Nutritional Sciences 9(3), August, 2006, pp.159-166
ⓒ 2006 The Korean Nutrition Society

Antioxidative and Anti-aging Effects of Sancho (*Zanthoxylum schinifolium*) Extract in Rats Fed High Fat Diet*

Mi Jin Jang, Mi Hee Woo[1], Soon Jae Rhee, Sung Hee Cho§

Department of Food Science and Nutrition, Catholic University of Daegu, Gyeongsan, Gyeongbuk 712-702, Korea
[1]Departmnt of pharmacy, Catholic University of Daegu, Gyeongsan, Gyeongbuk 712-702, Korea

This study was performed to investigate antioxidative and anti-aging action of extracts from Sancho (*Zanthoxylum schinifolium*) leaves. Two extracts were obtained by 80% methanol extraction followed by subsequent fractionation with methylene chloride (MC) and n-butanol (B) and fed at one or three levels to rats on normal level (5%) of fat (control) and high fat (20%) in diets. Male Sprague-Dawley rats weighing about 100 g were divided into ten groups such as control diet group (C), control diet+0.50%B group (CB), control diet+0.50%MC group (CMC), high-fat diet group (HF), high-fat diet+0.25%B group (HBL), high-fat diet+0.50%B group (HBM), high-fat diet+0.75%B group (HBH), high-fat diet+0.25%MC group (HMCL), high-fat diet+0.50%MC group (HMCM) and high-fat diet+0.75%MC group (HMCH) and fed each diet for four weeks. The effects of the extracts on antioxidant enzyme activities and indices of lipid peroxidation and aging were seen only in high fat diet groups. Hepatic superoxide dismutase and aryleaterase activities were not changed by Sancho extracts. But glutathione peroxidase, catalase and paraxonase activities were significantly restored by both MC and B at the level of 0.75%. Lipid peroxide which was increased by high fat diet was significantly reduced by B and MC at the level of 0.25% and over. Lipofuscin fluorescence and cabonyl value were increased by high fat diet were reduced byB and MC at the level of 0.5% and over. It is concluded that the Sancho extracts can be utilized as functional ingredients of health foods for reducing oxidative stress.

Key words: *Zanthoxylum schinifolium*, Antioxidative enzyme, Lipofuscin. Carbonyl value

Received May 25, 2006; Revised August 4, 2006; Accepted August 7, 2006

INTRODUCTION

In the 21st century, technological aspects of disease treatment and prevention have been improved through the mechanism of chronic diseases under the clear scientific basis, but the quality of life is still in the 20th century due to the lack of improvements in poor dietary habits.[1] Therefore, the importance of foods that we eat everyday has been more and more emphasized and studies on the prevention of chronic diseases using natural foods have been increased.[2-5]

Among substances in natural foods, quercetin,[6,7] phenols[8], flavone derivatives[9,10] and tocopherol[11,12] have antioxidative actions that can delay lipid oxidation and prevent cancer and cardiovascular diseases. Mun et al.[13] reported that quercetin-3-O-β-D-galactopyranoside, an active ingredient of Sancho(*Zanthoxylum schinifolium*), inhibited the peroxidation of unsaturated fatty acids and eliminated typical fishy smell in fish. In the preliminary study, Jang et al.[14] observed strong inhibitory effect on lipid peroxidation and DPPH radical elimination effect of the root, stem, and leaf of Sancho in various extracts such as methanol, methylene chloride, ethyl acetate, n-butanol, water extract and water-layer precipitate. Thus, the extract of Sancho has been expected to have antioxidative effects but most studies on Sancho have been about enhanced energy metabolism, improved appetite, abdominal pain and diuretic symptoms[15,16], and studies on antioxidative and anti-aging action by Sancho have been insufficient.

Thus, this study was performed to investigate an physiological action of Sancho for the prevention of aging and chronic diseases such as arteriosclerosis, hypertension, and cancer and by observing the antioxidant enzyme

* This study was supported by technology Development Program for Agriculture and Forestry, Ministry of Agriculture and Forestry, Republic of Korea
§ To whom correspondence should be addressed.
(E-mail : shcho@cu.ac.kr)

activity, lipid peroxidation, in the liver of rats after providing various concentrations of extracts of Sancho leaves to high-fat diet. The extracts used were methylene chloride and *n*-butanol fractions that showed relatively excellent effects among various extracts obtained from Sancho leaves on the basis of in vitro preliminary study[14].

MATERIALS AND NETHODS

1. Preparation of Sancho Extracts

The leaves of Sancho(*Zanthoxylum schinifolium*) were purchased from Dong-A Wood Co. located in Jain-myeon, Gyeongsan, Gyeongsangbuk-do in mid-June of 2004 and then freeze-dried and powdered for experimental samples.

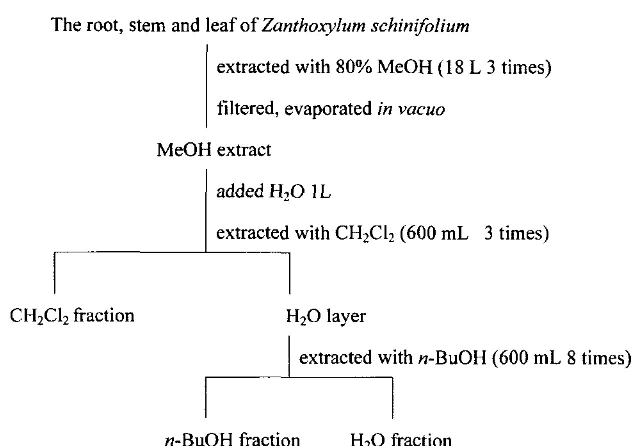

Fig. 1. Extraction and fractionation procedures from leaf of *Zanthoxylyum schinifolium*

The powder of dry leaves were extracted with 80% methanol and concentrated by rotary vacuum evaporator to obtain methanol concentrate. This concentrate was dissolved in 1 L of water and extracted with 600 mL of CH_2Cl_2 three times and concentrated to obtain CH_2Cl_2 fraction. Then the water layer was extracted again with 600 mL *n*-BuOH eight times and concentrated to obtain *n*-butanol fraction. (Fig. 1)

2. Animals and Diets

Experimental animals used in this study were male Sprague-Dawley rats with body weight of about 100 g and acclimated to the environment with commercial rat chow for one week. The experimental group was divided into normal diet group fed 5% fat (17), the control group, and high-fat diet group fed 20% in diet, the comparison group, by randomized complete block design, and then high-fat diet group was divided again by the administered concentration of dietary ingredients to examine the effect of *n*-butanol extract (B) and methylene chloride extract (MC) of Sancho leaves. Therefore, the experimental group of this study was divided into 10 groups such as control diet group (C), control diet + 0.50%B group (CB), control diet + 0.50%MC group (CMC), high-fat diet group (HF), high-fat diet + 0.25%B group (HBL), high-fat diet + 0.50%B group (HBM), high-fat diet + 0.75%B group (HBH), high-fat diet + 0.25%MC group (HMCL), high-fat diet + 0.50%MC group (HMCM) and high-fat diet + 0.75%MC group (HMCH). Experiment diets of the ten groups were shown in Table 1. Each group had 10 rats and physiologically active substance was orally

Table 1. Experimental diet and groups.

(g/1000 g)

Ingredients \ Group[1]	C	CB	CMC	HF	HBL	HBM	HBH	HMCL	HMCM	HMCH
Con starch		150					100			
Casein		200					240			
Sucrose		500					340			
Mineral mix		35					42			
Vitamin mix		10					12			
Corn oil		50					200			
Cellulose		50					60			
DL-methionine		3					3.6			
Choline-chloride		2					2.4			
Buthanol[2]	-	5.0	-	-	2.5	5.0	7.5	-	-	-
Methylene-chloride[3]	-	-	5.0	-	-	-	-	2.5	5.0	7.5

[1] C: normal level of fat (5%, ref.17) group, CB · CMC: normal level of fat with 0.5% butanol and methylene chloride fractions of Sancho extract administered groups, HF: high fa (20%) diet group, HBL · HBM · HBH: high fat diet and 0.25%, 0.50%, 0.75% BuOH fraction administered groups, HMCL · HMCM · HMCH: high fat diet and 0.25%, 0.50%, 0.75% methylene chloride fraction administered groups

[2] Buthanol: buthanol fraction of *Zanthoxylum schinifolium*

[3] Methylene chloride: methylene chloride fraction of *Zanthoxylum schinifolium*

administered everyday for 4 weeks. The body weights of experimental animals were measured once in three days during the study period.

3. Preparation of Samples

After the study period, experimental animals were under light ether anesthesia and the blood was drained from the abdominal aorta. The liver tissue was evenly selected from each lobe and 10% (w/v) homogenate of it was prepared with 0.25 M sucrose/0.5 mM EDTA/5 mM HEPES solution using a Potter-Elvejhem homogenizer. The portion of homogenate was centrifuged at 8,000×g for 20 minutes and the supernatant was used for the quantitative analysis of lipid peroxides, lipofuscin and carbonyl values. Then the remaining portion was centrifuged at 10,000×g for 30 minutes. After obtaining the resultant precipitate(mitochondria), a certain portion of its supernatant was obtained and mixed with 0.4 parts of ethanol : chloroform cold mixture (5:3) and shaken for 2 minutes and then centrifuged at 10,000×g and the portion of supernatant was centrifuged again at 105,000×g for 30 minutes. The final supernatant (cytosol) was measured for the activities of superoxide dismutase, glutathione peroxidase and catalase and the resultant pellet (microsome) was used for measuring paraoxonase and arylesterase activity, and lipofuscin contents. Both mitochondria and microsomal pellets were used for the measurement of carbonyl values. All experimental condition was maintained and performed at 4℃.

4. Measurements of Superoxide Dismutase, Glutathione Peroxidase and Catalase Activity

The superoxide dismutase (SOD) activity was measured according to the method of Marklund and Marklund[18] which used the color production by the autoxidation of pyrogallol in alkaline condition. That is, the reaction was started by adding 0.1 ml of enzyme solution to 1.5 ml of tris-HCl buffer (50 mM Tris / 10 mM EDTA, pH 8.5) and then by adding 0.1 ml of 7.2 mM pyrogallol and then reacted at 25℃ for exactly 10 minutes. The reaction was stopped by adding 0.05 ml of 1N-HCl and the absorbance of oxidized pyrogallol was measured at 420 nm. Then 1 unit of enzyme activity was decided as the amount of enzyme that inhibited 50% of the oxidation of pyrogallol in the reaction solution. The glutathione peroxidase (GSH-px) activity was measured by the method of Lawerence and Burk.[19] That is, 1.72 ml of 0.1 M potassium phosphate buffer, pH 7.0, containing 1 mM EDTA and 0.05 ml of enzyme solution and 0.3 ml of 0.2 mM NADPH, 100 units of glutathione reductase, 0.3 ml of 1 mM sodium azide and 0.3 ml of 1 mM reduced glutathione (GSH) solution were mixed and then 0.3 ml of 0.25 mM H_2O_2 solution to start the reaction at 25℃ and then measured at 340 nm. Then, 1 unit of enzyme activity was decided as the amount of enzyme that produced 1 nmol of oxidized type NADPH during 1 minute. The catalase activity was measured by the method of Aebi et al.[20] in which the H_2O_2 concentration was obtained by the molar absorptivity of H_2O_2 using the changes in the absorption of H_2O_2 at 240 nm for 5 minutes.

5. Measurements of Paraoxonase and Arylesterase Activity

The paraoxonase (PON) activity was measured according to the method of Mackness.[21] A 50 µl fraction of microsomal suspension of liver tissue was mixed with 0.1 M Tris-HCl buffer (pH 8.0) including the reactant 2 mM CaCl2 and 5.5 mM paraoxon (0,0-diethyl-0-*p*-nitrophenylphosphate, Sigma Chemical Co.), the substrate, to make 1 ml of total reactant solution. The changes in absorbance of *p*-nitrophenyl (extinction coefficient: 17,000 $M^{-1}cm^{-1}$) were measured that was produced for 90 seconds at 37℃, 405 nm just after mixing with the enzyme source. The arylesterase activity was measured according to the method of La Du[22] after the same buffer used in the measurement of PON activity was mixed with the substrate, 1 mM phenylacetate (Sigma Chemical Co.), and the enzyme source, 100 µl of microsomal suspension to make 1 ml of total reactant solution, and the absorbance was measured at 37℃, 270 nm for 90 seconds.

6. Measurements of Lipid Peroxide, Lipofuscin and Carbonyl Values

The quantitative analysis of lipid peroxides in liver tissue was performed by using the method of Satoh[23] in which TBARS (thiobarbituric acid reactive substances) was measured. A 0.5 ml of supernatant obtained by centrifuging ground liver tissue at 8,000×g was mixed well with 2.5 ml of 10% TCA solution and left at room temperature for 10 minutes and then centrifuged at 1,500×g for 10 minutes. The supernatant was discarded and the precipitate was rinsed one time with 0.05 M sulfuric acid and the resulting precipitate was mixed well with 2.5 ml of 0.05 M sulfuric acid and 3.0 ml of 0.6% TBA, and then heated at 95℃ in a water bath for 30 minutes and cooled immediately. Then 3.0 ml of *n*-butanol : pyridine mixture (15:1, v/v) was added to this solution and mixed well and centrifuged at 1,500×g for 10minutes. The absorbance of its supernatant was measured at 530 nm. For the reference standard, 1,1,3,3-tetramethoxypropane was used. The measurement of lipofuscin, a wear-and-tear or

aging pigment which has been known to be produced by combining of malondialdehyde (MDA) and protein in the body, was measured according to the method of Fletcher.[24] Carbonyl value was measured by detemining the content of oxidized protein in the microsome of liver tissue according to the method of Levine et al.[25]

7. Quantitative Analysis of Protein

For the measurement of the protein content of each sample, bovine serum albumin was used as reference standard and the quantitative analysis of each protein was performed by using the Lowry method.[26]

8. Statistical Analysis

Statistical analysis of all experimental results was performed by using the analysis of variance to test the difference between the means of each treatment group and if the result (ANOVA test) showed the significance, the significance among groups was analyzed by Tukey's HSD test.[27]

RESULTS AND DISCUSSION

1. Body Weight Changes of Animals

Body weight changes of experimental animals before and after the feeding period were shown in Table. 2. In normal diet groups, there was no significant difference by the administration of the two types of Sancho leaf extract (CB and CMC groups) compared to the control group. But in high fat diet groups, especially HMCL and HMCH groups, administration of the extracts tended to reduce the body weight gains that were higher than the normal control group. But the reduction of body weight appeared to be due to lower food intakes in the groups fed the Sancho extracts so that FER was not affected by feeding the Sancho extracts either in control and high fat groups. Two *in vivo* studies[28,29] were done with mouse fed Sancho methanol extract but did not reportbody weight changes.

Table 2. Body weight gain, food intake and food efficiency ratio (FER) of ten experimental groups.

Groups[1]	Food intake (g/day)	Body weight gai (g/27 days)	FER
C	19.61±1.10[ab2)3)]	97.17± 8.16[c]	0.20±0.03[a]
CB	20.45±1.43[a]	102.57± 8.30[bc]	0.20±0.01[a]
CMC	19.14±1.05[ab]	100.50± 7.12[bc]	0.19±0.02[a]
HF	20.05±0.57[a]	130.40± 3.44[a]	0.15±0.00[b]
HBL	16.74±0.84[b]	122.83± 5.38[a]	0.14±0.01[b]
HBM	18.64±0.71[ab]	121.57±14.85[ab]	0.16±0.02[ab]
HBH	18.50±1.17[ab]	124.67± 5.39[a]	0.15±0.01[ab]
HMCL	17.34±0.52[b]	119.50± 4.37[b]	0.15±0.01[b]
HMCM	17.33±1.83[ab]	110.00±11.43[ab]	0.16±0.01[ab]
HMCH	16.31±1.42[b]	103.33± 5.50[bc]	0.16±0.02[ab]

[1] C: normal level of fat (5%, ref.17)group, CB · CMC: normal level of fat with 0.5% butanol and methylene chloride fractions of Sancho extract administered groups, HF high fat (20%) diet group, HBL · HBM · HBH: high fat diet and 0.25%, 0.50%, 0.75% BuOH fraction administered groups, HMCL · HMCM · HMCH: high fat diet and 0.25%, 0.50%, 0.75% methylene chloride fraction administered groups
[2] All values are mean±SD (n=8)
[3] Values within a column with different superscripts are significantly different at $p<0.05$ by Tukey's test

2. SOD, GSH-px and Catalase Activities

Superoxide dismutase (SOD), glutathione peroxidase (GSH-px) and catalase are the major enzymes for antioxidative defense mechanism in the body. SOD protects the body from reactive oxygen by reducing superoxide radical to H_2O_2, and GSH-px detoxifies organic hydroperoxide as well as hydrogen peroxide using reduced gluthatione while catalase turns hydrogen peroxide into H_2O.[30] As shown in Table 3, the SOD activity was not significantly different among normal diet groups (C, CB and CMC groups). The enzyme activity was decreased in high-fat diet (HF) group compared to the control group but it was not significantly increased in the groups administrated *n*-butanol or methylene chloride extracts of Sancho leaves at three levels (HBL, HBM, HBH, HMCL, HMCM and HMCH groups) compared with HF group. The GSH-px activity was not changed either by the administration of the active substances of Sancho leaves(CB and CMC groups) as for the SOD activity. But the decreased enzyme activity by high fat diet (HF group) tended to be increased by the administration of the active substances of Sancho leaves and the increase was significant in HBH, HMCM and HMCH groups. Changes of the catalase activity were almost same as those of the GSH-pxactivity. These results suggest that the active substances present in the extracts of Sancho leaves are effective in anitioxidative acition in the body via modification of GSH-px and catalase activities rather than SOD activity.

Anioxidant substances reported in various plant have been shown to modify the three enzyme activities but not in the same manner. Green tea, persimmon leaves and safflower seed were reported to increase SOD and GSH-px activities[31] and Kimchi increased all three enzyme activites.[32] But various mulberry products were tested in diabetic rats, the SOD activity was not affected whereas GSH-pxand catalase activities were increased.[33]

Table 3. Effects of n-butanol and methylene chloride fractions of Zanthoxylyum schinifolium administration on the hepatic superoxide dismutase (SOD), glutathione peroxidase (GSH-px) and catalase activities in the normal and high fat diet fed rats.

Groups[1)]	SOD (unit/mg protein/min)	GSH-px (nmol NADPH/min/mg protein)	Catalase (±μmol/min/mg protein)
C	4.11±0.57[2)a3)]	151±15.72[a]	57.07±4.48[a]
CB	3.81±0.13[a]	141±17.38[a]	56.45±6.53[a]
CMC	3.90±0.22[a]	146±10.41[a]	52.05±5.57[a]
HF	2.81±0.20[b]	101±10.62[c]	21.22±4.09[c]
HBL	2.92±0.31[b]	111± 8.52[bc]	24.92±1.01[c]
HBM	2.97±0.21[b]	119±15.61[bc]	30.97±3.46[b]
HBH	3.08±0.18[b]	121±11.92[ab]	39.85±2.84[b]
HMCL	2.82±0.13[b]	121±17.59[bc]	27.90±3.37[c]
HMCM	3.00±0.22[b]	121±11.54[ab]	34.93±5.18[bc]
HMCH	3.15±0.20[b]	131±20.48[a]	37.03±2.32[b]

1) C: normal level of fat (5%, ref.17) group, CB · CMC: normal level of fat with 0.5% butanol and methylene chloride fractions of Sancho extract administered groups, HF high fat (20%) diet group, HBL · HBM · HBH: high fat diet and 0.25%, 0.50%, 0.75% BuOH fraction administered groups, HMCL · HMCM · HMCH: high fat diet and 0.25%, 0.50%, 0.75% methylene chloride fraction administered groups
2) All values are mean±SD (*n*=8)
3) Values within a column with different superscripts are significantly different at p<0.05 by Tukey's test

3. Paraoxonase and Arylesterase

The paraoxonase (PON), a HDL-binding enzyme that inhibits the oxidation of serum HDL and LDL is originated from microsomal fraction of the liver cell and arylesterase, which has been known to be derived from the same cell.[34)] Both enzymes are known to play roles in prevention of atherosclerosis. As shown in Table 4. the PON and arylesterase activities were higher in three C- groups fed normal level of fat in diet than H- groups of high fat diet. There was no changes in the activities of the both enzymes by administration of *n*-butanol or methylene chloride extracts of Sancho leaves (CB and CMCgroups vs C group). But in the high fat group (HF) the PON activity was decreased and restored back to those of C-groups when high level (0.75%) of the extracts was administered (HBH and HMCH groups). But the arylesterase activity was not significantly changed by the extracts even at the high levels in high fat fed groups.

Table 4. Effects of n-butanol and methylene chloride fractions of Zanthoxylyum schinifoliumadministration on the hepatic microsomal paraoxonase (PON) and arylesterase activities in the normal and high fat diet fed rats.

(umol/min/mg protein)

Groups[1)]	Paraoxonase	Arylesterase
C	68.6±7.9[2)a3)]	7.5±0.1[a]
CB	65.6±9.6[a]	7.0±0.2[a]
CMC	67.6±8.3[a]	6.8±0.1[a]
HF	25.6±3.6[c]	2.9±0.6[b]
HBL	30.6±4.5[c]	3.5±0.6[b]
HBM	45.6±3.6[bc]	3.9±0.5[b]
HBH	58.6±4.6[ab]	3.1±0.3[b]
HMCL	35.6±2.6[c]	3.9±0.3[b]
HMCM	43.6±5.2[bc]	4.0±0.6[b]
HMCH	60.6±9.0[ab]	4.4±0.9[b]

1) C: normal level of fat (5%, ref.17) group, CB · CMC: normal level of fat with 0.5% butanol and methylene chloride fractions of Sancho extract administered groups, HF high fat (20%) diet group, HBL · HBM · HBH: high fat diet and 0.25%, 0.50%, 0.75% BuOH fraction administered groups, HMCL · HMCM · HMCH: high fat diet and 0.25%, 0.50%, 0.75% methylene chloride fraction administered groups
2) All values are mean±SD (n=8)
3) Values within a column with different superscripts are significantly different at p<0.05 by Tukey's test

4. TBARS Content

Accumulation of lipid peroxides in the liver has become the index of oxidative damage of the tissue and has been reported to increase under the conditions of chronic diseases such as diabetes.[35)] As shown in Fig. 2, the level of lipid peroxide measured as thiobarbituric acid reactive substances (TBARS) was increased as dietary fat was high (C group vs HF group). The increased contents of TBARS was reduced significantly by the administration of both *n*-butanol and methylene chloride extracts of Sancho leaves even at the level of 0.25% (HBL) and further lowered as the levels were higher. As compared to the changes in antioxidative enzyme activities, the response of TBARS was greater at the same level of the extracts of Sancho leaves. This result indicates that the active substances in the extracts play direct antioxidant roles in preventing production of lipid peroxide by scavenging free radicals or reduction of peroxide in addition to modification of antioxidative enzyme activity. Recently five types of polyphenol were purified and identified from *n*-butanol fractions and four related compounds from methylene chloride fractions of of Sancho leaves.[36)] It is regarded that these compounds are major ones playing direct action on reducing the TBARS contents.

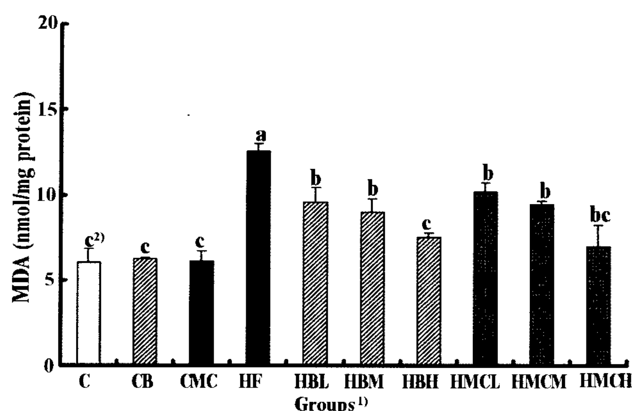

Fig. 2. Effects of *n*-butanol and methylene chloride fractions of *Zanthoxylyum schinifolium* administration on the hepatic TBARS values in the high fat diet fed rats.

1) C: normal level of fat (5%, ref.17) group, CB · CMC: normal level of fat with 0.5% butanol and methylene chloride fractions of Sancho extract administered groups, HF high fat (20%) diet group, HBL · HBM · HBH: high fat diet and 0.25%, 0.50%, 0.75% BuOH fraction administered groups, HMCL · HMCM · HMCH: high fat diet and 0.25%, 0.50%, 0.75% methylene chloride fraction administered groups.

2) All values are mean±SD (n=8) and those with different letters are significantly different at $p<0.05$ by Tukey's test

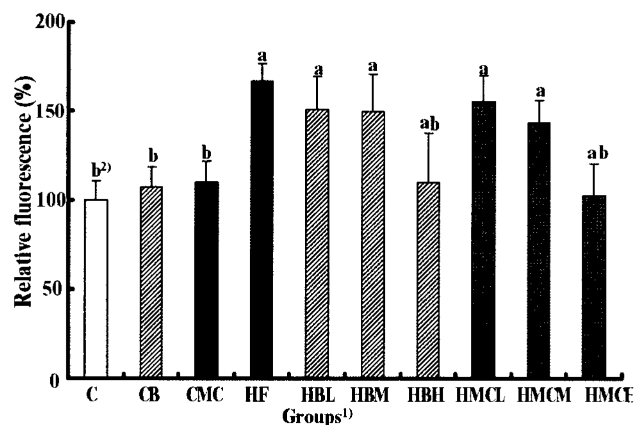

Fig. 3. Effects of *n*-butanol and methylene chloride fractions of *Zanthoxylyum schinifolium* administration on the hepatic lipofuscin fluorescence (%) in the normal (C) and high (H) fat diet fed rats.

1) C: normal level of fat (5%, ref.17) group, CB · CMC: normal level of fat with 0.5% butanol and methylene chloride fractions of Sancho extract administered groups, HF high fat (20%) diet group, HBL · HBM · HBH: high fat diet and 0.25%, 0.50%, 0.75% BuOH fraction administered groups, and HMCL · HMCM · HMCH: high fat diet and 0.25%, 0.50%, 0.75% methylene chloride fraction administered groups.

2) All values are mean±SD (n=8) and those with different letters are significantly different at $p<0.05$ by Tukey's test.

5. Lipofuscin Fluorescence and Carbonyl Value

Lipofuscin and carbonyl value are believed as an important indice of aging and carbonyl value, in particular,.

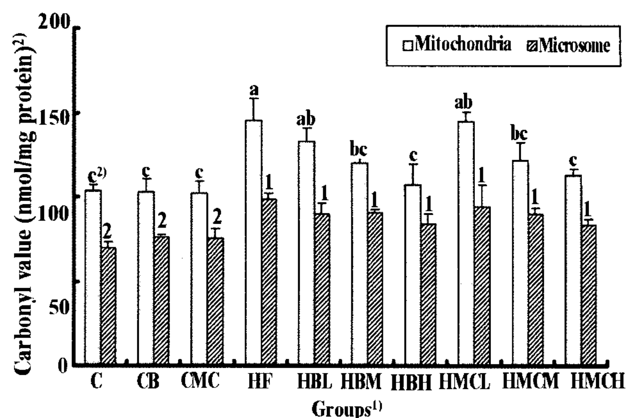

Fig. 4. Effects of n-butanol and methylene chloride fractions of Zanthoxylyum schinifolium administration on the hepatic mitochondria and microsomal carbonyl values in the high fat diet fed rats

1) C: normal level of fat (5%, ref.17) group, CB · CMC: normal level of fat with 0.5% butanol and methylene chloride fractions of Sancho extract administered groups, HF high fat (20%) diet group, HBL · HBM · HBH: high fat diet and 0.25%, 0.50%, 0.75% BuOH fraction administered groups, HMCL · HMCM · HMCH: high fat diet and 0.25%, 0.50%, 0.75% methylene chloride fraction administered groups

2) All values are meanSD (n=8) and those with different letters are significantly different at $p<0.05$ by Tukey's test

represents protein oxidation.[37,38] Lipofuscin contents of liver tissue were shown in Fig. 3 as relative values to that of the fluorescence absorbance of the control group and carbonyl values of mitochondria and microsome fractions of the liver tissue are shown in Fig. 4. Both lipofuscin and carbonyl value were increased by high fat diets. Llipofuscin levels were not significantly decreased by the extracts of Sancho leaves below the administration level of 0.75% (HBH and HMCH), while mitochondrial carbonyl values were reduced by 0.5% and higher levels of the extracts of Sancho leaves. Less sensitive effect by the extracts on reducing lipofuscin and carbonyl values than TBARS contents may be due to more complicated mechanisms involved in the formation of the formers than the latter in the liver. However, our previous study using mulberry and silk worm showed the different results.[33] Therefore, the effects appears to be more related to the active substances in each study and the effects of the Sancho leaf extracts shown in this study seem to be ascribed to characteristics of the nine polyphenols present in the extracts.[36]

In summary, the two types of extract obtained from Sancho leaf (*Zanthoxylum schinifolium*) had the obvious antioxidative effects under the condition of high fat diet enhancing an oxidative stress *in vivo* and the safety of the each extract should be evaluated in detail to utilize them for development of new functional foods in the future.

Literature cited

1) Choi HM. NUTRITION & HEALTH. Life Science Publication Co., 2002
2) Shin DH. Antioxidation substances in mulberry leaf. *J Korean Oil Chemists Soc* 16(3):27-31, 1998
3) Kodama T, Ishida H, Kokubo T, Yamakawa T, Noguchi H. Glucosylation of quercetin by a cell suspension culture of vitis species. *Agric Biol Chem* 54:3238-3288, 1990
4) Chae JY, Lee JY, Hoang IS, Whangbo D, Choi PW, Lee WC, Kim JW, Kim SY, Choi SW, Rhee SJ. Analysis of functional components of leaf of different mulberry cultivars. *J Korean Soc Food Sci Nutr* 32:15-21, 2003
5) Frielovich I. The biology of oxygen radicals: The superoxide radical as an agent of oxygen toxicity: Superoxide dismutase provides an important defense. *Sci* 201:875-880, 1978
6) Naitoh K. Studies on the micro constituent in mulberry leaves part 2. Isolation of rutin and quercetin from mulberry leaves. *Nippon Nogei Kagaku Kaishi* 42:422-425, 1968
7) Shin KH, Young HS, Lee TW, Choi JS. Studies on the chemical component and antioxidative effects of solanum lyratum. *Kor J Pharmacogn* 26:130-138, 1995
8) Nakayama T. Suppression of hydroperoxide induced cytotoxicity by polyphenol. *University of Shizuoka Japan* 7:1991-1993, 1994
9) Chi YS, Jong HG, Son KH, Chang HW, Kang SS, Kim HP. Effects of naturally occurring prenylated flavonoids on enzymes metabolizing arachidonic acid: cylcooxygenases and lipoxygenases. *Biochemical pharmacology* 62:1185-1191, 2001
10) Kwak CS, Lee SH. Carboxylesterase, arylesterase and cholinesterase activities in cholestatic rat liver induced by common bile duct *ligation. Korean Biodchem J* 25(3):251-261, 1992
11) Park KY, Kang CS, Lee YS, Lee YH, Lee YS. Tocotrienol and tocopherol content in various plant seeds. *Korean J Crop Sci* 49:207-210, 2004
12) Cha WS, Shin HR, Park JH, Oh SL, Lee WY, Chun SS, Choo TW, Cho YJ. Antioxidant activity of phenol compounds from Mulberry fruits. *Korean J Food Preservation* 11:383-387, 2004
13) Mun SI, Ryu HS, Lee HJ, Choi JS. Further screening for antioxidant activity of vegetable plants and its active principles from *Zanthoxylum schinifolium. J Korean Soc Food Nutr* 23:466-471, 1994
14) Jang MJ, Woo MH, Kim YH, Jun DY, Rhee SJ. Effects of antioxidative, DPPH radical scavenging activity and antithrombogenic by the extract of sancho (*Zanthoxylum schinifolium*). *J Korean Nutr* 38:386-394, 2006
15) Lee MS, Chung MS. Analysis of volatile flavor components from Zanthoxylum schinifoliumand sensory evaluation as natural spice. *Korean J Soc Food Sci* 16:216-220, 2000
16) Lim SJ, Han HK, Ko JH. Effects of edible and medicinal plants intake on blood glucose, glycogen and protein levels in streptozotocin induced diabetic rats. *J Korean Nutr* 36:981-989, 2003
17) Reeves PG, Nielsen FH, Fahey Jr GC. AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc writing committee on the freformulation of the AIN-76A dodent diet. *J Nutr* 123:1939-1951, 1993
18) Marklund S, Marklund G. Involvement of the superoxide anion radical in the antioxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem* 47:469-474, 1974
19) Richard AL, Raymond FB. Glutathione peroxidase activity in selenium-deficient rat liver. *Biochem and BiopHysical Research Communications* 71(4):952-958, 1976
20) Aebi H, Wyss SR, Scherz B, Skvaril F. Heterogeneity of erythrocyte catalase II. Isolation and Characterization of normal and variant erythrocyte catalase and their subunits. *Eur J Biochem* 48:137-145, 1974
21) Mackness MI, Harty D, Bhatnagar D, Winocour PH, Arrol S, Ishola M, Durrington PN. Serum paraoxonase activity in familial hypercholesterolemia and insulin-dependent diabetes mellitus. *Atherosclerosis* 86:193-199, 1991
22) La Du BN, Exkerson HW. The polymorpHic paraoxonase/ arylesterase isozymes of human serum. *Fed Proc* 43:2338-2341, 1992
23) Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clinica Chemica Acta* 90:37-43, 1978
24) Fletcher BL, Dillard CJ, Tappel AL. Measurement of fluorescent lipid peroxidation products in biological systems and tissues. *Analytical Biochemistry* 52:1-9, 1973
25) Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, Ahn B, Shaltiel S, Stadtman ER. Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol* 186:464-478, 1990
26) Oliver HL, Nira JR, Lewis F, Rose JR. Protein measurement with the folin phenol reagent. *J Biol Chem* 193:265-275, 1951
27) Sreel RGD, Torrie JH. Principles and procedures of satistics. McGrow Hill New York, USA, 1990
28) Mun SI, Ryu HS, Choi JS. Inhibition effects of *Zanthoxylum schinifolium* and its active principle on lipid peroxidation and liver damage in carbon tetrachloride-treated mice. *J Korean Soc Food Nutr* 26:943-951, 1997
29) Mun SI. Effects of *Zanthoxylum schinifolium* and its active principle on serum lipid levels in carbon tetrachloride-treated mice. *Korean J Food & Nutr* 13:249-254, 2000
30) Halliwell B, Gutteridge MC. Free radicals inbiology and medicine. pp.166-170, Oxford University Press Oxford, 1985
31) Kim MJ, Cho SY, Jang JY, Park JY, Park EM, Lee MK, Kim DJ. Effect of water extract of green tea, persimmon leaf and safflower seed on heme synthesis and erythrocyte antioxidant enzyme activities in lead-administered rats. *J Korean Soc Food Sci Nutr* 32:191-196, 2003
32) Kim JH, Kwon MJ, Lee SY, Ryu JD, Moon GS, Cheigh HS, Song YO. The effect of kimchi intake on production of free radicals and anti-oxidative enzyme activities in the liver of SAM. *J Korean Soc Food Sci Nutr* 31:109-116, 2002
33) Kwon RH, Jung MA, Rhee SJ, Choi SW, Cho SH. Antioxidative

effects and improvement of lipid metabolism of mulberry fruit, mulberry leaves and silkworm powder with different mixing ratios in streptozotocin-induced diabetic rats. *Korean J Nutr* 39:91-99, 2006

34) Kim WS, Kim YS. Paraoxogenase polymorphism as a new genetic marker of atherosclerosis. *Korean Society for Lipid and Atherosclerosis* 6:81-86, 1996

35) Griesmacher A, Kindhauser M, Andert SE, Schreiner W, Toma C, Knoebl P, Pietschmann P, Prager R, Schnack C, Schernthaner G. Enhanced serum levels of thiobarbituric-acid-reacive substances in diabetes mellitus. *Am J Med* 98:469-475. 1995

36) Fang Z. Bioactive constituents from the leaves of *Zanthoxylum schinifolium*. MS thesis, Dept. of Pharmacy, Daegu catholic University, 2006

37) Choi JH, Kim DI, Park SH, Kim DW, Lee KG, Yeo JH, Kim JM, Lee YW. Effects of silk fibroin powder on lipofuscin, acetylcholine and Its related enzyme activities in brain of SD rats. *Korean J Seric Sci* 42:120-125, 2000

38) Fridovich I. Bioligical effects of the superoxide radical. *Arch Biochem Biophys* 247:1-15, 1986