Protective effects of traditional Uighur medicine-seeds of *Nigella glandulifera* Freyn extracts against ccl_4-induced acute hepatic injury in mice

Dilinuer Maimaitiyiming[1], Mayila Kamilijiang[2], Xiadiya Xiaerfuding[3], Shi Wen Hui[4], Meiliwan Abudureyimu[1], Hajinisha[1] and Ainiwaer Aikemu[5,6]*

[1]Department of Comprehensive Cardiac Medicine, Hospital of Xinjiang Medical University, Urumqi, Xinjiang, China
[2]Key Laboratory of Molecular Biology and Endemic Diseases, Xinjiang Medical University, Urumqi, Xinjiang, China
[3]Teaching and Research Section for Health Service Management, Xinjiang Medical University, Urumqi, Xinjiang, China
[4]Department of animal experiment, Urumqi general hospital of Lanzhou Military, Urumqi, Xinjiang, China
[5]Department of Drug Analysis, Xinjiang Medical University, Urumqi, Xinjiang, China
[6]Central laboratory, Xinjiang Medical University, Urumqi, Xinjiang, China

Abstract: To explore the protective effects of Traditional Uighur medicine Seeds of *Nigella glandulifera* Freyn (SNF) extracts against CCl_4-induced acute hepatic injury in mice. Hepatic injury mice models induced by intraperitoneal injection of 0.1% CCl_4 olive oil were established. Liver and spleen coefficient, Serum ALT and AST activities, SOD, GSH-Px activities and MDA content in hepatic homogenate were measured and the hepatic histological changes were observed by optical microscope. Serum activities of ALT ($P<0.01$) and AST ($P<0.05$) in Alcohol extraction group was decreased; Activity of hepatic homogenate SOD increased in Alcohol extraction group and Water extraction group significantly ($P<0.05$). Content of MDA was decreased in Alcohol extraction group ($P<0.01$). Water extracts of SNF have obvious protective effects on hepatic injury induced by CCl_4 in mice.

Keywords: Traditional Uighur medicine, seeds of *Nigella glandulifera* Freyn, carbon tetrachloride (CCl_4), acute liver injury.

INTRODUCTION

Traditional Uighur Medicine is an important part of Traditional Chinese Medicine, based on which, various new drugs have been under research and developed. Uighur Medicine system was established by Uighur people during their struggles against disease in history. Comparing with traditional chemicals, Uighur Medicine is widely available, inexpensive and has numerous types and quantity and little side effects. What is more, Uighur Medicine has the characteristics of lots of links, multiple targets and multi-level comprehensive effects making it had some advantages in treatment of liver disease (Traditional Uighur Medicine Ingredients Standard (First Volume 1993). Therefore, in the present age, searching for ideal anti-hepatitis drugs from Traditional Uighur medicine is becoming one of the most important topics. *Nigella glandulifera* Freyn is accumulated to Raunnculaceae and Nigella plants, the seed of it is generally used as drug. At present, three kinds of seeds are used as drugs (Nie Lingyun *et al*., 2001; Chen Liguo *et al*., 1989; Li Yikui *et al*., 2006). *Nigella glandulifera* Preyn et Sint is a kind of traditional Uygur's herb in Xinjiang China, which is also named as Syadan. *Nigella glandulifera* is widely planted in some regions of Xinjiang, such as in Turpan, Shan Shan (Ali *et al*., 2008).

**Corresponding author:* e-mail: ainiwa@sina.com

The seeds of *Nigella glandulifera* Freyn are widely distributed in Xinjiang region of China and have been used in traditional Uighur medicine for the treatment of diseases such as diuretic, brain and kidney supplement, blood circulation improvement, detoxification, galactagogue, menstruation improvement, anti-inflammatory and anti-aging for a long period of time (Ali *et al*., 2008; Liu *et al*., 2004; Nguyen *et al*., 2007; Nguyen *et al*., 2007; Zhou *et al*., 2010).

Liver is one of the most important organs of human body which has the function of digestion, detoxification and excretion. The structure and function of liver is complex and it is vulnerable to a variety of vulnerable to a variety of pathogens, toxins and immune pathological involvement. Hepatic disease is one of the common diseases which can do serious harm to human health. Improper treatment of hepatic injury would lead to various complications such as infections, hepatic cutting surface bile leakage and secondary hemorrhage. Moreover, serious damages to liver would even lead to liver fibrosis, cirrhosis and cancer, etc (Wei *et al*., 2000; Liu *et al*., 2000). Among them the most common diseases are acute and chronic hepatitis resulted from hepatic inflammatory reaction caused by viruses, chemical drugs, ethanol and immune responses, etc. In addition, fatty liver and the hepatic injury caused by autoimmune diseases have higher incidence. Liver also plays a key role in the metabolism process of carbohydrate, lipid, protein,

vitamins and hormones, etc. Clinical manifestations of acute and chronic hepatic injury include fatigue, anorexia, weight loss, hepatalgia and abdominal distension. Abnormal results of hepatic function tests such as increase of serum aminotransferase and aspartate aminotransferase (AST), decrease of liver tissue glutathione peroxidase (Gpx) and superoxide dismutase (SOD), increase of malonaldehyde and NO could be observed by the measurement of serum biochemistry (Behne D *et al*., 1983; Huang P at al., 2000; Look MP *et al*., 1997).

MATERIALS AND METHODS

Materials and reagents

Uyghur Medicine Seeds of *Nigella glandulifera* Freyn (Xinjiang Uygur Hospital , batch number: 20100701); Bifendate dripping pills (size: 1.5 mg / pill, Beijing Xiehe Pharmaceutical Factory, batch number 10120101); Carbon tetrachloride (CCl_4, A/R, Tianjin Yongjingxi Chemical Co., Ltd., batch number 20100901; prepare with 0.1% CCl_4 olive oil solution); Glutathione per oxidase (GSH-Px), Super oxide dismutase (SOD) and malondialdehyde (MDA) reagent kit (purchased from Nanjing Jiancheng Bioengineering Institute).

Experimental animals

Kunming mice, ♀♂half amount for each, weight (20±2) g. provided by the Experimental Animal Center of Xinjiang Medical University were used.

Experimental instruments

LD5-2A low-speed centrifuge (Beijing Medical Centrifuge Factory); DY89-1 electric glass homogenate Machine (Ningbo Xinzhi Biotechnology Co., Ltd.); BS-320 automatic biochemical analyzer (Shenzhen Mindray Medical Electronics Co., Ltd.); HH-S4 digital display constant temperature water bath (Jintan medical instrument factory); BS224s electronic balance (Beijing Sartorius Instrument System Co., Ltd.); 721 spectrophotometer (Shanghai 3rd Analytical Instrument Factory).

Animal grouping and treatment

50 mice were randomly divided into 5 groups according to body weight and sex. 10 mice/group, which were normal group, model group, positive control group (bifendate group, $0.2g \cdot kg^{-1} \cdot d^{-1}$), Seeds of *Nigella glandulifera* Freyn Ethanol extract (SNFEE) group ($2g \cdot kg^{-1} \cdot d^{-1}$), Seeds of *Nigella glandulifera* Freyn Water extract (SNFWE) group ($2g \cdot kg^{-1} \cdot d^{-1}$). Normal group and model group were given distilled water, other groups were given the test drugs. qd ×14d daily morning and afternoon after weighing.

Establishment of animal model (Sanmugapriya E et al., 2006)

One hour after the last administration, except normal control group, all other groups were given intraperitoneal injection of 1% CCl_4 olive oil solution, 10 $ml \cdot kg^{-1}$, normal control group was given intraperitoneal injection of equal volume of olive oil. Then all mice were fasted and given water only, after 24 hours, all mice were weighed, the blood, liver and spleen specimens were collected for correlation detection.

Animal handling and observing

Blood were collected from each mice eyeballs and the plasma was placed in refrigerator for half an hour at 4°C. Biochemical analyses were performed in serum supernatant obtained after centrifugation of total blood at 3000r·min-1 for 10min. BS-320 automatic biochemical analyzer was used in determination of the following biochemical parameters: Alanine amino transferase (ALT), aspartate aminotransferase (AST); Killed mice and removed the liver and spleen, rinsed with 4°C saline, used filter paper to dry up, weighed, and calculated liver and spleen coefficient (Organ weight (g)/ weight (g) × 100n% =organ coefficient (%)). Take same parts of the right lobe of the liver in mice liver organization for 0.5g; Put them into 4.5mL of ice saline solution to made 10% liver homogenate centrifuged for 10 minutes at 2500r·min-1, supernatant for the determination of hepatic GSH-Px and SOD activity and MDA content according to the instructions. Fragments of the same part of the left lobe of mice liver were fixed in 10% formalin for his to pathological examination.

STATISTICAL ANALYSIS

SPSS 17.0 software was used, the measurement data was expressed as mean ±SD, test of significance of difference between groups were used single coefficient analysis of variance. Differences were considered significant for $P<0.05$.

RESULTS

There was no statistically significant difference between normal control group and model group ($P>0.05$), Results are shown in table 1.

Compared with the normal control group CCl_4 liver injury model group, Water extraction group, serum ALT and AST activity was significantly increased ($P<0.01$). Compared with model group, positive control group, Water extraction group, serum ALT activity was decreased, the difference was statistically significant ($P<0.01$~0.05). Results are shown in table 2.

Compared with normal control group, CCl_4 liver injury model mice liver tissue SOD activity was significantly decreased ($P<0.01$), GSH-Px activity decreased ($P<0.05$), MDA content was significantly increased ($P<0.01$); Compared with model group, alcohol extraction group can make the liver SOD activity increased ($P<0.05$); positive control group, alcohol extraction group can make the liver MDA content significantly lower ($P<0.01$);

The results in table 3.

Histological examination of liver pathology Histo pathological examination can reflect the degree of liver injury objectively. Results of this study show that, SNFWE of Uighur medicine alleviates liver cell edema, steatosis, spotty necrosis and inflammatory cell infiltration significantly, in addition, regeneration of liver cell is also obvious. From acute liver injury pathological observations we found that regeneration of liver cell was observed in every tissue section except in normal control group. Regeneration of liver cells indicate that liver protection is achieved by promoting regeneration and repairing of liver cell; therefore it can be inferred that SNFWE of Uighur Medicine has some protective effects on liver cells. As shown in fig. 1.

DISCUSSION

Some studies have revealed that liver injury is a complex process involved various factors. Free radicals in excess and lipid per-oxidation play a pivotal role in the process of liver disease (Yin XL *et al*., 2003). Excessive generation or inefficient clearance of free radicals indicates that dynamic balance *in vivo* is destroyed (McAnulty SR *et al*., 2004) and Adhesion, aggregation of excessive free radicals in the micro-vessels would result in microcirculation disturbance. MDA, as the end-product of lipid per-oxidation (Kandil FE *et al*., 2002), can cause necrosis and swelling of cell and bring severe damages to the cell membrane structure, and contents of MDA are usually measured to determine the extent of lipid per-oxidation *in vivo*. Therefore, MDA can indirectly reflect the degree of cell injury (Mustafa Atalay *et al*., 2003).

In this study, we found that regardless of acute experiment, SNFWE can reduce contents of MDA at different degrees. SOD is one of the main materials involved in antioxidant enzyme system which can clear oxygen free radicals caused by biological oxidation in the body, and it plays an essential role in the balance of oxidation and anti-oxidation, which indicates that SOD may play the role of protecting liver(Okado-Matsumoto *et al*.,2001). In this study we found that SNFWE can increase the activity of SOD ($P<0.05$). GSH-PX is an important component of the endogenous antioxidant system and it plays an important role in the protection of liver cell from immune oxidative damage. Our study indicates that SNFWE can reduce the content of MDA in liver, inhibit per-oxidation and reduce the damage of MDA to the body significantly; meanwhile it can increase the activity of SOD, GSH-PX. Our results show that SNFWE may protect liver cells by scavenging free radicals, increasing activities of free-radical scavenging enzyme and anti-lipid per-oxidation. When

Table 1: SNF extract on mice with acute CCl_4 liver damage liver, spleen coefficient ($x \pm s$, $n = 10$)

Group	Dose ($g \cdot kg^{-1}$)	Liver coefficient (%)	Spleen coefficient (%)
Normal group	—	5.40±0.28	0.74±0.18
Model group	—	5.67±0.58	0.46±0.08
Positive control group	0.2	5.08±0.61**	0.36±0.06
SNFEE	2	5.87±0.51	0.53±0.04
SNFWE	2	5.01±0.34	0.42±0.08

Table 2: SNF extract on mice serum ALT, AST activity ($x \pm s$, $n = 10$)

Group	Dose ($g \cdot kg^{-1}$)	ALT /$U \cdot L^{-1}$	AST /$U \cdot L^{-1}$
Normal control	—	28.39±7.64	149.92±16.59
Model	—	769.39±438.57△△	608.80±179.96△△
Positive control	0.2	106.53±99.05**	199.60±105.36**
SNFEE	2	339.31±271.23△**	347.52±147.15*
SNFWE	2	458.76±212.13△△	507.69±293.58△△

Table 3: SNF extract on liver tissue of SOD, GSH-Px activity and MDA content ($x \pm s$, $n=10$)

Group	Dose $g \cdot kg^{-1}$	SOD $C/U \cdot (mgPro)^{-1}$	GSH-Px $C/U \cdot (mgPro)^{-1}$	MDA $C/U \cdot (mgPro)^{-1}$
Normal control	—	38.39±1.17	384.67±125.89	3.21±1.61
Model	—	20.72±3.57△△	223.58±57.14△	15.17±6.02△△
Positive control	0.2	29.34±1.50	348.76±77.39	5.71±3.07**
SNFEE	2	36.12±5.85*	321.07±128.32	9.82±3.21△△**
SNFWE	2	34.58±2.31*	298.33±154.26	11.74±4.53△△

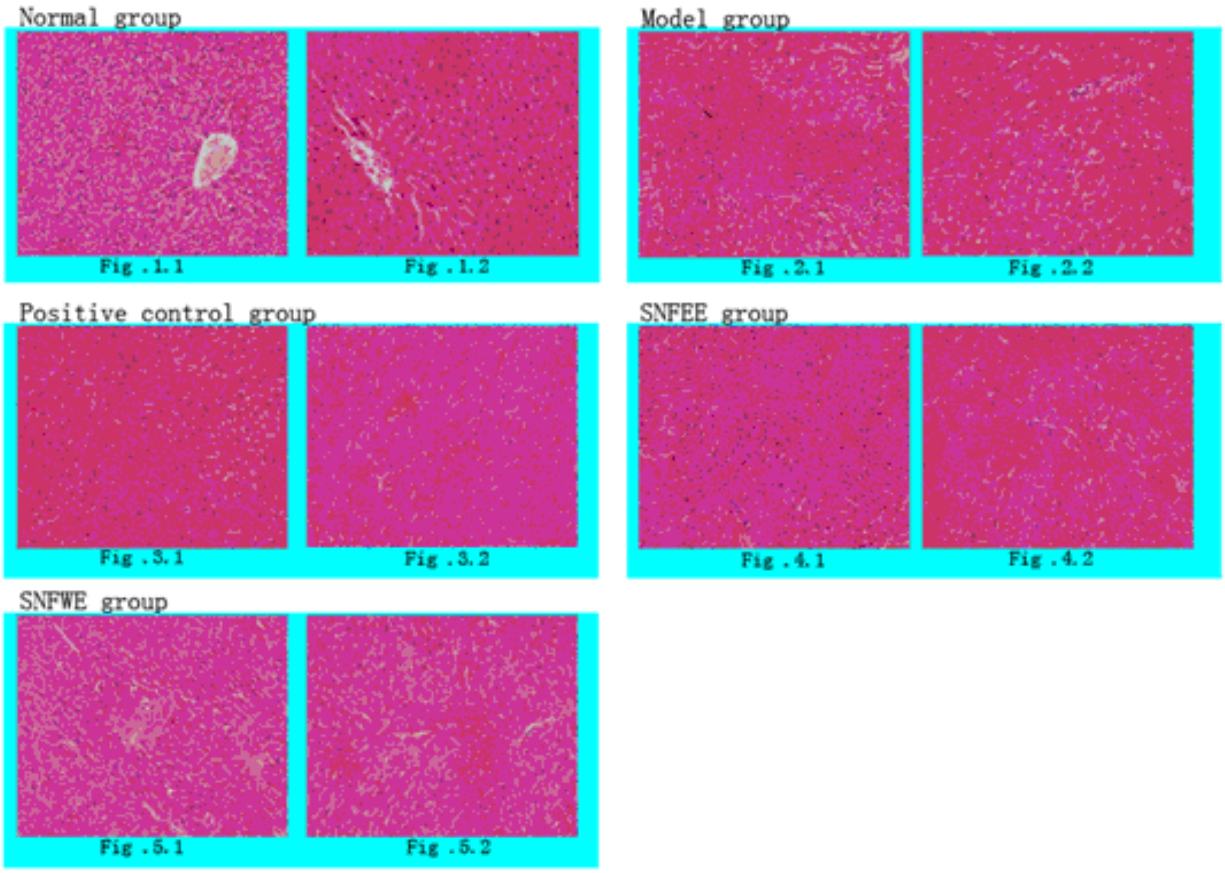

Fig. 1: The effect of SNFWE on acute liver injury pathology

the liver cell is damaged to some extent, activity of ALT and AST can increase significantly and cause liver cell necrosis. This is because ALT and AST exists mainly in liver cells, when liver cell degenerated or cell membrane was broken, ALT and AST which exist in the plasma membrane flow into the blood, thus indexes described above would increase. Many studies based on this research have found that SNFWE can significantly reduce the activity of ALT and AST in serum which indicates that SNFWE has the effect of anti-liver injury.

Histopathological examination can reflect the degree of liver injury objectively. Results of this study show that, SNFWE of Uighur medicine alleviates liver cell edema, steatosis, spotty necrosis and inflammatory cell infiltration significantly. Besides that, regeneration of liver cell is also obvious. From acute liver injury pathological observations we found that regeneration of liver cell was observed in every tissue section except in normal control group. Regeneration of liver cells indicates that liver protection is achieved by promoting regeneration and repairing of liver cell; therefore it can be inferred that SNFWE of Uighur Medicine has some protective effects on liver cells.

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