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The anti-oxidant activities of processed fruits and vegetables in APAP induced oxidative stress in BALB/c mice

Hyun-Kyoung Kim†

† *Professor , Department of Food Science and Engineering, Seowon University, Cheongju, Korea*
Kimhk4@seowon.ac.kr

Abstract

There is a strong connection between the diet rich in antioxidants and the decreased incidence of cardiovascular and cancerous diseases. Diets that are rich in anti-oxidants particularly include fruits and vegetables containing the high amounts of vitamin A-E, carotenoids, and minerals. Different processing conditions applied for vegetables and plants results in the alteration of the nutrients present in them. Therefore the rationale of our study was to compare the antioxidant effects of different processed vegetables and plants and to see that which one of them showed best anti-oxidant activity. For this purpose, we have used acetaminophen induced oxidative stress model in mice to check the effects of processed apple, pear, carrot, cabbage, broccoli and radish. Our results have shown that the administration of these samples effectively decreased the expression of parameters related with oxidative stress like ALT, AST, catalase, superoxide dismutase, GPx and 8-OHdG. Moreover they also significantly protected the mice livers from APAP induced damage as shown by histological changes. Therefore our results have demonstrated the effects of processed fruits and vegetables in mice model of oxidative stress.

Keywords*: Anti-oxidant activity, Vegetables, fruits, APAP, mice*

1. INTRODUCTION

There is vast epidemiological evidence on the connection between the consumption of diets rich in fruits and vegetables and the reduced risk of particularly cardiovascular and cancerous diseases [4, 5]. Since oxidation is related to the presence of free radicals, therefore different studies have shown that free radicals present in the human organism cause oxidative damage to different molecules, such as lipids, proteins and nucleic acids and thus are involved in the initiation phase of some degenerative and chronic diseases [6, 7]. As a consequence, the antioxidant compounds that comprise both of natural and synthetic origin, capable of neutralizing free radicals may play a major role in the prevention of certain diseases, such as cancer, cataracts, cerebral pathologies and rheumatoid arthritis [6, 8].

Fruits and vegetables contain different antioxidant compounds, such as vitamin C, vitamin E and carotenoids, whose activities have been established in recent years. However, these compounds are not the only ones contributing to the antioxidant activity of fruit and vegetables. Recent work shows that the presence of polyphenol compounds, such as flavonoids (in fruits and vegetables) also contribute to beneficial effects of this group of foods [9-11]. And besides polyphenols, there are many other compounds present in fruits and vegetables that are responsible for their remarkable anti-oxidant activity. Epidemiological studies have

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Corresponding Author: : kimhk4@seowon.ac.kr
Tel:+82-43-299-8474, Fax: +82-43-299-8470
Prof., Department of Food Science and Engineering, Seowon University, Cheongju, Korea

indicated the relationship between the plant antioxidants and reduction of chronic diseases [8, 12]. Ascorbic acid that is a major antioxidant is widely consumed in today's world as an antioxidant source in the form of tablets, syrups and other food supplement forms. However, some studies have indicated that the intake of Vit. C through this form may contribute towards its pro-oxidant properties [13]. Proper processing of natural food products is the main aim of scientific communities these days to preserve the maximum amount of nutrients in them.

Therefore in our study we have geared to investigate the antioxidant properties of processed apple, pear, carrot, cabbage, broccoli and radish extracts in vivo in acetaminophen (APAP) induced mouse model of oxidative stress. Our results have strongly indicated that the administration of these samples to mice decreased the levels of plasma ALT, AST, catalase, SOD, GPx and 8-OHdG. Furthermore, these samples also protected the mice livers from the harmful effects of APAP. Together our results have shown that the processing of these fruits and vegetables have posed a positive effect in securing the mice from anti-oxidative stress.

2. EXPERIMENT MATERIALS AND METHODS

2.1. Preparation of Processed fruits and vegetables Extract

Fruits and vegetables purchased from an agricultural and marine products market was washed, cut to a size of about 0.5 cm × 0.5 cm × 0.5 cm, and then freeze-dried. The freeze-dried sample was placed in the inner chamber of a heat-treatment apparatus (Jisco, Seoul, Korea), which was designed and manufactured to be capable of resisting even a pressure of 10 kg/cm2 or higher. Water was placed in the outer chamber, and the sample was heat-treated at a temperature of 140 to 150°C for 6 hours. The apparatus could prevent the sample from coming into direct contact with water and also prevent the carbonization of the sample by direct heat transfer, from water contained in the outer chamber.

The heat-treated sample was cooled, and then crushed using a crusher, and a 10-fold volume (v/v) of distilled water was added, followed by extraction at 60°C for 2 hours. The extract was filtered, and then freeze-dried before use.

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2.2. Experimental animals and sample administration

Male BALB/c mice, 6–8 weeks old, (19-22g), were purchased from Charles River, Orient Biotechnology, Gyeonggi-do, South Korea. The mice were housed in a specific-pathogen-free barrier facility at 21 ± 2°C with a relative humidity of 60 ± 10% under a 12-h light and dark cycle. Feed and water were provided ad libitum. All animal care and experimental procedures were carried out in accordance with internationally accepted guidelines on the use of laboratory animals (IACUC) and the protocols were approved by the Animal Care Committee of the College of Veterinary Medicine, Kyungpook National University, Daegu, South Korea. The mice were divided into 9 groups with each group containing animals (n=6). Group 1 was the control group with vehicle treatment only, Group 2 was the APAP challenged group at 400mg/kg i.p at single dose, Group 3 was the positive control N-acetylcysteine (NAC) group as previously reported [15] at 75mg/kg orally for 7 days, Group 4 was the processed apple supplemented group, Group 5 was the processed pear supplemented group, Group 6 was the processed carrot supplemented group, Group 7 was the processed broccoli supplemented group, Group 8 was the processed cabbage supplemented group and Group 9 was the processed radish supplemented group. All animals were fed with the fruits and vegetables extracts for 7 days and then 2 hrs after last sample administration; APAP was injected i.p to all mice except the control group. Then 24 hr later, blood and liver tissue was harvested for estimation of different oxidative parameters.

2.3. Assessment of serum 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels

For 8-OHdG measurement and biochemical analysis, blood samples were collected from mice under isoflurane respiratory anesthesia and placed in serum tubes. The 8-OHdG assay kit (Cayman chemical, Michigan, USA) was used for the measurement of 8-OHdG levels. The content of 8-OHdG in the samples were measured based on the competition of 8-OHdG and AChE enzyme-labeled 8-OHdG for 8-OHdG monoclonal antibody.

2.4. Preparation of liver homogenate

After euthanasia, the liver tissue was immediately extracted and stored in physiological saline on ice until homogenization. The buffer that was used for homogenization of liver tissue consisted of 10 mmol Tris-HCl and 1 mmol ethylenediaminetetraacetic acid (EDTA, pH 7.4). Whole liver tissues were homogenized in 20 mL of homogenization buffer, and then 10% homogenate was prepared relative to the liver weight. After centrifugation at 1,500 rpm for 30 min at 4℃, the supernatant was separated and stored at -80℃ until further analysis.

2.5. Measurement of antioxidant enzyme levels in liver tissue

The activities of all antioxidant enzymes were analyzed using specific enzyme assay kits (Cayman chemical, Michigan, USA). Superoxide dismutase (SOD) activity (Cu/Zn, Mn, and FeSOD) was measured by diluting the samples to 1: 1000. Catalase (CAT) activity was measured by 4-amino-3-hydrazino-5- mercapto-1,2,4-triazole (Purpald), and the sample was diluted to 1: 1000. Glutathione peroxidase (GPx) assay was performed in the presence of glutathione (GSH) and oxidized glutathione (GSSG), and GPx activity was evaluated by differences in absorbance at different NADPH levels (sample dilution of 1:20).

2.6. Evaluation of liver damage by serum biochemical analysis

The blood levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) in mice were measured using a biochemical analyzer.

2.7. Hematoxylin & Eosin (H&E) staining for liver tissue

The liver tissue was harvested from mice after euthanasia and was processed for paraffin embedded H&E staining.

2.8. Statistical analysis

Data are presented as mean ± SEM. One-way ANOVA and Dunnett's test were applied for the statistical evaluation of the data. Differences with ***$p < 0.001$ were considered significant. Respective significant marks other than the above mentioned are described in the figure legends.

3. RESULT AND DISCUSSION

3.1. Amelioration in the activity of ALT and AST by fruits and vegetables samples:

Alanine transaminase (ALT) is a transaminase enzyme. It is found in plasma and in various body tissues, but is most common in the liver. It catalyzes the two parts of the alanine cycle. Serum ALT level, serum AST (aspartate transaminase) level, and their ratio (AST/ALT ratio) are commonly measured clinically as biomarkers for liver health. ALT is commonly measured clinically as a part of a diagnostic evaluation of hepatocellular injury, to determine liver health [13, 14]. As shown in Fig. 1A, all the samples but most predominantly processed apple and processed radish samples reduced the levels of ALT in the serum of APAP mice. Similarly serum AST levels were also remarkably reduced by apple and cabbage samples as shown in Fig. 1B.

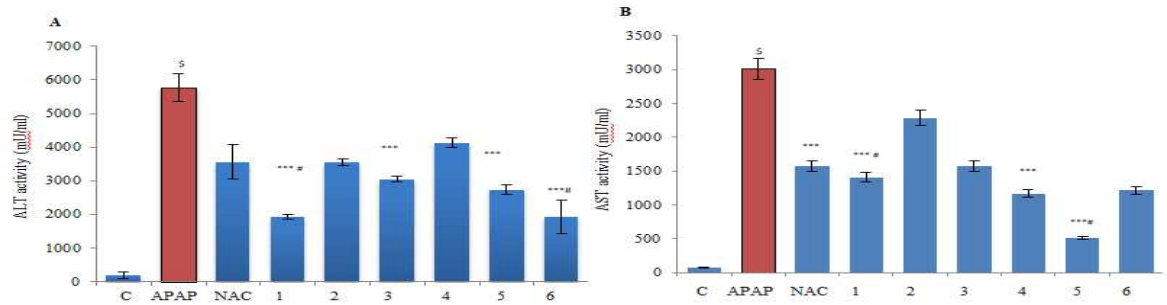


Figure. 1. (A) Alanine aminotransferase (ALT) and (B) aspartate transaminase (AST) levels in the serum.

#indicates p<0.05 as compared to the control group, *** indicates p<0.001 compared to acetaminophen, and # indicates p<0.01 as compared to the positive control, NAC. 1= Apple, 2= Pear, 3= Carrot, 4= Broccoli, 5= Cabbage and 6= Radish.

3.2. Restoration of the levels of SOD, GPx and catalase activity by fruits and vegetables samples:

Superoxide dismutase (SOD) is an enzyme that alternately catalyzes the dismutation (or partitioning) of the superoxide (O^{2-}) radical into either ordinary molecular oxygen (O_2) or hydrogen peroxide (H_2O_2). It is produced as a by-product of oxygen metabolism and, if not regulated, causes many types of cell damage. Thus, SOD is an important antioxidant defense in nearly all living cells exposed to oxygen [18]. As can be seen in Fig. 2A, APAP caused SOD levels to decrease significantly when compared to control group, and then most predominantly, processed apple, processed carrot and processed cabbage samples restored the SOD levels back when compared to APAP group.

Glutathione peroxidase (GPx) is the general name of an enzyme family with peroxidase activity whose main biological role is to protect the organism from oxidative damage. The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water [19]. Therefore, GPx serves as important anti-oxidant enzyme against oxidative stress in cells. As shown in Fig. 2B, GPx levels was predominantly decreased in APAP group while processed apple and processed broccoli samples restored the levels of this enzyme when compared to both the APAP and positive control NAC group.

Catalase (CAT) is a common enzyme found in nearly all living organisms exposed to oxygen (such as bacteria, plants, and animals). It catalyzes the decomposition of hydrogen peroxide to water and oxygen. It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS). Hydrogen peroxide is a harmful byproduct of many normal metabolic processes; to prevent damage to cells and tissues, it must be quickly converted into other, less dangerous substances. To this end, catalase is frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less-reactive gaseous oxygen and water molecules [23, 24]. It is clearly indicated in Fig. 2C, that CAT activity which was significantly decreased in APAP group was highly increased for processed apple and processed cabbage treated groups both when compared to APAP and positive control groups.

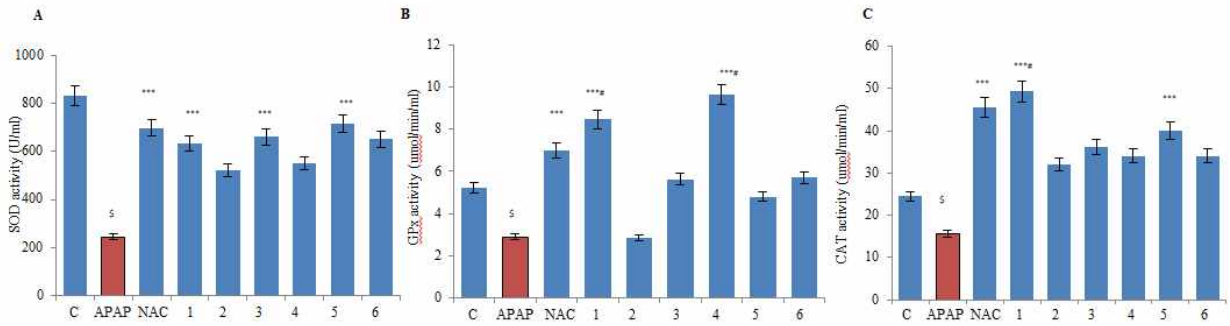
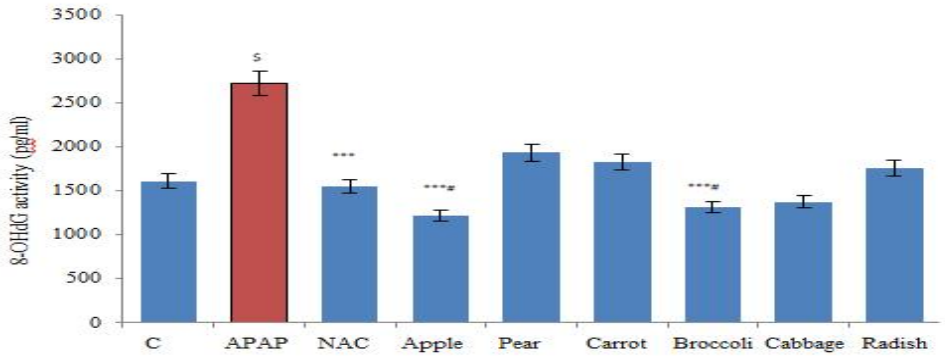


Figure. 2. (A) Superoxide dismutase (SOD), (B) glutathione peroxidase (GPx) and (C) catalase activity (CAT) in the serum of mice. #indicates p<0.05 as compared to the control group, ***indicates p<0.001 compared to acetaminophen, and # indicates p<0.01 as compared to the positive control, NAC. 1= Apple, 2= Pear, 3= Carrot, 4= Broccoli, 5= Cabbage and 6= Radish.

3.3. Reduction in the levels 8-OHdG by fruits and vegetables samples

8-Oxo-2'-deoxyguanosine (8-OHdG) is an oxidized derivative of deoxyguanosine. It is one of the major products of DNA oxidation. Concentrations of 8-oxo-dG within a cell are a measurement of oxidative stress [19]. As can be seen from Fig. 3, the 8-OHdG levels that were extremely elevated for APAP oxidative stress group were reduced significantly by processed apple and processed broccoli treated groups when they were

compared with both APAP and positive control, NAC groups.



A Figure. 3. 8-Oxo-2'-dexoxyguanosine (8-OHdG) levels in the serum of mice. #indicates $p < 0.05$ as compared to the control group, *** indicates $p<0.001$ compared to acetaminophen, and # indicates $p<0.01$ as compared to the positive control, NAC.

3.4. Effects of fruits and vegetables samples on APAP induced hepatic damage

As it is indicated in materials and methods section that liver tissues from all the groups were harvested for examining the damage in morphology induced by APAP and if the samples treatment had affected the damage caused by APAP. From Fig. 4B, it is clearly visible that APAP induced severe hepatocellular damage like, the hepatic lobules showed extensive centrilobular coagulative necrosis with increased eosinophilia. Severe hemorrhage was observed mostly in the hepatic lobule. The sinusoids were dilated and endothelium of the central veins was destroyed. The centrilobular hepatocytes showed severe ballooning degeneration. The sinusoids were heavily congested with red blood cells and lymphocytes. The cell boundaries were ill defined and most nuclei were darkly stained. The amount of heterochromatin increased at the periphery of the nuclei. The nuclei showed extensive karyolysis, pyknosis and karyorrhexis neutrophil accumulation, presence of hemorrhage and parenchymal cell injury as shown by arrows in figure and as reported previously [20, 21]. However, these all damages were almost reversed by the treatment of mice with positive control NAC and predominantly processed apple and processed cabbage samples.

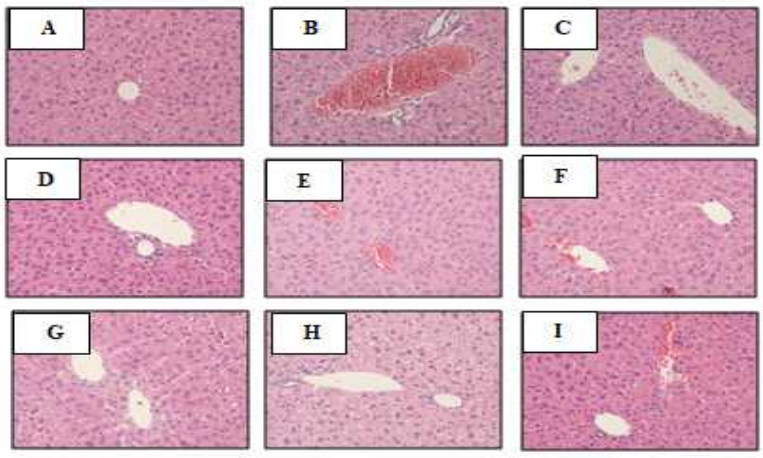


Figure. 4. Hematoxylin & Eosin (H&E) staining of sections of the liver of mice in group (A) control, (B) acetaminophen, (C) NAC, (D) apple, (E) pear, (F) carrot, (G) broccoli, (H) cabbage and (I) radish. Images were taken at a magnification of 20x under a microscope.

5. CONCLUSION

There is a strong connection between the diet rich in antioxidants and the decreased incidence of cardiovascular and cancerous diseases. Diets that are rich in anti-oxidants particularly include fruits and vegetables containing the high amounts of vitamin A-E, carotenoids, and minerals. Different processing conditions applied for vegetables and plants results in the alteration of the nutrients present in them. Therefore the rationale of our study was to compare the antioxidant effects of different processed vegetables and plants and to see that which one of them showed best anti-oxidant activity. For this purpose, we have used acetaminophen induced oxidative stress model in mice to check the effects of processed apple, pear, carrot, cabbage, broccoli and radish. Our results have shown that the administration of these samples effectively decreased the expression of parameters related with oxidative stress like ALT, AST, catalase, superoxide dismutase, GPx and 8-OHdG. Moreover they also significantly protected the mice livers from APAP induced damage as shown by histological changes. Therefore our results have demonstrated the effects of processed fruits and vegetables in mice model of oxidative stress.

In a nutshell, the above mentioned results indicate that the processed apple sample possess the best anti-oxidant activities among all the other samples as it was most effective for all the parameters related to oxidative stress. Moreover it also restored the damage induced by APAP as evident from H&E staining. Therefore future studies at more detailed mechanistic levels will reveal the factors involved in the antioxidant activity of processed apple sample.

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