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Method Development for Evaluation of Hepato-Protection Functionality of Health Food

CHIEN-MING HU AND HUI-WEN CHENG[1]*

Institute of Pharmaceutical Sciences, Taipei Medical College, 250, Wu Hsing Street, Taipei 110-31, Taiwan, R.O.C.

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ABSTRACT

Proper regulation of the acclaimed functionality and safety of the health food is a consensus among the health authorities of every nation. The objective of this study is focused on the methods development for safety and functionality of hepatic injury prevention of health food using CCl_4-induced hepatotoxicity as model. The result from this study could provide some useful information for setting a feasible and reasonable evaluating method for regulatory purpose. In this study, we have shown that in the untreated rats, administration of CCl_4 at 0.05 and 0.20 mL/ kg, BW caused the increase of SGOT activities from 118 ± 25 to 328 ± 42 and 670 ± 95 (U/L), respectively; SGPT activities from 48 ± 5 to 170 ± 18 and 535 ± 123 (U/L), respectively. In the rats treated with silymarin for 7 days at 140 mg/ kg, BW prior the administration of CCl_4 could attenuate the liver injury induced by CCl_4 at 0.05 and 0.20 mL/ kg, BW. Similar results were obtained when pretreated with *Ganoderma lucidum* and red ginseng. However, none of the pretreatment can effectively prevent or reduce the liver injury induced by CCl_4 at higher dosage (0.40 and 0.80 mL/ kg, BW). In addition, we have also found that no toxic effects were observed when pretreated with silymarin, *Ganoderma lucidum* and red ginseng for 7 and 30 days as judged by the pathological examination and serum enzyme activities. The results of this study might provide some useful information for the Department of Health with a scientific and objective means to evaluate the safety and functionality for the hepatic injury prevention of health foods.

Key words: silymarin, *Ganoderma lucidum*, red ginseng extract, functionality, safety, hepatotoxicity

INTRODUCTION

Foods possessing health-promoting functions are becoming popular around the world and have received great attention by the health authorities of most countries as people are becoming aware of their health enhancement. However, lack of medical knowledge by the public, untruthful sales, and exaggerated propaganda have made the health food market chaotic. As a result, health food quality varies greatly. In such circumstances, not only is the function for health promotion not guaranteed, but also, in some cases, it even jeopardizes health[1-7]. According to statistical data from the Department of Health, hepatic-related diseases, such as chronic hepatic disease, liver cirrhosis, and liver cancer rank in the top ten for causing deaths in Taiwan. Therefore, most of the commercially available health-promoting foods are likely to emphasize their ability to prevent hepatic injury. Health foods have been integrated into our lives. Thus, to develop a method to evaluate the safety and functionality of hepatic injury prevention by health foods in order to assist the health authorities to promote people's health is necessary and needs to be done quickly.

Some chemicals such as ethanol[8-10], carbon tetrachloride (CCl_4)[11], and acetaminophen[12], have been reported to cause hepatotoxicity. Carbon tetrachloride is capable of causing liver necrosis, changing the activities of metabolic enzymes in liver[13], as well as increasing the hepatic lipid peroxidation[14]. It has been shown that the hazardous factor for inducing liver disease could affect the mode of action of the hepatic metabolic enzyme and increase the hepatic lipid peroxidation, resulting in fatty liver, liver cirrhosis, and liver necrosis. Therefore, CCl_4 was used as a liver damage-inducing agent in this study to evaluate the hepato-protection property of some selected health foods.

Ganoderma lucidum and ginseng have been claimed by traditional health food practitioners as being able to improve health, enhance strength, and prevent tumors. Some literatures have reported that *Ganoderma lucidum* exhibits the ability to prevent tumors[15], inhibit histamine-release[16], decrease cholesterol synthesis[17], and protect against hepato-related diseases[18,19]. Ginseng has been shown to carry the liver-protection activity via a cytochrom P450 inhibition to reduce CCl_4-induced hepatic lipid peroxidation[20,21]. However, a valid method to evaluate the functionality of health food has not yet been established. To take the CCl_4-induced hepatotoxicity study as an example, a single medication was usually used to test for the performance on reduction of CCl_4-induced hepatotoxicity. Determination of CCl_4 dosage and induction time was dependent on the experimental design. In this study, the CCl_4 dosage was tested and optimized to evaluate the hepato-protection functionality of health food. The results of this study could provide a scientific and objective method to evaluate the functionality of hepato-prevention by health foods and could be useful information for related authorities in establishing health food related regulations.

* Author for correspondence. Tel: 02-27361661 ext. 686; Fax: 02-23775875.

MATERIALS AND METHODS

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I. *Materials*

Carbon tetrachloride (of purity >99.5%) was obtained from Merck (F. R. Germany). Corn oil was purchased from Sigma (St. Louis, MO, USA) and silymarin (70 mg/capsule) was purchased from N. V. Sanico. *Ganoderma lucidum* (mycelium, 500 mg/capsule) was obtained from Sheng Foong Pharmaceutical Co., Ltd. Red ginseng concentrate (1g) extracted from 2g red ginseng was purchased from Korea T. & Ginseng Corp.

II. *Methods*

(I) *Treatment of Animals*

Male Wistar rats (100-120 g) purchased from the animal center at the medical school at National Taiwan University were randomly divided into 3 groups with 5 animals in each group. The experimental group was administrated with *Ganoderma lucidum* and ginseng, the positive control group was administrated with silymarin, and the normal control group was administrated with water. Before administration, rats were acclimated in an animal feeding center for one week. During acclimation and experimentation, an animal-feeding standard of water and food (from Purina Mills, Inc., St. Louis, MO) and light exposure was strictly followed. Rats in the experimental group were administrated with 10 mL of *Ganoderma lucidum* (500 mg/kg) solution or 10 mL of red ginseng (1g/kg) solution. Rats in the positive control and normal control groups were administrated with 10 mL of silymarin (140 mg/kg) solution and water, respectively. The administration was conducted by gavage once a day for 7 consecutive days followed by treatment with CCl_4. The activities of the following hepatic enzymes were measured as biochemical parameters of liver functionality: glutamic oxaloacetic transaminase (SGOT), glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH). After 7 and 30 consecutive days of administration of the drug, the body, liver, kidney weights, and SGOT, SGPT, ALP, LDH, and blood urea nitrogen (BUN) were measured to investigate the toxic effect of repeated drug treatment on the animals.

(II) *Method for Inducing Acute Liver Injury by CCl_4*

Four dosages, 0.05, 0.20, 0.40, and 0.80 mL/kg, of CCl_4 in 5 mL of corn oil were tested to induce acute liver injury in rats. The experimental group was intraperitoneally treated with CCl_4 while the vehicle control group received only corn oil. Rats were then sacrificed after 24 hr and blood from the peritoneal artery was collected.

(III) *Clinical Biochemistry Test*

After drug treatment, the rats were anesthetized with diethyl ether, and blood (~8 mL) from the peritoneal artery was collected and transferred to a test tube containing STT gel, which is for separating serum from blood cells and preventing hematolysis. Blood in the test tube was left to stand until coagulation and then centrifuged at 2500 rpm (CR5B2 Hitachi, Ltd., Tokyo, Japan) for 12 min. The upper layer was collected and tested for serum biochemistry using an automatic analyzer (Model 7450, Hitachi, Ltd., Tokyo, Japan).

(IV) *Statistical Analysis*

The unpair Student's *t*-test ($P<0.05$) was used to compare the differences of CCl_4- and corn oil-induced serum biochemical parameters concurrent with silymarin, *Ganoderma lucidum*, red ginseng concentrate, or water administration. The CCl_4-induced serum biochemical parameters in experiment and control groups were also compared.

RESULTS

I. *The Toxic Effect on Animal as Repeating Treatment with Drug*

After oral administration with silymarin, *Ganoderma lucidum*, or red ginseng concentrate for 7 and 30 days, the rats were sacrificed via a peritoneal artery bloodletting. The blood was then tested for serum enzyme activities and the weights of livers and kidneys were measured. A statistical study showed that there was no significant difference in body, liver, and kidney weights, nor on serum enzyme activities including SGOT, SGPT, ALP, LDH, and BUN (Table 1).

II. *Effect of Silymarin on Hepatotoxicity Induced by Different Dosage of CCl_4*

After administration with silymarin for 7 days, rats were then treated with four dosages of CCl_4 (0.05, 0.20, 0.40, and 0.80 mL/kg) to induce hepatotoxicity. Results showed that SGOT and SGPT activities were increased by increasing the concentrations of CCl_4 as shown in Figure 1. A significant difference in SGOT and SGPT activities between untreated rats and the rats treated with silymarin was observed at low dosages (0.05 and 0.20 mL/kg) of CCl_4 induction. However, when treated with higher dosages (0.40 and 0.80 mL/kg) of CCl_4 there was no significant difference observed between these two groups.

III. *Effect of Silymarin, Ganoderma lucidum, and Red Ginseng Concentrate on Hepatotoxicity Induced by CCl_4 at Low Dosages*

Rats were treated with silymarin, *Ganoderma lucidum*, or red ginseng concentrate (the dosages of which refer to the description in Materials and Methods) prior to intraperitoneal administration with CCl_4 at 0.05 and 0.20 mL/kg for 24 hr. The SGOT and SGPT activities were significantly increased for the rats treated with drugs concurrent with CCl_4 administration as compared to vehicle control group, indicating CCl_4 at lower dosages is still capable of inducing hepatotoxicity.

Table 1. Effects of silymarin, *Ganoderma lucidum* and red ginseng on serum biochemical parameters, and body, liver, kidney weight in rats

	Untreated		Silymarin		*Ganoderma lucidum*		Red ginseng	
	7 days	30 days	7 days	30 days	7 days	30 days	7 days	30 days
Body weight (g)	216.9 ± 3.7	350.5 ± 13.2	213.2 ± 5.2	365.8 ± 20.3	216.3 ± 3.0	342.2 ± 16.7	221.5 ± 4.6	344.1 ± 14.6
Liver/Body weight (%)	5.35 ± 0.18	4.11 ± 0.20	4.69 ± 0.19	4.13 ± 0.33	5.18 ± 0.14	4.06 ± 0.15	5.24 ± 0.23	3.89 ± 0.49
Kidney/Body weight (%)	0.83 ± 0.05	0.80 ± 0.20	0.80 ± 0.05	0.79 ± 0.25	0.83 ± 0.05	0.80 ± 0.20	0.86 ± 0.09	5.35 ± 0.18
SGOT (U/L)	118 ± 25	136 ± 7	123 ± 21	125 ± 11	119 ± 19	130 ± 13	130 ± 30	116 ± 5
SGPT (U/L)	48 ± 5	42 ± 4	52 ± 7	49 ± 6	43 ± 5	57 ± 8	54 ± 10	54 ± 6
ALP (U/L)	496 ± 104	407 ± 26	514 ± 87	550 ± 43	523 ± 46	510 ± 39	476 ± 48	523 ± 38
LDH (U/L)	915 ± 245	719 ± 64	897 ± 220	856 ± 55	880 ± 129	897 ± 34	921 ± 164	713 ± 27
BUN (U/L)	19.7 ± 2.7	19.7 ± 2.7	17.6 ± 1.6	17.9 ± 1.6	18.7 ± 2.1	20.4 ± 3.1	16.8 ± 2.3	18.3 ± 2.0

Rats were treated with silymarin, *Ganoderma lucidum* and red ginseng for 7 and 30 days according to the procedures outlined in "Materials and Methods".The data were expressed as mean ± s.e.m. (n>5) and analyzed with the student's *t*-test.A p value of 0.05 or less is considered statistically significant.

Table 2. Effect of silymarin, *Ganoderma lucidum* and red ginseng administration on SGOT and SGPT activities in rats treated with low dosage of CCl_4

serum enzyme	Untreated		Silymarin		*Ganoderma lucidum*		Red ginseng	
activities (U/L)	Corn oil	CCl_4	Corn oil	CCl_4	Corn oil	CCl_4	Corn oil	CCl_4
SGOT	118 ± 25	328[a] ± 42	123 ± 21	221[a,b] ± 39	119 ± 19	198 [a,b] ± 17	130 ± 30	220[a,b] ± 37
SGPT	48 ± 5	170[a] ± 18	52 ± 7	90[a,b] ± 26	43 ± 5	90 [a,b] ± 12	54 ± 10	95[a,b] ± 16

Rats were treated with silymarin, *Ganoderma lucidum* and red ginseng, and then with 0.05 mL CCl_4/Kg, BW according to the procedures states in "Materials and Methods". The data were expressed as mean ± s.e.m. (n>5) and analyzed with the student's *t*-test. A p value of 0.05 or less is considered statistically significant.

[a]comparing the CCl_4 and corn oil treated of each group.

[b]comparing the CCl_4-treated rats of untreated and silymarin, *Ganoderma lucidum* and red ginseng treated group.

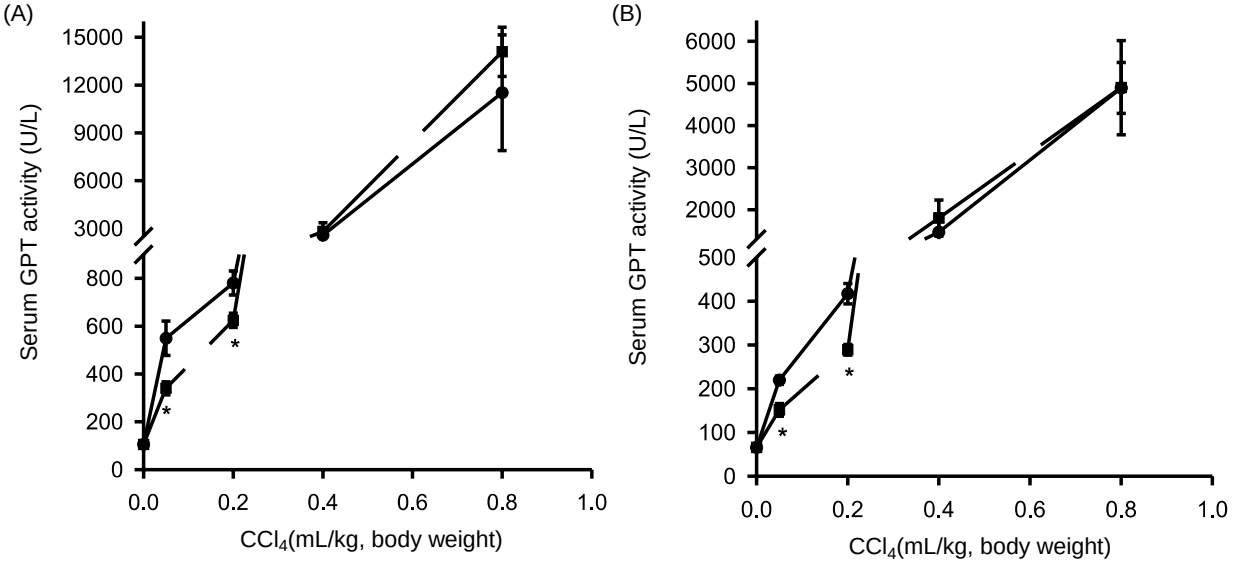

Figure 1. Effect of silymarin on CCl_4-induced liver injury.The CCl_4-induced liver injury were evaluated by the increase of SGOT (A) and SGPT (B) activities in serum from treated animals according to the procedures stated in Methods.Various concentrations of CCl_4 were injected i.p. to induce liver injury after the animals were treated with vehicle (●) or silymarin (■).The data were expressed as mean ± s.e.m. (n = 5). A p value of 0.05 or less is considered statistically significant, *p <0.05.

Moreover, SGOT and SGPT activities in both experiment and positive control groups at low dose CCl_4 exposure were significantly decreased as compared to the normal control group (Table 2 and 3).

IV. *Effect of Red Ginseng Concentrate on Hepatotoxicity Induced by High Dosages of CCl_4*

The hepatotoxicity in rats was induced by CCl_4 at 0.8 mL/kg for 24 hr and blood from the peritoneal artery was tested for serum biochemistry. As shown in Table 4, the SGOT and SGPT activities were significantly increased for rats treated with CCl_4 as compared to those treated with corn oil, indicating hepatotoxicity was successfully induced by CCl_4. However, at such a high dosage of CCl_4 induction, the serum enzyme activities in rats treated with red ginseng concentrate (experiment group) showed no difference to those rats treated with water (normal control group).

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Table 3. Effect of silymarin, *Ganoderma lucidum* and red ginseng administration on SGOT and SGPT activities in rats treated with medium dosage of CCl_4

serum enzyme	Untreated		Silymarin		*Ganoderma lucidum*		Red ginseng	
activities (U/L)	Corn oil	CCl_4	Corn oil	CCl_4	Corn oil	CCl_4	Corn oil	CCl_4
SGOT	118 ± 25	670[a] ± 95	123 ± 21	452[a,b] ± 43	119 ± 19	497[a,b] ± 62	130 ± 30	481[a,b] ± 51
SGPT	48 ± 5	535[a] ± 123	52 ± 7	217[a,b] ± 40	43 ± 5	253[a,b] ± 28	54 ± 10	240[a,b] ± 33

Rats were treated silymarin, *Ganoderma lucidum* and red ginseng and then with 0.20 mL CCl_4/Kg, BW according to the procedures states in "Materials and Methods". The data were expressed as mean ± s.e.m. (n>5) and analyzed with the student's *t*-test. A p value of 0.05 or less is considered statistically significant.

[a]comparing the CCl_4 and corn oil treated of each group.

[b]comparing the CCl_4-treated rats of untreated and silymarin, *Ganoderma lucidum* and red ginseng treated group.

Table 4. Effect of red ginseng administration on SGOT and SGPT activities in rats treated with high dosage of CCl_4

serum enzyme activities (U/L)	Untreated		Red ginseng	
	Corn oil	CCl_4	Corn oil	CCl_4
SGOT	157 ± 18.6	7098 ± 3117[a]	197 ± 42	7330 ± 125[a]
SGPT	57 ± 9	4400 ± 1253[a]	60 ± 5	3297 ± 990[a]

Rats were treated with red ginseng and then with 0.80 mL CCl_4/ Kg, BW according to the procedures states in "Materials and Methods". The data were expressed as mean ± s.e.m. (n>5) and analyzed with the Student's *t*-test. A p value of 0.05 or less is considered statistically significant.

[a]comparing the CCl_4 and corn oil treated of each group.

DISCUSSION

Health foods have become a part of daily life as people have become aware of their benefits. However, health food management and researchers have not been able to keep pace with this growing market. The purpose of this study was to establish a method to evaluate the CCl_4-induced hepatotoxicity reduction ability (an index for hepato-protection) of health foods. Silymarin was used as a positive control in this study because it has been reported to clinically cure various hepatic diseases[22-24]. These include acute and chronic hepatitis caused by viruses, hepatic disease induced by alcohol, and hepatitis and liver necrosis caused by drugs or toxicants. Silymarin has been shown to have the ability to prevent CCl_4-induced liver cirrhosis[25] and to reduce CCl_4-induced SGPT[26,27]. However, at high dosages (0.40 and 0.80 mL/kg) of CCl_4 induction, SGOT and SGPT were greatly increased in both positive and normal control groups, but showing no significant different between these two groups. This result indicates a test model using a high dosage of CCl_4 is not suitable for evaluation of hepato-protection drugs.

As mentioned above, the positive control group showed no difference from the normal control group in SGOT and SGPT activities induced by high-dose CCl_4. The same result was observed between the experimental group treated with red ginseng concentrate, and the normal control group. This could be due to a high-dose CCl_4 exposure which can cause severe liver damage and is hardly recovered by the hepato-protection drug. Moreover, a high standard deviation was observed in SGOT and SGPT at high-dose CCl_4 exposure. This could result in a variation in the evaluation of the liver protective functionality of health foods. The health food claimed for hepato-protection functionality should not be overly expected to cure hepatic disease, since it is not a drug. An experimental model using a high-dose CCl_4 induction could cause severe liver damage that could be hardly recovered by drugs, and therefore this model is not suggested to evaluate the hepato-protection functionality of health food.

Gnaoderma lucidum and red ginseng as well as silymarin exhibit the ability to reduce hepatotoxicity, in terms of SGOT and SGPT activities, induced by low-dose CCl_4 (0.05 and 0.20 mL/kg). This result shows an experimental model using a low-dose CCl_4 exposure is not only able to induce hepatotoxicity but also able to successfully evaluate the hepato-protection ability of health food. SGOT and SGPT are the two index enzymes for evaluation of hepato-protection functionality because they are abundant in liver and their concentrations change significantly as liver injury occurs. ALP and LDH are another two enzymes in liver in large amounts. Clinically, ALP could be well expressed in the patient with bile duct and bone diseases[30], while trace plasma excretion could increase LDH concentration in serums[31]. In this study, we have found that these two enzymes are not suitable for evaluating hepato-protection functionality.

In Taiwan, silymarin has been approved as an adjuvant for treatment of chronic hepatitis, liver cirrhosis, and fatty liver. In this study, we have demonstrated that silymarin is capable of significantly reducing SGOT and SGPT activities. However, as compared to the vehicle group, which is treated with corn oil only, silymarin could not entirely recover the CCl_4-induced hepatotoxicity by reducing SGOT and SGPT activities to normal levels. Therefore, the positive control group should be used as a reference to compare the CCl_4-induced SGOT and SGPT activities between experimental and normal control groups. Based on our study, we have concluded that CCl_4 is a good hepatotoxicity inducer. A model using a low-dose CCl_4 induction and by monitoring the SGOT and SGPT activities in serum could effectively evaluate the hepato-protection functionality of health food. However, we must stress that the CCl_4-induced model could only be applied to the evaluation of the protective effect on liver injuries caused by drugs or toxicants, but not on chronic liver disease caused by viruses. The virus infection model needs to perform an animal test in which gorillas are used as

a subject.

The study on the toxic effect of long-term administration of health foods on organs (especially liver) showed no pathological changes in the liver, kidney, pancreas, or spleen (data not shown). The body, liver, and kidney weights, and serum biochemical parameters were not significantly changed. These results indicate that there is no adverse effect on liver after a long-term administration of silymarin, *Ganoderma lucidum*, and red ginseng concentrate.

The research on safety evaluation of health food is of great importance. The need for a proper safety standard is an international consensus. This safety standard should be established on the basis of scientific data in which safety dosage information is obtained via a toxicological test. Nevertheless, some questions still remain to be solved in the evaluation of the functionality of health food. It is agreed by most countries that health food should not be claimed for curing purposes but only for strengthening health, such as promoting liver function, preventing liver injury, improving digestion and absorption, promoting growth, adjusting blood lipid composition, and reducing blood sugar. The evaluation method established in this study could be a reference method to evaluate health foods which claim for hepato-protection functions. Moreover, other health food with a specific function should also be scientifically validated and approved by related authorities prior to marketing approval. Thus, the health-promoting functionality of commercially available health food would be guaranteed.

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保健食品之保肝功能評估方法模式之探討

胡健民　鄭慧文*

私立台北醫學院藥學研究所
台北市吳興街250號

摘　要

對於保健食品作一適當的管理與規範是世界各國政府的共識，本研究目的是將針對宣稱具保肝功效的保健食品，在陽性對照組-水飛薊的相較下，建立一套以四氯化碳誘導其肝毒性來評估保健食品的保肝功效。本研究方法是以大白鼠做爲實驗動物，著重於保護肝臟作用檢驗方法（每天1次，連續餵食7天，並以四氯化碳誘導其肝臟毒性）與保健食品對肝臟慢性毒理之評估（每天1次，連續餵食30天），以了解相關的評估方法是否確能驗證保健食品的機能性及安全性。在保護肝臟作用檢驗方法之評估方面，以低劑量四氯化碳0.05及0.20 mL/大鼠體重（公斤）誘導下，對照組（水）動物血清酵素之SGOT值由118± 25分別上升至328 ± 42及670 ± 95（U/L），SGPT值由48 ± 5分別上升至170 ± 18及535 ± 123（U/L）；陽性對照組－餵食水飛薊140 mg/大鼠體重（公斤）之SGOT值由123 ± 21分別上升至221 ± 39及452 ± 43（U/L），SGPT值由52 ± 7分別上升至90 ± 26及217 ± 40（U/L）。對照組與陽性對照組在此兩種四氯化碳濃度的誘導下，其血清酵素中SGOT及SGPT值的上升程度皆有統計上的差異，由此結果顯示，水飛薊確實有保護肝臟並減輕其受四氯化碳之傷害。再者，以靈芝及紅參精試驗之，其結果也都顯示具保護肝臟之作用。在保健食品對肝臟之慢性毒理評估方面，經長期餵予動物保健食品後，分析其血清酵素之結果，我們發現在本研究中所使用的劑量下，水飛薊、靈芝及紅參精並不會造成實驗動物之肝毒性。本研究之成果將可以提供一個具有科學性與客觀性的方法來評估保健食品之保肝功，以作爲政府決策單位訂定法規及管理規範保健食品事宜之參考。

關鍵詞：水飛薊，靈芝，紅參精，機能性，安全性，肝毒性