Hypolipidemic Action of hydroxycinnamic Acids from Cabbage (*Brassica oleracea L. var. capitata*) on Hypercholesterolaemic Rat in Relation to Its Antioxidant Activity

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Abstract Cabbage extract containing chlorogenic acid has notable scavenging activity against hydroxyl radicals, superoxide anion radicals, DPPH radicals as well as potent reducing power. Oral administration of the extract (240 mg/kg BW/day) to hypercholesterolaemic rats reduced by high–fat diet for 30 days lowered their blood TC, TG, and LDL–C levels by 36.3, 43.5 and 45.8%, respectively. However, the blood HDL–C levels in the same treated rats increased by 17.1%. Treatment of hypercholesterolaemic rats with the extract significantly increased the GSH level along with enhanced SOD, CAT activities in liver tissues. Furthermore, the extract significantly decreased hepatic MDA as well as GPx and GR activities in extract–treated rats. It can therefore be concluded that the extract has a high hypolipidaemic activity and this may be attributed to its antioxidative potential.

Keywords: cabbage, hydroxycinnamic acids, Hypercholesterolaemic, Antioxidant capacity, Hypolipidemic action

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1. Introduction

Oxidative stress is currently suggested as a mechanism underlying hypercholesterolaemia, which is one of the major risk factors for coronary heart disease [1]. It has been shown that hypercholesterolaemia increases the blood level malondialdehyde (MDA), one of the lipid peroxidation products. In addition, the level of reactive oxygen species (ROS), produced by polymorphonuclear leucocytes also increases along with decrease in activities of many tissue antioxidant enzymes [2].

Biological antioxidants are natural compounds which can prevent the uncontrolled formation of free radicals and activated oxygen species, or inhibit their reaction with biological structures. These compounds include antioxidative enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) and non–enzymatic antioxidants, such as glutathione (GSH), vitamin C and vitamin E [2]. The efficiency of this defense system is apparently weakened in hypercholesterolaemia condition resulting in ineffective scavenging of free radicals which may lead to tissue damages.

Dietary fat intake has been shown to be important in the development of human obesity and there are also experimental studies showing that high–fat diet can be associated with increased oxidative stress in mammals and more recently literature data have indicated that high–fat diet may increase the incidence of cardiovascular diseases [3]. Of particular importance, patients with cardiovascular diseases have been reported to eat a diet higher in fat than the general population [4], suggesting that high–fat diet may accelerate the development of the disease.

Recently, studies have shown that medicinal plants intake especially fruits and vegetables by rats resulted in an increase in the antioxidant enzyme activities and high density lipoprotein cholesterol (HDL–C), and a decrease in lipid peroxidation, which may reduce the risk of heart disease [2].

Phenolic antioxidants have been claimed to have beneficial health functions for retarding aging and preventing cancer and cardiovascular diseases [5]. The interest in phenolic antioxidants has increased remarkably over the last decade because of their protective effects against different diseases, including cardiovascular, inflammatory and neurological diseases, as well as cancers. The generally accepted mechanism is that free radical–scavenging activity of polyphenols contributes to reduce

the oxidative stress and to prevent the development of diseases [6].

Cabbage (Brassica oleracea L. var. capitata) is rich in phenolic compounds such as chlorogenic acid as well as vanillic acid, caffeic acid, gallic acid and protocatechuic acid, which belong to hydroxycinnamic acids and have similar bioactivity and functional group to chlorogenic acid [7,8]. The phenolic compounds have been found to be favorable to improves glucose tolerance and decreases plasma and hepatic lipids in obese and hyperlipidemic rats [9,10]. They have been also reported to remove particularly toxic reactive species by scavenging alkylperoxyl radicals and limit low–density lipid (LDL) oxidation, the major determinant of the initial events in atherosclerosis [11]. However, little has been reported on the mechanism and the phenolic compounds attributed to its hypolipidemic action.

The objective of this study was to examine the influence of cabbage extract containing high hydroxycinnamic acids on the activities of the antioxidant enzymes including CAT, SOD, GPx and GR as well as on the levels of MDA and GSH in the livers of rats fed with high–fat diet, to determine in vitro the antioxidant capacity of the compounds and to elucidate the mechanisms responsible for such an effect.

2. Materials and Methods

2.1. Chemicals

Caffeic acid, gallic acid, protocatechuic acid, vanillic acid and chlorogenic acid were purchased from Sigma company. All other chemicals and reagents were of the highest grade commercially available and purchased from Guoyao Chemical Reagent Co., Ltd. (Chengdu, China).

2.2. Cabbage Extact

Cabbage (Brassica oleracea L. var. capitata) was kindly supplied by Chonghong Food Co., Ltd, Xinghua, Jiangsu, China. 100.0 g of cabbage dried in air was crushed and refluxed in $CHCl_3$ for 2 h. The filter residue was extracted with methanol for one hour and then filtrated. The filter liquor was processed with $Pb(OAc)_2$ and then filtrated. The solid obtained was treated with 5% H_2SO_4 (pH 2.0). After removal of the solid substrates, the liquid was concentrated in vacuum and then extracted with ethyl acetate for three times. After recovery of ethyl acetate, the product obtained was lyophilized under vacuum. A yield of 2.67 g was obtained. The purified phenolic compounds were further analyzed using high performance liquid chromatography (HPLC).

HPLC determination was carried out by a HPLC/Diode Array (HP1100, a diode array detector G 1513−87311, Agilent Technologies, Wilmington, America) with detection at 280 nm (scanning between 220 and 450 nm). Firstly, sample was filtered (0.22 μm), and injected then (5 μl) onto a C18 column (415 cm × 4.6 mm, 5 μm, Agilent Technologies, Wilmington, America). The mobile phase composition was optimized and the best composition obtained was methanol: water: acetic acid (20:80:1.0, v/v). The flow−rate was 1.5 ml/min. Retention times and spectra were compared to pure standards of chlorogenic acid, caffeic acid, gallic acid, protocatechuic acid and

vanillic acid. HPLC analysis showed that the contents of chlorogenic acid, caffeic acid, vanillic acid, gallic acid and protocatechuic acid in the extract were 81.6, 6.3, 4.1 2.1 and 1.2%, respectively. The purified phenolic compounds were stored at 4°C for further investigation of bioactivities.

2.3. Animal Modeling, Group and Treatment

A total of 30 male Wistar rats weighing from 200 to 250 g were purchased from Experiment Animal Academe, Medical College of Sichuan Province, China and were housed under 22±2 oC ambient temperature and 70 % relatively humidity with a 12 h light :12 h dark cycle. Chow, with high–fat content, was made from normal pulverized chow (47%), cholesterol (2%), sodium cholate (1%), dextrose (40%), olive oil (10%) and water (15%). After the adaptation period, the rats were randomly divided into three groups (each group consisting of 10 animals): the first group of rats (control group, Group I) was fed the normal diet. The second and third groups of animals (Group II and Group III) were fed high–fat diet. Rats in Group I and II were not given the extract but instead received equal volumes of water every day for the same period. Group III was given, in addition to high–fat diet, a daily dose of the plant extract (240 mg/kg) by oral gavages for 30 consecutive days.

Twenty four hours before killing, all food was removed. Animals were anesthetized by diethyl ether, and blood and liver samples were collected for assay. Blood samples were centrifuged at 1500 g/min at 4 oC for 10 min to obtain serum. The serum obtained was used to estimate levels of total cholesterol (TC), triglyceride (TG) and low density lipoprotein cholesterol (LDL–C) as well as AST, ALT and ALP. The liver samples were cut into small pieces and homogenized in Tris–HCl buffer (0.025 M, pH 7.5) with a homogenizer to give a 10% (w/v) liver homogenate. The homogenates were then centrifuged at 12,257 g for 15 min at 4 oC (Beckman). The supernatant obtained was used for estimating CAT, SOD, GPx and GR as well as MDA and GSH. All experiments were carried out according to the guidelines for the care and use of experimental animals and approved by institutional animal ethical committee.

2.4. Antioxidant Activity in Vitro

2.4.1. Hydroxyl Radical–scavenging Activity

The analysis of hydroxyl radical–scavenging activity was carried out according to the method of Yanhong et al. [5]. 0.1 ml of extract solution in methanol (varying concentration from 20 to 100 μg/ml) was mixed with 0.8 ml of reaction buffer (0.2 M KH_2PO_4/KOH buffer, pH 7.2, 1.6 μmol deoxyribose, 0.1 μmol iron ammonium sulphate and 0.1 μmol ethylenediaminetetraacetic acid (EDTA)). 0.1 ml of 0.01 M H_2O_2 was then added to the reaction solution. The solution was incubated for 10 min at 37°C prior to the addition of 0.5 ml of 1% thiobarbituric acid and 1 ml of 2.8% trichloroacetic acid. The mixture was boiled for 10 min and cooled rapidly. The absorbance of the mixture was detected at 532 nm. The blank was prepared using distilled water instead of sample. Vitamin C was used as a control. All the tests were performed in triplicate. The scavenging activity on hydroxyl radical was expressed using the following equation: Hydroxyl radical

scavenging activity (%) = [1–(A1–A2) / A0] × 100, where A0 is the absorbance of blank; A1 is the absorbance of the mixture with sample; A2 is the absorbance of the mixture without sample.

2.4.2. Superoxide Anion Radical–scavenging Activity

The superoxide anion radical scavenging activity was evaluated according to the method of Yu et al. [12], with a slight modification. Superoxide anion radicals, generated in the phenazine methosulfate–reduced form of nicotinamide adenine dinucleotide (PMS–NADH) system by oxidation of NADH, were determined by the reduction of nitro blue tetrazolium (NBT). In this experiment, superoxide anion radicals were generated in 1.25 ml of Tris–HCl buffer (16 mM, pH 8.0) containing 0.25 ml of NBT (300 μM), 0.25 ml of NADH (468 μM) and extract solution (varying concentration from 20 to 100 μg/ml). The reaction was started by adding 0.25 ml of PMS (60 μM) solution to the mixture. The reaction mixture was incubated at room temperature for 5 min and the absorbance was detected at 560 nm by a spectrophotometer (UV–2100, Unico Corporation, Shanghai, China) against a blank. Decreased absorbance of the reaction mixture indicated increased superoxide anion radical–scavenging activity. Vitamin C was used as a control. The scavenging of the superoxide anion radicals was calculated by the following equation: Scavenging activity (%) = (A0–A1) / A0 × 100, where A0 is the absorbance of the control and A1 is the absorbance of the mixture with sample.

2.4.3. DPPH Radical–scavenging Activity

The 2,2−diphenyl−1−picrylhydrazyl (DPPH) radical–scavenging activity was determined according to the method of Blois [13]. The extract was dissolved in methanol (varying concentration from 0 to 40 μg/ml. 2 ml of extract solution were mixed with 1 ml of 0.2 mM DPPH in methanol. The mixture was shaken vigorously and maintained for 30 min in the dark. The absorbance was detected at 517 nm. The absorbance of the control was obtained by replacing the sample with methanol. Vitamin C was used as a control. The scavenging activity was calculated using the following equation: Scavenging activity (%) = (A0–A1) / A0 × 100, where A0 is the absorbance of the control and A1 is the absorbance of the mixture with sample.

2.4.4. Reducing Power

The reducing power of extract was determined according to the method of Ahmadi, Kadivar, and Shahedi [14]. 2 ml of sample solution (varying from 0 to 40 μg/ml), 2 ml of phosphate buffer (0.2 M, pH 6.6) and 10 mg/ml of potassium ferricyanide were mixed and incubated at 50 oC for 20 min, and then added 2 ml of trichloroacetic acid (100 mg/ml). 2 ml of the mixture was blended with 2 ml of distilled water and 0.4 ml of 0.1% (w/v) ferric chloride. After incubation for 10 min, the absorbance was detected at 700 nm. Increase in absorbance indicates increase in reducing power. Vitamin C was used as a control. Distilled water was used as a blank.

2.5. Biochemical Parameters

2.5.1. Serum Lipid and Hepatic Enzymes

TC, TG, HDL–C and LDL–C in serum were determined using enzymatic kits (Bioengineering Co., Ltd, China) according to the manufacture's instructions. ALP, AST and ALT were assayed using the corresponding commercial kits (Bioengineering Co., Ltd, China).

2.5.2. Hepatic Lipid Peroxidation

Hepatic lipid peroxidation was expressed using MDA level as index. MDA levels were measured by the double heating method [15]. The method is based on spectrophotometeric measurement of the purple color generated by the reaction of thiobarbituric acid (TBA) with MDA. 0.5 ml of liver homogenate was mixed with 2.5 ml of tricholoroacetic acid (TCA) (10%, w/v) solution followed by boiling in a water bath for 15 min. After cooling to room temperature, the samples were centrifuged at 3000 rpm for 10 min and 2 ml of each sample supernatant was transferred to a test tube containing 1 ml of TBA solution (0.67%, w/v). Each tube was then placed in a boiling water bath for 15 min. After cooling to room temperature, the absorbance was measured at 532 nm with respect to the blank solution. The concentration of MDA was calculated based on the absorbance coefficient of the TBA–MDA complex (ε =1.56×105 /cm/M) and it was expressed as nmol/mg protein.

2.5.3. Reduced Glutathione

GSH was detected according to the method of Jollow et al. [16]. An aliquot of 0.5 ml of each tissue homogenate was precipitated with 1ml of sulphosalicylic acid (4% w/v). The precipitate was removed by centrifugation. 1 ml of the filtered sample was mixed with 0.1 ml 5,5'−Dithiobis− (2−nitrobenzoic acid) (DTNB) (4 mg/ml) and 0.9 ml phosphate buffer (0.1 M, pH 7.4). The yellow colour developed was read at 412 nm. Reduced glutathione was expressed as μg/mg of protein.

2.5.4. Superoxide Dismutase

SOD activity was measured based on the extent inhibition of amino blue tetrazolium formazan formation in the mixture of mixture of nicotinamide adenine dinucleotide, phenazine methosulphate and nitroblue tetrazolium (NADH–PMS– NBT), according to method of Kakkar et al. [17]. Assay mixture contained 0.1 ml of supernatant, 1.2 ml of sodium pyrophosphate buffer (pH 8.3; 0.052 M), 0.1 ml of phenazine methosulphate (186 μM), 0.3 ml of nitroblue tetrazolium (300 μM) and 0.2 ml of NADH (750 μM). Reaction was started by addition of NADH. After incubation at 30 oC for 90 s, the reaction was stopped by addition of 0.1 ml of glacial acetic acid. Reaction mixture was stirred vigorously with 4.0 ml of n–butanol. Colour intensity of the chromogen in the butanol was measured spectrophotometrically at 560 nm. One unit of enzyme activity was defined as that amount of enzyme which caused 50% inhibition of NBT reduction/mg protein.

2.5.5. Catalase Activity

CAT activity was measured by the method of Aebi [18]. An aliquot (5 μl) of each tissue supernatant was added to a cuvette containing 1.995ml of 50 mM phosphate buffer (pH 7.0). Reaction was started by addition of 1.0ml of

freshly prepared 30 mM H2O2. The rate of decomposition of H2O2 was measured spectrophotometrically at 240 nm. Activity of CAT was expressed as $\times 10-1$ k/mg protein, where k represents the rate constant of the first order reaction of CAT.

2.5.6. Glutathione Reductase

Liver GR was assayed by a reaction mixture containing 0.99 ml of 100 mM potassium phosphate buffer (pH 7.0), 1.1 mM MgCl2, 5mM oxidized glutathione disulfide (GSSG) and 0.1 mM β−nicotinamide adenine dinucleotide 2'−phosphate reduced tetrasodium salt (NADPH). 10 µl of liver homogenate was added to trigger the NADPH conversion reaction. Changes in absorbance were monitored at 340 nm for 5min at 25 oC. The specific enzyme activity of GR was expressed as nmol NADPH oxidized to NADP+ (/min mg) protein with 6.22×106 (/cm/M) as the molar extinction coefficient of NADPH [16].

2.5.7. Glutathione Peroxidase

Liver GPx was assayed in a 1 ml cuvette containing 0.89 ml of 100 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, 1 mM NaN3, 0.2 mM NADPH, 1 U/ml GSH reductase and 1 mM GSH. 10 µl of each liver homogenate was added to make a total volume of 0.9 ml. The reaction was initiated by the addition of 100 µl of 2.5 mM H2O2, and the conversion of NADPH to NADP+ was monitored with a spectrophotometer at 340 nm for 3 min. GPx activity was expressed as nmoles of NADPH oxidized to NADP+ (/min mg) protein, using amolar extinction coefficient of 6.22×106 (/cm/M) for NADPH [19].

2.6. Statistical Analysis

All values are expressed as mean ± S.D. The significance of differences between the means of the treated and untreated groups have been calculated by unpaired Student's t–test and P–values less than 0.05 were considered significant.

3. Results

3.1. Antioxidant Activity in Vitro

Figure 1 showed that cabbage extract had significant scavenging activities on hydroxyl radicals as well as superoxide anion radicals in a dose–dependent manner. Compared with vitamin C, which is considered to be a potent hydroxyl radical–scavenger, cabbage extract showed in significant difference (P > 0.05) with regard to scavenging activities of hydroxyl radical and superoxide anion radical. Moreover, when the tested concentration was above 40 µg/ml, the extract showed higher scavenging activity of radicals than did vitamin C.

Figure 2A shows the scavenging effect of cabbage extract on DPPH free radicals. The extract exhibited a strong ability to quench DPPH radicals. The scavenging effect increased with increasing concentrations used in the test. The DPPH radical–scavenging activity of cabbage extract at low concentration was significantly higher (P < 0.05) than that of vitamin C, a commercial antioxidant used. It may also be noted in Figure 2B that cabbage

extract had a dose–dependent reducing power. When a relatively high concentration (no less than 15 µg/ml) was used, no significant difference (P > 0.05) was observed between the reducing power of cabbage extract and vitamin C. In the food industry. This indicated that cabbage extract was a good antioxidant with strong DPPH radical–scavenging activity.
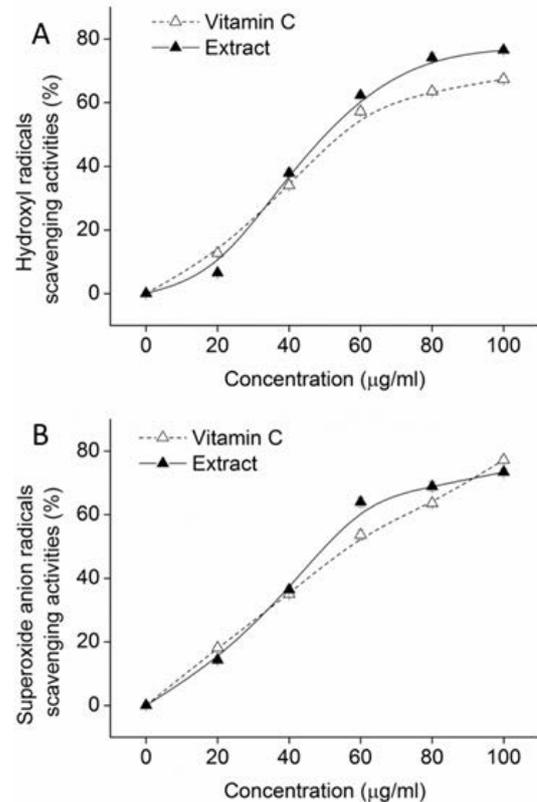

Figure 1. Hydroxyl radical− (A) and superoxide anion radical−scavenging activities (B) of cabbage extract and vitamin C
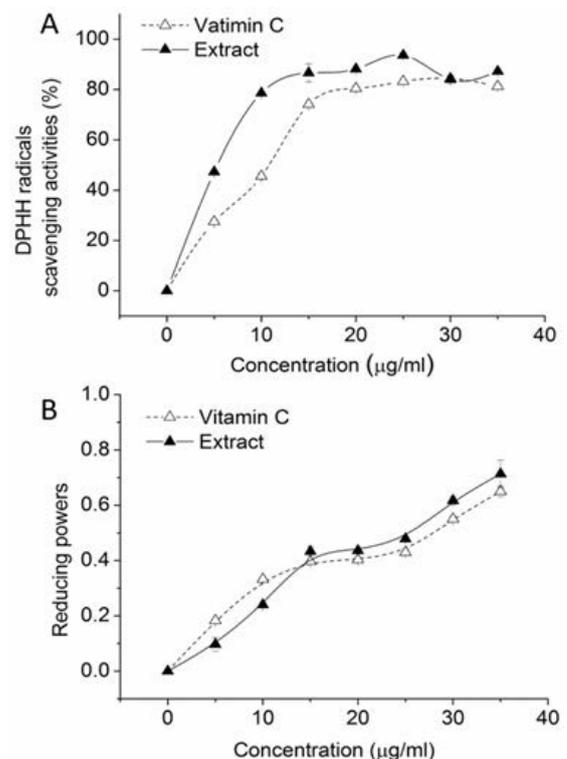

Figure 2. DPPH radical−scavenging activities (A) and reducing powers (B) of cabbage extract and vitamin C

3.2. Effect of Cabbage Extract on Serum Lipid and Hepatic Enzymes

The effect of oral administration of cabbage extract on serum lipid of rats fed high fat diet are summarized in Figure 3. Keeping the rats on a high–fat diet significantly increased the TC, TG and LDL–C levels in serum of Groups II (rats fed with high–fat diet) and III (rats fed with high−fat diet plus extract) as compared to Group I (rats fed with normal diet). The serum levels of TC, TG and LDL–C decreased in rats treated with cabbage extract by 36.3, 43.5 and 45.8%, respectively, whereas the level of serum HDL–C increased by 17.1% as compared to Group II (P < 0.05).
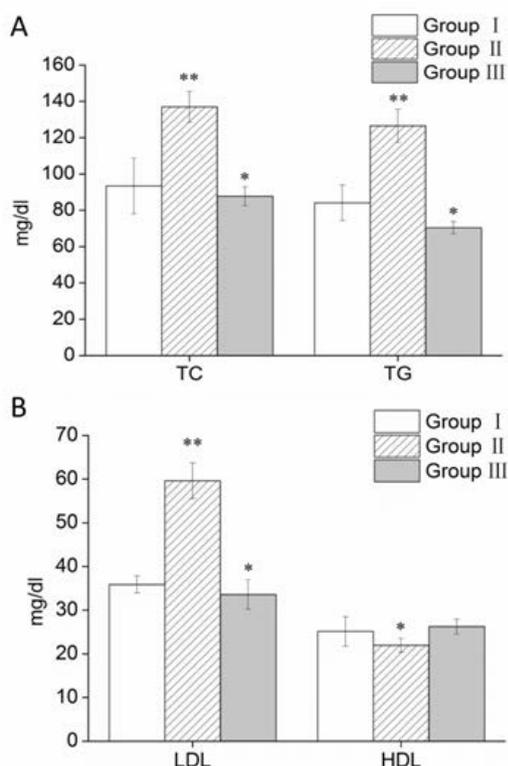

Figure 3. Effects of cabbage extract on the serum lipid level (A: TC, TG; B: LDL,HDL). Values are mean±S.D of 10 parallel measurements. **P < 0.01, compared with Group I; * P < 0.05, compared with Group II. Group I, normal diet–fed rats; Group II, high–fat diet–fed rats; Group III, high–fat diet plus cabbage extract–fed rats

ALP, AST and ALT serum levels were performed to assess liver function. As can be observed in Figure 4, animals fed with high–fat diet exhibited an elevation in serum ALP, AST and ALT compared to normal group. Administration of cabbage extract reduced ALP, AST and ALT activities compared to animals of Group II. Treatment with the extract significantly reduced these enzymes, implying that the plant has executed a protective effect against high–fat diet–induced liver damage.

3.3. Effect of Cabbage Extract on Liver Oxidative Status

Figure 5 shows that MDA level of the liver was significantly decreased upon crude extract therapy in Group III, whereas high–fat diet rats (Group II) showed enhanced levels of lipid peroxidation. It may be noted in Figure 5, the GSH level was lower among the high–fat diet rats (Group II).

However, treatment of the hypercholesterolaemic rats with cabbage extract significantly increased the GSH content.
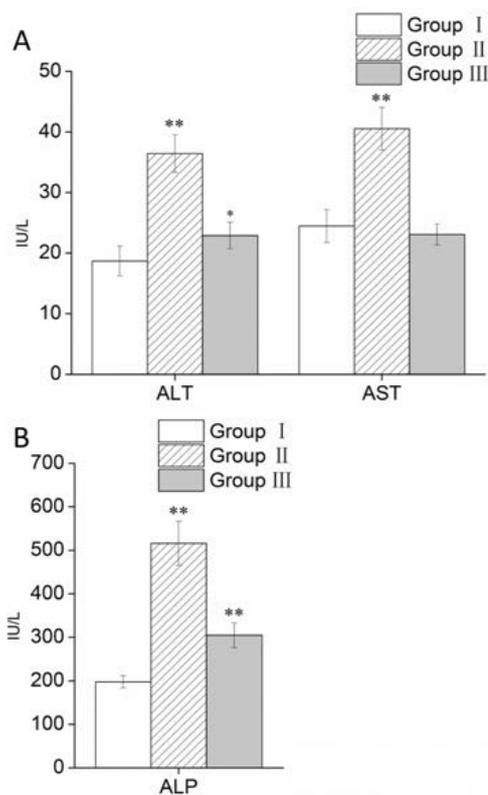

Figure 4. Effects of cabbage extract on the hepatic enzyme levels (A: ALT, AST; B: ALP). Values are mean±S.D of 10 parallel measurements. ** P < 0.01, compared with Group I; * P < 0.05, compared with Group II. Group I, normal diet–fed rats; Group II, high–fat diet–fed rats; Group III, high–fat diet plus cabbage extract–fed rats.

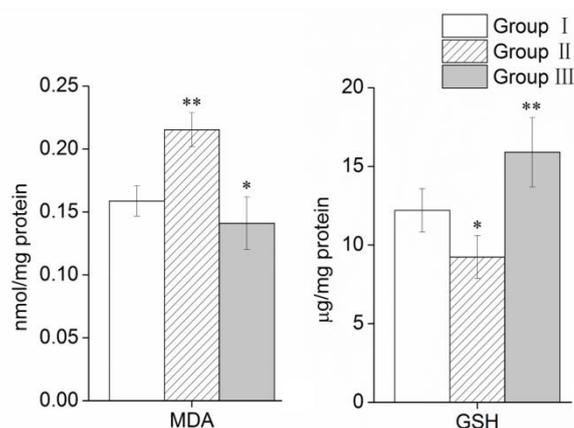**Figure 5.** Effects of cabbage extract on hepatic levels of MDA (nmol/mg protein) and GSH (µg/mg protein) in rats fed high–fat diet. Values are mean±S.D of 10 parallel measurements. ** P < 0.01, compared with Group I; * P < 0.05, compared with Group II. Group I, normal diet–fed rats; Group II, high–fat diet–fed rats; Group III, high–fat diet plus cabbage extract–fed rats

Figure 6 shows the CAT and SOD activities of the liver tissues. SOD and CAT activities of liver tissues have significantly decreased after 30 days of high–fat diet administration, relative to control group. However, cabbage extract administration at a dose of 240 mg/kg resulted in a significant increase in both CAT and SOD activities. Figure 6 also revealed the changes in hepatic GPx and GR activities after 30 days of high–fat diet

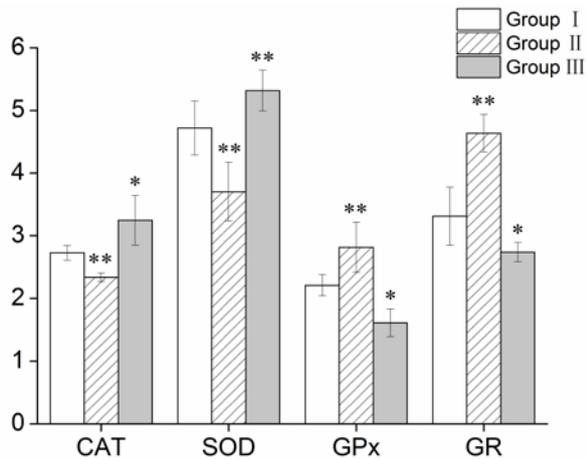consumption. Activities of GPx and GR had significantly increased compared to rats of the control group.

Figure 6. Effects of cabbage extract on hepatic levels of CAT ($\times 10^{-1}$ K/mg protein), SOD (U/mg protein), GR (nmol/min/mg protein) and GPx (nmol/min/mg protein) in rats fed high–fat diet. Values are mean±S.D of 10 parallel measurements. ** $P < 0.01$, compared with Group I; * $P < 0.05$, compared with Group II. Group I, normal diet–fed rats; Group II, high–fat diet–fed rats; Group III, high–fat diet plus cabbage extract–fed rats

4. Discussion

Despite the depth of knowledge concerning the pathogenesis of atherosclerosis, a potential therapeutic approach remained to be disclosed. There is growing evidence that excess generation of highly reactive free radicals, largely due to hypercholesterolaemia and hyperlipedaemia, causes oxidative stress, which further exacerbates the development and progression of atherogenesis.

In recent years, applications of dietary plants with antioxidative property have been the center of focus for improving the life quality of patients with hypercholesterolaemia who suffer from severe oxidative stress [6]. Antioxidants can interfere with the oxidation process by reacting with free radicals, chelating free catalytic metals and also by acting as oxygen scavengers. Thus, antioxidant defense systems have coevolved with aerobicmetabolismto counteract oxidative damage from ROS.

Most studies have attributed the deleterious effects of high dietary fat intake on lipoprotein–lipid such as an increase in serum LDL, triacylglycerol, cholesterol levels and a decrease in high–density lipoprotein level, and on liver function such as increase in serum ALP, AST and ALT levels [20]. Epidemiological evidence from many studies overwhelmingly supports the fact that supplement of antioxidants is significantly associated with a reduction in the level of oxidized lipoprotein. In our study, the administration of cabbage extract to rats fed on a high–fat diet significantly restored these abnormal lipid peroxidation and live function indices to near normal level, indicating that the extract has significant hypocholesterolaemic and liver protective effects.

In an effort to evaluate the mode of action, we examined the effects of cabbage extract on some of the hepatic oxidative parameters of the experimental animals. The characterization of specific oxidants responsible for modification of biomolecules in disease processes has been challenging. Biomarkers of ROS reactions have the potential not only to determine the extent of oxidative injury, but also to predict the potential efficiency of therapeutic strategies aimed at reducing the oxidative stress [21]. A decrease in lipid peroxidation leads to the reduction of atherosclerosis caused by hypercholesterolaemia [22]. The content of MDA in rats fed the high–fat diet was elevated as compared to rats fed the normal diet, suggesting that hypercholesterolaemia could enhance the process of lipid peroxidation. A possible explanation may lie in the finding that hypercholesterolaemia could elevate the cholesterol content of platelets, polymorphonuclear cells, leukocytes and endothelial cells. This initiates a series of reactions which may lead to generation of oxygen free radicals, thus speeding up the course of lipid peroxidation [22]. Moreover, end products of lipid peroxidation, such as MDA and 4–hydroxynonenal, are also protein inactivating agents, possibly due to Schiff base reaction. Through theses peroxides, the binding of apolipoprotein B (apo B) to its receptor is perturbed, and this may lead to deposition of LDL in vascular wall [23]. Our results demonstrated that oral administration of cabbage extract prevented the high–fat diet–induced elevation of MDA and resulted in a significantly decreased content of MDA in the liver homogenates.

The biological effects of ROS are controlled in vivo by a wide spectrum of enzymatic and non–enzymatic defense mechanisms such as SOD which catalyzes dismutation of superoxide anions into hydrogen peroxide (H_2O_2) and CAT and GPx which detoxify H_2O_2 and convert lipid hydroperoxides to nontoxic alcohols. The most important hepatic detoxification elements are GPx, GR and GSH [24]. Under oxidative stress, GSH is largely consumed by GSH–related enzymes, thereby resulting in induction of some intoxication [25]. In the present study, high–fat diet enhanced the activities of glutathione–related enzymes and decreased the glutathione content. The levels of GPx and GR were increased in compensatory manner in an attempt to maintain homeostasis, whereas cabbage extract reversed these effects. It is conceivable that the effect of cabbage extract may initially be due to a reduction in hepatic peroxidative activities followed by inhibition of the activities of GSH–related enzymes, thereby leading to restoration of glutathione content in high–fat diet.

SOD and CAT are the major enzymes dealing with ROS in most cells. Both enzymes play an important role in the elimination of ROS derived from the redox processes of xenobiotics in liver tissues. Different studies have shown that hypercholesterolaemia diminishes the antioxidant defence system and decreases the activity of SOD and CAT, thereby elevating the lipid peroxide content [26]. Interestingly, it has been suggested that CAT and SOD are easily inactivated by lipid peroxides or ROS. This may account for lower SOD and CAT activities in the livers of high–fat diet rats.

GSH is a reactive non–protein thiol in living organisms which performs a key role in coordinating innate antioxidant defence mechanisms. It is involved in the maintenance of normal structure and function of cells, probably through its redox and detoxification reactions [27]. Thus, the decrease in liver GSH level in the hypercholesterolaemic rats might initiate a set of reactions

with the generated free radicals which are largely consumed by GSH–related enzymes. In our study, a dramatic rise in liver GSH level was observed in the cabbage extract–treated rats. This probably indicates that the cabbage extract can either increase the biosynthesis of GSH or reduce the extent of oxidative stress leading to less GSH degradation, or it may have both effects.

Phytochemicals, especially the phenolics found in fruits and vegetables, have been proposed as the major bioactive compounds providing the health benefits associated with diets rich in plant–foods. Many of the biological action of phenolics have been attributed to their powerful antioxidant properties. They can act in several ways, including direct quenching of ROS, chelation of metal ions and regeneration of membrane–bound antioxidants. The antioxidant potency of the extract was also evaluated. Among the various methods used to evaluate the total antioxidant activity of vegetables or other plants, the hydroxyl radicals, superoxide anion radicals and DPPH radical scavenging determinations as well as reducing power assay are the most common applied methods. In the present study, cabbage extract showed higher hydroxyl radical, superoxide anion radical and DPPH radical scavenging activities as well stronger reducing capability.

Numerous studies have been shown the beneficial effect of phenolic compounds against oxidative stress injuries in hypercholesterolaemic patients [28]. Mateos reported that, fruit powders rich in phenolic compounds are very effective in reducing lipid peroxidation in hypercholesterolaemic rats [29]. Furthermore, oral feeding of green tea reduces hyperlipidemia and enhances the activities of SOD and CAT in rats [30]. These studies revealed that beneficial effects of phenolic compounds maybe mediated by one or more mechanisms such as by inhibiting lipid peoxidation, platelet aggregation and enhancing of antioxidant defense. Experimental data indicate that phenolic compounds may offer an indirect protection by activating endogenous defense systems. Several reports have described the positive effects of different classes of phenolic compounds on γ–glutamyl cysteine synthetase (the rate limiting enzyme of glutathione synthetic pathway) activity [31]. Treatment of hepatic cell line HepG2 with different plant–derived compounds has increased γ–glutamyl cysteine synthetase activity and consequently leading to higher GSH concentration [32]. In our study, a dramatic increase in the levels of liver GSH was observed in the rats treated with cabbage extract, which contain 81.6% of chlorogenic acid, 6.3% of caffeic acid, 4.1% of vanillic acid, 2.1% of gallic acid and 1.2% of protocatechuic acid. Regarding the high total phenolics of cabbage extract, they might be responsible for suppressing the extent of lipid peroxidation and enhancing antioxidant capacity in the liver of rats fed high–fat diet.

5. Conclusion

In conclusion, our findings clearly demonstrate the beneficial effect of cabbage extract on hepatic antioxidant status of rats fed high–fat diet. Cabbage extract modulates antioxidant enzyme activities as well as suppressing lipid peroxidation and increasing glutathione levels of the liver. The administration of phenolic extract from cabbage with strong antioxidant activity significantly decreased the blood lipid levels and mediate oxidative stress (activities of antioxidant enzymes and levels of non–enzymic antioxidants) of rat fed high–fat diet. It can therefore be concluded that the extract has a high hypolipidaemic activity and this may be attributed to its antioxidative potential.

Abbreviations

DPPH, 2,2−diphenyl−1−picrylhydrazyl; TC, total cholesterol; TG, triglyceride; LDL−C, low density lipoprotein cholesterol; HDL−C, high density lipoprotein cholesterol; GSH, Glutathione; SOD, superoxide dismutase, CAT, catalase; MDA, malondialdehyde; GPx, glutathione peroxidase; GR, glutathione reductase; TBA, thiobarbituric acid; TCA, tricholoroacetic acid; DTNB, 5,5′−Dithiobis− (2−nitrobenzoic acid); NADH–PMS–NBT, mixture of nicotinamide adenine dinucleotide, phenazine methosulphate and nitroblue tetrazolium; NADH, β−Nicotinamide adenine dinucleotide; NADPH, β−Nicotinamide adenine dinucleotide 2′−phosphate reduced tetrasodium salt; GSSG, glutathione disulfide; EDTA, Ethylenediaminetetraacetic acid.

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