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Ineffectiveness of Cholic Acid and Cyprinol Sulfate on the Toxicity of Streptozotocin in Rats

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ABSTRACT

The possible effects of bile acid and bile alcohol on the visual acuity of streptozotocin (STZ)-induced hyperglycemia male Wistar rats were investigated. Forty-eight rats were divided into two groups, STZ and non-STZ, and intraperitoneally injected with STZ (60 mg/kg body weight in 0.6 mL of saline) and saline (0.6 mL) on day 1, respectively. After 4 days of STZ treatment, all the rats were tested for glycosuria and then divided into four groups. Each group had 6 rats and the rats were treated orally every 3 days with saline and the non-toxic dosages of cholic acid (37 mg/kg) and cholic acid (74 mg/kg) combined with cyprinol sulfate (37 mg/kg). At the end of the 29-day experiment, the results revealed that the relative ratios of liver and kidney weight to body weight, the concentrations of RBC, hemoglobin and hematocrit in the blood, as well as the levels of AST, ALT, ALP, BUN and creatinine in the plasma of the rats were not significantly different. However, the levels of plasma glucose and liver thiobarbituric acid-relative substances (TBARS) in the rats treated with STZ were higher, while the levels of vitamin A in the cornea were contrarily lower than those of the non-STZ rats. The values of three indicators (plasma glucose, liver TBARS and vitamin A) related to visual acuity were not significantly influenced when the rats were treated with cholic acid alone or combined with cyprinol sulfate in the STZ or non-STZ group. The results indicated that both cholic acid and cyprinol sulfate could not influence the toxicity of STZ in rats.

Key words: cholic acid, cyprinol sulfate, bile acid, streptozotocin, toxicity

INTRODUCTION

In traditional Chinese medicine, the gallbladder is an important crude drug, which includes the bile of bears, cows, grass carps (*ctenopharyngodon idellus*), common carps (*cyprinus carpio*), snakes and chickens. The bile is thought to induce antitussive and hypotensive actions and enhance visual acuity. However, a survey of toxic fish in China by Ng and Kum[(1)] showed that the number of cases of food poisoning from the ingestion of fish gallbladders is only second to eating pufferfish. Fresh-water fish are bred in many areas of the world as a another source of food is a concern for people eating fish gallbladders. The extraction of toxic substances from grass carp bile has been performed[(2-5)] and it is generally agreed that poisoning occurs through the action of a group of bile alcohols or acids found in the bile of this family of fish[(6)]. There have been many studies on the structures and pharmacological effects of bile and gallstone components, especially the bile of bear and snake, in which C-24 bile acids such as cholic acid and chenodeoxycholic acid are major components[(7)]. In contrast, less work has been done on the chemical elucidation of bile alcohol, such as cyprinol, which is a major component of the *Cyprinidae* family[(6,8)]. The major component by weight (> 94%) in the bile of common carps and grass carps was cyprinol sulfate, while cholic acid, chenodeoxycholic acid and lithocholic acid accounted for less than 5%[(9,13)]. It has been reported that the ingestion of bile juices, especially carp bile juice, causes severe toxic effects in humans[(3)]. In some severe cases, the ingestion of raw carp bile causes death of experimental animals, due to a decrease in blood pressure and an increase in plasma potassium, hydrogen ions, blood urea nitrogen and hematocrit[(14)]. The toxin in carp bile is identified as 5α-cyprinol sulfate (5α-cholestane-3α, 7α, 12α, 26, 27-pentol 26 [or 27]-sulfate), an alcohol specific to carp bile[(10)]. Cyprinol sulfate is suggested to be the causative agent of

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common carp bile toxicity[9,10]. The bile from various fishes, such as sharks, carps, mackerels and crucians, has also been used as a crude drug to treat dyspnea due to disorders of the throat and pharynx and eye disease, among others, and as an analgesic[1]. The animal gallbladder is considered a bitter-cold medicine that enters the liver, gallbladder, spleen and stomach channels to detoxify and clear heat. It is used for delirium, extensive burns, high fever and convulsions. Topically, it alleviates skin pain and is used for reducing swelling and trauma caused by sprains and fractures[11].

To our knowledge, no pharmacological study for the visual acuity of these compounds has been reported yet. During our toxicological studies on the bile alcohol of grass carp and bile acids of laboratory animals, it is of great interest to study the pharmacological use of bile acid and bile alcohol in the improvement of visual acuity. It has been reported that the visual acuity of diabetic rats could be induced by streptozotocin (STZ)[12-15]. Diabetes mellitus is one of the endocrine disorders characterized by hyperglycemia complications affecting the eyes. The underlying mechanism of diabetic complications remains unclear, though possible events such as the activation of oxidative stress have been suggested[16-22]. In recent years, much attention has been focused on the role of oxidative stress and it has been suggested that oxidative stress may constitute the key and common event in the pathogenesis of different diabetic complications[22]. In fact, increasing the generation of reactive oxygen intermediates (ROS) such as superoxide anion, hydroxyl anion and peroxynitrite anion has been shown to occur in diabetes in association with hyperglycemia[23]. Therefore, the aim of this study was to examine if there is protective function of bile acid and bile alcohol on visual acuity using STZ induced hyperglycemic rat model. In a previous report[14], 148 mg/kg each of cholic acid and cyprinol sulfate was found to be toxic on the liver and kidney in rats. In this study, the protective effect of bile alcohol and bile acid in the safe dosage range of 37 to 74 mg/kg was investigated on visual acuity in STZ-induced hyperglycemia rats.

MATERIALS AND METHODS

I. *Preparation of Carp Bile Powder and Chemicals*

About 10 L of fresh gall bladders of the grass carp (*Ctenopharyngodon idellus*) was collected from Taoyuan County. The bile juices were filtered and freeze-dried. The grass carp bile powder (1,500 g) was stored at -18°C before use. Cholic acid and STZ (98%) were obtained from Sigma (St. Louis, MO, USA). All other chemicals used in this study are of analytical grade.

II. *Animals*

Male Wistar rats, 4 weeks old, were purchased from the National Laboratory Animal Center and housed individually in stainless steel wire bottom cages in a controlled environment (25°C, 50-60% humidity, 12 h of light per day) for 2 weeks. The animals were fed a laboratory diet (Laboratory Rodent Diet 5001; PMI Feeds, Richmond, VA, USA). The weight of rats were 270 ± 10 g at the start of the experiment. Tap water was supplied in free access. This study was approved by the Institutional Animal Care and Use Committee (IACUC No. 940) of Chung Shan Medical University.

III. *Cyprinol Sulfate from the Grass Carp Bile Powder*

A portion (20 g) of grass carp bile powder was extracted thrice with 60 mL of ethanol. The combined extract was defatted with dichloromethane and evaporated to dryness. The residue was extracted thrice with distilled water/n-butanol (1 : 1). The n-butanol layer was collected and evaporated to dryness. The n-butanol extract was dissolved with methanol and applied onto a PHP-LH 20 and silica gel column (20 × 2 cm) according to the method of Hwang *et al*.[24]. The purified grass carp bile salt (7.6 g) was dissolved and crystallized in acetonitrile. The crystal was evaporated to dryness. The crystallized 5α-cyprinol sulfate was kept in a desiccator and stored in a cold room before use[14,25-27].

IV. *Animal Test*

Forty-eight male Wistar rats were fasted for 12 h before the start of the experiment. The rats were then divided into two groups, STZ and non-STZ. In the STZ group, the rats were treated with STZ (60 mg/kg in 0.6 mL saline solution with 10 mM of citrate buffer, pH = 4.5, i.p.). The urine was collected and urine glucose was tested by using Ames reagent strips (Sanko Co. Ltd., Tokyo, Japan) after 4 days. In the non-STZ group, the rats treated with the same amount of saline solution. On day 5, the rats in each group were selected based on the urine glucose of 263 mg/dL and then divided into four groups. Each group had 6 rats and the rats were treated orally for every 3-day treatment with 0.6 mL saline solution (control group), 37 mg/kg body weight of cholic acid, 74 mg/kg body weight of cholic acid and 37 mg/kg body weight of cyprinol sulfate in 0.6 mL of saline, respectively. The concentrations of cholic acid (10 mg), cholic acid (20 mg) and cyprinol sulfate (10 mg) were 0.024×10^{-6} M, 0.049×10^{-6} M and 0.019×10^{-6} M, respectively. On days of 5 and 17, blood was obtained by tail vein puncture after 6 h of administration. On day 29, the rats were weighed and anesthetized with diethyl ether. Blood was obtained by heart puncture with syringes. The conical cornea, liver and kidney of rats were quickly excised. A part of liver and conical cornea was stored at -20°C for thiobarbituric-reactive substances (TBARS), vitamin A and retinol-binding-protein (RBP) determination.

The blood was analyzed using a CELL DYN 500 Hematology Analyzer (Sequoia-Turner, USA) to determine the levels of red blood cells (RBC), hematocrit, white blood cells (WBC) and hemoglobin (Hb). Plasma was collected by the centrifugation (1,000 *g* × 15 min) of blood and analyzed using a Merck VITALAB Selectra Biochemical Autoanalyzer (Merck, Germany) to determine the levels of glucose, blood urea nitrogen (BUN), creatinine, aspartate aminotransferase

(AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). The levels of vitamin A and retinol-binding-protein (RBP) in the plasma were also determined.

V. *TBARS Production*

Lipid peroxidation activities in the liver were assayed by the measurement of malondialdehyde (MDA), an end-product of peroxidized fatty acids, and thiobarbituric acid (TBA) reaction product. The sample of 20% liver homogenate was mixed with 1.0 mL of 0.4% TBA in 0.2 N HCl and 0.15 mL of 0.2% BHT in 95% ethanol. The samples were then incubated in a 90°C water-bath for 45 min. After incubation, the TBAMDA adduct was extracted with isobutanol. The isobutanol extract was mixed with methanol (2 : 1) prior to injection into the HPLC system[28]. The supernatant was examined by using the HPLC system at an excitation wavelength of 515 nm and an emission wavelength of 550 nm on a Hitachi Fluorescence Detector (Tokyo, Japan).

VI. *Measurement of Vitamin A Level*

About 0.1 g (mL) of sample (conical cornea, plasma and liver) was extracted thrice with chloroform/methanol solvent (2 : 1, v/v) in a dark room. The extracts were combined and evaporated to remove the organic solvents. Then an equal volume of 25% KOH in methanol solution containing 1,000 ppm each of vitamin C and BHT was added and heated in a 80°C water-bath for 30 min. After soap treatment, diethyl ether/petroleum ether (1 : 1, v/v) was added to the extract. The extract was evaporated by nitrogen gas and the residue was dissolved with 1 mL of hexane/isopropanol (95 : 5, v/v)[29]. The solution was examined for vitamin A level at a wavelength of 325 nm by using HPLC (Hitachi, Tokyo, Japan).

VII. *Measurement of Retinol-Binding-Protein (RBP) Level*

Each tissue sample (plasma and liver) was homogenized in 9 volumes of PBS (pH 7.5) containing 0.5% Triton X-100, 2.5 mM of EDTA and 1 mM of phenylmethylsulfonyl fluoride using a Polytron homogenizer for 60 s and sonicated for 15 s according to the method of Sparks *et al*.[30]. After the homogenates were centrifuged at 105,000 ×*g* for 1 h (4°C), the clear supernatant fraction (cytosol) was frozen at -20°C and used for the assay of RBP. RBP was purified to a fluorescence spectral and immunological homogeneity from the plasma of 3-month-old Sprague-Dawley rats as described by McGuire and Chytil[31], and monospecific IgG polyclonal rabbit anti-rat RBP anti-serum was prepared. RBP was quantified by sandwich-type enzyme-linked immunosorbent assay (ELISA, ALPCO Diagnostics, Salem, NH, USA) according to the procedure reported by Goda *et al*.[32]

VIII. *Measurement of Glutathione (GSH) Levels*

Glutathione (GSH) reacted non-enzymatically with 5,5'dithiobis (2-nitrobenzoic acid)(DTNB) to yield glutathione disulfide (GSSG) and 2-nitro-5-thiobenzoic acid (TNB). GSSG was then reduced enzymatically by NADPH and glutathione reductase (GR) to regenerate GSH. Concentrations of DNTB, NADPH and GR were chosen such that the rate of the overall reaction was linearly proportional to the concentration of total glutathione. The rate of formation of TNB was followed spectrophotometrically, and assay was calibrated using a standard solution of TNB. If the sample was reacted with 2-vinylpyridine, GSH was derivatized, and only GSSG was detected during subsequent assay[33].

IX. *Statistical Analysis*

A two-tailed Student's *t*-Test[34] was used to determine if the two population means were equal. A common application of this test is to determine if a new process or treatment is superior to a current process or treatment. A p value of less than 0.05 was considered statistically significant.

RESULTS

After STZ treatment, all the rats were tested positive for glycosuria (263 mg/dL)[48]. The effect of STZ on the glucose level in the plasma of rats is shown in Table 1. The glucose level in the plasma of rats increased significantly when the rats were treated with STZ. After the 29-day treatment with cholic acid and cyprinol sulfate, glucose levels did not decrease significantly. The results indicated that STZ could induce diabetic rats, and cholic acid and cyprinol sulfate did not reduce the symptoms of diabetes. The effects of cholic acid and cyprinol sulfate on the levels of vitamin A in the conical cornea, plasma and liver of rats treated with and without STZ are shown in Table 2. After the 29-day treatment with bile salt, the levels of vitamin A in the conical cornea, plasma and liver of rats treated without STZ were about 70 ng/g, 1.25 ng/mL and 430 ng/g, respectively. In comparison,

Table 1. Effects of bile salts on plasma glucose levels (mg/dL) of rats treated with or without streptozotocin after 29 days[1]

Treatment	Day	Control (0.6 mL of saline)	Cholic acid (mg/kg in 0.6 mL of saline)		Cyprinol sulfate (37 mg/kg in 0.6 mL of saline)
			37	74	
Without streptozotocin					
	5	89 ± 15^a	88 ± 22^a	88 ± 23^a	88 ± 23^a
	17	87 ± 23^a	86 ± 17^a	88 ± 28^a	87 ± 20^a
	29	87 ± 21^a	87 ± 21^a	87 ± 23^a	88 ± 24^a
With streptozotocin					
	5	264 ± 25^b	278 ± 33^b	262 ± 32^b	268 ± 33^b
	17	263 ± 31^b	258 ± 28^b	275 ± 28^b	258 ± 25^b
	29	265 ± 24^b	257 ± 24^b	254 ± 33^b	257 ± 26^b

[1]Data represents mean ± S.D. (n = 6). The values in the same row are not significantly different, while those in the same column with different superscripts (a - b) are significantly different ($p < 0.05$).

the vitamin A levels for the rats were treated with STZ were lower, at about 20 ng/g, 0.5 ng/mL and 310 ng/g, respectively. The results indicated that STZ induced glycosuria and visual damage in the rats, but cholic acid and cyprinol sulfate did not influence the STZ-induced toxicity in rats. The effects of cholic acid and cyprinol sulfate on the body weight and ratios of kidney and liver weights to body weight are shown in Table 3. After the 29-day treatment, the body weight and the ratios of kidney and liver weights to body weight of rats in the STZ and non-STZ groups were not affected by any dose of cholic acid and cyprinol sulfate treatments.

In biochemistry, the activities of AST, ALT and ALP in plasma are generally tested as indicators for liver functions, and the levels of creatinine and BUN in the plasma are tested as indicators for kidney function[35-37]. The effects of cholic acid and cyprinol sulfate on the BUN and creatinine levels and AST, ALT and ALP activities in the plasma of rats are shown in Tables 4 and 5. On the interval days of 5, 17 and 29, BUN and creatinine levels and the activities of AST, ALT and ALP in the plasma of rats were not significantly changed. Hence, 74 mg/kg of cholic acid, 37 mg/kg of cyprinol sulfate, and STZ were found to have no harmful effect on the functions of liver and kidney. In a previous report[25], 148 mg/kg of cholic acid and cyprinol sulfate was known to be toxic. In addition, the effects of cholic acid and cyprinol sulfate on the RBC, WBC, hematocrit and hemoglobin levels in blood in the rats treated with or without STZ were not found during the 29-day experimental period (data not shown). These data supported the above results that less than 74 mg/kg of cholic acid and less than 37 mg/kg of cyprinol sulfate did not have any adverse effect on the liver and kidney of rats.

The effects of cholic acid and cyprinol sulfate on the RBP of plasma and liver in the rats after the 29-day experimental period are shown in Table 6. It was found that STZ and bile salts (cholic acid and cyprinol sulfate) did not affect the concentration of RBP in the plasma and liver. However, STZ significantly increased the TBARS level, but not the GSH level in the liver of rats (Table 7). In addition, cholic acid and cyprinol sulfate did not influence the liver oxidation caused by STZ.

Table 2. Effects of bile salts on vitamin A levels in the conical cornea (ng/g), plasma (ng/mL) and liver (ng/g) of rats treated with or without streptozotocin after 29 days[1]

Treatment	Control (0.6 mL of saline)	Cholic acid (mg/kg in 0.6 mL of saline)		Cyprinol sulfate (37 mg/kg in 0.6 mL of saline)
		37	74	
		In the conical cornea		
Without streptozotocin	69.6 ± 4.2[a]	62.1 ± 5.1[a]	66.3 ± 4.4[a]	65.5 ± 3.3[a]
With streptozotocin	22.4 ± 1.4[b]	20.1 ± 1.9[b]	22.2 ± 1.8[b]	22.1 ± 1.5[b]
		In the plasma		
Without streptozotocin	1.25 ± 0.02[a]	1.23 ± 0.03[a]	1.24 ± 0.04[a]	1.23 ± 0.02[a]
With streptozotocin	0.53 ± 0.03[b]	0.54 ± 0.02[b]	0.55 ± 0.02[b]	0.54 ± 0.03[b]
		In the liver		
Without streptozotocin	422 ± 53[a]	427 ± 49[a]	430 ± 50[a]	420 ± 52[a]
With streptozotocin	312 ± 38[b]	316 ± 41[b]	313 ± 38[b]	316 ± 37[b]

[1] Data represents mean ± S.D. (n = 6). The values in the same row are not significantly different, while those in the same column with different superscripts (a - b) are significantly different ($p < 0.05$).

Table 3. Effects of bile salts on body weight and the ratios of kidney and liver weight to body weight of rats treated with or without streptozotocin after 29 days[1]

Treatment	Control (0.6 mL of saline)	Cholic acid (mg/kg in 0.6 mL of saline)		Cyprinol sulfate (37 mg/kg in 0.6 mL of saline)
		37	74	
Without streptozotocin				
Body weight (g)	260 ± 19	264 ± 22	274 ± 21	273 ± 22
Ratios of kidney weights to body weight (%)	0.54 ± 0.03	0.54 ± 0.03	0.57 ± 0.04	0.54 ± 0.05
Ratios of liver weights to body weight (%)	2.2 ± 0.3	2.2 ± 0.4	2.1 ± 0.3	2.4 ± 0.3
With streptozotocin				
Body weight (g)	271 ± 19	265 ± 21	265 ± 20	272 ± 20
Ratios of kidney weights to body weight (%)	0.52 ± 0.04	0.52 ± 0.03	0.51 ± 0.04	0.53 ± 0.05
Ratios of liver weights to body weight (%)	2.3 ± 0.2	2.4 ± 0.4	2.5 ± 0.3	2.1 ± 0.4

[1] Data represents mean ± S.D. (n = 6). The values in the same row and column are not significantly different ($p > 0.05$).

Table 4. Effects of cholic acid and cyprinol sulfate on the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in the plasma of rats treated with or without streptozotocin after 29 days[1]

Treatment	Day	Control (0.6 mL of saline)	Cholic acid (mg/kg in 0.6 mL of saline)		Cyprinol sulfate (37 mg/kg in 0.6 mL of saline)
			37	74	
			For AST (U/L)		
Without streptozotocin	5	124 ± 22	115 ± 13	120 ± 15	118 ± 19
	17	121 ± 20	121 ± 17	124 ± 14	120 ± 15
	29	127 ± 22	125 ± 16	121 ± 17	125 ± 18
With streptozotocin	5	124 ± 23	119 ± 17	123 ± 25	124 ± 16
	17	122 ± 18	127 ± 15	128 ± 19	123 ± 18
	29	119 ± 19	123 ± 14	124 ± 16	124 ± 17
			For ALT (U/L)		
Without streptozotocin	5	36 ± 12	39 ± 14	39 ± 15	39 ± 13
	17	38 ± 13	34 ± 13	36 ± 15	36 ± 17
	29	36 ± 14	36 ± 117	39 ± 16	41 ± 21
With streptozotocin	5	35 ± 16	35 ± 22	34 ± 19	37 ± 23
	17	35 ± 20	37 ± 20	23 ± 24	37 ± 25
	29	37 ± 18	34 ± 19	39 ± 19	38 ± 17
			For ALP (U/L)		
Without streptozotocin	5	269 ± 22	269 ± 21	273 ± 15	264 ± 19
	17	267 ± 21	271 ± 17	273 ± 16	271 ± 16
	29	277 ± 26	271 ± 16	278 ± 18	269 ± 14
With streptozotocin	5	264 ± 23	264 ± 24	353 ± 27	255 ± 21
	17	251 ± 24	282 ± 26	261 ± 12	267 ± 18
	29	261 ± 23	274 ± 27	262 ± 24	260 ± 18

[1] Data represents mean ± S.D. (n = 6). The values in the same row and column are not significantly different ($p > 0.05$).

Table 5. Effects of bile salts on the levels of blood urea nitrogen (BUN) and creatinine in the plasma of rats treated with or without streptozotocin after 29 days[1]

Treatment	Day	Control (0.6 mL of saline)	Cholic acid (mg/kg in 0.6 mL of saline)		Cyprinol sulfate (37 mg/kg in 0.6 mL of saline)
			37	74	
			BUN (mg/dL)		
Without streptozotocin	5	13.1 ± 1.3	13.1 ± 1.4	12.4 ± 1.4	12.4 ± 1.5
	17	12.6 ± 1.2	12.5 ± 1.2	12.2 ± 1.5	12.6 ± 1.7
	29	12.7 ± 1.5	12.9 ± 1.4	12.5 ± 1.3	12.8 ± 1.6
With streptozotocin	5	12.8 ± 1.4	12.4 ± 1.6	13.4 ± 1.4	12.7 ± 1.7
	17	12.5 ± 1.7	13.1 ± 1.3	12.6 ± 1.4	13.2 ± 1.6
	29	12.4 ± 1.6	12.7 ± 1.5	12.4 ± 1.6	12.6 ± 1.8
			Creatinine (mg/dL)		
Without streptozotocin	5	0.41 ± 0.04	0.39 ± 0.04	0.38 ± 0.04	0.40 ± 0.02
	17	0.39 ± 0.04	0.38 ± 0.03	0.40 ± 0.03	0.40 ± 0.03
	29	0.41 ± 0.03	0.40 ± 0.03	0.39 ± 0.04	0.41 ± 0.03
With streptozotocin	5	0.40 ± 0.03	0.37 ± 0.03	0.39 ± 0.04	0.40 ± 0.03
	17	0.42 ± 0.03	0.40 ± 0.03	0.42 ± 0.03	0.41 ± 0.03
	29	0.40 ± 0.03	0.39 ± 0.03	0.41 ± 0.02	0.39 ± 0.03

[1] Data represents mean ± S.D. (n = 6). The values in the same row and column are not significantly different ($p > 0.05$).

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Table 6. Effects of bile salts on the levels of retinol binding protein (RBP) in the plasma and liver of rats treated with or without streptozotocin after 29 days[1]

Treatment	Day	Control (0.6 mL of saline)	Cholic acid (mg/kg in 0.6 mL of saline)		Cyprinol sulfate (37 mg/kg in 0.6 mL of saline)
			37	74	
			Plasma (μg/mL)		
Without streptozotocin	5	23.3 ± 3.2[a]	22.6 ± 3.3[a]	23.5 ± 3.2[a]	23.5 ± 3.5[a]
	17	25.1 ± 3.6[a]	23.5 ± 3.2[a]	23.2 ± 3.5[a]	23.6 ± 3.3[a]
	29	24.7 ± 3.3[a]	23.9 ± 3.1[a]	23.6 ± 3.3[a]	23.8 ± 3.6[a]
With streptozotocin	5	13.5 ± 3.5[b]	13.2 ± 3.6[b]	13.3 ± 3.5[b]	13.7 ± 3.7[b]
	17	13.1 ± 3.7[b]	13.6 ± 3.3[b]	12.7 ± 3.3[b]	13.5 ± 3.6[b]
	29	13.3 ± 3.6[b]	13.7 ± 3.5[b]	12.8 ± 3.6[b]	13.6 ± 3.8[b]
			Liver tissue (μg/g)		
Without streptozotocin	29	41.3 ± 8.5[a]	42.3 ± 8.3[a]	42.5 ± 8.6[a]	43.5 ± 8.2[a]
With streptozotocin	29	25.5 ± 4.7[b]	25.2 ± 4.9[b]	25.3 ± 4.5[b]	24.3 ± 4.7[b]

[1] Data represents mean ± S.D. (n = 6). The values in the same row are not significantly different, while those in the same column with different superscripts (a - b) are significantly different ($p < 0.05$).

Table 7. Effects of bile salts on the levels of thiobarbituric acid-reactive substances (TBARS) and glutathione (GSH) in the liver of rats treated with or without streptozotocin after 29 days[1]

Treatment	Control (0.6 mL of saline)	Cholic acid (mg/kg in 0.6 mL of saline)		Cyprinol sulfate (37 mg/kg in 0.6 mL of saline)
		37	74	
		TBARS (nmol/g wet liver)		
Without streptozotocin	22.3 ± 1.4[a]	22.7 ± 1.7[a]	21.1 ± 1.5[a]	22.2 ± 1.2[a]
With streptozotocin	32.5 ± 1.6[b]	30.8 ± 1.2[b]	31.2 ± 1.4[b]	30.2 ± 1.4[b]
		GSH (nmol/g wet liver)		
Without streptozotocin	24.7 ± 2.1	26.5 ± 1.5	24.8 ± 1.7	24.3 ± 1.4
With streptozotocin	25.4 ± 1.7	25.7 ± 1.8	24.4 ± 1.5	25.1 ± 1.7

[1] Data represents mean ± S.D. (n = 6). The values in the same row are not significantly different, while those in the same column with different superscripts (a - b) are significantly different ($p < 0.05$).

DISCUSSION

Renal failure, liver dysfunction and cardiovascular and gastrointestinal impairment are featured as the common symptoms of poisoning by grass carp bile juice[38-40]. In a previous study, rats administered with 0.6 mL of grass carp bile juice were also intoxicated and displayed renal failure and liver dysfunction. For this reason, rats could be used as an animal model to evaluate the safe dosage of grass carp bile juice[14].

Diabetes mellitus (DM) is a metabolic disease characterized by inadequate production or utilization of insulin. It is the major cause of retinopathy leading to blindness in adults[41]. Prolonged vitamin A deficiency can lead to night blindness due to impaired production of rhodopsin, the compound in the retina responsible for detecting small amounts of light. Xerophthalmia, a condition characterized by changes to the conjunctiva and cornea of the eye, also results from prolonged vitamin A deficiency and is a major cause of blindness in developing nations[42]. Despite this common clinical consequence of vitamin A deficiency and DM, it is only very recently that some significant studies have been carried out linking the two conditions.

Streptozotocin (STZ)-induced diabetic rats have been associated with a decrease in plasma and an increase in hepatic vitamin A concentrations compared to nondiabetic animals[13-15,48]. It was of interest that the diabetic animals had lower plasma concentrations of retinol despite its increased food intake by 50%. A decrease in the concentration of 11-cis-retinal, a constituent of rhodopsin in the retina of the eye was also reported in parallel with a decrease in circulatory level of retinol in diabetic rats[49-51].

Recent studies have shown that plasma concentration of vitamin A (retinol) and its carrier proteins, retinol-binding protein (RBP) and transthyretin (TTR) are decreased in human subjects with insulin-dependent diabetes mellitus (IDDM)[44-47]. Rats made diabetic with STZ have also been shown to have reduced levels of plasma vitamin A[12,13,15].

The decreases in plasma retinol, RBP and TTR concentrations were a response to diabetes[12,13,15,52]. Then, the increase of free oxidative products is suggested to cause eye damage[53-56]. As the vitamin A levels in the conical cornea, plasma and liver of rats were significantly reduced, rats with eye damage could be induced by STZ (60 mg/kg, i.p.) in this study. After the STZ treatment, the glucose test was all positive in the urine of rats. The levels of glucose in the plasma of rats treated with STZ were also found to be higher than those of rats treated without STZ. Other indicators, including the activities of AST, ALT and ALP in the plasma (liver functional indicators), the concentrations of BUN and creatinine in the plasma (kidney functional indicators), as well as the blood and histological characteristics of liver and kidney were found to be not affected by STZ. After the 29-day experimental period, similar results were obtained the STZ and non-STZ group rats. The results indicated that 74 mg/kg of cholic acid and 37 mg/kg cyprinol sulfate did not have an adverse effect on liver and kidney functions. Furthermore, vitamin A levels in the conical cornea, plasma and liver, TBARS level in the liver, and glucose level in the plasma were not affected in the STZ and non-STZ groups when the rats were treated with or without cholic acid and cyprinol sulfate. Cholic acid and cyprinol sulfate did not induce toxicity in mild diabetic rats.

Eye damage can be induced by STZ due to a decrease in the levels of plasma vitamin A, RBP and TTR[12,13,15,52-56,65]. Eye damage in diabetic rats could be induced by STZ, followed by the assessment of the protective effects of treatment with bile alcohol and bile acid on the eye. The results suggested the protective effect of bile salts on visual acuity, as there was no enhancement of visual damage induced by STZ. Cholic acid and cyprinol sulfate did not alter STZ-induced vitamin A levels in the conical cornea, plasma and liver. Meanwhile, cholic acid and cyprinol sulfate did not influence hepatic lipid peroxidation and glucose level in the plasma of rats. Hence, cholic acid and cyprinol sulfate have no effect on the toxicity of STZ in rats. The results of this study did not support the use of bile salts as a medicinal material for curing STZ-induced damage. Further study on other medicinal functions is required. The animal bile juice had been proven to be effective on special diseases. For example, there were some evidence that bear bile juice could cure various cholestatic disorders[66-67]. However, due to the occasional poisoning cases from the consumption of animal bile juices, it is suggested that consumers should not consume animal bile juices or believe in their medicinal effects.

Until now, animal gallbladders are believed by Asians to have a medicinal action on the enhancement of visual acuity. However, from the results obtained in this study, the ameliorating effect of cholic acid and cyprinol sulfate on STZ-induced visual damage was not found. Moreover, oxidative stress in liver and glycosuria in the rats induced by STZ were also not affected by cholic acid and cyprinol sulfate. Hence, bile acids and alcohols could not influence the toxicity of STZ in rats.

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