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Effect of Colored Potato Flakes Against Acetaminophen-induced Liver Damage in Rats

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Abstract We examined the hepatoprotective effects of colored potato flakes on acetaminophen (AAP)-induced liver damage in rats. F344/DuCrj (8 week-old) rats were fed a cholesterol-free diet with 54.9486 g of α-corn starch/100 g diet and were orally treated with 25% colored flakes of *Kitamurasaki* (KM: light purple), *Northern Ruby* (NR: red), and *Shadow Queen* (SQ: medium purple) potatoes co-administered with AAP (0.5 g/100 g diet) for 4 weeks. The hepatic thiobarbituric acid-rea ctive substances (TBARS) values in the KM, NR, and SQ groups were significantly lower ($p<0.05$) than those in the control groups with and without AAP. Furthermore, the hepatic catalase, Mn-superoxide dismutase (SOD), and Cu/Zn-SOD mRNA levels in the KM, NR, and SQ groups were higher than those in the control groups with and without AAP. The present findings suggest that colored potato flakes are useful as a prophylactic agent against oxidative liver damage.

Keywords: acetaminophen, catalase, color potato flake, Cu/Zn-superoxide dismutase, Mn-superoxide dismutase, TBARS value

Introduction

It is known that liver injury occurs via direct injurious attack by a wide variety of primary hepatotoxins, including alcohol, aflatoxins, heavy metals, and drugs. Among these, acetaminophen (AAP) is a commonly used analgesic-antipyretic drug, but overdoses can result in hepatic failure in both humans (1) and experimental animals (2, 3). This hepatotoxicity is likely related to the oxidative stress in the cell, ultimately leading to its demise. Generally, a major metabolite, *N*-acetal-*p*-benzoquinone imine is detoxified by conjugation with glutathione (GSH), and binds to other important cellular macromolecules (4). The involvement of oxidative stress has been found in the AAP-induced free radical-mediated pathway, since the hepatic lipid peroxidation level is elevated after AAP treatment both *in vivo* (2) and *in vitro* (5).

In epidemiological studies, the consumption of anthocyanins through fruit- and vegetable-based intake has been shown to be connected to improved health (6-8). The positive effects of these pigments could be related to their potent antioxidative activities demonstrated in various studies (9, 10). Recently, Han *et al.* (11) have reported the protective action of an adzuki extract against acetaminophen-induced hepatotoxicity via a hepatic GSH-mediated antioxidation/detoxification system in rat liver after 4 weeks of feeding. However, few *in vivo* nutraceutical studies have been published on the hepatoprotective activity of colored potatoes against chemically induced liver damage. Furthermore, at present, the mechanism of colored potato flake protection against AAP-induced liver damage remains unclear.

Therefore, the present study was conducted to clarify the hepatoprotective effects of colored potato flakes against AAP-induced toxicity, and to elucidate the mechanisms underlying these protective effects in rats.

Materials and Methods

Preparation of colored potato flakes *Kitamurasaki* (KM: light purple), *Northern Ruby* (NR: red), and *Shadow Queen* (SQ: medium purple) potato flakes were a kind gift from Somatech Center, House Foods Corporation, Japan. The preparation of potato flakes was as follows: KM, NR, and SQ potatoes were thoroughly washed with water and air dried on filter paper, and then they were peeled and sliced. The sliced potatoes were treated with steam blanching at 100°C to minimize enzymatic reactions that bring out degrading anthocyanins. Next, they were mashed and dried in a drum dryer at 120°C, and finally ground into flakes.

Animals and diets Male F344/DuCrj rats (7 week-old) were purchased from Charles River Japan (Yokohama, Japan). All animals were housed individually in cages on a 12-hr light/dark cycle. Temperature was maintained at 23± 1°C with 60±5% relative humidity. Animals were randomly assigned into 5 groups, each of 5 animals. There was no significant difference in body weights between the groups at the start of the experiment. The composition of

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each diet is shown in Table 1. Diets were based on the AIN-93G semipurified rodent diet (12). Rats were fed KM, NR, or SQ potato flakes at 250 g/kg diet with acetaminophen (AAP) at 0.5 g/100 g diet for 4 weeks (11, 13). Control rats were fed a diet with cornstarch instead of potato flakes for 4 weeks with and without AAP. The rats were allowed free access to experimental diets and water for the 4 weeks. Body weight and feed consumption were recorded weekly and daily, and blood samples were taken every week. This experimental design was approved by the Animal Experiment Committee of Obihiro University of Agriculture and Veterinary Medicine. All animal procedures described conformed to standard principles in *Guide for the Care and Use of Laboratory Animals* (14).

Analytical procedures Blood samples (1 mL) were collected between 8:00 and 10:00 from the jugular veins of fasting rats anaesthetized with sodium pentobarbital. At the end of the experimental period of 4 weeks, blood samples (1 mL) were collected. The samples were taken into tubes without an anticoagulant. After the samples stood at room temperature for 2 hr, serum was prepared by centrifugation at 1500×g for 20 min. At the end of the experimental period of 4 weeks the rats were anesthetized with diethyl either. Then the liver was quickly removed, washed with cold saline, dehydrated on filter paper, and weighed before freezing for storage.

Chemical analysis Triglyceride and free fatty acid concentrations, and alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in the serum were evaluated with commercially available kits for the TDX system (Abbott Laboratory Co., Irving, TX, USA). Lipid peroxidation in the hepatic homogenate was assessed by the thiobarbituric acid-reactive substances (TBARS) method (15). The reaction mixture contained 0.2 mL of homogenate, 0.2 mL of 0.8% SDS, 1.5 mL of 20% acetic acid solution (pH 3.5), and 1.5 mL of 0.5% aqueous solution of TBA. The mixture was heated for 60 min in a boiling water bath. After cooling, 4 mL of *n*-butanol was added and mixed vigorously. The organic phases were separated by centrifugation, and absorbance was measured using a spectrophotometer at 532 nm. Protein content was determined as described by Lowry *et al.* (16).

RNA isolation, reverse transcription-polymerase chain reaction (RT-PCR), and Southern blot analysis As described previously (17), total RNA in the liver was isolated using ISOGEN (Nippon Gene, Tokyo, Japan) according to the User's manual. The amounts of mRNA encoding catalase, glutathione reductase (GSH-R), Mn-superoxide dismutase (Mn-SOD), Cu/Zn-superoxide dismutase (Cu/Zn-SOD), and glycelaldehyde-3-phosphate dehydrogenase (GAPDH, as an internal standard) were estimated by semi-quantitative RT-PCR and subsequent Southern blot analysis (16). The set of oligonucleotide primers for the PCR of GAPDH was as reported previously (17). Those for catalase, GSH-R, Mn-SOD, and Cu/Zn-SOD were: catalase, sense primer 5'-CTG GTT AAT GCG AAT GGA GA-3', anti-sense 5'-TGG GGT AGT AGT TGG GAC CAC-3'; GSH-R, sense 5'-GCC GCA GCG TTA TTG TGG GTG-3', anti-sense 5'-CGA TAG GCG GGT GGC TGA AGA C-3'; Mn-SOD, sense 5'-CGC TGT CAC TGT CAT CAT AAG-3', anti-sense 5'-GTC CGG TGC AGG GCG TCA TTC-3'; Cu/Zn-SOD, sense 5'-GTC CGG TGC AGG GCG TCA TTC-3', anti-sense 5'-CAA TCA CAC CAC AAG CCA AGC-3'. Probes with digoxigenin (DIG) were hybridized to blots. DIG was detected by using an anti-DIG antibody (Boehringer Mannheim, Mannheim, Germany). The probe for GAPDH was reported previously (6, 8). The other probes used were: catalase, 5'-AGT TGG CCA CGC GAG

Table 1. Compositons of the experimental diets (g/kg)

	Dietary group				
	Control without AAP	Control with AAP	KM	NR	SQ
Casein	200	200	200	200	200
Sucrose	100	100	100	100	100
Mineral mixture[1)]	35	35	35	35	35
Vitamin mixture[2)]	10	10	10	10	10
α-Corn starch	549.486	544.486	294.486	294.486	294.486
Kitamurasaki flake	0	0	250	0	0
Northern Ruby flake	0	0	0	250	0
Shadow Queen flake	0	0	0	0	250
L-Cystine	3	3	3	3	3
Cellulose power	50	50	50	50	50
Soybean oil	50	50	50	50	50
Choline bitartrate	2.5	2.5	2.5	2.5	2.5
tert-Butyl hydroquinone	0.014	0.014	0.014	0.014	0.014
Acetaminophen (AAP)	0	5	5	5	5

[1,2)]These diets were based on the AIN-93G diet composition.

CAC GGT AGG GAC AGT TGA CAG TTA TCT TCA GAT AGT TTG-3'; GSH-R, 5'-ACC ACA TGG AGT TCC AAG CCC GAT GAG GTC TTC TTT ACT TCC TTA ACC TGT GAG-3'; Mn-SOD, 5'-CAG TCA GGA GCC TAG CTT GGG TCT GTT GAT TTG TTC AGT AGT GAG GTA GAC CC-3'; and Cu/Zn-SOD, 5'-AGC AGC CAC ATT GCC CAG GTC TCC AAC ATG CCT CTC TTC ATC CGC TGG ACC GCC-3'. The probe was 3'-tailing labeled with digoxigenin, using a DIG oligonucleotide tailing kit (Boehringer Mannheim). Prehybridization, hybridization, and detection were carried out with a DIG luminescent detection kit (Boehringer Mannheim) as recommended by the manufacturer. The relative quantity of mRNA was estimated by densitometry scanning with x-ray film. The intensity of bands was quantitatively analyzed using NIH image version 1.63.

Statistical analysis Data are presented as means and standard deviations. The significance of differences among all groups was determined by ANOVA with Duncan's multiple-range test (SAS Institute, Cary, NC, USA).

Results and Discussion

Polyphenol concentrations of colored potato flakes The contents of total phenols, flavonoids, and anthocyanins from potato flakes are shown in Table 2. The contents of polyphenols in KM, NR, and SQ potato flakes were 241.7, 460.1, and 505.4 mg/100 g of powder, respectively. The contents of flavonoids in KM, NR, and SQ potato flakes were 65.7, 91.9, and 131.4 mg/100 g of powder, respectively. Moreover, the contents of total monomeric anthocyanins in KM, NR, and SQ potato flakes were 55.5, 92.0, and 213.8 mg/100 g powder, respectively.

Food intake, rat growth, cecum weight, and liver weight The food intake, rat growth, cecum weight, and liver weight are shown in Table 3. There were no significant differences in the food intake, body weight, and cecum weight among the groups. However, the liver weight in the NR group was significantly higher ($p<0.05$) than in the other groups.

Serum lipid concentrations and enzyme activities Figure 1 shows the serum triglyceride and free fatty acid concentrations in rats. The triglyceride concentration in the control group with AAP was significantly high ($p<0.05$) among the groups. On the other hand, the triglyceride concentrations in the KM, NR, and SQ groups were significantly lower ($p<0.05$) than in the control group with AAP, and that in the KM group was significantly lower ($p<0.05$) than in the control groups with and without AAP. The free fatty acid concentrations in the KM, NR, and SQ groups were significantly lower ($p<0.05$) than in the

Table 2. Micronutrient and antioxidant contents in colored potato flakes

g/100 g powder	*Kitamurasaki* flake (Light purple)	*Northern Ruby* flake (Red)	*Shadow Queen* flake (Medium purple)
Water	5.2	4.0	5.7
Protein	5.8	8.7	7.6
Lipid	0.4	0.5	0.5
Carbohydrate	84.6	82.4	82.0
Soluble fiber	2.6	2.5	2.3
Insoluble fiber	3.2	3.6	3.5
Ash	4.0	4.4	4.2
Polyphenols (gallic acid mg/100 g)	241.7	460.1	505.4
Anthocyanin (mg/ 100 g)	55.5	92.0	213.8
Flavonoid (catechin mg/100 g)	65.7	91.9	131.4
Calorie (kcal/100 g)	365	369	363

Table 3. Body weight, food intake, liver weight, and cecum weight in rats fed color potato flakes for 4 weeks[1)]

Components	Dietary group				
	Control without AAP	Control with AAP	KM	NR	SQ
Initial body weight (g)	185±6	187±6	185±5	186±4	187±7
Final body weight (g)	249±10	250±6	249±10	251±14	250±8
Body weight gain (g/4 week)	64±8	63±9	64±7	65±11	63±4
Food intake (g/4 week)	435±25	444±32	436±26	441±44	451±36
Liver weight (wet g/100 g B.W.)	341±0.16^{b}	376±0.37^{b}	3.74±0.43^{b}	417±0.62^{a}	367±0.22^{b}
Cecum weigh (wet g)	364±0.44	4.52±0.70	4.23±0.58	440±0.70	4.24±0.57

[1)]Values are expressed as means±SD for 5 rats.
^{a,b}Means within the same rows bearing different superscript roman letters are significantly different ($p<0.05$).

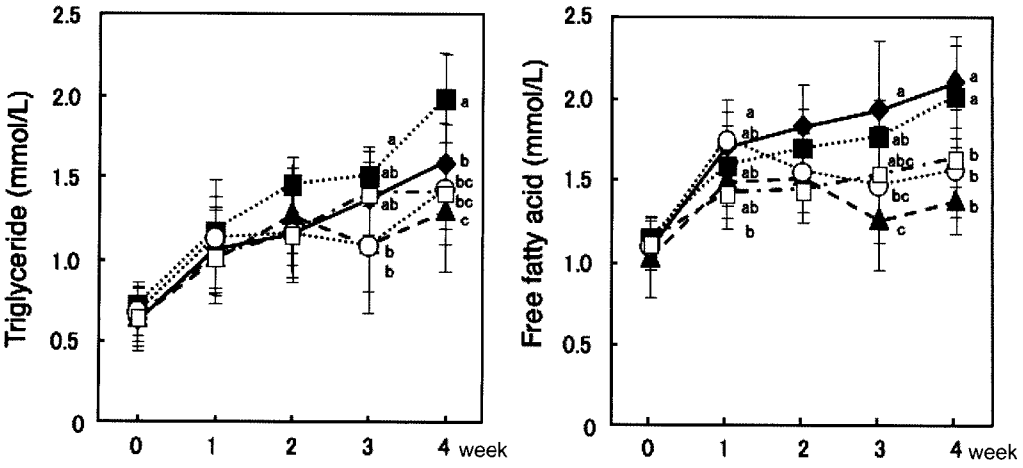

Fig. 1. Serum triglyceride and free fatty acid concentrations in rats fed colored potato flakes and acetaminophen for 4 weeks. Values are means for 5 rats, with standard deviations indicated by bars. Means values (a, b, c) were significantly different ($p<0.05$), as determined by analysis of variance with Duncan's multiple-range test. Control without acetaminophen (◆); control with acetaminophen (■); *Kitamurasaki* potato flakes (▲); *Northern Ruby* flakes (○); *Shadow Queen* flakes (□).

control groups. The liver protective effect of purple sweet potato anthocyanin via depression of serum AST activity has been reported (18). However, administration of AAP to rats at repeated doses at 0.5 g/100 g of diet for 4 weeks did not affect serum ALT and AST compared with the control group without AAP in the present study (not shown data). On the other hand, the administration of AAP increased the serum triglyceride concentration compared with the other treatment groups. Oda *et al.* (19) reported expression of the HMG-CoA reductase gene and malic enzyme gene induced by polychlorinated biphenyls in primary cultured rat hepatocytes and showed that the action of polychlorinated biphenyls on the gene expression relating to lipid metabolism was directed at hepatocytes. In this study, expression of the malic enzyme gene may have been induced by AAP, which might account for the higher serum triglyceride concentration in the control group with AAP. The AAP-induced alteration in hepatic antioxidant status might therefore be a manifestation of increased tissue oxidative stress caused by AAP metabolism (4). On the other hand, the KM, NR, and SQ potato flakes decreased the serum triglyceride concentration as compared with the control group with AAP. However, there was no difference in the serum free fatty acid concentration between the control groups with and without AAP. Thus, it may be that AAP did not affect lipase activity such as that of lipoprotein lipase or hepatic triglyceride lipase, though the reason for was not clear in the present study. Furthermore, the free fatty acid concentrations in the KM, NR, and SQ groups decreased as compared with both control groups. Potato starch contains abundant phosphate and phosphorylated oligosaccharides from potato starch increased the short chain fatty acid concentration in the cecal content (20). Thus, the phosphorylated oligosaccharides from the potato flake starches in the small intestine may have affected the lipid metabolism in the present study.

Liver TBARS concentration The TBARS concentrations in the KM, NR, and SQ groups were significantly lower ($p<0.05$) in the control groups with and without AAP, but there was no difference in the TBARS concentration among the colored potato flake groups (Fig. 2). Since biological membranes contain highly oxidizable polyunsaturated fatty acids, they are particularly vulnerable to radical attack. Thus, treatment with the pigmented substances of colored potato flakes appears to be helpful in conditions related to oxidative stress induced by AAP metabolites. In the present study, the contents of polyphenol in KM, NR, and SQ potato flakes were 241.7, 460.1, and 505.4 mg/100 g of color potato flake powder, respectively, and the rats fed about 604, 1,150, or 1,264 mg of KM, NR, or SQ polyphenol, about 139, 230, or 535

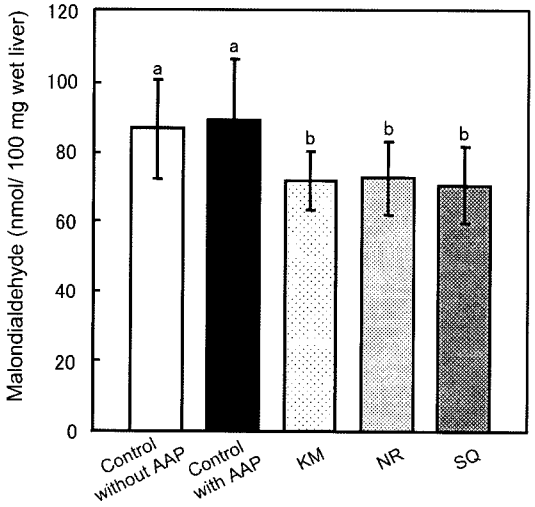

Fig. 2. Hepatic TBARS concentrations in rats fed colored potato flakes and acetaminophen for 4 weeks. Values are means for 5 rats, with standard deviations indicated by bars. Means values (a, b) were significantly different ($p<0.05$), as determined by analysis of variance with Duncan's multiple-range test. AAP, acetaminophen; KM, *Kitamurasaki* potato flakes; NR, *Northern Ruby* flakes; SQ, *Shadow Queen* flakes.

mg of KM, NR, or SQ anthocyanin, and about 164, 230, or 329 mg of KM, NR, or SQ flavonoid, per kg diet, respectively. Thus, the intake of at least 600 mg of polyphenol, 140 mg of anthocyanin, 160 mg of flavonoid, per kg of diet may have suppressed the oxidation of lipids in rats in the present study. Tsuda *et al.* (21, 22) reported that 2 g of dietary cyanidin 3-*O*-β-D-glucoside per kg of diet significantly suppressed serum and liver lipid peroxidation provoked by oxidative stress. The pigmented substances of colored potato flakes may have more antioxidative function than cyanidin 3-*O*-β-D-glucoside.

Hepatic antioxidant enzyme mRNAs Figure 3 and 4 show the hepatic catalase, Mn-SOD, Cu/Zn-SOD, and GSH-R mRNA levels in rats. The catalase mRNA levels in the KM, NR, and SQ groups were significantly higher ($p<0.05$) in the control groups with and without AAP, but there was no significant difference in the catalase mRNA level among the colored potato flake groups. The Mn-SOD mRNA levels in the colored potato flake groups tended to be higher than in the control groups, and that in the SQ group was significantly higher ($p<0.05$) than in the control groups. The Cu/Zn-SOD mRNA levels in the colored potato flake groups also tended to be higher and

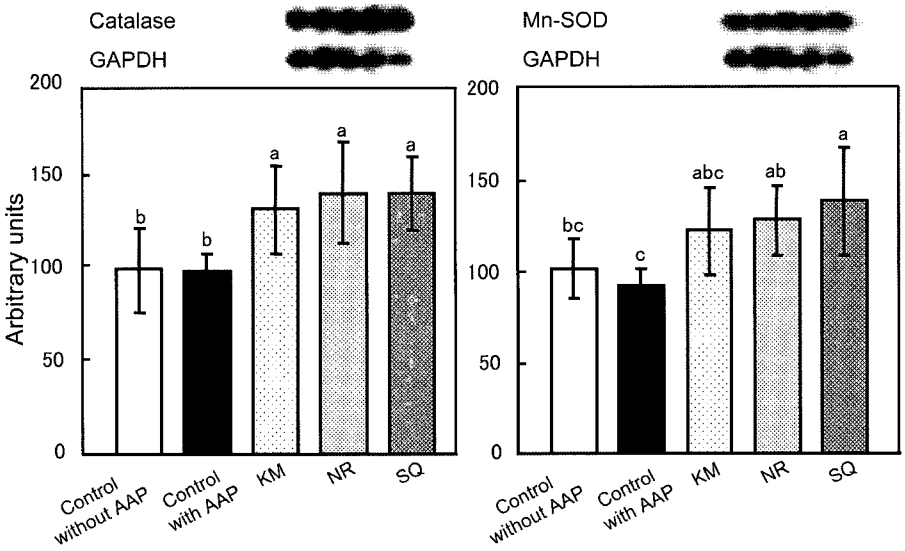

Fig. 3. Hepatic catalase and Mn-SOD mRNA levels in rats fed colored potato flakes and acetaminophen for 4 weeks. Values are means for 5 rats, with standard deviations indicated by bars. Means values (a, b, c) were significantly different ($p<0.05$), as determined by analysis of variance with Duncan's multiple-range test. AAP, acetaminophen; KM, *Kitamurasaki* potato flakes; NR, *Northern Ruby* flakes; SQ, *Shadow Queen* flakes; SOD, superoxide dismutase.

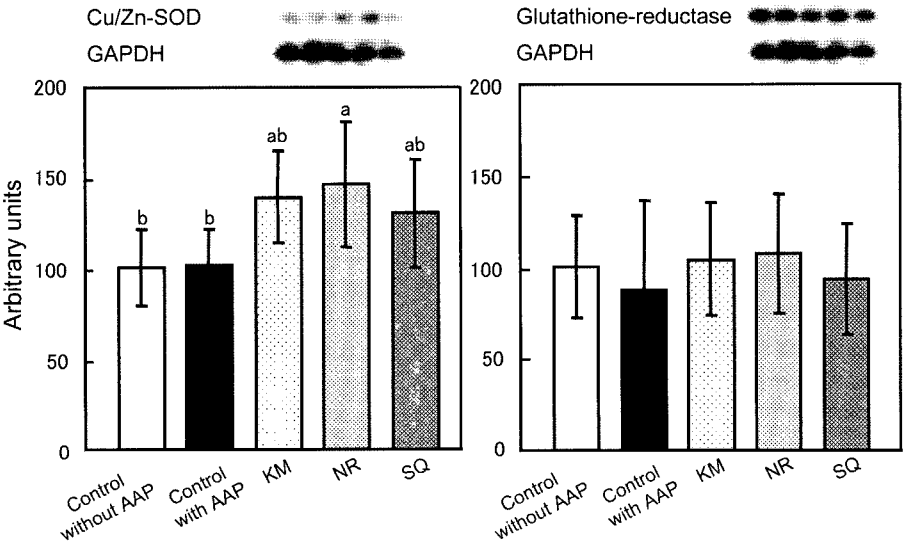

Fig. 4. Hepatic Cu/Zn-SOD and glutathione reductase mRNA levels in rats fed colored potato flakes and acetaminophen for 4 weeks. Values are means for 5 rats, with standard deviations indicated by bars. Means values (a, b) were significantly different ($p<0.05$), as determined by analysis of variance with Duncan's multiple-range test. AAP, acetaminophen; KM, *Kitamurasaki* potato flakes; NR, *Northern Ruby* flakes; SQ, *Shadow Queen* flakes; SOD, superoxide dismutase; GSH, glutathione.

that in the NR group was significantly higher ($p<0.05$) than in the control groups. There was no significant difference in the GSH-R mRNA level among the groups. The hepatic antioxidant enzyme mRNA levels in the colored potato flake groups tended to increase as compared with both control groups, and the results agreed with a previous experiment (23). These enzymes are the key enzymes to catalyze the metabolism of xenobiotics; therefore, the increased activity of these antioxidant enzymes will promote the efficiency of detoxification. Moreover, phenolic acids elevated the antioxidant enzyme activities and mRNA levels under oxidative stress (24-26). Yeh and Yen (26) have indicated that phenolic acids significantly induce phase II hepatic antioxidant enzymes and increase the antioxidant status of the rat liver, and these phenolic acids seem to selectively induce hepatic mRNA transcripts for Cu/Zn-SOD, glutathione peroxidase and catalase, likely through up-regulation of gene transcription as well as the nuclear factor-E2-related factor transcription factor. There may be two possible mechanisms for the antioxidative function. One is that the pigmented substances of colored potato flakes may have an antioxidative mechanism similar to that described above. The other possibility is that the active components of the pigmented substances of colored potato flakes scavenge the free radicals derived from AAP metabolites efficiently before the radicals attack the hepatic membrane to inhibit the chain initiation, and thereby protect the membrane from oxidative damage (27). However, we did not obtain sufficient data to conclusively explain the antioxidative mechanisms in this experiment.

In conclusion, present study suggests that pigmented substances from flakes of colored potatoes such as KM, NR, and SQ have antioxidant functions against acetaminophen-induced oxidative stress in rats. In addition, colored potato flakes may improve the antioxidant potentials in rats by enhancing hepatic Mn-SOD, Cu/Zn-SOD, and catalase mRNA expression. The intake of at least 600 mg of polyphenol, 140 mg of anthocyanin, 160 mg of flavonoid, per kg of diet may have suppressed the oxidation of lipids in rats in the present study. However, the hepatoprotective mechanism and the active components of the colored potato flakes require further investigation.

Acknowledgments

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