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Effects of Chicken Extract on Antioxidative Status and Liver Protection under Oxidative Stress

Hsiang-Chi PENG and Shyh-Hsiang LIN*

School of Nutrition and Health Sciences, Taipei Medical University, 250 Wu-Hsing Street, Taipei, Taiwan 110

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Summary Chicken extract contains carnosine and anserine, both of which possess some antioxidant abilities The objective of this study was to investigate the protective effects of chicken extract in male Sprague-Dawley (SD) rats under induced oxidative stress Carbon tetrachloride was used as the oxidative stress inducer Glutamic-oxalacetatic transaminase (GOT), glutamic-pyruvic transaminase (GPT), total antioxidant status (TAS), thiobarbituric-reactive substances (TBARS), iron content, and the activities of antioxidant enzymes were determined We concluded that under oxidative stress, the intake of chicken extract was helpful in promoting the activities of antioxidant enzymes and in protecting the liver from oxidative damage

Key Words chicken extract, oxidative stress, antioxidative enzymes

Many diseases are related to oxidative damage caused by free radicals (*1, 2*) The role of food is not only to provide nutrients and calories but also to protect our bodies from chronic or oxidative damage Chicken extract is one of the traditional foods that have been consumed in Asian countries for centuries It is made through the process of high-pressure steaming of deboned chickens, then defatting, and concentrating the extract (*3*) Chicken extract is rich in amino acids, peptides, and minerals It has been found that consuming chicken extract is helpful for emotional control and improving working efficiency (*4*) Wade and Tucker (*5*) indicated that carnosine and anserine in chicken extract both possess antioxidative abilities Anserine and carnosine are composed of histidine and alanine The only difference between them is that a methyl group is attached to the imidazole ring of anserine Chan et al (*6*) found that carnosine, alone or together with vitamin E, reduced the thiobarbituric-acid reactive substances (TBARS) in the muscle of rats It was also found that the addition of carnosine to the diet slowed down the aging process of senescence-accelerated mice (*7*) Carbon tetrachloride (CCl_4) is a lipid-soluble chemical that induces the generation of free radicals The introduction of CCl_4 may induce a decrease in the activities of antioxidative enzymes, depletion of glutathione, and release of ferric ions, which result in lipid oxidation in cells (*8*) The indexes of peroxidation induced by CCl_4 are plasma TBARS concentration and ferric peroxidation (*9*) Injection of CCl_4 resulted in decreases of antioxidants in plasma The objective of this study was to investigate the effect of chicken extract consumption on the plasma antioxidant status in Sprague-Dawley (SD) rats under an induced oxidative stress by CCl_4

* To whom correspondence should be addressed
E-mail lin5611@tmu edu tw

MATERIALS AND METHODS

The chicken extract used in this experiment was produced locally

Animals Fifty-eight 6-wk-old healthy male Sprague-Dawley (SD) rats (National Laboratory Animal Center, Taiwan) were randomly assigned into four groups according to different diets Guidelines for the ethical care and treatment of animals from the Animal Care Committee at Taipei Medical University were strictly followed Rats were individually housed and maintained in a temperature controlled (23±2°C) room with a 12-h light/dark cycle They were fed chow diet for 1 wk before switching to the experimental diet Water and food were available ad libitum Fasting blood samples from the tail vein were collected in tubes containing heparin on the last day of weeks 2, 4, and 6 The blood was then centrifuged for 15 min at 1,400 ×*g* to separate the plasma and erythrocytes, which were separately stored at −80°C Starting from the 4th wk, rats received an abdominal injection of CCl_4 (0 2 mL/100 g body weight) once a week until the end of the experiment The body weight of each rat was recorded every 2 days At the end of wk 8, rats were killed and liver samples were collected, weighed, and stored at −80°C The control diet was AIN76 (A) Three experimental diets were prepared by mixing 1 5 (B), 3 0 (C), and 7 5 mL/kg-d (D) of chicken extract with the control diet (Table 1) The average calories between each diet did not significantly differ

Glutamic-oxalacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) determinations GOT and GPT were measured using commercial kits (GOT and GPT reagent L-type, Wako)

Antioxidative status Total antioxidative ability (TAS) was determined using a commercial (NX2332, Randox) kit Reduced glutathione (GSH) was determined using a

Table 1 The compositions of the control and experimental diets[1]

Ingredients	A*	B	C	D
Corn starch (%)	52 7	52 7	52 7	52 7
Casein (%)	20 0	20 0	20 0	20 0
Cellulose (%)	7 0	7 0	7 0	7 0
Soybean oil (%)	6 0	6 0	6 0	6 0
Mineral mixture (%)	6 0	6 0	6 0	6 0
Vitamin mixtute (%)	2 0	2 0	2 0	2 0
Sucrose (%)	6 0	6 0	6 0	6 0
Methionine (%)	0 3	0 3	0 3	0 3
Chicken extract (mL/kg-d)	0	1 5	3 0	7 5

[1] The total energy of each diet was not significantly different ($p<0$ 05) * A, control group, B, 1 5 mL/kg-d, C, 3 0 mL/kg-d, D, 7 5 mL/kg-d

commercial kit (cat no 354102, CalBiochem)

Activities of antioxidant enzymes The activities of antioxidation enzymes were determined using commercial kits superoxide dismutase (SOD) no 574600, Calbiochem, glutathion peroxidase (GPx) RS504, Randox, and glutathione reductase (GR) GR2368, Randox

Thiobarbituric acid reactive substances (TBARS) A blank (0 1 mL of water), a standard (0 1 mL of 4 2 μM malonaldehyde), or 0 1 mL of a sample was mixed well with the reagent (a mixture of 4 mL of 22% sulfuric acid, 0 5 mL of 10% phosphotungstic acid, and 1 mL of 0 67% 2-thiobarbituric acid) Then the mixture was heated in a water-bath at 95°C for 1 h After being cooled with ice water, 2 mL of butanol was added and the mixture was centrifuged at 3,000×g for 15 min The optical density of the pinkish upper part was determined using a fluorescence spectrometer with excitation at 515 nm and emission at 555 nm

Statistics All results are presented as the mean±SD Two-way analysis of variance (ANOVA) and the least significant difference test were performed using SAS® 6 13 to analyze the time and diet effects

RESULTS AND DISCUSSION

Body weights of rats The amounts of chicken extract fed to rats were equivalent to about 75, 150, and 375 mL for a normal adult in a day The average body weight of each group increased before the injection of CCl_4 in wk 4 The average body weight of those rats fed 7 5 mL/kg-d of chicken extract was the lowest and started to decrease ($p<0$ 05) from wk 4 On the other hand, the average body weight for the rats fed 1 5 mL/kg-d of chicken extract was the highest after wk 4 ($p<0$ 05) The ratio of intake to weight gain in group D (7 5 mL/kg-d) was also the lowest ($p<0$ 05) In our observations during the experiment, those rats fed 7 5 mL/kg-d had a more yellowish hair color than others This could have been due to a nutritional imbalance or to an unpleasant flavor caused by too much chicken extract in the diets

Antioxidative status in plasma As shown in Table 2, the Fe(II) concentrations increased in chicken extract-fed groups through wk 6 to wk 8 ($p<0$ 05) The groups fed higher amounts of chicken extract had higher concentrations of Fe(II) compared to the control group On the other hand, Fe(III) concentrations decreased in all chicken extract-fed groups The ratio of Fe(II) to Fe(III) in the chicken extract-fed groups tended to increase while that in the control group remained unchanged throughout the experimental period The higher the ratio of Fe(II) to Fe(III), the more lipid oxidation that had occurred (*10*) Because chicken extract is a rich source of iron, intake of chicken extract may have coun-

Table 2 The effect of chicken extract on the concentrations of plasma ferrous, ferric irons and the ratio of ferrous to ferric iron in rats treated with CCl_4 under oxidative stress

	Wk 0	Wk 2	Wk 4	Wk 6	Wk 8
Fe^{2+}(μM)					
A*	8 97±1 84^{a}	10 72±2 08^{ab}	10 97±1 74^{b}	8 93±2 59$^{a\,1}$	10 40±1 71$^{ab\,1}$
B	9 30±1 27^{a}	10 55±0 90^{bc}	9 47±0 80^{ab}	10 54±0 92$^{bc\,1}$	11 20±1 47$^{c\,12}$
C	9 64±1 74^{a}	11 13±1 13^{b}	10 13±1 23^{ab}	12 76±0 96$^{c\,2}$	12 47±1 38$^{c\,2}$
D	8 97±1 94^{a}	11 38±1 78^{b}	11 05±2 40^{b}	14 69±1 32$^{c\,3}$	14 51±1 01$^{c\,3}$
Fe^{3+}(μM)					
A	12 28±3 20^{a}	12 58±4 72^{a}	13 97±5 33^{a}	12 52±4 62$^{a\,1}$	11 13±1 11^{a}
B	15 41±4 64^{a}	12 14±3 89^{ab}	11 68±3 96^{bc}	8 23±2 99$^{c\,2}$	10 07±2 45^{bc}
C	12 66±3 24^{a}	12 18±4 68^{a}	11 03±2 18^{ab}	7 15±1 20$^{c\,2}$	9 19±1 94^{bc}
D	13 32±3 12^{a}	12 74±3 83^{a}	12 51±2 13^{a}	9 62±1 86$^{b\,12}$	10 57±2 22^{ab}
Fe^{2+}/Fe^{3+}					
A	0 78±0 25^{a}	0 96±0 40^{a}	0 92±0 50^{a}	0 85±0 50$^{a\,1}$	0 94±0 13$^{a\,1}$
B	0 66±0 25^{a}	0 95±0 29^{ab}	0 91±0 36^{bc}	1 41±0 44$^{c\,2}$	1 21±0 48$^{bc\,12}$
C	0 83±0 32^{a}	1 03±0 37^{a}	0 95±0 23^{a}	1 83±0 32$^{b\,2}$	1 39±0 20$^{c\,2}$
D	0 69±0 18^{a}	0 95±0 27^{a}	0 91±0 29^{a}	1 58±0 34$^{b\,12}$	1 44±0 45$^{b\,2}$

Data are presented as the mean±SD (n=8) $^{a\,b\,c}$ Within a row, values with the same letters do not significantly differ $^{1\,2}$ Within a column, values with the same numbers do not significantly differ * A, control group, B, 1 5 mL/kg-d, C, 3 0 mL/kg-d, D, 7 5 mL/kg-d

Table 3 The dosage effect of chicken extract on the activities of erythrocyte superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione reductase (GR) in rats treated with CCl_4 under oxidative stress

	Wk 0	Wk 2	Wk 4	Wk 6	Wk 8
SOD (U-525/g protein)					
A*	305 2±55 9^{ab}	342 9±59 9$^{a\,12}$	215 2±20 0$^{c\,1}$	176 8±56 3$^{c\,1}$	278 9±53 6$^{b\,1}$
B	329 6±54 6^{a}	339 2±46 7$^{a\,2}$	263 3±28 9$^{b\,2}$	316 4±72 9$^{ab\,2}$	363 4±52 4$^{a\,2}$
C	320 2±45 3^{a}	322 6±68 3$^{a\,2}$	226 4±14 7$^{b\,1}$	347 3±71 5$^{a\,2}$	339 4±32 8$^{a\,2}$
D	324 6±75 8^{a}	396 4±34 7$^{b\,1}$	237 3±26 3$^{c\,1}$	367 3± 39 6$^{a\,2}$	348 6±27 3$^{a\,2}$
Catalase (KU/mg protein)					
A*	0 064±0 018^{a}	0 065±0 015$^{a\,1}$	0 064±0 019$^{a\,1}$	0 057±0 010$^{a\,1}$	0 071±0 013$^{a\,1}$
B	0 061±0 009^{a}	0 068±0 008$^{a\,1}$	0 087±0 098$^{b\,2}$	0 068±0 020$^{a\,12}$	0 098±0 011$^{b\,2}$
C	0 070±0 011^{a}	0 085±0 012$^{ab\,2}$	0 103±0 027$^{c\,2}$	0 078±0 014$^{a\,2}$	0 096±0 007$^{bc\,2}$
D	0 064±0 009^{a}	0 081±0 007$^{b\,2}$	0 087±0 009$^{b\,2}$	0 082±0 008$^{b\,2}$	0 103±0 025$^{c\,2}$
GPx (U/g protein)					
A	212 9±23 2$^{a\,1}$	203 0±4 2$^{ab\,12}$	189 5±21 3$^{bc\,1}$	188 9±25 5$^{bc\,1}$	180 9±11 0$^{c\,1}$
B	218 0±29 9$^{a\,1}$	203 6±6 4$^{ab\,12}$	198 5±23 6$^{ab\,1}$	197 8±25 8$^{ab\,1}$	193 8±11 9$^{b\,2}$
C	214 1±32 8$^{a\,1}$	191 7±13 5$^{b\,2}$	204 3±23 1$^{ab\,1}$	192 8±9 9$^{b\,1}$	202 2±15 1$^{ab\,2}$
D	198 2±13 9$^{ab\,1}$	211 1±19 7$^{a\,2}$	194 3±15 1$^{b\,1}$	198 5±11 6$^{ab\,1}$	195 4±13 5$^{b\,2}$
GR (U/g protein)					
A	28 0±2 0^{a}	28 6±0 7$^{a\,1}$	27 7±1 5^{a}	29 3±2 9$^{a\,1}$	28 8±1 0$^{a\,1}$
B	27 9±1 9^{a}	33 8±2 7$^{b\,2}$	35 6±3 0$^{b\,2}$	33 3±2 8$^{b\,2}$	36 0±2 5$^{b\,2}$
C	27 3±0 7^{a}	37 8±4 1$^{b\,3}$	35 4±1 6$^{bc\,2}$	34 6±2 2$^{c\,2}$	36 9±1 9$^{bc\,2}$
D	27 6±0 9^{a}	28 0±1 2$^{a\,1}$	30 6±1 3$^{b\,3}$	34 6±3 5$^{c\,2}$	35 4±3 3$^{c\,2}$

Data are presented as the mean±SD (n=8) $^{a\,b\,c}$ Within a row, values with the same letters do not significantly differ $^{1\,2\,3}$ Within a column, values with the same numbers do not significantly differ * A, control group, B, 1 5 mL/kg-d, C, 3 0 mL/kg-d, D, 7 5 mL/kg-d

teracted the reduction in Fe-related lipid oxidation by carnosine and anserine in this study

Table 3 shows the results for the activities of superoxide dismutase (SOD), erythrocyte catalase, glutathione peroxidase (GPx), and glutathione reductase (GR) in the plasma of rats during the experiment The injection of CCl_4 induced the generation of peroxyl radicals Before the CCl_4 injection, SOD activities in all groups did not differ significantly After the injection of CCl_4, the rats fed chicken extract had higher SOD activities than those in the control group ($p<0$ 05) CCl_4 reduced the SOD activity in the control group ($p<0$ 05) The SOD activities in the chicken extract-fed group recovered to normal in wk 6 The function of SOD in the body is converting reactive oxygen species (ROS) to hydrogen peroxide Tras et al (*11*) found that feeding chickens a normal diet with additional antioxidants increased the activity of SOD The carnosine and anserine in chicken extract are antioxidants that could increase or maintain the SOD activity in rats when attacked by pro-oxidants such as CCl_4 On the contrary, CCl_4 did not affect the activity of catalase, GPx or GR, while chicken extract effectively increased their activities At the end of the feeding period, the rats fed chicken extract had higher activities of catalase, GPx or GR, than did the control group ($p<0$ 05) Lalonde et al (*13*) and Marzatico et al (*14*) found that under oxidative stress, the activity of catalase decreased due to the free redicals produced It was also found that the presence of antioxidants in the plasma increased the activities of GPx and other antioxidative enzymes in mice (*15, 16*) Thus, the antioxidative ingredients may help scavenge peroxyl free radicals and increase the activities of antioxidative enzymes

Results for the total antioxidative status (TAS) in rats were inconsistent (data not shown) TAS is affected by anti- or pro-oxidative substances According to previous results, both the activities of antioxidative enzymes and the contents pro-oxidative materials such Fe were increased, which may be the reason that we did not see a clear result on TAS in the experiment Kadiiska et al (*17*) indicated that TAS was not a proper index for evaluating the antioxidative condition in plasma since the major target organ that CCl_4 affects is the liver

In the liver Liver samples were collected at the beginning and the end of the feeding period The peroxyl radicals generated by CCl_4 caused liver damage Before the injection of CCl_4, no significant differences in GOT or GPT were observed (data not shown) After the introduction of CCl_4, both GOT and GPT in rats of the control group went up in wk 6 ($p<0$ 05) while those in rats of the experimental groups remained stable At the end of wk 8, both GOT and GPT in the control group decreased, but GPT in the control group was still significantly higher ($p<0$ 05) than in the experimental groups In addition, the activities of SOD, catalase, GPx, and GR in chicken extract-fed groups were not affected by CCl_4 (Table 4) This may indicate that the intake of chicken extract had a protective effect on the liver from oxidation damages Antioxidants increase or maintain the activities of antioxidative enzymes (*16*) Thus, the amino acid- or dipeptides-rich chicken extract did provide the antioxidative effect in the livers

Table 4 The dosage effect of chicken extract on the activities of antioxidative enzymes in the liver of rats treated with CCl_4 under oxidative stress

	A*	B	C	D
SOD (U-525/g protein)				
Baseline	1,298 7±312 1	1,298 7±312 1	1,298 7±312 1	1,298 7±312 1
Week 8	1,517 2±206 9	1,286 0±401 3	1,687 2±215 1[1]	1,708 7±241 7[1]
Catalase (KU/mg protein)				
Baseline	1 2±0 3	1 1±0 3	1 2±0 3	1 2±0 3
Week 8	1 1±0 3	1 0±0 2	1 1±0 2	1 0±0 1
GPx (U/g protein)				
Baseline	560 4±66 4	560 4±66 4	560 4±66 4	560 4±66 4
Week 8	509 1±15 4	530 2±46 3	574 6±69 6	578 6±116 0
GR (U/g protein)				
Baseline	797 3±34 4	797 3±334 4	797 3±334 4	797 3±334 4
Week 8	755 2±80 0	811 0±93 2	763 7±124 7	775 4±154 3

Data are presented as the mean±SD (n=8) [1]significantly different from the baseline *A, control group, B, 1 5 mL/kg-d C, 3 0 mL/kg-d, D, 7 5 mL/kg-d

Table 5 The dosage effect of chicken extract on the concentration of liver TBARS (mM/L) in rats treated with CCl_4 under oxidative stress

	Wk 0	Wk 2	Wk 4	Wk 6	Wk 8
A*	27 2±4 7^{a}	42 0±4 9$^{b\,1}$	29 9±8 1^{a}	49 5±6 2^{c}	43 5±4 9$^{bc\,1}$
B	27 7±2 1^{a}	38 6±3 3$^{b\,12}$	28 3±5 1^{a}	48 5±3 8^{c}	48 7±6 0$^{c\,2}$
C	27 3±3 6^{a}	35 6±3 0$^{b\,2}$	31 8±4 2^{ab}	51 1±9 3^{c}	49 8±4 3$^{c\,2}$
D	27 8±3 6^{a}	35 1±3 5$^{b\,2}$	31 4±5 4^{ab}	43 6±3 9^{c}	35 2±2 9$^{b\,3}$

Data are presented as the mean±SD (n=8) $^{a\,b\,c}$ Within a row, values with the same letters do not significantly differ $^{1\,2}$ Within a column, values with the same numbers do not significantly differ * A, control group, B, 1 5 mL/kg-d, C, 3 0 mL/kg-d, D, 7 5 mL/kg-d

The result (Table 5) of thiobarbituric acid-reactive substances (TBARS) in the liver indicated that consuming chicken extract tended to inhibit the lipid oxidation in the liver, although not significantly At the end of the experiment, the rats fed 7 5 mL/kg-d of chicken extract tended to have a lower TABRS value CCl_4 in rats caused lipid peroxidation As the oxidative stress in the body increased, the TBARS in the body also increased (*18, 19*) The TBARS decreased if antioxidants such as carnosine and anserine in chicken extract appear in the body (*20*)

The above results indicated that consumption of chicken extract inserted a protective effect on the liver from oxidative damage by increasing or maintaining the activities of antioxidative enzymes under an oxidative stress In addition, chicken extract is rich in proteins and amino acids, and thus, it may help with liver reconstitution Consuming a moderate amount of chicken extract did not affect the growth of rats even under oxidative stress Chicken extract positively increased the activities of antioxidative enzymes in plasma, was helpful in protecting the liver from oxidative damage, and maintained the activities of antioxidative enzymes in the liver despite the presence of oxidative stress

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