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Hepatoprotective Effect of Blue Honeysuckle on Rat Hypothyroidism

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Abstract : This study was to determine whether blue honeysuckle lyophilized concentrated powder (BH) has positive effects on hypothyroidism and related liver damage in propylthiouracil-induced Hypothyroidism. Levothyroxine (LT4)-treated group was administrated by intraperitoneal injection with LT4, while BH or Flos Lonicerae lyophilized aqueous extract (LF)-treated groups were orally administrated for 42 days which two weeks pri or to PTU injection. The changes in organ weight, serum AST & ALT, serum lipid level, liver defense system were measured and the histopathology of the liver was observed. The oral administrations of 125, 250, and 500 mg/kg of BH showed favorable effects compared to LF on hypothyroidism-related liver damages through the enhance antioxidant defense system.

Key words **:** blue honeysuckle, hypothyroidism, Levothyroxine, liver, rat.

Introduction

Hypothyroidism is estimated to affect 2.0-5.0% of the global population; subclinical hypothyroidism is estimated to occur in 4.0-8.5%. The rates of hypothyroidism were increased with aging, and subclinical hypothyroidism in older women was estimated as 21% (2,3).

Blue honeysuckle (*Lonicera caerulea L., Caprifoliaceae*) is a member of *Caprifoliaceae* family, widely harvested in Russia, China, and Japan. Berries are known to have phenolic-rich compounds which include biphenyls, flavonoids ad phenolic acids. It is widely known that berry phenolics exhibit antioxidant properties (1).

The positive effects of anthocyanins and polyphenolics on various chronic diseases such as coronary heart disease, cancer, arthritis and age-related hormonal imbalances are reported to be associated with their antioxidant properties (4,10).

The purpose of this study was to evaluate blue honeysuckle lyophilized concentrated powder (BH) has positive effects on hypothyroidism and related liver damage in propylthiouracil-induced Hypothyroidism.

Materials and Methods

Animals

Fifty-six specific-pathogen-free (SPF), VAF outbred male, Crl:CD (Sprague-Dawley, SD) rats (6-weeks-old upon receipt; Orient Bio, Seungnam, Korea) were used. All experimental procedures were approved by the Institutional Animal Care and Use Committee in Daegu Haany University.

Preparations of test substances

A solution of concentrated blue honeysuckle was supplied by H&K Bioscience Co., Ltd. (Seoul, Korea). BH was prepared as following; 200 g of 63°Bx concentrated blue honeysuckle solution was diluted to 25°Bx using distilled water. Then, completely lyophilized with freeze dryer (Operon FDB-5503, Kimpo, Korea). Total volume of 124.4 g (yield = 62.2%) of BH was obtained. Lonicerae Flos harvested in China, was purchased from a local herb shop (Omniherb, Yeoungcheon, Korea). A total of 500 g dried Lonicerae Flos was boiled in 5,000 ml of distilled water for 3 h and brew three times at 60°C, evaporated using an automated flask evaporator (Eyela N-1110, Tokyo, Japan), and completely lyophilized. Total 119.0 g (yield = 23.8%) of LF was obtained. BH and LF were stored at −20°C before use. LT4 (Sigma-Aldrich, St. Louise, MO, USA) was used as a positive control material.

Induction of hypothyroidism

Hypothyroidism and related liver damages were induced by daily subcutaneous application of PTU 10 mg/kg for 28 days as 1 ml/kg dissolved formula in saline. PTU treatment was initiated from two weeks after the first BH or LF oral administration and the day of the first LT4 treatment. All test materials were then treated in case of simultaneously treated days, respectively. In intact control group, only saline was subcutaneously administered in a volume of 1 ml/kg.

Experimental groups

Experimental groups were divided to seven groups (n = 8)

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1. Intact control: Saline treated instead of propylthiouracil (PTU) and distilled water orally administered to rats
2. PTU control: PTU (10 mg/kg) subcutaneously administered and distilled water orally administered to rats
3. LT4: PTU subcutaneously administered and LT4 0.5 mg/kg intraperitoneally treated to rats
4. LF 250: PTU subcutaneously administered and LF 250 mg/kg orally administered to rats
5. BH 500: PTU subcutaneously administered and BH 500 mg/kg orally administered to rats
6. BH 250: PTU subcutaneously administered and BH 250 mg/kg orally administered to rats
7. BH 125: PTU subcutaneously administered and BH 125 mg/kg orally administered to rats

Blood collection and serum separation

At 42 days after treatment, about 6 ml of blood was sampled from vena cava in each rat, and collected bloods were collected in clotting-activated serum tubes. After clotting, they were centrifuged at 3,000 rpm for 10 min at 4°C to separate the serum. These samples were stored in an ultra-deep freezer (Sanyo, Tokyo, Japan) under −150°C before analysis.

Serum biochemistry

Serum AST, ALT, TC and TG levels were measured using automated blood analyzer (Hemagen Analyst, Hemagen Diagnostic, Columbia, MD, USA), and serum HDL and LDL were also detected by other typed using automated blood analyzer (AU400, Olympus, Tokyo, Japan), respectively.

Lipid peroxidation and antioxidant defense systems

The malondialdehyde (MDA) contents for lipid peroxidation, H_2O_2, contents, CAT and SOD enzyme activities in rat hepatic tissues were assessed, respectively. Separated hepatic tissues were weighed and homogenized in ice-cold 0.01 M Tris-HCl (pH 7.4), and then centrifugated, at 800 g for 10 min and then mitochondria fractions were separated by again centrifugated, at 12,000 g for 15 min of supernatants. Total amounts of protein were measured using bovine serum albumin Invitrogen, Carlsbad, CA, USA) as internal standard. The concentrations of hepatic lipid peroxidation were determined by estimating MDA using the thiobarbituric acid test at absorbance 525 nm, as nM of MDA/mg protein. Hepatic contents of H_2O_2 were spectrophotometrically (OPTIZEN POP, Mecasys, Daejeon, Korea) detected using horseradish peroxidase (Sigma-Aldrich, St. Louise, MO, USA) and phenol red (Wako, Osaka, Japan) as nM/mg protein. Decomposition of H_2O_2 in the presence of CAT was followed at 240 nm. CAT activity was defined as the amount of enzyme required to decompose 1 nM of H_2O_2 per minute, at 25°C and pH 7.8. Results were expressed as U/mg protein. Measurements of SOD activities were made. SOD estimation was based on the generation of superoxide radicals produced by xanthine and xanthine oxidase, which react with nitrotetrazolium blue to form formazan dye. SOD activity was then measured at 560 nm by the degree of inhibition of this reaction, and was expressed as U/mg protein. One unit of SOD enzymatic activity is equal to the amount of enzyme that diminishes the initial absorbance of nitroblue tetrazolium by 50% during 1 min.

Histopathology

After measuring of organ weights, Left lateral lobes of liver were sampled and then crossly trimmed and fixed in 10% neutral buffered formalin after measuring of organ weights. Paraffin embedded samples were prepared as 3-4 μm serial sections, and stained with hematoxylin and eosin (H&E) for microscopic examination. The changes on the hepatocyte numbers were observed in a restricted view field of liver (nuclei numbers/mm^2)

Statistical analyses

Measured values were expressed as mean ± SD. Kruskal-Wallis H test followed by the Mann-Whitney U (MW) test was conducted to determine the significant differences with SPSS program (Release 14.0K, SPSS Inc., USA).

Results

Effects on the thyroid gland weights

Significant ($p < 0.01$) increases of absolute thyroid gland weights were detected in PTU control as compared with intact control, related to noticeable hypertrophic changes of thyroid glands at gross inspections. However, these increases of thyroid gland weights were significantly ($p < 0.01$) inhibited by treatment of all test substances including LT4 0.5 mg/kg, intraperitoneally, in the both absolute weights, respectively. Especially, BH 500 and 250 mg/kg treated rats also showed significant ($p < 0.01$) decreases of the absolute thyroid gland weights as compared with LF 250 mg/kg treated rats, respectively. BH 125 mg/kg treated rats also showed non-significant decreases of the thyroid gland weights as compared with those of LF 250 mg/kg treated rats, in this experiment

Table 1. Changes on Absolute Organ Weights in Intact and PTU-induced Hypothyroid Rats

Groups	Absolute organ weights (g)	
	Thyroid gland	Liver
Controls		
Intact	0.012 ± 0.003	12.228 ± 1.049
PTU	0.035 ± 0.004[a]	6.961 ± 0.393[e]
Reference		
LT4 0.5 mg/kg	0.012 ± 0.003[c]	10.833 ± 0.589[ef]
LF 250 mg/kg	0.026 ± 0.004[ac]	7.994 ± 0.232[ef]
BH treated		
500 mg/kg	0.018 ± 0.004[bcd]	9.334 ± 0.574[efh]
250 mg/kg	0.020 ± 0.005[acd]	9.026 ± 0.506[efh]
125 mg/kg	0.024 ± 0.006[ac]	8.342 ± 0.804[ef]

Values are expressed as Mean ± SD. PTU = Propylthiouracil, 6-n-propyl-2-thiouracil; LT4 = Levothyroxine; LF = Flos Lonicerae lyophilized aqueous extracts; BH = Blue honeysuckle concentrated and lyophilized powder. a $p < 0.01$ as compared with intact control by LSD test, b $p < 0.01$ and c $p < 0.05$ as compared with PTU control by LSD test, d $p < 0.01$ and e $p < 0.05$ as compared with LF 250 mg/kg by LSD test, f $p < 0.01$ and g $p < 0.05$ as compared with intact control by MW test, h $p < 0.01$ and i $p < 0.05$ as compared with PTU control by MW test, j $p < 0.01$ and k $p < 0.05$ as compared with LF 250 mg/kg by MW test.

Table 2. Changes on the Liver Lipid Peroxidation and Antioxidant Defense Systems in Intact and PTU-induced Hypothyroid Rats

Groups	Lipid peroxidation	Antioxidant defense system		
	Malondialdehyde (nM/mg protein)	H_2O_2 (nM/mg protein)	SOD (U/mg protein)	Catalase (U/mg protein)
Controls				
Intact	3.15 ± 0.35	118.63 ± 28.18	54.25 ± 11.72	368.75 ± 112.57
PTU	2.83 ± 0.70	254.88 ± 35.46[a]	98.13 ± 19.56[a]	143.25 ± 20.91[e]
Reference				
LT4 0.5 mg/kg	2.89 ± 0.74	113.75 ± 37.27[b]	58.75 ± 16.03[b]	230.63 ± 38.92[fg]
LF 250 mg/kg	2.82 ± 1.02	184.63 ± 31.64[ab]	73.25 ± 13.61[ab]	182.38 ± 16.66[eg]
BH treated				
500 mg/kg	2.72 ± 0.68	117.38 ± 20.06[bc]	51.25 ± 10.55[bc]	274.00 ± 32.18[gh]
250 mg/kg	2.84 ± 0.78	135.88 ± 22.43[bc]	54.50 ± 11.64[bd]	237.50 ± 37.95[fgh]
125 mg/kg	2.81 ± 0.73	167.13 ± 27.26[ab]	65.13 ± 12.23[b]	190.88 ± 31.19[eg]

Values are expressed as Mean ± SD. PTU = Propylthiouracil, 6-n-propyl-2-thiouracil; LT4 = Levothyroxine; LF = Flos Lonicerae lyophilized aqueous extracts; BH = Blue honeysuckle concentrated and lyophilized powder. a $p < 0.01$ as compared with intact control by LSD test, b $p < 0.01$ and c $p < 0.05$ as compared with PTU control by LSD test, d $p < 0.01$ and e $p < 0.05$ as compared with LF 250 mg/kg by LSD test, f $p < 0.01$ and g $p < 0.05$ as compared with intact control by MW test, h $p < 0.01$ and i $p < 0.05$ as compared with PTU control by MW test, j $p < 0.01$ and k $p < 0.05$ as compared with LF 250 mg/kg by MW test.

(Table 1).

Effects on the liver weights

Significant ($p < 0.01$) decreases of absolute hepatic weights were detected in PTU control as compared with intact control, but these decreases of liver weights were significantly ($p < 0.01$ or $p < 0.05$) inhibited by treatment of LT4, LF 250 mg/kg, BH 500, 250 and 125 mg/kg, in the both absolute weights, respectively. Especially, BH 500 and 250 mg/kg treated rats also showed significant ($p < 0.01$ or $p < 0.05$) increases of the absolute liver weights as compared with LF 250 mg/kg treated rats, respectively. BH 125 mg/kg treated rats also showed marked increases of the hepatic weights as compared with those of LF 250 mg/kg treated rats without significances, in this experiment (Table 1).

Effects on the liver lipid peroxidation and antioxidant defense systems

Although slight and non-significant decreases of liver lipid peroxidation, the slight reduction of hepatic MDA contents were demonstrated in PTU control rats, significant ($p < 0.01$) increases of hepatic H_2O_2 contents and SOD activities and more profound decreases of CAT activities were detected in PTU control as compared with intact control, respectively. However, these PTU treatment related abnormal changes on the hepatic antioxidant defense systems were significantly ($p < 0.01$) normalized by treatment of LT4, LF 250 mg/kg and all three different dosages of BH, 500, 250 and 125 mg/kg, respectively. Especially, BH 500 and 250 mg/kg treated rats also showed significant ($p < 0.01$ or $p < 0.05$) decreases of hepatic H_2O_2 contents and SOD activities and increases of CAT activities as compared with LF 250 mg/kg treated rats, respectively. BH 125 mg/kg treated rats also showed non-significant decreases of hepatic H_2O_2 contents and SOD activities and increases of CAT activities as compared with those of LF 250 mg/kg treated rats in this experiment (Table 2).

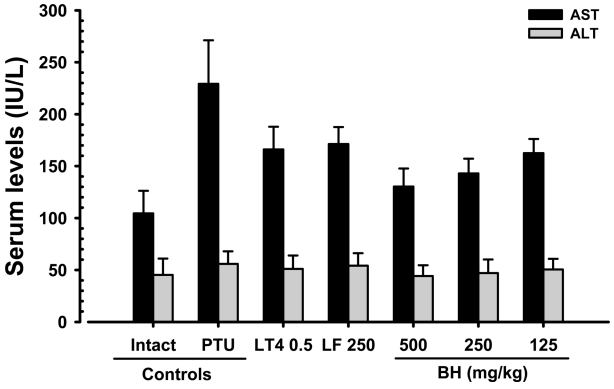

Fig 1. Serum AST and ALT Levels in Intact and PTU-induced Hypothyroid Rats. Values are expressed as Mean ± SD.

Effects on the serum AST and ALT levels

Significant ($p < 0.01$) increases of serum AST levels were detected in PTU control as compared with intact control, but these PTU treatment related serum AST elevations were significantly ($p < 0.01$) inhibited by treatment of LT4, LF 250 mg/kg and all three different dosages of BH, 500, 250 and 125 mg/kg, respectively. Especially, BH 500 and 250 mg/kg treated rats also showed significant ($p < 0.01$) decreases of serum AST levels as compared with LF 250 mg/kg treated rats, respectively. BH 125 mg/kg treated rats also showed non-significant decreases of serum AST levels as compared with those of LF 250 mg/kg treated rats. In this experiment, PTU treatment did not influenced on the serum ALT levels except for slight non-significant increases, and no significant changes on the serum ALT levels were noticed in all test substance treated rats as compared with PTU control rats (Fig 1).

Effects on the serum lipid levels

Significant ($p < 0.01$) increases of serum HDL levels and decreases of serum TG levels were detected in PTU control as compared with intact control, but these PTU treatment

Table 3. Changes on the Serum Lipid Levels in Intact and PTU-induced Hypothyroid Rats

Groups	Serum lipid levels (mg/dl)			
	Total cholesterol	LDL	HDL	Triglyceride
Controls				
Intact	58.75 ± 12.49	16.73 ± 2.86	22.32 ± 4.97	94.35 ± 11.20
PTU	58.75 ± 12.49	17.40 ± 1.77	66.01 ± 14.75[a]	37.35 ± 8.91[a]
Reference				
LT4 0.5 mg/kg	66.48 ± 10.60	16.55 ± 2.23	29.63 ± 11.26[b]	81.89 ± 12.74[b]
LF 250 mg/kg	62.53 ± 12.38	16.88 ± 1.50	43.60 ± 8.56[ab]	56.19 ± 11.37[ab]
BH treated				
500 mg/kg	61.43 ± 9.95	16.21 ± 1.51	27.55 ± 4.23[bc]	86.80 ± 16.55[bc]
250 mg/kg	59.39 ± 13.96	16.50 ± 2.23	31.99 ± 7.85[bd]	74.35 ± 12.64[abc]
125 mg/kg	62.68 ± 12.90	16.90 ± 1.23	41.76 ± 12.75[ab]	62.86 ± 13.54[ab]

Values are expressed as Mean ± SD. PTU = Propylthiouracil, 6-n-propyl-2-thiouracil; LT4 = Levothyroxine; LF = Flos Lonicerae lyophilized aqueous extracts; BH = Blue honeysuckle concentrated and lyophilized powder. a $p < 0.01$ as compared with intact control by LSD test, b $p < 0.01$ and c $p < 0.05$ as compared with PTU control by LSD test, d $p < 0.01$ and e $p < 0.05$ as compared with LF 250 mg/kg by LSD test.

related abnormal changes on the serum HDL and TG levels were significantly ($p < 0.01$) normalized by treatment of LT4, LF 250 mg/kg and all three different dosages of BH, 500, 250 and 125 mg/kg, respectively. Especially, BH 500 and 250 mg/kg treated rats also showed significant ($p < 0.01$ or $p < 0.05$) decreases of serum HDL levels and increases of TG levels as compared with LF 250 mg/kg treated rats, respectively. BH 125 mg/kg treated rats also showed noticeable decreases of serum HDL levels and increases of TG levels as compared with those of LF 250 mg/kg treated rats without significances. In this experiment, PTU treatment did not influenced on the serum TC and LDL levels, and no significant changes on the serum TC and LDL levels were noticed in all test substance treated rats as compared with PTU control rats (Table 3).

Effects on the hepatic tissue histopathology

Dramatic hepatocyte swellings due to noticeable lipid droplet depositions were demonstrated in PTU control rats, and accordingly, liver cell numbers were significant ($p < 0.01$) decreases in PTU control rats as compared with intact control rats at histomorphometrical analysis. However, these PTU treatment-related abnormal hepatic histopathological changes, the decreases of liver cell numbers were markedly normalized by treatment of LT4, LF 250 mg/kg and all three different dosages of BH, 500, 250 and 125 mg/kg, respectively. Especially, BH 500 and 250 mg/kg treated rats also showed significant ($p < 0.01$) increases of liver cell numbers as compared with LF 250 mg/kg treated rats, respectively. BH 125 mg/kg treated rats also showed non-significant increases of liver cell numbers as compared with those of LF 250 mg/kg treated rats, in this experiment (Table 4, Fig 2).

Table 4. Changes on the Histopathology-Histomorphometry of the Liver in Intact and PTU-induced Hypothyroid Rats

Groups \ Items	Liver
	Cell numbers (nuclei/mm^2)
Controls	
Intact	1314.38 ± 253.53
PTU	609.38 ± 136.74[f]
Reference	
LT4 0.5 mg/kg	855.63 ± 108.24[fg]
LF 250 mg/kg	742.50 ± 37.11[f]
BH treated	
500 mg/kg	983.38 ± 107.84[fgh]
250 mg/kg	908.38 ± 86.52[fgh]
125 mg/kg	805.88 ± 107.04[fg]

Discussion

Hypothyroidisms induce various organ damages especially, on the liver (7). Blue honeysuckle (honeyberry) is a fruit of *Lonicera caerulea var. edulis*, and it has been showed various favorable pharmacological effects including hepatoprotective effects (15), modulation of lipid and glucose metabolisms, anti-inflammatory effects, through potent anti-oxidative effects (1).

It has been well established that thyroid hormone affects blood lipid concentration, hepatic metabolism and cholesterol synthesis (2). Common abnormalities of lipoprotein metabolism associated with hypothyroidism are elevated levels of TC and LDL, and these elevated TC and LDL can be induced the lethal cardiovascular disorders as side effects of hypothyroidism (4a). These abnormal blood lipid concentration and increases of the risk of cardiovascular disorders in hypothyroidism, ameliorated by treatment of LT4 (2). However, various changes on the serum lipid levels have been induced depend upon the status of thyroid hormone levels - degrees and time of hypothyroidism with existences of complications, and especially, they were also directly related to body antioxidant defense system (11,12). In the present study, PTU treatment did not influenced on the serum TC and LDL but induced noticeable and significant elevations of the serum HDL and decreases of TG levels, respectively. However, these PTU treatment-related abnormal changes on the

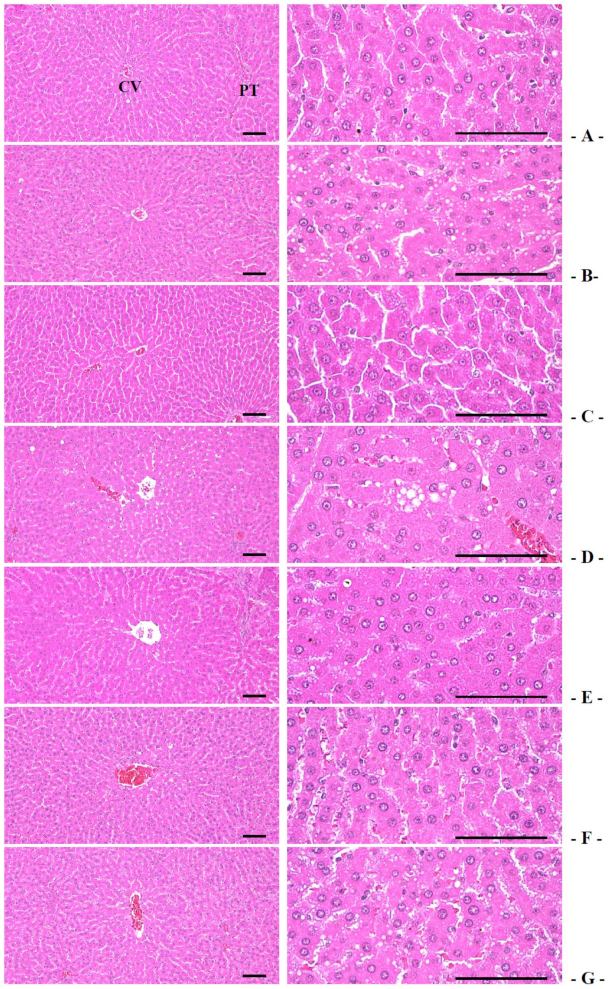

Fig 2. Representative Histological Images of the Liver, Taken from Intact and PTU-induced Hypothyroid Rats. A = Intact control rats, B = PTU control rats, C = LT4 0.5 mg/kg treated rats, D = LF 250 mg/kg treated rats, E = BH 500 mg/kg treated rats, F = BH 250 mg/kg treated rats, G = BH 125 mg/kg treated rats. CV = Central vein, PT = Portal Triad. Scale bars = 120 μm.

serum HDL and TG levels were significantly normalized by treatment of LT4, LF 250 mg/kg and all three different dosages of BH, 500, 250 and 125 mg/kg, respectively. These normalization of serum HDL and TG levels are considered as secondary changes from thyroid protective effects, re-storage of thyroid hormones and related normalization of liver metabolisms by treatment of test substances. Especially, BH 500 and 250 mg/kg treated rats also showed significant decreases of the serum HDL levels and increases of serum TG levels as compared with LF 250 mg/kg treated rats. In addition, BH 125 mg/kg treated rats also showed more favorable inhibitory effects on the PTU-induced serum HDL and TG level changes as compared with those of LF 250 mg/kg treated rats, without significances. These results are considered as direct evidences that BH can be ameliorated the PTU-induced serum lipid changes, at least 125 mg/kg dose levels in this study, more favorably than those of equal dosage of LF. In this experiment, no significant changes on the serum TC and LDL levels were noticed in all test substance treated rats as compared with PTU control rats, respectively.

Liver is a major target organ for thyroid hormone with important biological and medical implications (7), liver damages accompanied with hypothyroidism (3). Clinical diagnosis of disease and damage to the structural integrity of liver is commonly assessed by monitoring the status of serum AST and ALT activities (3). Higher activities of these enzymes in serum have been found in response to oxidative stress induced by hyperthyroidism (2), and increased serum levels of AST and ALT have also been observed in about 30% of hyperthyroid patients treated with PTU (6). Anyway, some researchers indicated the elevation of serum AST levels without serum ALT level changes in PTU treated rats, and it also have been reported that decreases of live cell numbers were induced by PTU treatment, related to micro-architectural hypertrophy of hepatocytes (11,12), and also in the present study; PTU treatment augmented the serum AST levels with unaltered ALT levels, and decreases of live cell counts. However, these PTU treatment related abnormal changes on the serum AST levels and hepatic histopathological inspections were significantly inhibited by treatment of LT4, LF 250 mg/kg and all three different dosages of BH, 500, 250 and 125 mg/kg, and especially, BH 250 and 500 mg/kg treated rats also showed significant and more favorable hepatoprotective effects as compared with LF 250 mg/kg treated rats, and also markedly in BH 125 mg/kg treated rats without significances, suggesting 125, 250 and 500 mg/kg of BH showed favorable hepatoprotective effects on PTU-induced hypothyroidism related liver damages, dose-dependently and more favorably than those of equal dosage of LF in this experiment. Earlier, it also reported that disorder of body antioxidant defense systems were also involved in liver damages in hypothyroidisms (1,11,12); hypothyroidism induced decreases of basic body metabolisms and internal respirations, and then inhibit the lipid peroxidation and slightly increased the endogenous antioxidant enzymes, SOD activities with more profound decreases of another endogenous antioxidant enzymes, SOD activities and intracellular deposition of harmful ROS, the H_2O_2 in hepatic tissues. Since hypothyroidisms and related organ damages have been ameliorated by treatment of potent antioxidants (1,11,12), many efforts have been trials to find the potent and less toxic natural origin antioxidants, can be applied to refined or treat the hypothyroidisms (1). BH also has been showed various organ protective effects through augment of body antioxidant defense systems (1), and powerful free radical scavenger effects of betaine, detected in this study as specific ingredient of BH, for the first time, upon our knowledge, also have been well-documented by other researchers. In the present study, significant increases of hepatic H_2O_2 contents and SOD activities and more profound decreases of CAT activities in mitochondria fractions were detected in PTU control, did not accompanied the noticeable changes on the liver lipid peroxidation, well corresponded to the previous studies (1,11,12), respectively. However, these PTU treatment-related abnormal changes on the hepatic antioxidant defense systems were significantly normalized by treatment of LT4, LF 250 mg/kg and all three different dosages of BH, 500, 250 and 125 mg/kg, respectively. Especially, BH 500 and 250 mg/kg treated rats also showed significant decreases of hepatic H_2O_2 contents and SOD activities and

increases of CAT activities as compared with LF 250 mg/kg treated rats, and also non-significantly in BH 125 mg/kg treated rats.

Conclusion

The results obtained in this study suggest that oral administration of 125, 250 and 500 mg/kg of BH showed favorable effects on the hypothyroidism and related liver damages through enhance antioxidant defense system in liver, more favorably than those of equal dosage of LF. All three different dosages of BH treated rats showed dose-dependent favorable inhibitory effects on the PTU-induced hypothyroidism and related hepatic damages in this experiment.

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References

1. Amin A, Hamza AA. Oxidative stress mediates drug-induced hepatotoxicity in rats: a possible role of DNA fragmentation. Toxicology 2005; 208: 367-375.
2. Das K, Chainy GB. Modulation of rat liver mitochondrial antioxidant defence system by thyroid hormone. Biochim Biophys Acta 2001; 1537: 1-13.
3. Devdhar M, Ousman YH, Burman KD. Hypothyroidism. Endocrinol Metab Clin North Am 2007; 36: 595-615, v.
4. Dory L, Roheim PS. Rat plasma lipoproteins and apolipoproteins in experimental hypothyroidism. J Lipid Res 1981; 22: 287-296.
5. Feng X, Jiang Y, Meltzer P, Yen PM. Thyroid hormone regulation of hepatic genes in vivo detected by complementary DNA microarray. Mol Endocrinol 2000; 14: 947-955.
6. Frost PH, Havel RJ. Rationale for use of non-high-density lipoprotein cholesterol rather than low-density lipoprotein cholesterol as a tool for lipoprotein cholesterol screening and assessment of risk and therapy. Am J Cardiol 1998; 81: 26b-31b.
7. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. J Nutr Biochem 2002; 13: 572-584.
8. Hertog MG, Kromhout D, Aravanis C, Blackburn H, Buzina R, Fidanza F, Giampaoli S, Jansen A, Menotti A, Nedeljkovic S, et al. Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. Arch Intern Med 1995; 155: 381-386.
9. Ito M, Arishima T, Kudo T, Nishihara E, Ohye H, Kubota S, Fukata S, Amino N, Kuma K, Sasaki I, Hiraiwa T, Hanafusa T, Takamatsu J, Miyauchi A. Effect of levo-thyroxine replacement on non-high-density lipoprotein cholesterol in hypothyroid patients. J Clin Endocrinol Metab 2007; 92: 608-611.
10. Jung YS, Kim SJ, Kwon DY, Ahn CW, Kim YS, Choi DW, Kim YC. Alleviation of alcoholic liver injury by betaine involves an enhancement of antioxidant defense via regulation of sulfur amino acid metabolism. Food Chem Toxicol 2013; 62: 292-298.
11. Jurikova T, Rop O, Mlcek J, Sochor J, Balla S, Szekeres L, Hegedusova A, Hubalek J, Adam V, Kizek R. Phenolic Profile of Edible Honeysuckle Berries (Genus Lonicera) and Their Biological Effects. Molecules 2012; 17: 61-79.
12. Laycock MA, Pascuzzi RM. The neuromuscular effects of hypothyroidism. Semin Neurol 1991; 11: 288-294.
13. Palikova I, Valentova K, Oborna I, Ulrichova J. Protectivity of blue honeysuckle extract against oxidative human endothelial cells and rat hepatocyte damage. J Agric Food Chem 2009; 57: 6584-6589.
14. Sarandol E, Tas S, Dirican M, Serdar Z. Oxidative stress and serum paraoxonase activity in experimental hypothyroidism: effect of vitamin E supplementation. Cell Biochem Funct 2005; 23: 1-8.
15. Subudhi U, Das K, Paital B, Bhanja S, Chainy GBN. Supplementation of curcumin and vitamin E enhances oxidative stress, but restores hepatic histoarchitecture in hypothyroid rats. Life Sci 2009; 84: 372-379.
16. Subudhi U, Das K, Paital B, Bhanja S, Chainy GBN. Alleviation of enhanced oxidative stress and oxygen consumption of L-thyroxine induced hyperthyroid rat liver mitochondria by vitamin E and curcumin. Chem Biol Interact 2008; 173: 105-114.
17. Surks MI, Ortiz E, Daniels GH, Sawin CT, Col NF, Cobin RH, Franklyn JA, Hershman JM, Burman KD, Denke MA, Gorman C, Cooper RS, Weissman NJ. Subclinical thyroid disease: scientific review and guidelines for diagnosis and management. JAMA 2004; 291: 228-238.
18. Wang L, Chen L, Tan Y, Wei J, Chang Y, Jin T, Zhu H. Betaine supplement alleviates hepatic triglyceride accumulation of apolipoprotein E deficient mice via reducing methylation of peroxisomal proliferator-activated receptor alpha promoter. Lipids Health Dis 2013; 12: 34.
19. Williams KV, Nayak S, Becker D, Reyes J, Burmeister LA. Fifty years of experience with propylthiouracil-associated hepatotoxicity: what have we learned? J Clin Endocrinol Metab 1997; 82: 1727-1733.
20. Wu S, He X, Wu X, Qin S, He J, Zhang S, Hou D-X. Inhibitory effects of blue honeysuckle (Lonicera caerulea L) on adjuvant-induced arthritis in rats: Crosstalk of anti-inflammatory and antioxidant effects. Journal of Functional Foods 2015; 17: 514-523.