doi.org/10.36721/PJPS.2021.34.2.REG.649-656.1

Protective effect of *Phaeoporus obliquus* polysaccharide against acute liver injury induced by carbon tetrachloride and alcohol in mice

Zhang Lei[1], Ren Xiao-hong[2], He Jing[3], Li Wan-nan[4], Hao Jing-wei[1], Jing Yun-rong[1], Chai Jun-rong[1], Sun Xiao-wei[1] and Wan Chun-lei[1]*

[1]College of Life Science and Technology, Mudanjiang Normal University, Mudanjiang, PR China
[2]Shanghai Institute of Medicine, Chinese Academy of Sciences, Shanghai, RP China
[3]Department of Medical Biology, Wannan Medical College, Wu Hu, RP China
[4]Edmond H Fischer Signal Transduction Laboratory, College of Life Sciences, Jilin University, Chang Chun, RP China

Abstract: Studied the optimum extraction process of polysaccharide from *Phaeoporus obliquus* and the effect of *Phaeoporus obliquus* polysaccharide on carbon tetrachloride (CCl_4)- or alcohol-induced acute liver injury in mice. The main factor in influencing the extraction rate of *Phaeoporus obliquus* polysaccharide were extraction power and time, which was a kind of pyran glucose by infrared spectroscopy. CCl_4 and alcohol were employed respectively to establish CCl_4 and alcohol-induced acute liver injury mouse models. Compared with model groups mice, *Phaeoporus obliquus* polysaccharide treatment at the doses of 100mg/kg and 200mg/kg exhibited an obvious reduction liver index, ALP, ALT, AST levels, MDA content and TNF-α level ($p<0.01$) and SOD activity was increased, which was in a dose-dependent manner. Compared with the model group, the necrosis degree of hepatocytes was obviously reduced and the small fat droplets were formed in some cytoplasm, especially in high dose group, which the liver cells recovered to the level of normal group. Rt-PCR results showed that the expression of CYP2E1 mRNA in liver tissues of *Phaeoporus obliquus* polysaccharide groups were significantly reduced, and the difference were statistically significant compared with the model group ($p<0.05$). These results demonstrated that *Phaeoporus obliquus* polysaccharide has significantly hepatoprotective effect on CCl_4 and alcohol-induced acute liver injury in mice.

Keywords: *Phaeoporus obliquus* polysaccharide, CCl_4, alcohol, acute liver injury.

INTRODUCTION

Liver is important organ in human body, which has some functions such as digestion, absorption, metabolism, detoxification and hematopoietic. It is a important place of storage and metabolism. Liver injury refers to the various causes of the abnormal function of liver, which is the basis of acute liver failure. Severe or persistent liver damage eventually lead to liver failure (Peng *et al.* 2015; DAY, 2007). Liver disease is a worldwide disease with the characteristics of high prevalence and high fatality rate. So it is important to develop drugs with better efficacy and safety to prevent liver injury.

Phaeoporus obliquus (*Phaeoporus obliquus j. Schroet*), also called chaga, has been praised as "ganoderma lucidum", and which is a very rare medicinal fungi. It has been found that its chemical composition includes polysaccharide compounds, aromatic substances, polyphenolic compounds, triterpenoids and other substances (Yusoo *et al.*, 2001; Yong *et al.*, 2005; Yong *et al.*, 2006; Song *et al.*, 2004), which have important medicinal value. Since the 16th century, *Phaeoporus obliquus* has obvious effect on diabetes, high blood pressure, anticancer, prevention AIDS and so on (Huang 2002 and Jin *et al.*, 2004). However, related studies have not been reported about the treatment of liver injury. In this study, we extracted the polysaccharide from *Phaeoporus obliquus* with orthogonal experiment, and the treatment of liver injury possible mechanism was also investigated. We also explored the treatment of liver injury potency and tried to get a better understanding about its mechanism in mouse model.

*Corresponding author: e-mail: swxziz@126.com

MATERIALS AND METHODS

Phaeoporus obliquus were purchased from Sui Fen He city Market (Hei Long Jiang, China). ALT' AST and ALP was tested by chayang hospital in qiqihar, heilongjiang province. ethyl alcohol' sopropanol and carbon tetrachloride were purchased from Beijing chemical plant. Red Star Er Guotou (52°) were purchased from Beijing hongxing co. LTD. SOD and MDA were purchased from Shanghai source leaf biology. TNF-αElisa Kit was purchased from RD Company, TRizol Reagent was purchased from Intro-gen life technologies Company; RT-PCR Kit was purchased from Fermentas Company ,HE dye kit (Solarbio), Infrared spectrometer, Bruker Germany.

Extraction and determination of Phaeoporus obliquus polysaccharide

Phaeoporus obliquus 5g power dissolved in 30% ethanol after sifted it. We disposed the *Phaeoporus obliquus* power in different time, temperature, liquid ratio and ultrasonic power. Polysaccharide were precipitated with

80% ethanol and the precipitate were centrifuged at 4000×g for 7 minutes at 4°C, then the supernatants were collected individually. The crude polysaccharide obtained after reduced pressure and concentrated to dry after soaking. Determined the content by standard curve method.

Effects of single factor test index on the extraction of Phaeoporus obliquus polysaccharide

Design of *Phaeoporus obliquus* polysaccharide in birch extracts variable conditions of single factor experiments were extraction temperature, extraction time and solid-liquid ratio and ultrasonic frequency, in order to determine the level of various factors in the orthogonal experiment.

Optimize the extraction by orthogonal test

On the basis of single factor test, orthogonal experiment was carried out by using orthogonal test table L9 (3^4) and the factors and levels were shown in table 1.

Qualitative analysis was performed by infrared spectroscopy

The extracted polysaccharides were analyzed by infrared spectrometer in order to know the possible functional groups.

Establishment of acute liver injury mouse model

One hundred male Kun Ming mice were randomly used in the studies, which were 8 weeks old and 18-22 g weight. All the mice were housed five to a cage in a 12:12 hours light/dark cycle at ambient temperature of 22°C-25°C. Ten mice were fed with ordinary chow as a normal group and the other 90 mice were established acute liver injury mouse model. These mice were separated into six groups (fifteen mice per group), including CCl_4 and alcoholic mode group received 0.9% saline [vehicle], CCl_4 *Phaeoporus obliquus* polysaccharide low-dose group received 100mg/kg, CCl_4 *Phaeoporus obliquus* polysaccharide high dose group received 200mg/kg and CCl_4 Bifendate Pills group received 60mg/kg doses of Bifendate Pills. Alcoholic *Phaeoporus obliquus* polysaccharide low-dose group received 100mg/kg and Alcoholic *Phaeoporus obliquus* polysaccharide high dose group received 200mg/kg, VE group received 60mg/kg doses of Vitamine E (VE) group . Continuous gavage for 20 days. After 2h of the last dose, except the normal control group, the mice were injected with the same amount of normal saline and all the mice in the CCl_4 groups were injected with 0.1% CCl_4 peanut oil solution of 10mL/kg and Alcoholic groups of lavage 56% alcool 15mL/kg. The mice were fasted after the last delivery, but had free access to water.

Tissue collection and HE staining

At the end of week 8 of treatments, mice were sacrificed. The liver was dissected immediately and made of paraffin section. HE staining morphology was used to observe the morphology of mice liver cells.

Expression of CYP2E1 in liver tissues was detected by RT-PCR

RNA was extracted by TRIzol method from the liver tissues 100mg in every group and the first chain cDNA was synthesized after reverse transcription. The CYP2E1 fragment was amplified with cDNA as template. The upstream primers of CYP2E1 were 5'-ctcctgtcactatccatctg-3' and the downstream primers were 5' -catttctgatggcttgc-3' and the internal reference was made by gate-actin.

Ethical approval

The ethical approval was taken from College of Life Science, Jilin University vide Certificate No.SY0809.

STATISTICAL ANALYSIS

Data were presented as mean ± SD. Statistical analysis was conducted using Student's t-test or one-way ANOVA with GraphPad Prism 5 software. A probability value of $p<0.05$ was considered statistically significant.

RESULTS

The single factor experiment of Phaeoporus obliquus polysaccharide extraction

The standard curve equation was y=0.0504x+0.0011, R^2=0.9991, which prepared by using glucose solution with a certain concentration gradient. We analyzed the extraction rate of *Phaeoporus obliquus* polysaccharide under different conditions by change the extraction time, ultrasonic power, extraction temperature and the ratio of material liquid respectively. The results showed that the extraction rate of *Phaeoporus obliquus* polysaccharide was maximum in the condition of ultrasonic power was 40% for 30 min, extraction temperature was 40°C and solid-liquid ratio was 1:15 (fig. 1). Therefore, We selected to further optimize the range of material liquid ratio in the condition of the extraction time was 20-40min, ultrasonic power was 30%-50%, extraction temperature was 30-50, and material to liquid ratio was 1:10-1:20.

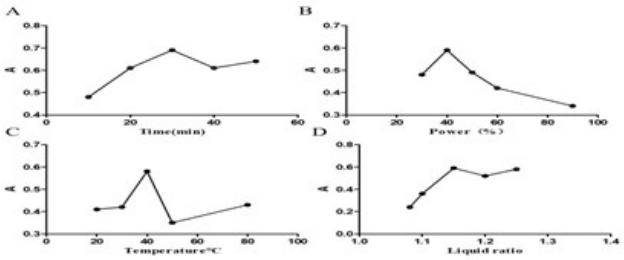

Fig. 1: The effect of single factor on the extraction rate of *Phaeoporus obliquus* saponins. A: Time; B: Ultrasonic power; C: Temperature; D: Tolid-liquid ratio.

Orthogonal optimization experiment of the extraction of Phaeoporus obliquus polysaccharide

Orthogonal experiment design and result Phaeoporus obliquus polysaccharide

The optimum extraction process of *Phaeoporus obliquus* polysaccharide was determined by orthogonal test. Orthogonal experiment results show that the primary and secondary order of factors, which effected on *Phaeoporus obliquus* polysaccharide extraction quantity, was B>A>C>D. It means that the extraction of *Phaeoporus obliquus* polysaccharide was the largest at the optimum ultrasonic power and ultrasonic temperature ($p<0.05$), and followed by the effect of the extraction temperature and the ratio of material to liquid. Integrated the above conditions, the best extraction conditions of *Phaeoporus obliquus* polysaccharide was A2B3C3D3. The *Phaeoporus obliquus* polysaccharide was determinated at temperature 50°C and the ultrasonic power was 60% and the ethanol concentration was 30% at the temperature of 1:20 (table 2).

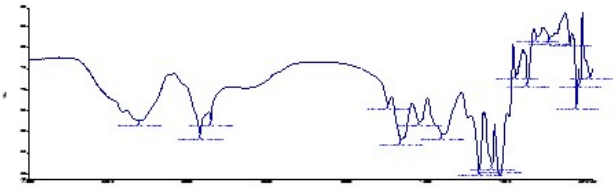

Fig. 2: IR spectra of *Phaeoporus obliquus* saponins

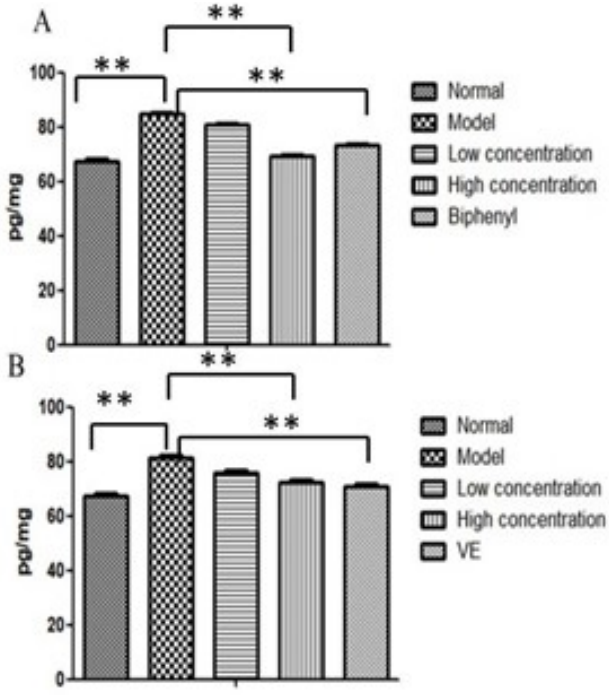

Fig. 3: The Level of TNF-α in acute liver injury mice (A: bycarbon tetrachloride, B: alcohol)

Qualitative analysis was performed by infrared spectroscopy

Infrared spectral analysis (fig. 2) showed that polysaccharides had strong O-H retractable vibration absorption peak at 3299cm-1 and C-H bond retractable vibration absorption peaks at 2,918 cm^{-1}. There was an obvious peak by C-O telescopic vibration of intramolecular C-O-C macrocyclic polyethers and O-H variable angular vibration of C-O-H at 1,08cm^{-1}.There were C-O-C and O-H absorption peaks of the pyran glucose ring At 1081, 1034cm^{-1}. These results all indicated that the analyzed substance was mainly a polysaccharide compound.

Effects of Phaeoporus obliquus polysaccharide treatment on liver index in acute liver injury in mice induced by Carbon Tetrachloride and Ethanol

In normal group, everyday water-drinking amount and urine volume were normal, the body weight increased rapidly, the skin was shiny and the activity was flexible. The mice showed an excited state after giving ethanol solution, and they were paralyzed. After 30min, they were drowsy and drunk, breathing rapidly and convulsively, which lasted for about 1h. It was very obvious that the model mice were generally in poor condition, with low spirits, dry and dull hair and decreased activity. The condition of *Phaeoporus obliquus* polysaccharide group was better than the model group and the high dose groups were looked well. Compared with normal mice, the liver index in model group mice increased significantly, which showed that CCl_4 and alcohol could cause significant enlargement of liver in mice. Compared with the model group, the liver index in *Phaeoporus obliquus* polysaccharide and the biphenyl group and VE were significantly reduced. These results suggest that *Phaeoporus obliquus* polysaccharide had alleviating effect on the liver enlargement caused by CCl_4 and alcohol.

Effects of Phaeoporus obliquus polysaccharide treatment on AST, ALT and ALP levels

Hepatocytes are the main survival sites for transaminases. When liver cells become infected with inflammation, poisoning or necrosis and the liver cells are damaged, and the transaminase is released into the blood, which caused the serum transaminase to rise. The AST, ALT and ALP levels in model group were significantly increased, compared with normal mice ($p<0.01$). The AST, ALT and ALP levels in high dose *Phaeoporus obliquus* polysaccharide -treated mice and biphenyl group and VE were significantly decreased, compared with model mice ($p<0.01$). However which the low-dose group mice declined slightly (table 3,4). Different concentrations of *Phaeoporus obliquus* polysaccharide have obvious effect, which shows obvious dose-dependent decreased in transaminase levels.

Effects of Phaeoporus obliquus polysaccharide treatment on liver tissue oxidative stress in mice

The SOD level in serum significantly decreased in the model group compared with normal group and the MDA level increased significantly. These results confirmed the successful establishment of the liver damage model.

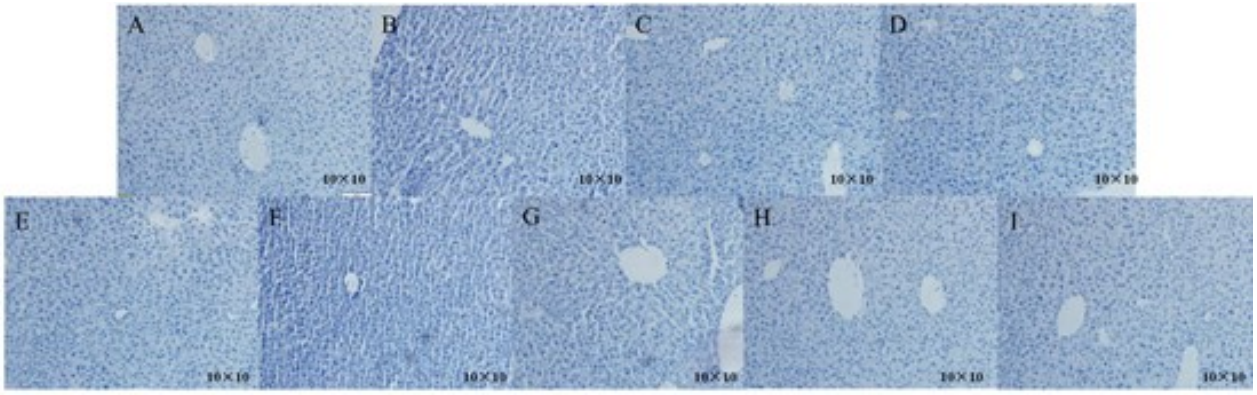

Fig. 4: Effect of *Phaeoporus obliquus* polysaccharide on hepatic histopathological change in acute liver injury mice
Note: A：Normal group; B：Carbon tetrachloride model group; C：Low concentration group; D： High concentration group; E： Biphenyl group; F：Alcohol model group; G：Low concentration group; H： High concentration group; I： VE group

Table 1: The levels and factors of orthogonal test

Level	Factors			
	Time (min)	Solid-liquid ratio	Temperature (^{o}C)	Ultrasonic frequency (%)
1	20	1:10	30	40
2	30	1:15	40	50
3	40	1:20	50	60

Table 2: The results of orthogonal test (n=3)

	Time (min)	Solid-liquid ratio	Temperature(^{o}C)	Power (%)	Extraction ratio
1	20	1:10	30	40	0.567
2	20	1:15	40	50	0.583
3	20	1:20	50	60	0.563
4	30	1:10	40	60	0.650
5	30	1:15	50	40	0.610
6	30	1:20	30	50	0.688
7	40	1:10	50	50	0.623
8	40	1:15	30	60	0.660
9	40	1:20	40	40	0.391
K_1	0.569	0.577	0.608	0.620	
K_2	0.650	0.572	0.599	0.577	
K_3	0.603	0.672	0.615	0.625	
Range	0.081	0. 1	0.016	0.048	
Important order B>A>C>D Optimization levels A2B3C3D3					

Compared with model group, SOD level decreased significantly. The level of SOD in high dose group, biphenyl group and VE group were higher than those in low dose group, which was in a dose-dependent manner. The level of SOD in high-dose group treated mice exhibited no statistically difference at the end of treatment, compared with normal mice and biphenyl -treated mice. Daily treatments of *Phaeoporus obliquus* polysaccharide decreased the blood MDA levels gradually in mice compared with model group. These results suggest that *Phaeoporus obliquus* polysaccharide have a protective effect on the oxidative damage of liver tissue.

Effects of Phaeoporus obliquus polysaccharide treatment on TNF-α in mice with acute liver injury

Tumor necrosis factor is a factor that can kill certain tumor cells or cause blood necrosis *in vivo* tumor tissue. The hepatic inflammatory factors (TNF-α) level in model group was significantly increased, compared with normal mice ($p<0.01$). Compared with model mice the TNF-α level in high dose *Phaeoporus obliquus* polysaccharide-treated mice was significantly decreased ($p<0.01$). Both the biphenyl group and VE group were also significantly decreased. However the TNF-α level of low-dose group mice declined slightly (fig. 3). These results outlined

Table 3: Effects of blood index in acute liver injury mice induced by carbon tetrachloride

Group	Dose (mg/kg)	Liver index	AST (U/L)	ALT (U/L)	ALP (U/L)
Normal		3.24±0.10	64.37±0.67	97.17±1.06	79.83±1.40
Model		4.92±0.23Δ	155.77±3.73Δ	200.2±1.18Δ	167.63±2.93Δ
Low concentration	100	4.73±0.78**	133.13±4.15**	171.4±1.05**	135.6±3.24**
High concentration	200	4.35±0.06**	114.2±1.73**	154.5±2.46**	109.03±1.39**
Biphenyl	100	4.23±0.04**	88.97±1.37**	134.93±2.42**	97.1±1.51**

Table 4: Effects of blood index in acute liver injury mice induced by alcohol

Group	Dose (mg/kg)	Liver index	AST (U/L)	ALT (U/L)	ALP (U/L)
Normal		3.24±0.10	64.37±0.67	97.17±1.06	79.83±1.40
Model		4.81±0.06Δ	155.1±4.32Δ	184.67±1.84Δ	147±2.98Δ
Low concentration	100	4.47±0.06*	125.2±2.95**	145.77±2.35*	116.87±0.65*
High concentration	200	4.08±0.13**	102.2±1.83**	125.37±2.08**	96.63±1.73**
VE	100	3.78±0.11**	88.4±0.90**	118.6±1.61**	92.13±1.89**

Table 5: Effects of liver tissue oxidative stress in acute liver injury mice induced bycarbon tetrachloride

Group	Dose /(mg/kg)	SOD (U/mg pro)	MDA (nmol/mg pro)
Normal		30.9±1.42	4.52±0.14
Model		21.3±0.56Δ	12.2±0.75Δ
Low concentration	100	25.87±0.25*	9.95±0.23*
High concentration	200	28.2±0.70**	7.05±0.23**
Biphenyl	100	29.83±1.02**	6.11±0.07**

Table 6: Effects of liver tissue oxidative stress in acute liver injury mice induced alcohol

Group	Dose/(mg/kg)	SOD (U/mg pro)	MDA (nmol/mg pro)
Normal		30.9±1.42	4.52±0.14
Model		19.3±0.75Δ	10.8±0.36Δ
Low concentration	100	23.07±0.65*	8.78±0.16
High concentration	200	26.1±0.89**	5.93±0.15**
VE	100	31.63±0.91**	5.95±0.16**

Note: Δp<0. 01, compared with the control group, *: p<0. 05,**:p<0. 01, compared with model group.

Phaeoporus obliquus polysaccharide could improve the level of inflammation in liver injury.

Effects of Phaeoporus obliquus polysaccharide on liver structure in mice

Observation of liver cells by HE staining morphology showed that, the liver tissue structure was very clear and the liver cells were arranged radially and no abnormal pathological changes were found in the hepatic sinusoids and hepatocytes. The mice liver cord was arranged disordered and most of the liver cells were swollen in the model group. There was obvious infiltration of inflammatory cells appeared. In the biphenyl and VE groups, the hepatocytes of mice were not swollen and there were only a small number of inflammatory cells in the portal area. Compared with the model group, the degree of hepatocyte lesion in the *Phaeoporus obliquus* polysaccharide groups, biphenyl group and VE groups was lighter and the liver cells swelling and fatty degeneration were not obvious. The necrosis degree of hepatocytes was obviously reduced and the small fat droplets were formed in some cytoplasm, especially in high dose group, which the liver cells recovered to the level of normal group (fig. 4).

Effects of Phaeoporus obliquus polysaccharide on expression of CYP2E1 in mice

Rt-PCR results showed that the expression of CYP2E1 mRNA in normal liver tissues was low and in the model group was significantly increased. Compared with the normal group, which the difference in model group was statistically significant (p<0.01). The expression of CYP2E1 mRNA in liver tissues of *Phaeoporus obliquus* polysaccharide groups were significantly reduced and the difference were statistically significant compared with the model group (p<0.05) (fig.5).

DISCUSSION

Liver injury is a common clinical disease which is harmful to human health. It refers to the invasion of the liver by external factors, which can be divided into pathological liver injury and chemical liver injury. Acute liver injury induced by CCl_4 and alcoholic are the most commonly used models (Qiu *et al.*, 2009 and Chen *et al.*, 2019). The mechanism of liver injury is related to CCl_4 itself and its free radical metabolites. CCl_4 after entering the body, which eventually metabolism into 2 - trichloromethyl (CCl_3) and oxygen free radical freedom groups - CCl_3O_2.The process consumes a large amount of antioxidants and cause airframe oxidative stress state. Oxidative stress is an important link in CCl_4 induced liver injury (Park *et al.*, 2010), Body subjected to harmful stimulation, Excessive production of highly reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) *in vivo*. The degree of oxidation exceeds the removal rate of the oxide. Oxidation system and anti-oxidation system are out of balance, leading to tissue damage (Gonzalez 2005).The activity of SOD reflects the ability of scavenging oxygen free radical indirectly. GSH-PX can scavenge lipid peroxides induced by reactive oxygen species and OH. It protects the integrity of cell membrane structure and function, and MDA indirectly reflects the severity of free radical attack. Alcoholic liver injury (ALD) refers to the liver disease caused by long-term or large-scale consumption of alcohol-containing beverages, which is a common cause of liver cirrhosis. In recent years, the incidence of alcoholic liver injury has increased significantly, which has attracted more and more attention from the society and the medical profession. The mechanism of acute alcoholic liver injury is that ethanol is absorbed by the body and can be dehydrogenate to acetaldehyde and acetate under the catalysis of ethanol dehydrogenase and produce reactive oxygen free radicals, resulting in liver cell damage caused by lipid peroxidation in the liver cell membrane (Mishra *et al.*, 2011 and Vidičević *et al.*, 2020). When the concentration of GSH in hepatocytes decreased, the scavenging effect of GSH was weakened, which indirectly promoted lipid peroxidation and aggravated the damage of hepatocytes (Govinder *et al.*, 1999). SOD, CAT and gsh-px are the three most important antioxidant enzymes, which constitute the most important antioxidant enzyme defense system in organism. It plays an important role in anti-oxidative damage by effectively removing reactive oxygen species and ending free radical chain reaction (Balasubramaniyan *et al.*, 2003 and Wang *et al.*, 2015). When these enzymes are reduced. It can lead to free radical accumulation and loss of cell membrane integrity and function. In this study compared with model groups mice, *Phaeoporus obliquus* polysaccharide treatment at the doses of 100mg/kg and 200mg/kg exhibited an obvious reduction liver index, ALP, ALT, AST levels and MDA content and SOD activity was increased, which was in a dose-dependent manner.

TNF- is a mononuclear factor mainly produced by monocytes and macrophages and plays an important role in the process of liver injury (Zhao *et al.*, 2017 and Zheng *et al.*, 2018). Studies have shown that the production of TNF- is regulated by NF-κB and the increase in its release promotes the phosphorylation of NF-κB, makes NF-κB activated, and continues the complex loop regulation of inflammatory response, in order to continuous amplification of the initial inflammatory signal (Rodrigues *et al.*, 2017 and Liu *et al.*, 2019). At the same time, as the first medium of liver injury, the presence of TNF- induces many secondary mediators related to liver necrosis, such as NO, IL-1, IL-6, IL-8, SIL-2R. In addition, as the second medium, IL-1 enhances the liver damage effect of TNF- alpha. Even if a small amount of TNF- is present, tissue damage will occur (Liu *et al.*, 2017 and Zeng *et al.*, 2017). Our results showed that the level of TNF-α in model groups were increased significantly, after each dose group mice liver damage degree show significantly reduce state. *Phaeoporus obliquus* polysaccharide in acute liver injury in mice induced by CCl_4 and alcoholic improvement effect, and its mechanism may be related to reduce inflammation factors.

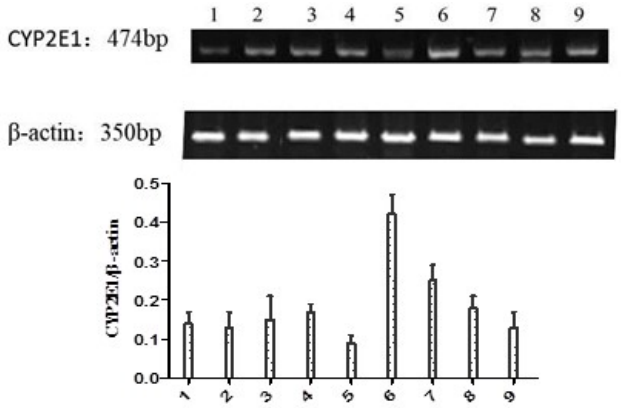

Fig. 5: The expression of CYP2E1 mRNA in liver tissues of mice in each group (1: biphenyl group, 2:high dose group (CCl_4), 3: low dose group (CCl_4),4: CCl_4 model group, 5:Normal group, 6: alcohol model group, 7: low dose group(alcohol),8: high dose group(alcohol), 9: VE groups)

Over expression of CYP2E1 can cause hepatotoxicity and mitochondrial damage, and reactive oxygen species produced by CYP2E1 can activate hepatic stellate cells to form hepatic fibrosis through diffusion (Wang *et al.*, 2010 and Wu *et al.*, 2005). In this study, the expression of CYP2E1 in liver tissue of model group was higher than that of normal group, but the expression level of CYP2E1 in *Phaeoporus obliquus* polysaccharide groups was significantly lower than that in normal group. The results

suggest that the anti-acute liver injury effect of *Phaeoporus obliquus* polysaccharide may be related to its inhibition of CYP2E1 over expression to regulate oxidative metabolism.

Although we have proved the relieves acute liver damage effects of *Phaeoporus obliquus* polysaccharide on Acute liver injury induced by CCl_4 and alcoholic, there are also some limitations for this study, such as there are many other pathways affecting the acute liver damage effects. Therefore, if applying *Phaeoporus obliquus* polysaccharide into clinical therapy, the side effects of *Phaeoporus obliquus* polysaccharide must be investigated in future studies.

CONCLUSIONS

Through Optimizing extraction of *Phaeoporus obliquus* polysaccharide, which is one of pyran glucose, it was applied to mice with liver damage induced by CCl_4 and alcoholic. Compared with model groups mice, *Phaeoporus obliquus* polysaccharide treatment at the doses of 100mg/kg and 200mg/kg exhibited an obvious reduction liver index, ALP, ALT, AST levels, MDA content and TNF-α level and SOD activity was increased, which was in a dose-dependent manner. Compared with the model group, the necrosis degree of hepatocytes was obviously reduced, and the small fat droplets were formed in some cytoplasm, especially in high dose group, which the liver cells recovered to the level of normal group. Rt-PCR results showed that the expression of CYP2E1 mRNA in liver tissues of *Phaeoporus obliquus* polysaccharide groups were significantly reduced, and the difference were statistically significant compared with the model group. These results demonstrated that *Phaeoporus obliquus* polysaccharide has significantly hepato-protective effect on CCl_4 and alcohol-induced acute liver injury in mice.

ACKNOWLEDGEMENTS

This work was supported by the Postdoctoral Project of Human Resources and Social Security Department of Heilongjiang Province [grant number LBH-Q19190] and Basic research expenses for provincial colleges and universities [grant number 1355ZD006]. Thanks for the help from College of life sciences, Jilin University.

REFERENCES

Balasubramaniyan V, Kalaivani Sailaja J and Nalini N (2003). Role of leptin on alcohol-induced oxidative stress in swiss mice. *Pharm. Res.,* **47**(3): 211-216.

Chen YS, Chen QZ, Wang ZJ and Hua C (2019). Anti-inflammatory and hepatoprotective effects of *Ganoderma lucidum* polysaccharides against carbon tetrachloride-induced liver injury in Kunming mice. *Pharmacology,* **103**(3-4): 143-150.

Day CP (2007). Treatment of alcoholic liver disease. *Liver Transp.*, **13**(2): 69-75.

Gonzalez FJ (2005). Role of cytochromes P450 in chemical toxicity and oxidative stress: Studies with CYP2E1 (2005). *Mutat Res.*, **569**(1): 101-110.

Govinder JSF and Prahlad KS (1999). Beneficial effects of S-adenosyl-l-methionine on aminolevulinic acid dehydratase, glutathione and lipid peroxidation during acute lead: Ethanol administration in mice. *Alcohol,* **18**(2): 103-108.

Guang J, En-yue Y and Chun-zan H (2004). Experimental study on the anti tumor effect of polysaccharides of *Holococcus inulata. Med. J. Yanbian Univ.,* **27**(4): 257-259.

Liu B, Li J, Yi R, Mu J, Zhou X and Zhao X (2019). Preventive effect of alkaloids from *Lotus plumule* on acute liver injury in mice. *Foods,* **8**: 36.

Liu J, Fu Y and Zhang H (2017). The hepatoprotective effect of the probiotic *Clostridium butyricum* against carbon tetrachloride-induced acute liver damage in mice. *Food Funct.,* **8**(11): 4042-4 052.

Liu Peng, Zhang Lei, Xie Xiao-na, Wang Deli, Sun Jing, Wang Yong-sen, Wang Zhi, Xing Shu, Ma Jun-feng, Li Wan-nan and Fu Xue-qi (2015). An extract of Perilla stem inhibits Src homology phosphatase-1(SHP)-1 and influences insulin signaling. *Pak. J. Pharm. Sci.,* **28**(2): 421-424.

Mishra A, Paul S and Swarnakar S (2011). Downregulation of matrix metalloproteinase-9 by melatonin during prevention of alcohol-induced liver injury in mice. *Biochimie.*, **93**(5): 854-866.

Nian-lai H (2002). Russia's mysterious folk medicinal fungus botulin. *Chi. Edible Fungus*, **2**(4): 7-8.

Park CM, Cha YS and Youn HJ (2010). Amelioration of oxidative stress by dandelion extract through CYP2E1 suppression against acute liver injury induced by carbon tetrachloride in Sprague-Dawley rats. *Phytother. Res.*, **24**(9): 1347-1353.

Qiu Ji and Gui Shuang-ying (2009). Protective effect of astragalus extract on acute chemical liver injury in mice. *Anhui Med.*, **13**(6): 603-605.

Rodrigues GB, Oliveira EE and Fjbm J (2017). Characterization and evaluation of nanoencapsulated diethylcarbamazine in model of acute hepatic inflammation. *Int. Immunopharmacol*., **50**(07): 330-337.

SašenkaVidičević , JelenaTošić , ŽeljkaStanojević , Aleksandra Isaković , DraganaMitić , Dušica Ristić , Dragana Dekanski (2019). Standardized *Olea europaea* L. leaf extract exhibits protective activity in carbon tetrachloride-induced acute liver injury in rats: The insight into potential mechanisms. *Arch Physiol Biochem. ,* **126**(5): 399-407.

Song HS, Lee YJ and Kim SK and Ki-Young M (2004). Down regulatory effect of AGI-1120 (α-glucosidase inhibitor) and Chaga mushoom (*Inonotus obliquus*) on

cellular NF-kB acti-vation and their antioxidant activity. *Kor. J. Phar-macogen*, **1**(136): 92-97.

Wang Y, Tang C and Zhang H (2015). Hepatoprotective effects of kaempferol 3-O-rutinoside and kaempferol 3-O-glucoside from *Carthamus tinctorius* L. on CCl4-induced oxidative liver injury in mice. *J. Food Drug Anal.*, **23**(2): 310-317.

Wang Yan, Li Jun and Wang Bao-wei (2010). The protective effect of hesperidin on nonalcoholic fatty liver and its mechanism in rats. *Acta Universitatis Med.Anhui*, **45**(5): 646-649.

Wu DF and Cederbaum AI (2005). Oxidative stress mediated toxicity exerted by ethanol-inducible CYP2E1. *Tox. App. Pha.,* **207**(2): 70-76.

Yong Cui, Dong-Seok Kim and Kyoung-Chan Park (2005). Antiox-idant effect of Inonotus obliquus. *J. Ethno-pha.,* **96**: 79-85.

Yong Ook Kim, Hae Woong Park and Jong Hoon Kim (2006). Anti-cancer effect and sructural characterization of endo-polysacchride from cultivated mycelia of Inonotusobliquus. *L. Sci.,* **79**: 72-80.

Yusoo Shin, Yutaka Tamai and Minoru Terazawa (2001). Chemical constituents of *Inonotus boliquus,* a new triterpene, 21, 24-cyclopentalanosta-3β,21,25-triol-8-ene from sclera-tium. *Jap. Wood Res. Soc.*, **47**: 313-316.

Zeng B, Su M and Chen Q (2017). Protective effect of a polysaccharide from *Anoectochilus roxburghii* against carbon tetrachloride-induced acute liver injury in mice. *J. Ethnopharmacol.,* **200**: 124-135.

Zhao XY, Li R and Liu Y (2017). Polydatin protects against carbon tetrachloride-induced liver fibrosis in mice. *Arch Biochem. Biophys.*, **629**: 1-7.

Zheng L, Yin L and Xu L (2018). Protective effect of dioscin against thioacetamide-induced acute liver injury via FXR/AMPK signaling pathway *in vivo*. *Biomed. Pharmacother.*, **97**(6): 481-488.