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The Potential Effect of Traditional Herbal Medicine on Bile Flow in CC14-Induced Liver Injury Model

Munkhjargal Radnaa[1,2], Tuul Khalzaibaast[3], Dorjbat Sosorburam[1], Bayasgalan Gombojav[4], Chimedragchaa Chimedtseren[5]

[1]Department of Mongolian Medicine Study, International School of Mongolian Medicine, Mongolian National University of Medical Sciences, Ulaanbaatar, Mongolia;
[2]Department of Traditional Medicine, The First Central Hospital of Mongolia, Ulaanbaatar, Mongolia;
[3]Mongolian Traditional Medicine and Training Center Manba Datsan, Ulaanbaatar, Mongolia;
[4]Department of Epidemiology and Biostatistics, School of Public Health, Mongolian National University of Medical Sciences, Ulaanbaatar, Mongolia;
[5]Institute of Traditional Medicine and Technology of Mongolia, Ulaanbaatar, Mongolia.

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Corresponding Author:
Tuul Khalzaibaast (M.D., Ph.D., Prof.)
Department of traditional Medicine
Otoch Manramba University
Post office-49 P.O BOX-235; Ulaanbaatar-133361, Mongolia
Tel: +976-80100038
Fax: +976-70158489
E-mail: khtuul2017@gmail.com
ORCID: https://orcid.org/0009-0004-4835-7743

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Objective: To investigate whether the modified Mongolian traditional medicine Yaman Serdeg-3 (NYS-3) effects the biliary excretion in the carbon tetrachloride-induced liver injury model. **Methods:** Forty-seven male and female Sprague-Dawley rats were divided into 5 groups: Group I healthy group (n=12); Group II healthy group + NYS-3 (n=9); Group III liver injury model (n=10); Group IV liver injury model + Chinese medicine Hua gan Pian (n=10); Group V liver injury model + NYS-3. Experimental groups II-V, received corresponding medicinal preparations for 2 weeks. The dose of Hua gan Pian (HGP) was 0.046g/ml while the dose of NYS-3 was 2.025g/ml. For the acute induction of liver injury, the rats were orally administered 40% CCl4 in olive oil (4 ml/kg) twelve hours after the last administration of preparations. **Results:** At the first 15-minute interval, the highest bile flow was observed in Group II rats, which were administered NYS-3. This phenomenon continued at the second 15-minute interval, also. However, at the third 15-minute interval, the bile flow decreased sharply in Group II. On the other hand, in the liver injury model, untreated or pre-treated rats had significantly different bile flows. For example, in the first half of the experiment, both non-treated and HGP pre-treated groups had similar bile flow, while, the group pre-treated with NYS-3 had significantly low bile flow. Then, after 45 minutes of the experiment, the bile flow became similar amounts in both pre-treated groups. **Conclusion:** We conclude that the NYS-3 administration had increased the bile flow at 3rd 15min of interval, which is higher than positive control group where liver injury model rats received HGP.

Keywords: Carbon, Organic Chemical, Liver Disease, Drug Induced Liver Injury, Bile Acid

Introduction

The liver is an essential metabolic organ that performs over 500 vital physiological functions such as detoxification, utilization of various nutrient cycles as well as secretion of proteins. Because of its rich blood supply and the primary location of body metabolism, the liver is more

susceptible to numerous pathogenic factors. According to worldwide statistics, approximately 2 million people die from various liver diseases (liver cancer, cirrhosis, and other complications) each year.

Numerous studies have demonstrated that the bile acid profile could serve as a potential diagnostic and therapeutic indicator for liver diseases.[1-5] Bile acids can be divided into primary and secondary bile acids. Cholic acid (CA) and chenodeoxycholic acids (CDCA) are the most abundant primary cholic acids synthesized in the liver. Whilst, the secondary bile acids include deoxycholic acid, lithocholic acid (LCA), ursodeoxycholic acid (UDCA) as well as taurochenodeoxycholic acids (TCDCA). These acids are derived from the primary bile acids via gut bacterial deconjugation, dehydrogenation, 7α-dehydroxylation, and epimerization. Wang, et al. demonstrated the quantitative profiles of serum bile acid in patients with hepatitis B-induced cirrhosis and revealed that the five bile acids, glycocholic acid (GCA), glycochenodeoxycholic acid (GCDCA), taurocholic acid (TCA), TCDCA, and glycoursodeoxycholic acid (GUDCA), were significantly altered among different stages of liver cirrhosis.[6] Allen, et al. showed that the hepatocytes treated with bile acids increased the expressions of proinflammatory mediators without the activation of Toll-like receptor [4.7] The rapidly increasing chronic liver disease NAFLD (non-alcoholic fatty liver disease) among adults and children is also significantly related to altered bile acid homeostasis. A recent cross-sectional study resulted in higher fecal bile acid, cholic acid (CA), chenodeoxycholic acid (CDCA) levels, and synthesis in patients with NAFLD compared to healthy individuals ($P<0.05$).[8]

The treatment of liver injury is important for the prevention, treatment, and recovery of a variety of liver diseases, and bile acids are one of the most sensitive indicators for the clinical diagnosis of liver diseases. In recent decades, the extract of natural products has been popular as an alternative therapy for tumors, cardiovascular diseases as well as metabolic disorders.[9-11] For example, Totum-070 (the blend of olive leaves, artichoke leaves, chrysanthemum, goji fruits, and black pepper) modulated dysbiosis associated with metabolic disorders, and increased the fecal short-chain acid concentrations.[12] Kim, et al. demonstrated that mice fed with 5% Platycodi Radix extract prevented hepatic steatosis and increased the bile acids, which was lower in non-treated high-fat diet mice.[13] Like this, multiple treatment strategies are aimed at alleviating hepatic functions and minimizing the severity of hepatic fibrosis using different herbal compounds.

The formal Yaman Serdeg-3 is the mixture of three medicinal plants Saxifraga hirculus, Hemerocallis minor Mill, and Smilax glabra. Roxb. Smilacaceae used in Mongolian Traditional Medicine for the liver and gallbladder, and it has been studied to support the excretion of bile. Saxifraga hirculus contains myricetin, quercetin, and its glycosides, while Hemerocallis minor Mill has a high amount of chlorogenic acid, quercetin as well as different types of polysaccharides. In traditional medicine, Hemerocallis minor Mill is used for decreasing blood pressure and blood cholesterol levels and increasing bile acid secretion. Further, Smilax glabra. Roxb. Smilacaceae is a widely distributed medicinal plant used for the treatment of diabetic vascular complications. It contains flavonoids, terpenoids, and mannose-binding lectin.

The present study was designed to investigate whether the modified Mongolian traditional medicine Yaman Serdeg-3 (NYS-3) effects the biliary excretion in the carbon tetrachloride-induced liver injury model. In the present study, we have modified Yaman Serdeg-3 (NYS-3) by adding 4th plant component, Saussurea amara (L.), a traditional Mongolian medicine used in the treatment of hepatic-biliary disorders.

Methods and Materials

Research design

The effect of the NYS-3 on mean biochemical indicators and bile acid as well as bile flow was measured on the 15th, 30th, 45th and 60th minutes of the experiment.

The NYS-3 is comprised of Saxifraga hirculus, Hemerocallis minor Mill, and Smilax glabra. Roxb. Smilacaceae and Saussurea amara (L.). The medical preparation of Yaman Serdeg-3 is manufactured by Traditional Medicine Manufacture Co., Ltd. Mongolia (The Standard Number MNS 5585:2006). Subsequently, small cut Saussurea amara (L.) was added with a 1:10 ratio and extracted with hot ethanol for 15 minutes, cooled, and filtered and evaporated ethanol.

Establishment of animal model and drug administration Animal care and experimental procedures were approved by the Ethics Committee of the Mongolian National University of Medicinal Sciences. Forty-seven male and female Sprague-Dawley rats in specific pathogen-free (SPF) grade weighing 200-250 g were purchased from the Institute of Laboratory Animal Science, Mongolia. The rats were housed under specific pathogen-free conditions with controlled lighting (12 h per day) and tempera-

ture (22 ± 2 °C), fed standard laboratory chow, and allowed water ad libitum. After 1 week of adaptive feeding, rats were divided into 5 groups: Group I healthy group (n=12); Group II healthy group + NYS-3 (n=9); Group III liver injury model (n=10); Group IV liver injury model + Chinese medicine HGP (n=10); Group V liver injury model + NYS-3. Experimental groups II-V, received corresponding medicinal preparations for 2 weeks. The dose of HGP was 0.046g/ml while the dose of NYS-3 was 2.025g/ml. For the chronic induction of liver injury, the rats were orally administered 40% CCl4 in olive oil (4 ml/kg) twelve hours after the last administration of preparations. After the anesthetization with pentobarbital sodium (40 mg/kg; Sigma-Aldrich, St. Louis, MO, USA), the distal portion of the obstructed biliary cannula was then exposed and cut to relieve the bile duct obstruction. The cannula was immediately attached to another piece of tubing and the skin incision was closed. Bile was collected continuously, in dim light, into weighed tubes over 15-minute periods for one hour.

Statistical Analysis

The average value of variables in the group 1 to 10 and control were compared using the mixed effect two-way ANOVA test. Tukey test was used as a multiple comparison. The Geisser-Greenhous correction was used to p-value. For repeated measures, the paired t-test was carried out. A critical p-value of < 0.05 was used. SPSS version 24 software (SPSS Inc., Chicago, IL, USA) was used for statistical analyses.

Ethical Statement

The animal study was carried out in accordance. Ethical approval to conduct the experiments was obtained from the Mongolian National University of Medical Sciences (No.2022/06/02). All efforts were made to minimize the number of animals used and their suffering.

Results

Changes in liver function in the present study are shown in Table 1. Compared to the healthy group 1, the body weight of the experimental animals of the 2nd group that was given-NYS-3increased by 1.10 times, compared to the liver injury model group, the body weight of the 4th group of HGP increased by 1.05 times, and especially the body weight of the 5th group of the NYS-3experiment increased by 1.11 times. The body weight of Group V was 351.49 ± 16.45, which is significantly recovered compared to the liver injury model Group III (315.91 ± 27.63) (P< 0.004).

Table 1. Effect of CC14 on serum biochemical indicators.

Parameters	Groups					Total	*P-value
	Group I Healthy group	Group II Healthy +NYS-3	Group III Liver injury model	Group IV Liver injury model + HGP	Group V Liver injury model + NYS-3		
	n = 12 Mean ± SD	n = 9 Mean ± SD	n = 10 Mean ± SD	n = 10 Mean ± SD	n = 6 Mean ± SD	N = 47 Mean ± SD	
Weight a, b	312.13 ± 28.03	343.6 ± 14.25	315.91 ± 27.63	334.63 ± 14.20	351.49 ± 16.45	324.32 ± 27.86	0.004
ALP c, d	162.8 ± 30.85	187.13 ± 39.77	245.78 ± 67.03	273.5 ± 81.80	309.4 ± 35.12	207.66 ± 71.48	0.01
ALT e	66.93 ± 16.48	75.11 ± 16.66	697.03 ± 143.47	682.71 ± 155.72	632.77 ± 129.61	430.91 ± 67.25	0.001
AST f	226.56 ± 52.37	240.09 ± 69.97	650.34 ± 181.53	553.52 ± 143.75	453.43 ± 121.19	424.81 ± 113.76	0.001
LDH g	1529.1 ± 425.95	1169.12 ± 385.79	2536.51 ± 355.97	1938.54 ± 880.49	1724.55± 720.84	1779.55 ± 553.79	0.001
BIL h	65.4	0.4 ± 0.41	0.299 ± 0.046	0.206 ± 0.054	0.264 ± 0.08	0.434 ± 0.472	0.045

**One-way ANOVA; Tukey test; multiple comparisons: aGroup I vs. Group IV, P< 0.032; bGroup I vs. Group V, P< 0.042; cGroup II vs. Group IV, P< 0.024; dGroup I vs. Group III, P< 0.035; eGroup II vs. Group V, P< 0.041; fGroup I vs. Group IV, P< 0.031; gGroup I vs. Group II, P< 0.012; hGroup I vs. Group V, P< 0.033.*

The effect of NYS-3 on biochemical ALP parameters in CCl4-induced liver injury model, compared to healthy group 1, the ALP level in the 2nd group given NYS-3 significantly increased by 1.14 times ($p< 0.001$), compared to the healthy group 1. In group p, the amount of ALP increased significantly by 1.5 times ($p< 0.001$). Compared with control group 3, ALP level was 1.25 times lower ($p< 0.001$) in HGP group 4, while ALP level was 1.11 times lower ($p< 0.001$) in group 5 of NYS-3. Comparing group 5 of NYS-3 with group 4 of HGP, the amount of ALP decreased by 1.31 times ($p< 0.001$).

In CCl4-induced liver disease model, considering the effect of NYS-3 on biochemical ALT parameters, compared to healthy group 1, ALT level increased significantly by 10.41 times ($p<0.001$) in pathological control group 3. Compared with control group 3, HGP group 4 had a 1.02-fold lower ALT level ($p< 0.001$), while NYS-3 experimental group 5 had a 1.10-fold lower ALT level ($p< 0.001$). Compared with NYS-3 group and HGP group 4, the ALT level decreased by 1.07 times ($p< 0.001$).

Considering the effect of NYS-3 on biochemical AST parameters in CCl4-induced liver disease model, compared to the healthy group 1, the AST level in group 2 given NYS-3 significantly increased by 1.05 times ($p< 0.001$), compared to the healthy group 1 ($p< 0.001$). In group p, AST increased significantly by 2.87 times ($p< 0.001$). Compared with control group 3, HGP group 4 had a 1.17-fold ($p< 0.001$) lower AST level, while NYS-3 experimental group 5 had a 1.43-fold ($p< 0.001$) lower AST level. Compared with group 5 of NYS-3 compared to group 4 of HGP, AST decreased by 1.22 times ($p< 0.001$).

Considering the effect of NYS-3 on biochemical LDH parameters in CCl4-induced liver disease model, LDH level was significantly decreased by 1.37 times ($p< 0.001$) in group 2 given NYS-3 compared to healthy group 1. In control group 3, LDH level increased significantly by 1.65 times ($p< 0.001$). Compared with control group 3, HGP group 4 had a 1.3-fold lower LDH ($p< 0.001$), while NYS-3 experimental group 5 had a 1.47-fold lower LDH level ($p<0.001$). Compared with group 5 of NYS-3 and group 4 of HGP, LDH decreased by 1.12 times ($p< 0.001$).

Considering the effect of NYS-3 on biochemical total bilirubin in CCl4-induced liver disease model, compared to the healthy group 1, the BIL level in the 2nd group given NYS-3 significantly increased by 1.37 times ($p< 0.001$), compared to the healthy group 1. In the group p, the amount of BIL decreased significantly by 2.44 times ($p< 0.001$). Compared with control group 3, HGP group 4 had a 1.45-fold lower BIL ($p< 0.001$), while NYS-3 experimental group 5 had a 1.13-fold lower BIL ($p< 0.001$). Comparing group 5 of NYS-3 with group 4 of HGP, BIL increased by 1.28 times ($p< 0.001$). See Table 1.

After 2 weeks of pre-treatment with NYS-3, bile acid flow was measured. Bile fluid is collected and measured every 15 minutes. Formula for calculating bile flow:

$$Bile\ flow\left[\frac{\mu L}{\text{min}\bullet 100\text{g}}\right]=\frac{(\text{EP2}-\text{EP1})*100000}{\text{rat weight}*15}$$

Note: EP2: Weight of mini tube after bile fluid is collected; EP1: Weight of mini tube before bile fluid is collected

Table 2 describes tube weight before and after the bile amounts. As seen here, before the bile flow, the tube weights of all groups were approximately the same. However, after the second 15-minute interval, the tube weight was significantly high in Group II. This phenomenon was also seen in 4th 15-minute interval.

The bile flow measurements are given in Table 3. At the first 15-minute interval, the highest bile flow was observed in Group II rats, which were administered NYS-3. Bile flow during the CCl4-induced pathological model showed that compared with the healthy group 1, the 2nd group given NYS-3 had a 3.07-fold increase in bile secretion in the first 15 minutes and a 2.32-fold increase in the second 15 minutes ($p< 0.001$). In the 3rd 15 minutes, compared to the healthy group, the bile secretion of the 2nd group decreased by 1.15 times, and in the 4th 15 minutes, the bile secretion of the 2nd group of NYS-3 increased by 1.48 times ($p < 0.001$).

Compared with the healthy group 1, the bile flow in the 3 pathological control groups decreased by 1.47 times from the first 15 minutes to the 4th 15 minutes. Compared with the control group, bile flow decreased by 1.06 times in the first 15 minutes ($p < 0.001$), 1.55-2.02 times in the 2nd and 3rd 15 minutes, and 3.18 times in the 4th 15 minutes.

Bile flow was reduced by 2.30 times in the group of NYS-3 compared to the pathological control. Compared with the group of NYS-3, the bile secretion was 2.62 times lower in the first 15 minutes, 1.11 times lower in the 2nd 15 minutes, and 1.71 times higher in the 3rd 15 minutes. In the last 15 minutes, compared with the HGP experimental group, the NYS-3 group had a 1.38-fold decrease in bile secretion.

Table 2. Effect of CC14 on bile acid.

Variables	Groups					Total	*P-value
	Group I a Healthy group	Group II Healthy +NYS-3	Group III [b] Liver injury model	Group IV Liver injury model + HGP	Group V [c] Liver injury model + NYS-3		
	N = 12 Mean ± SD	N = 9 Mean ± SD	N = 10 Mean ± SD	N = 10 Mean ± SD	N = 6 Mean ± SD	N = 47 Mean ± SD	
Ep1_015a,b,c	0.948 ± 0.021	0.971 ± 0.037	0.964 ± 0.012	0.960 ± 0.023	0.979 ± 0.044	0.958 ± 0.027	0.013
EP1_15_30	0.956 ± 0.031	0.943 ± 0.063	0.947 ± 0.025	0.960 ± 0.041	0.954 ± 0.011	0.953 ± 0.035	0.999
EP1_30_45	0.976 ± 0.046	0.948 ± 0.019	0.959 ± 0.039	0.957 ± 0.022	0.959 ± 0.014	0.965 ± 0.037	0.190
EP1_45_60	0.967 ± 0.029	0.951 ± 0.015	0.870 ± 0.300	0.968 ± 0.029	0.963 ± 0.013	0.949 ± 0.121	0.356
Ep2_015	1.07 ± 0.14	1.13 ± 0.19	1.18 ± 0.24	1.09 ± 0.15	1.09 ± 0.10	1.09 ± 0.16	0.155
Ep2_15_30a	1.10 ± 0.08	1.31 ± 0.25	1.01 ± 0.08	1.01 ± 0.07	0.99 ± 0.05	1.09 ± 0.14	0.000
Ep2_30_45b	1.11 ± 0.13	1.08 ± 0.14	1.03 ± 0.07	0.99 ± 0.05	1.03 ± 0.05	1.07 ± 0.11	0.002
EP2_45_60d	1.13 ± 0.17	1.23 ± 0.15	1.02 ± 0.07	1.01 ± 0.06	0.995 ± 0.037	1.09 ± 0.15	0.000

**One-way ANOVA; Tukey test; multiple comparisons: aGroup I vs. Group II, P< 0.013; bGroup I vs. Group III, P< 0.010; cGroup III vs. Group IV, P< 0.001; dGroup III vs. Group V, P< 0.034;*

Table 3. Effect of CC14 on bile flow.

Variables	Groups					Total	*P-value
	Group I a Healthy group	Group II Healthy +NYS-3	Group III [b] Liver injury model	Group IV Liver injury model + HGP	Group V [c] Liver injury model + NYS-3		
	N = 12 Mean ± SD	N = 9 Mean ± SD	N = 10 Mean ± SD	N = 10 Mean ± SD	N = 6 Mean ± SD	N = 47 Mean ± SD	
Flow 0-15	558.26 ± 625.18	1719.42 ± 865.37	460.56 ± 495.10	488.22 ± 433.38	186.33 ± 238.88	631.58 ± 713.45	0.034
Flow 15-30	687.84 ± 370.99	1598.13 ± 1008.64	332.47 ± 365.68	213.23 ± 195.35	191.18 ± 224.07	612.22 ± 617.08	0.000
Flow 30-45	669.34 ± 502.36	580.89 ± 591.49	354.49 ± 308.78	170.92 ± 180.89	292.31 ± 186.15	495.87 ± 463.91	0.001
Flow 45-60	824.55 ± 716.15	1222.87 ± 624.06	711.13 ± 1320.64	187.00 ± 186.11	135.16 ± 161.85	692.37 ± 806.07	0.007

*Two-way mixed ANOVA results: Interaction of time and treatment F (1.813, 316.59) = 22.165, P< 0.001; Main effect of time F (1.918, 336.59) = 325.31, P< 0.031; Main effect of CCC14 F (1,186) = 0.775, P= 0.616; *One-way ANOVA; Paired t-test: aFlow 0-15 vs. Flow 45-60, P=0.049; bFlow 0-15 vs. Flow 45-60, P=0.045; cFlow 0-15 vs. Flow 30-45, P=0.011.*

Discussion

Natural products, especially plant products, have a long history of medicinal use. They have various anti-inflammatory, antioxidant, and immunomodulatory compounds that show different physiological activities.[14-19] Specifically, natural substances such

as resveratrol, green tea, and turmeric have been a significant focus of studies investigating potential NAFLD treatments.

As described by Yang, et al. the Chinese herbal medicine CHM consisting of Chinese thorowax root, Scutellaria root, and white peony root is beneficial in regulating lipid metabolism and liver function.20 A literature survey conducted on herbs, their preparations, and ingredients with reported liver protection activities revealed that there are a total of 274 different species and hundreds of active ingredients. These ingredients can be roughly classified into two categories (1) the main ingredients, such as silybin, coumarin, glycyrrhizin, saikosaponin A, schisandrin A, flavonoids; and (2) sugars, amino acids, resins, tannins, and volatile oil. Several of these compounds have shown hepatoprotective activities.[21] Further, dried roots of Polygonum cuspidatum also can promote blood circulation and remove blood stasis. The active ingredients include resveratrol, emodin of anthraquinones, quercetin, polydatin, and its derivatives of flavonols, coumarin, and lignan. It has been demonstrated that resveratrol, polydatin, and emodin are the main active components and work together to exert a therapeutic effect on NAFLD.[22]

In the present study, we investigated Mongolian traditional medicine Yaman Serdeg-3 on bile flow in CCL4-induced liver injury model rats. The Yaman Serdeg-3 is widely used in traditional medicines to improve liver functions through the bile flow rise. It consists of three medicinal plants Saxifraga hirculus, Hemerocallis minor Mill, and Smilax glabra. Roxb. Smilacaceae. Saxifraga hirculus contains myricetin, quercetin, and its glycosides. In the study of Kayani, et al. Saxifraga hirculus is used significantly for respiratory disorders.[23] Further, Hemerocallis minor Mill has a high amount of chlorogenic acid, quercetin as well as different types of polysaccharides. Monoterpene derivatives of Hemerocallis minor Mill have depress-reducing activity, and caffeoylquinic acids show antioxidative actions.[24-25] Moreover, Smilax glabra. Roxb. Smilacaceae is a widely distributed medicinal plant used for the treatment of diabetic vascular complications. It contains flavonoids, terpenoids, and mannose-binding lectin. In the present study, we have modified Yaman Serdeg-3 by adding 4th plant component, Saussurea amara (L.), a traditional Mongolian medicine used in the treatment of hepatic-biliary disorders. As described here, the highest bile flow was observed in group II, where the experimental animals received HGP. However, the 3rd 15 minutes of interval, we have observed high bile flow at group V where the animals received the NYS-3. In the study of Glasl, et al. the methanolic extract exerted a dose-dependent increase in bile flow, while the aqueous crude extract as well as the ethyl acetate extract also showed a dose-dependent increase. However, high-concentration administration showed a continuous decrease in bile flow.[26] Further, as described by Accatino, et al. a plant-derived steroid diosgenin markedly increased cholesterol and lamellar structures in bile and attenuated the acute cholestatic effects of 17 alpha-ethynylestradiol. Also, diosgenin prevented the decrease of taurocholate maximum secretory rate and the increase of biliary alkaline phosphatase and Ca2+, Mg2+-EctoATPase (EctoATPase) excretion, as well as the increase of cholesterol/ phospholipids ratio, alkaline phosphatase activity. However, diosgenin did not prevent E-induced decrease of basal bile flow, bile salt, cholesterol, and phospholipid secretory rates nor the decrease of Na+K+-ATPase activity and Na+-taurocholate cotransporting polypeptide (Ntcp) content in isolated sinusoidal membranes.[27] Mongolian traditional medicinal plants Dianthus versicolor (Caryophyllaceae) and Lilium pumilum (Liliaceae) was also studied for the stimulation of bile flow in perfused rat liver model. It has become clear that aqueous and methanolic extracts increased the bile flow dose-dependently (between 9% and 30%), and no hepatotoxic effect was seen even during longer perfusions.[28]

We conclude that the NYS-3 administration had increased the bile flow at 3rd 15min of interval, which is higher that positive control group where liver injury model rats received Hua gan Pian. The present study had several limitations. First, we did not evaluate the adequate dose of Saussurea amara (L.) for the modification of Yaman Serdeg-3. As shown in the present study, we could not detect significant high bile flow in the rats, compared to positive control HGP. Second, recent studies have revealed that intestinal flora may be an essential bridge between liver autophagy and bile acid metabolism. However, we could not confirm the intestinal microflora in the present study. Further studies are needed to characterize the bioactive compounds of the NYS-3 and to determine its optimal dosage in order to contribute to the beneficial effects on bile flow.

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