2003. Vol. 24. No. 4. 11-18
Korean Journal of Oriental Medicine

Original Articles

Effects of *TongBiYeum* (TBY) on the Fatty Liver

Ki-Tae Lee, Yun-Sik Kim, In-Chan Seol

Department of Ciculatory Internal Medicine, College of Oriental Medicine, Daejeon University

Purpose : This study examined the effect of *Tongbiyeum* (TBY) on rats with fatty livers.

Methods : After administration of TBY extracts to rats with fatty livers, body weight, liver weight, serum, cholesterol, TG, HDL-cholesterol were measured, and histopathological changes were observed.

Results : In proportion to density, TBY decreased liver weight and serum TG levels and suppressed fat accumulation and liver cell death, but increased serum HDL levels in comparison with those of controls.

Conclusion : TBY might be considered to be used to control fatty liver by inhibiting absorption and storage of TG. *(Korean J of Oriental Med 2003;24(4):11-18)*

Key Words: *Tongbiyeum* (TBY), fatty liver, lipid, cholesterol, HDL

Introduction

Fatty liver is excessive accumulation of triglyceride in hepatocytes, the most common response of the liver to injury. Fatty liver occurs when triglyceride accumulation exceeds the normal 5% of liver weight[1].

Lipids are a diverse group of biomolecules. Molecules such as fats and oils, phospholipids, steroids, and the carotenoids, which differ widely in both structure and function, are all considered lipids[2].

Recently, due to a diet increasingly high in fat and sucrose, dangerous conditions of fatty liver and hypercholestrolemia have increased. In response, many studies of methods for treating fatty liver and hypercholesterolemia have been undertaken[3]. Growing interest in oriental medicine, which may have less toxicity, has led to an increasing number of non-pharmacological therapies for fatty liver[4].

Among these is *Tongbiyeum* (TBY), which has been used at Daejeon Oriental Medicine Hospital since 2001 for patients with weakness after stroke, numbness or pain and hyperlipidemia. We evaluated its therapeutic effects on patients by clinical and scientific basis of the effect on fatty liver under laboratory examination. These experimental models were performed with inducing by alcohol or oil[5]. As fatty liver increases according to augmentation of diet including sugar and sucrose, we need to seek out various methods of treating fatty liver. Therefore, this present study aimed to elucidate the effects of TBY on fatty liver induced by a sucrose-rich diet by determination of serum triglyceride as well as total and HDL-cholesterol level, and

Received 16 April 2003; revised 18 September 2003; accepted 1 October 2003

Correspondence to:In-Chan Seol, Department of Ciculatory Internal Medicine Daejeon University Hospital of Oriental Medicine Daehung-Dong, Joong-Gu, Taejeon, 301-724, South Korea; Tel: 82-42-229-6805, Fax: 82-42- 254-3403, E-mail: seolinch@dju.ac.kr

histological changes.

Materials and Methods

1. Materials

Medicinal herbs were purchased from Daejeon Oriental Medical Hospital. The composition of *Tongbiyeum* (TBY) formation is described in Table 1. After drying, 1 day' s dosage (162g) of the formulation for a human adult was mixed with 2 *l* of distilled water and left for 1h at room temperature, and the whole mixture was then boiled twice for 1h each time. The TBY extract was filtered and then freeze-dried. The yield of TBY extract was 7.25% (w/w) in terms of the dried medicinal herbs.

2. Experimental animals

Five-week-old male Sprague-Dawley rats were purchased from a commercial animal breeder (Daehan BioLink, Korea). After one week of acclimation, 40 rats were used for this experiment. The rats were housed in an environmentally controlled room at 22±2° C, relative humidity at 55±10% and 12 hrs light/dark and fed with commercial pellets (Samyang Feed Ltd., Korea) and tap water *ad libitum*.

Table 1. Prescription of *Tongbiyeum* (TBY)

General Name	Part used	Dosage(g)
Rehmannia glutinosa (熟地黃)	Radix	12
Paeonia lactiflora (白芍藥)	Radix	8
Cinnamomum cassia (桂枝)	Ramulus	8
Eucommia ulmoides (杜虫)	Cortex	4
Achyranthes bidentata (牛膝)	Radix	4
Angelica gigas (當歸)	Radix	4
Lycium chinense (枸杞子)	Fructus	4
Poria cocos (茯苓)	Hypha	4
Asarum sieboldii (細辛)	Radix	4
Angelica dahurica (白芷)	Radix	4
Aconitum carmichaeli (附子)	Radix	4
Glycyrrhiza uralensis (甘草)	Radix	4
Total amount		64

Forty Sprague-Dawley rats were divided into 5 groups of 8 animals each. Fatty liver was induced by feeding the animals a sucrose-rich diet for 5 weeks. The sucrose-rich diet was made at our laboratory and contained 65% sucrose (Samyangsa, Korea), while in the normal diet sucrose was replaced by sweet potato starch. Animals in the treatment groups were given the sucrose-rich diet *ad libitum* for 5 weeks, whereas rats in the normal group were given sweet potato starch (Cheiljedang, Korea). The rats in treatment groups were administrated orally with TBY (200㎎/10*㎖*/kg), TBY (600㎎/10*㎖*/㎏), or Lipidil (3.33㎎/10*㎖*/㎏) respectively. The rats in the control group were given 10*㎖*/kg of distilled water. On the last day, the animals fasted for 4hrs, after which whole blood was collected from abdominal aorta and liver, and spleen and thymus were removed, weighted and fixed in phosphate buffered formalin.

After being clotted for 1hr, the blood was centrifuged at 3000 rpm for 15min. to separate serum.

3. Hematological examinations

Under ether anesthesia, whole blood was collected from the abdominal vein, and transferred to vials treated with ethylenediamine tetraacetic acid (EDTA).

Hematological parameters, red blood cell (RBC), white blood cell (WBC), hematocrit (HCT), hemoglobin (HB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet (PLT) were analyzed using automated blood analyzer, Hemavet (CDC Technologies, Inc., Oxford, CT).

4. Serum biochemical analysis

The levels of serum aspartate transaminase (AST), alanine transaminase (ALT), total cholesterol (CHO), high-density lipoprotein cholesterol (HDL), and triglyceride (TG) were determined using Olympus

Optical Reply (Olympus Ltd., Japan).

5. Histopathological observations

For the histomorphological evaluation, a portion of liver tissue was removed and fixed in 10% phosphate buffered formalin. The paraplast-embedded liver sections (4㎛ in thickness) were stained with hematoxylin & eosin for histopathological examination.

6. Statistical analysis

Results were expressed as the mean ± standard deviation (S.D.). Statistical analysis of the data was carried out by Student's *t*-test. A difference from the respective control data at the levels of $p<0.05$, $p<0.01$ and $p<0.001$ was regarded as statistically significant.

Results

1. Clinical signs and changes in body weight

There were no deaths among the animals during the entire experimental period. Body weight gain of rats administered TBY 200㎎/㎏, TBY 600㎎/㎏, Lipidil and control were lower than the normal group. The body weight gain of rats in groups administered TBY 200㎎/㎏, TBY 600㎎/㎏ and Lipidil were higher than that of rats in the control group of sucrose-rich diet only at 5 weeks (Fig. 1).

2. Organ weights

At autopsy after the termination of treatment, hypertrophy of liver was observed in rats of all groups given a high sucrose diet. In groups given a sucrose-rich diet, absolute liver weight increased as compared with that in the group given a sweet potato starch diet, but not in the group administrated Lipidil. Absolute liver weight in the groups administered TBY 200㎎/㎏ and Lipidil was lower than that in the group given a sucrose-rich diet only (Fig. 2).

Relative liver weight increased in all groups given a sucrose-rich diet. Relative liver weight gain in groups administered TBY 200㎎/㎏, TBY 600㎎/㎏ and Lipidil decreased as compared with that in the group given a sucrose-rich diet only (Fig. 3).

3. Hematological parameters

As shown in Table 2, RBC counts were increased in

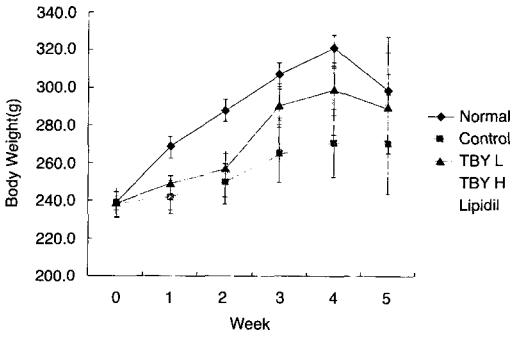

Fig. 1. Changes in the mean body weight of rats given a sucrose-rich diet.
Normal, given sweet potato starch; Control, given sucrose-rich diet only; TBY L, 200mg/kg TBY; TBY H, 600mg/kg TBY; Lipidil, 3.33mg/kg Lipidil.
The values are expressed as the mean ± S.D. (n=8).

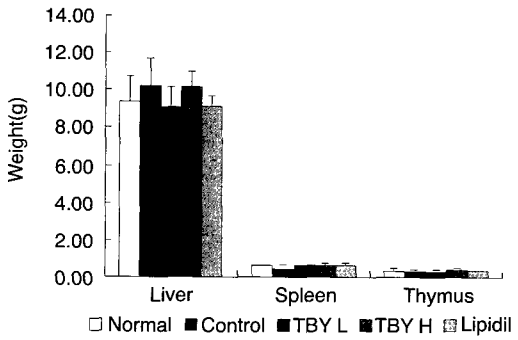

Fig. 2. Absolute organ weight of rats given a sucrose-rich diet.
Normal, given sweet potato starch; Control, given sucrose-rich diet only; TBY L, 200mg/kg TBY; TBY H, 600mg/kg TBY; Lipidil, 3.33mg/kg Lipidil.
The values are expressed as the mean ± S.D. (n=8).

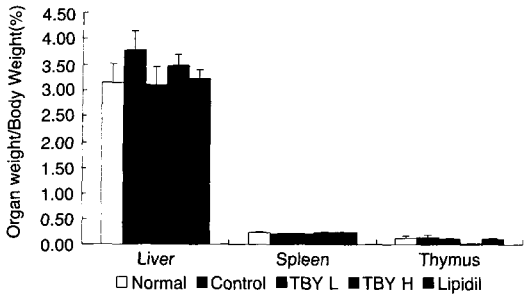

Fig. 3. Relative organ weight of rats given a sucrose-rich diet.
Normal, given sweet potato starch; Control, given sucrose-rich diet only; TBY L, 200mg/kg TBY; TBY H, 600mg/kg TBY; Lipidil, 3.33mg/kg Lipidil.
The values are expressed as the mean ± S.D. (n=8).

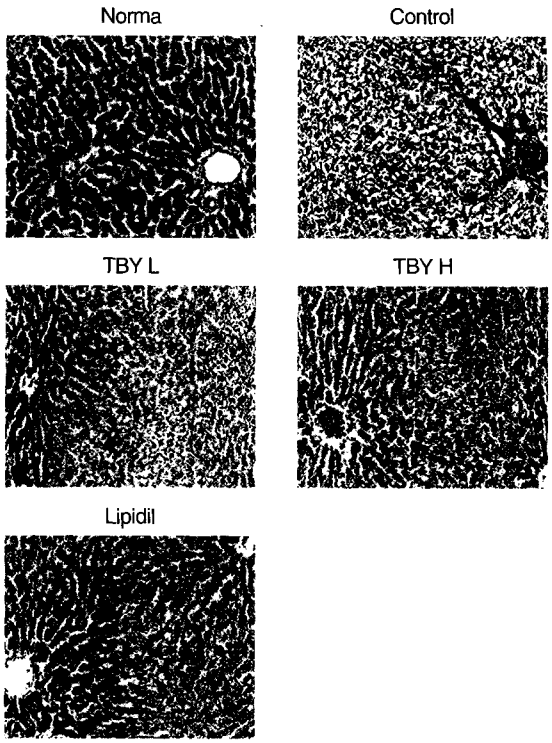

Fig. 4. Histopathological findings in liver of rats given a sucrose-rich diet.
H & E stained (×200) Normal, given sweet potato starch; Control, given sucrose-rich diet only; TBY L, 200mg/kg TBY; TBY H, 600mg/kg TBY; Lipidil, 3.33mg/kg Lipidil.

groups administrated TBY 200㎎/kg, TBY 600㎎/kg and Lipidil, and that in the group administrated TBY 200㎎/kg increased as compared with the group given a sucrose-rich diet only. HB counts increased in the group administrated TBY 200㎎/kg as compared with the group administrated Lipidil. Platelet counts in the groups given TBY 200㎎/kg, TBY 600㎎/kg decreased as compared with the group given a sucrose-rich diet only (Table 2).

4. Histopathological observations

After fatty liver was induced by a sucrose-rich diet for 5 weeks, macrovacuolar cytoplasmic alterations of hepatocytes were detected in the periportal area. These alterations were observed in all groups given a sucrose-rich diet, and were especially severe in those given a sucrose-rich diet only. Macrovacuolar and microvacuolar in the group administered TBY 600㎎/kg were less than that in the group administered TBY 200㎎/kg. An increase of the number of apoptosis was markedly observed in the group given a sucrose-rich diet only.

5. Serum biochemical analysis

As shown in Fig. 5 and Fig. 6, AST and ALT activities did not show significant differences through the experimental period. In the group administered TBY 600㎎/kg, at 5 weeks, serum cholesterol levels were reduced, while those in the group administered TBY 200㎎/kg increased (Fig. 7).

In the group administered TBY 600㎎/kg, TG levels at 5 weeks decreased as compared with those in the group given a sucrose-rich diet only while in the group administered TBY 200㎎/kg, TG levels decreased more comparatively (Fig. 8).

HDL levels increased at 5 weeks in the group given TBY 200㎎/kg as compared with those in the group given a sucrose-rich diet only (Fig. 9).

Table 2. Hematological Parameters of Rats given a Sucrose-rich Diet

	Normal	Control	TBY L	TBY H	Lipidil
WBC(102/㎕)	7.22± 2.65	10.01± 4.91	7.08± 2.88	5.07± 1.01	7.62± 2.60
RBC(104/㎕)	6.7 ± 1.789	7.50± 0.57	8.01± 0.51	7.76± 0.90	7.60± 0.18
HBg/dl	14.31± 1.80	13.26± 0.57	13.33± 0.59	12.90± 0.71	11.68± 3.38
HCT(%)	36.60± 9.47	36.87± 2.80	37.79± 1.59	36.56± 3.53	35.74± 5.70
MCV(fl)	53.99± 1.93	49.23± 1.49	47.23± 1.32	47.19± 1.25	47.00± 2.07
MCH(pg)	22.59± 7.07	17.76± 1.31	16.70± 1.12	16.73± 1.25	15.16± 3.41
MCHC(g/dl)	41.87± 13.04	36.09± 2.40	35.30± 1.55	35.47± 2.21	32.10± 6.22
PLT(104/㎕)	467.57±158.76	336.43±126.21	318.00±223.19	246.86±216.12	367.80±221.22

Normal, given sweet potato starch; Control, given sucrose-rich diet only; TBY L, 200mg/kg TBY; TBY H, 600mg/kg TBY; Lipidil, 3.33mg/kg Lipidil.
The values are expressed as the mean ± S.D. (n=8).
*: $p < 0.05$: significant differences compared with the group given sucrose-rich diet only.

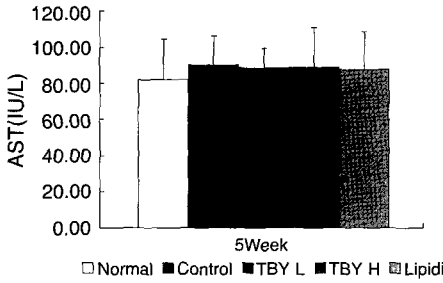

Fig. 5. *Serum aspartate transaminase (AST) activities of rats given sucrose-rich diet.*
Normal, given sweet potato starch; Control, given sucrose-rich diet only; TBY L, 200mg/kg TBY; TBY H, 600mg/kg TBY; Lipidil, 3.33mg/kg Lipidil.
The values were expressed as the mean ± S.D. (n=8).

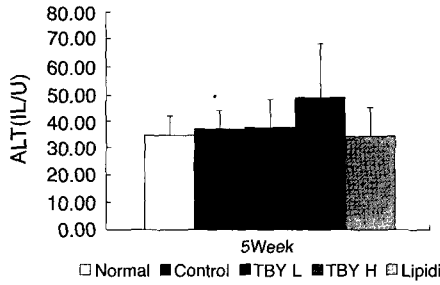

Fig. 6. Serum alanine transaminase (ALT) activities of rats given sucrose-rich diet.
Normal, given sweet potato starch; Control, given sucrose-rich diet only; TBY L, 200mg/kg TBY; TBY H, 600mg/kg TBY; Lipidil, 3.33mg/kg Lipidil.
The values were expressed as the mean ± S.D. (n=8).

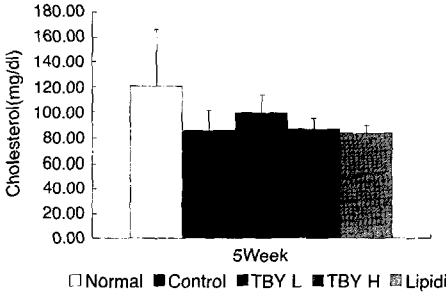

Fig. 7. Serum cholesterol (CHO) levels of rats given sucrose-rich diet.
Normal, given sweet potato starch; Control, given sucrose-rich diet only; TBY L, 200mg/kg TBY; TBY H, 600mg/kg TBY; Lipidil, 3.33mg/kg Lipidil.
The values were expressed as the mean ± S.D. (n=8).

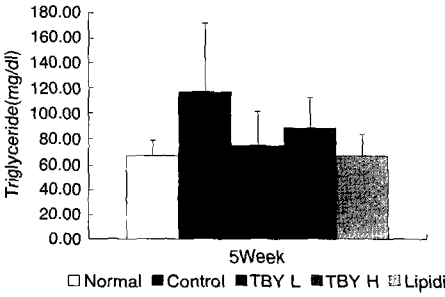

Fig. 8. Serum triglyceride (TG) levels of rats given sucrose-rich diet.
Normal, given sweet potato starch; Control, given sucrose-rich diet only; TBY L, 200mg/kg TBY; TBY H, 600mg/kg TBY; Lipidil, 3.33mg/kg Lipidil.
The values were expressed as the mean ± S.D. (n=8).

Discussion

The liver occupies a central position in lipid metabolism. A small, rapidly used pool of free fatty acids (FFA), absorbed from the diet or released into the blood from chylomicrons or fat cells, accommodates almost all of the energy requirements of a fasting animal. FFA are taken up by the liver to join the hepatic

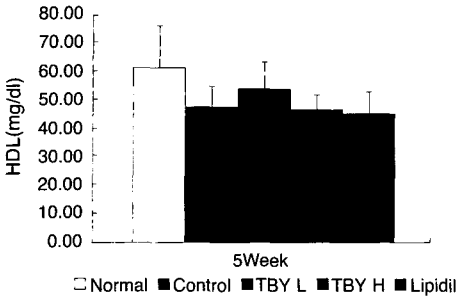

Fig. 9. Serum High Density Lipoprotein (HDL) levels of rats given sucrose-rich diet.
Normal, given sweet potato starch; Control, given sucrose-rich diet only; TBY L, 200mg/kg TBY; TBY H, 600mg/kg TBY; Lipidil, 3.33mg/kg Lipidil.
The values were expressed as the mean ± S.D. (n=8).

pool of FFA, a portion of which the liver synthesizes. Some FFA are oxidized to CO_2 in the liver for energy, but most are rapidly incorporated into complex lipids (e.g., triglycerides, phospholipids, glycolipids, cholesterol esters). Some of these complex lipids enter a slowly used pool that comprises the structural lipids of liver cells and their storage site. Most triglycerides enter an active pool where they combine with specific apoproteins to form lipoproteins, which are secreted into plasma. The liver is also responsible for lipid degradation.

Triglycerides accumulate in the liver because of increased input through synthesis from FFA or decreased export as VLDL from the hepatocytes. Increased triglycerides synthesis may result from increased delivery or availability of FFA, from acetylcoenzyme A, or from decreased oxidation of FFA in the liver. Reduced elimination of triglycerides involves depressed packaging with apolipoproteins, phospholipids, and cholesterol, resulting in decreased VLDL secretion.

The several possible mechanisms involved in the pathogenesis of the fatty liver may operate alone or together. In obesity, delivery of dietary fat or mobilization from adipose tissue is increased. Decreased oxidation of FFA may contribute to the fatty liver induced by carbon tetrachloride, yellow phosphorus, hypoxia, or certain vitamin deficiencies. Blocked production and secretion of lipoprotein is often the main cause of triglyceride accumulation in the liver. Impaired apolipoprotein synthesis is the most important pathogenetic factor in several types of toxic fatty liver and in the fatty liver produced by protein-calorie malnutrition. Toxic inhibition of protein synthesis can lead to fatty liver through inhibition of mRNA synthesis or translation[1].

Lipoproteins are classified according to their density. Chylomicrons, which are large lipoproteins of extremely low density, transport dietary triacylglycerols and cholesteryl esters from the intestines to the tissues. Very low lipoproteins (VLDL), synthesized in the liver, transport lipids to the tissues. As VLDL are transported through the body, they become depleted of triacylglycerides, as well as some apoproteins and phospholipids. Eventually, VLDL are converted to low-density lipoproteins (LDL). LDL carry cholesterol to tissues. LDL are engulfed by cells after binding to LDL receptors. The role of high-density lipoproteins (HDL), also produced in the liver, appears to be the scavenging of excessive cholesterol from cell membranes. Cholesterol esters are formed when the plasma enzyme lecithin:cholesterol acyltransferase (LCAT) transfers a fatty acid residue from lecithin. It is now believed that HDL transports these cholesteryl esters to the liver. The liver, the only organ that can dispose of excess cholesterol, converts most of it to bile acids.

Atherosclerosis is a chronic disease in which soft masses, called atheromas, accumulate on the inside of arteries. These deposits are also referred to as plaque. Disruption of vital organ functions, especially those of the brain, heart, and lungs caused by oxygen and nutrient deprivation, usually ensues. In coronary artery

disease, one of the most common consequences of atherosclerosis, this deprivation damages the heart muscle.

Most of the cholesterol found in plaque is obtained by the ingestion of LDL by foam cells. It is not surprising, therefore, that high plasma LDL levels are directly correlated with high risk for coronary artery disease. In contrast, a high plasma HDL level is considered to be associated with a low risk for coronary artery disease. Liver cells are the only cells that possess HDL receptors. Other high risk factors induced a high-fat diet, smoking, stress, and a sedentary lifestyle[2].

Recently, vascular disease has taken a dreadful toll, and recent mass research has shown that coronary events in patients with symptomatic vascular disease can be reduced with cholesterol lowering agents[8]. Because chemical therapeutics have serious organ toxicity, including adrenocortical degeneration, herbal remedies in many cultures over the world have become potential candidates for the same purpose with less toxicity[4,6].

The present studies demonstrate that TBY can reduce the rise in plasma cholesterol, TG levels and increase plasma HDL levels induced by a sucrose-rich diet and reverse pre-established hypercholesterolemia and hypertriglycemia.

TBY has been used in therapy for hyperlipidemia and sequella of CVA and has been studied clinically and experimentally. It has become clear that TBY protects from thrombosis and possesses a hypolipidemic effect[7,8].

In TBY groups, total cholesterol levels increased (Fig. 7) but TG levels decreased (Fig. 8), and HDL-cholesterol increased (Fig. 9). These show the decrease of other lipoprotein fractions including LDL and IDL. In the analysis of relative liver weight, TBY inhibited the weight gain induced by a sucrose-rich diet as compared with the control group. We could suggest that the accumulation of fatty acids in the hepatocyte were blocked by TBY. In the histological observations, lipid droplet and apoptotic change in the TBY treated group decreased more as compared with the control group. In the serum biochemical analysis, a difference of serum AST and ALT changes among groups was not shown, but TG decreased more and HDL level increased more compared with the control group. We infer that TBY is a considerable preventive remedy of CHD. From this study, we can suggest that TBY extract treats fatty liver by lowering serum TG levels and enhancing HDL level and by inhibiting lipid droplet accumulation and apoptotic change. Thus, *Tongbiyeum* (TBY) could be used for patients with fatty liver and needs to be developed for more specific therapeutics.

Conclusion

This study aimed to elucidate the effects of TBY on fatty liver and the results were as follows:

1. TBY treatment in proportion to the concentration inhibited the liver weight gain induced by a sucrose-rich diet as compared with the control group.
2. TBY treatment inhibited lipid droplet accumulation and apoptotic change in the liver as compared with the control group.
3. TBY treatment inhibited the increasing of serum TG induced by a sucrose-rich diet as compared with the control group.
4. TBY treatment increased HDL level induced by a sucrose-rich diet as compared with the control group. From this study, we can infer that TBY treats fatty liver by inhibiting the intestinal absorption and storage of exogenous and endogenous cholesterol.

References

1. Keryn A. G. Lane. The Merk Manual (17th/edition). 1999:366-367.
2. Trudy McKee, James R. McKee. Biochemistry, an Introduction (2nd Edition). 1999;216:233-235.
3. Jumana Saleh, Allan D. Sniderman and Katherine Cianflone. Regulation of plasma fatty acid metabolism. Clinia Chimica Acta. 1999;286:1-2:163-180.
4. Ghasi S, Nwobodo E, Ofili JO. Hypocholesterolemic effects of crude extract of leaf of Moringa oleifera Lam in high fat diet fed Wistar rats. Journal of Ethnopharmacology. 2000; (69):21-25.
5. Ki-Cheol Song, Chang-Gue Son. Effects of Gamichunggantang on Hyperlipidemia. College of Oriental Medicine, Graduate School of Daejeon University. 2002.
6. Youn-Seoung Ryu. Effects of Gamichunggantang on Fatty Liver induced by Sucrose-rich Diet. College of Oriental Medicine, Graduate School of Daejeon University. 2002.
7. Young Choi. Study on the Effect of *Tongbiyeum* on Brain damage and Thromboembolism. College of Oriental Medicine, Graduate School of Daejeon University. 2001.
8. Seong-Min Lim. The Study on the Effects of Samgiyeum and *Tongbiyeum* on Thrombosis and Brain Damage. College of Oriental Medicine, Graduate School of Daejeon University. 2001.