.w. p.o.) + 52 % (v/v) ethanol (12.5 mL/kg b.w. p.o.) for 6 days as a positive control; (4) Group 4, treated with OPAV (5 mL/kg b.w. p.o.) + 52 % (v/v) ethanol (12.5 mL/kg b.w. p.o.) for 6 days; (5) Group 5, treated with OPAV (10 mL/kg b.w. p.o.) + 52 % (v/v) ethanol (12.5 mL/kg b.w. p.o.) for 6 days; and (6) Group 6, treated with OPAV (15 mL/kg b.w. p.o.) + 52 % (v/v) ethanol (12.5 mL/kg b.w. p.o.) for 6 days. Thirty minutes after the last treatment, the mice were sacrificed, and their blood and liver were collected immediately.

c) BALB/c male mice were randomly divided into six groups of twelve animals each: (1) Group 1, treated with saline as a negative control; (2) Group 2, treated with saline and given a high-fat diet for 30 days; (3) Group 3, treated with simvastatin (7 mg/kg b.w. p.o.) + high-fat diet for 30 days as a positive control; (4) Group 4, treated with OPAV (5 mL/kg b.w. p.o.) + high-fat diet for 30 days; (5) Group 5, treated with OPAV (10 mL/kg b.w. p.o.) + high-fat diet for 30 days; and (6) Group 6, treated with OPAV (15 mL/kg b.w. p.o.) + high-fat diet for 30 days. Sixteen hours after the last treatment, the mice were sacrificed, and their blood and liver were collected immediately.

Assays of ALT, AST, MDA, SOD and GSH in mice serum

The blood of b) was kept at room temperature for 1 h and then centrifuged at 1500 × g for 10 min to obtain serum. The ALT, AST, MDA, and GSH levels and the SOD activity were measured using kits according to the manufacturer's instructions.

Assays of lipids in serum and liver

The TC, TG, LDL-C and HDL-C levels in serum of c) were measured using kits according to the manufacturer's instructions.

Lipids were extracted from liver tissue according to a method reported by Ding et al.[29] The contents of liver TC and TG were determined using kits.

Assays of TC and fat in excrement

The excrement of mice from c) was weighed after freeze-drying. The excrement fat content was determined via Soxhlet extraction. The excrement TC content was measured using a kit.

Histopathological examination

The liver tissues of c) were fixed in 10 % phosphate-buffered neutral formalin, dehydrated in graded (50-100 %) ethanol and embedded in paraffin. The sections were cut, stained with hematoxylin and eosin and examined using a light microscope (Novel XYL-1, Ningbo Yongxin Optics Co., Ltd., China).

Statistical analysis

The values are presented as the means ± SD. The data were evaluated by one-way analysis of variance followed by Duncan's multiple-range tests. *P*-values less than 0.05 and 0.01 were regarded as significant and very significant, respectively.

Results and discussion

Protective effect of OPAV against ethanol-induced acute liver injury in mice

As shown in Table 2, after 52 % ethanol feeding, the ethanol-induced intoxication status of mice occurred at ethanol doses of 12.5, 15.0, 17.5 and 20.0 mL/kg. The intoxication rates of mice at the ethanol doses of 12.5, 15.0, 17.5 and 20.0 mL/kg were 50 %, 80 %, 100 % and 100 %, respectively. The intoxication times of mice at the ethanol doses of 12.5, 15.0, 17.5 and 20.0 mL/kg were 68.6, 62.38, 18.35 and 9.90 min, respectively. Ethanol-induced mortality occurred at the ethanol doses of 15.0, 17.5 and 20.0 mL/kg. The mortality of mice at the ethanol doses of 15.0, 17.5 and 20.0 mL/kg were 20 %, 30 % and 60 %, respectively. Thus, only the ethanol dose of 12.5 mL/kg was used in the ethanol-induced acute liver injury model.

Table 2

Ethanol dosage experiment in acute alcoholism BALB/c mice (means ± SD, n = 10)

Dose (mL/kg)	Intoxication rate (%)	Intoxication time (min)	Mortality (%)
10,0	0	-	0
12,5	50	68,60±5,54	0
15,0	80	62,38±15,58	20
17,5	100	18,35±7,45	30
20,0	100	9,9±5,54	60

Acute ethanol consumption has been associated with ethanol-induced liver injury (1). The levels of AST and ALT after 6 days of treatment with 52 % (v/v) ethanol at the dose of 12.5 mL/kg b.w. p.o. are shown in Table 3. As shown in Table 3, treatment with 52 % (v/v) ethanol significantly elevated the release of AST and ALT. Treatment with the OPAV (5 mL/kg b.w. p.o., 10 mL/kg b.w. p.o. or 15 mL/kg b.w. p.o.) significantly reduced serum ALT ($p < 0.01$) and serum AST ($p < 0.01$) levels relative to the mice treated with 52 % (v/v) ethanol alone. Tiopronin (50 mg/kg b.w. p.o.) also significantly lowered serum AST ($p < 0.01$) and serum ALT ($p < 0.01$) levels compared with the ethanol-treated BALB/c mice (Table 3).

OPAV (5, 10 or 15 mL/kg b.w. p.o.) significantly decreased MDA (5, 10 or 15 mL/kg b.w. p.o.) levels and increased SOD and ADH activity (5, 10 or 15 mL/kg b.w. p.o.) compared with the ethanol group. Tiopronin (50 mg/kg b.w. p.o.) also significantly decreased MDA ($p < 0.01$) and increased SOD and ADH activity ($p < 0.01$) compared with the ethanol group (Table 3). MDA is a decomposition product of lipid hydroperoxides,[30] and the SOD system and ADH protect hepatic membranes from free radical injury via a number of mechanisms.[31] OPAV inhibited the ethanol-induced increase of hepatic MDA, suggesting that OPAV inhibits lipid peroxidation. Pretreatment with OPAV also elevated hepatic SOD and ADH activity in the mice with liver injury. The polysaccharides are well recognized for their potential to act as protective agents against common types of alcohol liver injury. OPAV contains many polysaccharides, particularly CGPS-1. This suggests that OPAV has potential hepatoprotective effects.

Table 3

Effect of OPAV on the levels of serum markers in BALB/c mice with ethanol-induced acute hepatic injury

Group	ALT (U/L)	AST (U/L)	MDA (nmol/mg protein)	SOD (U/mg protein)	ADH (U/mg protein)
Control	32,09±6,85$^{\#\#}$	51,70±1,35$^{\#\#}$	31,74±1,78$^{\#\#}$	131,83±8,68$^{\#\#}$	131,73±10,06$^{\#\#}$
Ethanol	51,83±2,68**	83,73±4,99**	36,68±1,63**	89,94±3,99**	77,78±5,72**
Tiopronin + ethanol	45,49±0,60**	60,41±2,60$^{\#\#}$	31,16±0,55$^{\#\#}$	111,51±1,65**$^{\#\#}$	111,95±18,24*$^{\#\#}$
OPAV (5 mL/kg/day) + ethanol	44,82±4,31*	72,47±1,68**	32.26±0.84$^{\#\#}$	106.26±1.06**$^{\#\#}$	91,46±2,96**
OPAV (10 mL/kg/day) + ethanol	64,57±8,59**$^{\#}$	78.92±3.34**	33,19±1,15$^{\#}$	105.61±1.49**$^{\#\#}$	109,49±6,59**$^{\#\#}$
OPAV (15 mL/kg/day) + ethanol	56,84±2,86**	77,47±16,66**	29,84±1,90$^{\#\#}$	109,27±6,33**$^{\#\#}$	124,82±11,71$^{\#\#}$

*p < 0.05 versus normal control group, **p < 0.01 versus normal control group. $^{\#}p$ < 0.05 versus model control group, $^{\#\#}p$ < 0.01 versus model control group. The results are the mean ± SD.

Lipid-lowering effect of OPAV

High-fat food also has the potential to cause liver damage and the accumulation of a large amount of fat in hepatocytes [2, 3]. Here, we investigated the effect of OPAV on the TC, TG, LDL-C, and HDL-C levels in the serum, TC and TG in the liver tissue, and TC and fat in the excrement of mice. The effect of simvastatin (7 mg/kg b.w. p.o.) on the TC, TG, LDL-C, HDL-C and fat levels was used as a positive control. After 30 days of high-fat diet treatment, the TC, TG and LDL-C levels in the serum were increased (6.14, 5.64 and 1.05 nmol/L, respectively), and the HDL-C levels (1.72 nmol/L) were decreased in the high-fat diet group compared with the control group, indicating that a high-fat diet leads to fat accumulation. However, the TC, TG and LDL-C levels were reduced, and the HDL-C level was increased in the high-fat diet group treated with simvastatin or OPAV (5, 10 or 15 mL/kg b.w. p.o.) compared with the high-fat diet group (Table 4).

Table 4

Effects of OPAV on the levels of serum markers in BALB/c mice fed a high-fat diet

Group	TC (mmol/L)	TG (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)
Control	3,76±0,99	2,46±0,79	0,94±0,20	2,77±0,45
High-fat diet	6,14±0,70**	5,64±0,30**	1,05±0,14	1,72±0,39**
Simvastatin + high-fat diet	4,23±0,61$^{\#\#}$	2,98±0,41$^{*\#\#}$	1,00±0,19	2,37±0,44$^{\#\#}$
OPAV (5 mL/kg/day) + high-fat diet	4,70±0,41$^{**\#\#}$	3,09±0,56$^{*\#\#}$	1,05±0,17	2,29±0,47$^{*\#\#}$
OPAV (10 mL/kg/day) + high-fat diet	4,38±0,51$^{*\#\#}$	3,04±0,70$^{*\#\#}$	0,97±0,15	2,32±0,58$^{*\#\#}$
OPAV (15 mL/kg/day) + high-fat-diet	4,17±0,63$^{\#\#}$	3,04±0,55$^{*\#\#}$	0,95±0,15	2,37±0,48$^{\#\#}$

*p < 0.05 versus normal control group, **p < 0.01 versus normal control group. $^{\#}p$ < 0.05 versus model control group, $^{\#\#}p$ < 0.01 versus model control group. The results are the mean ± SD.

In addition, after 30 days of high-fat diet treatment, the TC and TG levels in the liver tissue were increased (17.6 and 18.46 μmol/g, respectively), and the TC and fat levels in the excrement were decreased (25.24 and 51.30 μmol/g, respectively) in the high-fat diet group compared with the control group. The TC and TG levels in the liver tissue were reduced, and the TC and fat levels were increased in the high-fat diet group treated with simvastatin or OPAV (5, 10 or 15 mL/kg b.w. p.o.) compared with the high-fat diet group (Table 5).

Table 5

Effects of OPAV on the levels of serum markers in liver tissue and excrement in BALB/c mice fed a high-fat diet

Group	TC (μmol/g liver tissue)	TG (μmol/g liver tissue)	TC (μmol/g excrement)	Fat (mg/g excrement)
Control	12,76±1,21$^{\#\#}$	12,58±4,00$^{\#\#}$	4,22±0,75$^{\#\#}$	12,73±0,84$^{\#\#}$
High-fat diet	17,60±1,66**	18,46±1,73**	25,24±5,87**	51,30±1,41**
Simvastatin+ high-fat diet	14,47±1,15$^{\#\#}$	15,56±1,40	69,21±3,64**$^{\#\#}$	77,10±2,04**$^{\#\#}$
OPAV (5 mL/kg/day) + high-fat diet	16,95±1,33**	16,89±1,70**	66,63±3,57**$^{\#\#}$	88,00±5,37**$^{\#\#}$
OPAV (10 mL/kg/day) + high-fat diet	16,21±2,44**	16,80±2,98**	86,84±0,44**$^{\#\#}$	96,9±2,69**$^{\#\#}$
OPAV (15 mL/kg/day) + high-fat diet	15,93±0,78**	14,09±2,86$^{\#\#}$	95,81±7,68**$^{\#\#}$	98,57±3,29**$^{\#\#}$

*$p < 0.05$ versus normal control group, **$p < 0.01$ versus normal control group. $^{\#}p < 0.05$ versus model control group, $^{\#\#}p < 0.01$ versus model control group. The results are the mean ± SD.

The differences in mouse liver tissue slices from the control group, the high-fat diet group, the simvastatin + high-fat diet group and the various OPAV groups were also compared by optical microscopy. As shown in Fig. 1A, the liver sections from the controls showed normal hepatic cells, whereas abundant large fat vacuoles were observed in the high-fat diet group (Fig. 1B). In the simvastatin and OPAV (5, 10 and 15 mL/kg b.w. p.o.) groups, no fat vacuoles were found (Fig. 1C-F). These results suggest that OPAV prevents the high-fat diet from impairing hepatic function. A number of polysaccharides with preventive effects against fat-induced liver injury have been investigated [15, 28].

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References

1. Altamirano J. and Bataller R. Alcoholic liver disease: pathogenesis and new targets for therapy. Nat. Rev. *Gastroenterol Hepatol* 8: 491–501 (2011).

2. Li H., Zhang M. and Ma G. Hypolipidemic effect of the polysaccharide from *Pholiota nameko*. *Nutrition* 26: 556–562 (2010).

3. Wang C., Cui J. and Wei L. Effects of *Artemisia* capillary extract on hyperlipemia and liver fatty degeneration in rats. *Chin Arch Tradit Chin Med* 28:1738–1740 (2010).

4. Tamura A., Sasaki M., Yamashita H., Matsui-Yuasa I., Saku T., Hikima T., *et al*. Yerba-mate (*Ilex paraguariensis*) extract prevents ethanol-induced liver injury in rats. *J Funct Foods* 5: 1714–1723 (2013).

5. Wu M., Wu Y., Qu M., Li W. and Yan X. Evaluation of antioxidant activities of water-soluble polysaccharides from brown alga *Hizikia fusiformis*. *Int J Bio Macromol* 56: 28–33 (2013).

6. Mladenović D., Ninković M., Aleksić V., Šljivančanin T., Vučević D., Todorović V., *et al*. The effect of calorie restriction on acute ethanol-induced oxidative and nitrosative liver injury in rats. Environ. *Toxicol Pharmacol* 36: 296–302 (2013).

7. Li H-M, Guo P., Hu X., Xu L. and Zhang X-Z. Preparation of corn (*Zea mays*) peptides and their protective effect against alcohol-induced acute hepatic injury in NH mice. *Biotechnol Appl Biochem* 47: 169–174 (2007).

8. Li M., Lu Y., Hu Y., Zhai X., Xu W., Jing H., *et al*. Salvianolic acid B protects against acute ethanol-induced liver injury through SIRT1-mediated deacetylation of p53 in rats. *Toxicol Lett* 228: 67–74 (2014).

9. He H., Shi Y., Xu S., Zhang S. and Guo H. Hepatoprotective effect of *Ganoderma lucidum* peptides against alcohol-induced liver injury in mice. Chin. *Food Sci* 31: 213–216 (2010).

10. Tang C-C, Huang H-P, Lee Y-J, Tang Y-H and Wang C-J. Hepatoprotective effect of mulberry water extracts on ethanol-induced liver injury via anti-inflammation and inhibition of lipogenesis in c57bl/6j mice. *Food Chem Toxicol* 62: 786–796 (2013).

11. Yoon S-J, Koh E-J, Kim C-S, Zee O-P, Kwak J-H, Jeong W-J., *et al*. Agrimonia eupatoria protects against chronic ethanol-induced liver injury in rats. *Food Chem Toxicol* 50: 2335–2341 (2012).

12. Müller L.G., Pase C.S., Reckziegel P., Barcelos RCS, Boufleur N., Prado ACP., *et al*. Hepatoprotective effects of pecan nut shells on ethanol-induced liver damage. *Exp Toxicol Pathol* 65: 165–171 (2013).

13. Park J.E., Kim J.Y., Kim J., Kim Y.J., Kim M.J., Kwon S.W., *et al*. Pomegranate vinegar beverage reduces visceral fat accumulation in association with AMPK activation in overweight women: A double-blind, randomized, and placebo-controlled trial. *J Funct Foods* 8: 274–281 (2014).

14. Qiu T., Ma X., Ye M., Yuan R. and Wu Y. Purification, structure, lipid lowering and liver protecting effects of polysaccharide from *Lachnum* YM281. *Carbohydr Polym* 98: 922–930 (2013).

15. Chen Q., Yang R-J and Gu L-L. Studies on oyster glycogen (II) – the fine structure of oyster glycogen. *Chin Food Sci* 26: 43–46 (2005).

16. Shi X., Ma H., Tong C., Qu M., Jin Q. and Li W. Hepatoprotective effect of a polysaccharide from *Crassostrea gigas* on acute and chronic models of liver injury. *Int J Biol Macromol* 78: 142–148 (2015).

17. Fushimi T., Tayama K., Fukaya M., Kitakoshi K., Nakai N., Tsukamoto Y., *et al*. Acetic acid feeding enhances glycogen repletion in liver and skeletal muscle of rats. *J Nutr* 131: 1973–1977 (2001).

18. Ebihara K. and Nakajima A. Effect of acetic acid and vinegar on blood glucose and insulin responses to orally administered sucrose and starch. *Agr Biol Chem* 52: 1311–1312 (1988).

19. Kondo S., Tayama K., Tsukamoto Y., Ikeda K. and Yamori Y. Antihypertensive effects of acetic acid and vinegar on spontaneously hypertensive rats. *Biosci Biotechno Biochem* 65: 2690–2694 (2001).

20. Xu Q., Tao W. and Ao Z. Antioxidant activity of vinegar melanoidins. *Food Chem* 102: 841–849 (2007).

21. Zheng Y., Dong A., Yin H., Su G., Lin F. and Wang M. Development and application of nutrient salts for cider vinegar fermentation. *J Chin Inst Food Sci Technol* 15: 74–81 (2015).

22. Wang L., Yan X., Li Y., Li X., Liang X., Zai X., *et al*. Optimization of grape vinegar fermentation conditions by response surface methodology. *China Brewing* 33: 55–58 (2014).

23. Ubeda C., Callejón R.M., Hidalgo C., Torija M.J., Troncoso A.M. and Morales M.L. Employment of different processes for the production of strawberry vinegars: Effects on antioxidant activity, total phenols and monomeric anthocyanins. *LWT-Food Sci Technol* 52: 139–145 (2013).

24. Wakuda T., Azuma K., Saimoto H., Ifuku S., Morimoto M., Arifuku I., *et al*. Protective effects of galacturonic acid-rich vinegar brewed from Japanese pear in a dextran sodium sulfate-induced acute colitis model. *J Funct Foods* 5: 516–523 (2013).

25. Wu H-Y. Development of hawthorn vinegar peptide beverage. *Food Res Dev* 29: 18–20 (2008).

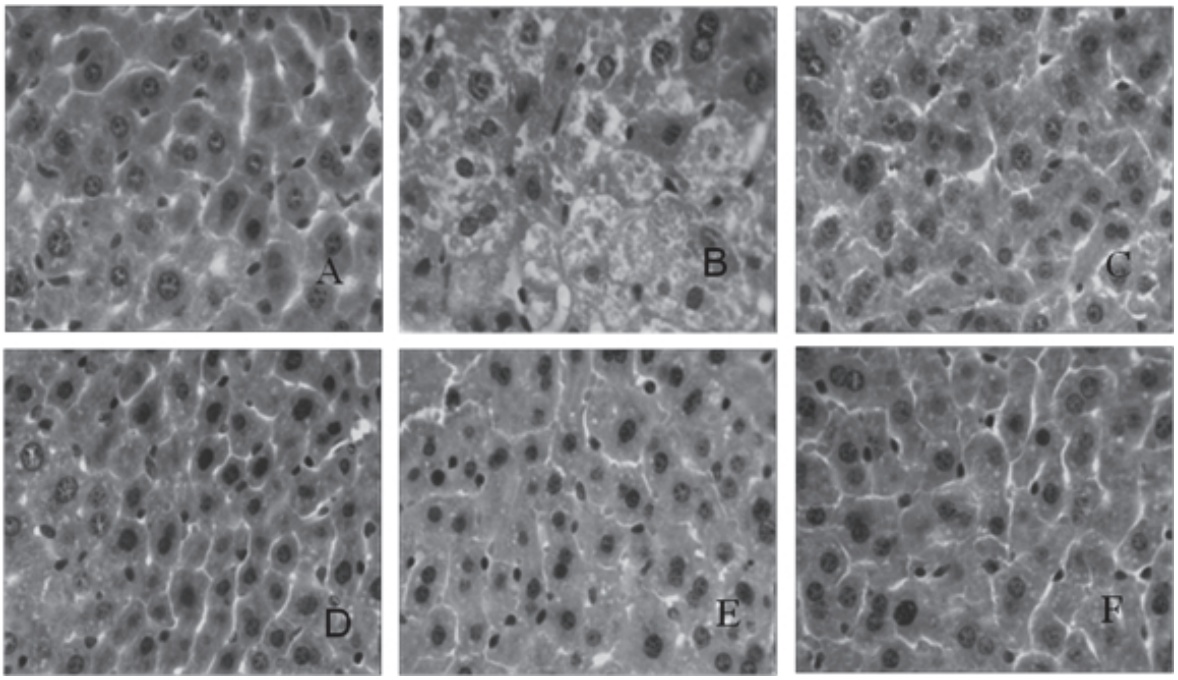
26. Kim S-H, Cho H-K and Shin H-S. Physicochemical properties and antioxidant activities of commercial vinegar drinks in Korea. *Food Sci Biotechnol* 21: 1729–1734 (2012).

27. Jacobs Jr DR, Gross MD and Tapsell LC. Food synergy: an operation concept for understanding nutrition. *Am J Clin Nutr* 89:1543–1548 (2009).

28. Ding H., Gao Y., Chao H. and Xia D. Effect of polysaccharide from moso bamboo leaves on blood lipid of mice with hyperlipemia. *Chin Food Sci* 31: 259–262 (2010).

29. Gutteridge J.M. Lipid peroxidation and antioxidants as biomarkers of tissue damage. *Clin Chem* 41: 1819–1828 (1995).

30. Proctor P.H. and McGinness J.E. The function of melanin. *JAMA Dermatol* 122: 507–508 (1986).



Effect of OPAV on high-fat diet-fed BALB/c mice: A control; B high-fat diet; C simvastatin + high-fat diet; D OPAV (5 mL/kg/day) + high-fat diet; E OPAV (10 mL/kg/day) + high-fat diet; F OPAV (15 mL/kg/day) + high-fat diet. Original magnification 40×

Сведения об авторах: Чанцин Тун, Синьяо Ли, Чэнчэн Цай, Сюань Ши, Вэй Ли, e-mail: aisingioro@hotmail.com.