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# CONSUMPTION OF BROCCOLI SPROUTS INCREASED THE ACTIVITY OF GLUTATHIONE-DEPENDENT ANTIOXIDANT ENZYMES IN MURINE LIVER

*M. V. IVANOCHKO, O. I. DEMIANCHUK, M. M. BAYLIAK, V. I. LUSHCHAK*

Vasyl Stefanyk Precarpathian National University,
Ivano-Frankivsk, Ukraine

*E-mail: marian.ivanochko.20@pnu.edu.ua*

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Consumption of hypercaloric food leads to increased production of reactive oxygen species (ROS) with the development of oxidative stress resulted in obesity and various metabolic disorders.

Broccoli sprouts may have a potential protective effect against obesity and its comorbidities because of high content of bioactive compounds, such as phenolic compounds and isotiocyanate sulforaphane. Sulforaphane is intensively produced in 3–5 day-old broccoli sprouts. This compound was found to be an activator of the Nuclear factor, erythroid 2 like 2 (Nrf2). Nrf2 is transcription factor that inhibits proinflammatory processes and stimulates antioxidant defense mechanisms. Nrf2 targets are glutathione-dependent genes of glutathione-S-transferase (GST) and glutathione peroxidase (GPx). These enzymes use glutathione to neutralize ROS. They form second line of antioxidant defense against oxidative stress while superoxide dismutase (SOD) and catalase are primaly antioxidant enzymes.

The purpose of this work was to investigate effects of the consumption of broccoli sprouts on the activity of antioxidant defense enzymes in the liver of mice fed with a cafeteria diet.

*Methods*. Eight-month-old C57BL/6J males were divided into 4 groups. First group (Control) consumed basal feed. Second group (Broccoli) consumed basal feed with 5% (w/w) 3-day-old broccoli sprouts (*Brassica oleracea var. Italica*, sort Calabrese). Third group (Cafeteria Diet) consumed a cafeteria diet which consisted of 70% (by mass) products of human ration (peanuts in chocolate, milk chocolate, chocolate cracker) and 30% of basal feed. Fourth group (Cafeteria Diet + Broccoli) consumed combination of a cafeteria diet and broccoli (5% of total food). After 16 weeks mice were euthanized according to bioethics norms with $CO_2$ anesthesia, murine liver was dissected and frozen in liquid nitrogen.

Frozen tissue was homozeniged in lysis buffer, centrifuged and resulted supernatants were used for analysis. Activities of enzymes were determined by using electrophoresis in polyacrylamide gel by the method of Ramesh et al. [1]. After electrophoresis we conducted dyeing of separate gels for detection of superoxide dismutase (SOD), glutathione-S-transferase (GST) or glutathione peroxidase (GPx) isoforms. Identification of SOD isoforms was carried out by the method of Beauchamp and Fridovich [2]. GST isoforms were detected by the method of Ricci et al. [3]. GPx isoforms were identified by the method of Lin et al. [4]. After photo fixation of gels, we determined activity of each isoform densitometrically using ImageJ software. Results were statistically analyzed by ANOVA followed by post-hoc Tukey's test. Data are presented as mean±SEM.

*Results*. In the hepatic tissue of all four groups of mice, two isoforms of SOD (SOD1 and SOD2) were detected in gels. The intensity of the bands of both isoforms was not significantly different between groups (Fig. 1). Three isoforms of GST (GST1, GST2, GST3) were detected in the liver samples. The activity of GST1 did not significantly differ between the experimental groups (Fig. 2).

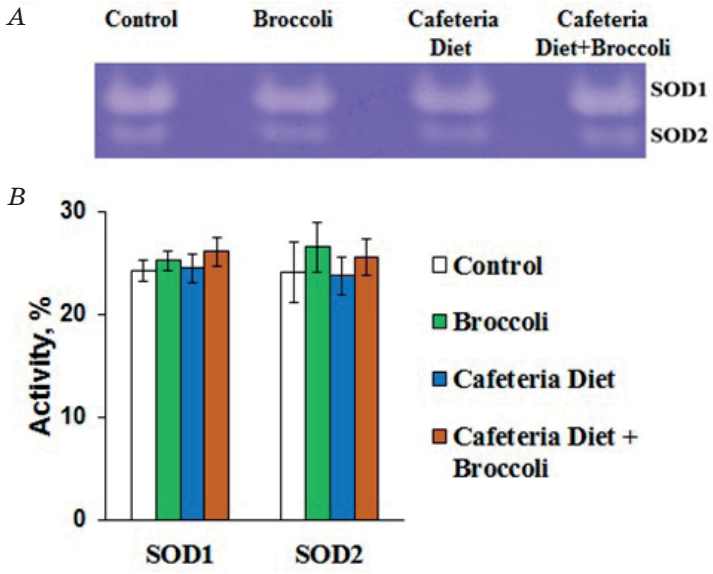


*Fig. 1.* **Electrophoretic dyed gel pattern (*A*) and the in-gel activity (*B*) of SOD isoforms in the murine liver $n = 5$**

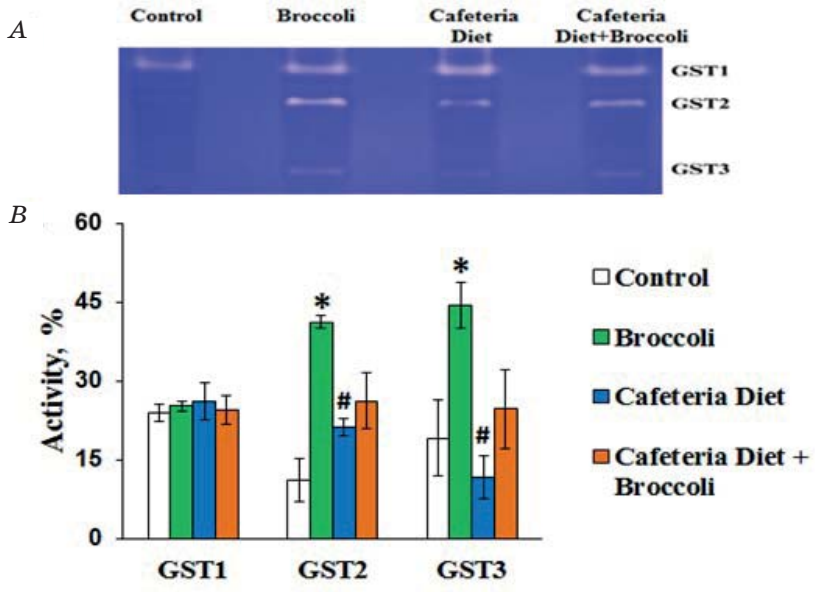


*Fig. 2.* **Electrophoretic dyed gel pattern (*A*) and the in-gel activity (*B*) of isoforms of glutathione-S-transferase (GST) in murine liver, $n = 3$:**
* — significantly different from the control group, # — significantly different from the Broccoli group

Activities of GST2 and GST3 forms were significantly higher in the group "Broccoli" compared to Control and Cafeteria Diet groups (Fig. 2).

We identified three isoforms of glutathione peroxidase (GPx1, GPx2, GPx3) in liver samples. The activity of GPx isoform 1 was not significantly different between the experimental groups (Fig. 3). The activity of GPx2 was significantly higher in the group of mice that consumed Broccoli and Cafeteria Diet + Broccoli compared to Control. GPx2 activity was significantly higher in the Broccoli group compared to the Cafeteria Diet group. Activity of GPx3 was significantly higher in the Broccoli group compared to the Control and Cafeteria Diet group (Fig. 3).

*Discussion.* We expected that both cafeteria diet and brocolli spouts would modulate antioxidant enzyme activities in mouse liver, as it was observed in rats fed high-calorie diet with antioxidant plants [1].

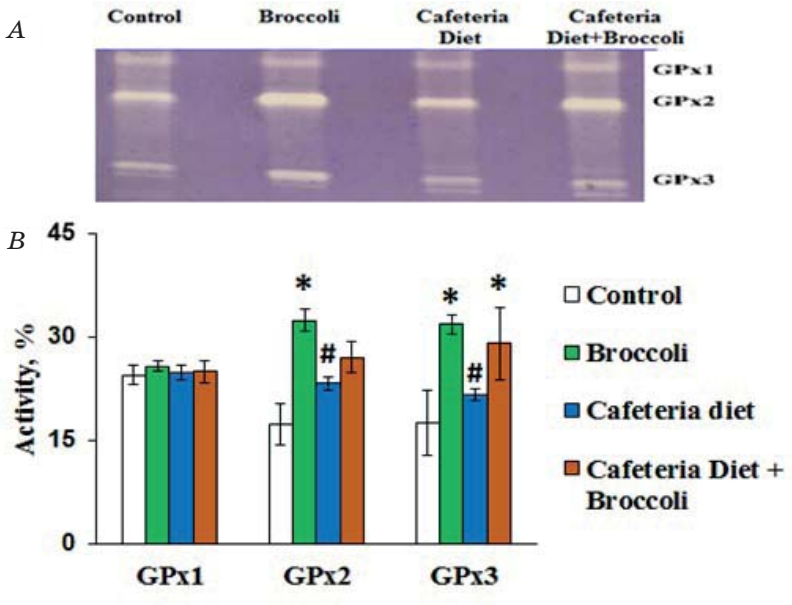


*Fig. 3.* **Electrophoretic dyed gel pattern (*A*) and the in-gel activity (*B*) of isoforms of glutathione peroxidase (GPx) in murine liver, $n = 4$:**
* — significantly different from the control group according, # — significantly different from the Broccoli group

In our study, enzymes activities were not significant different between control mice and mice fed with cafeteria diet. However, we found an improvement in the functioning of GST2 and GST3 in Broccoli group compared to Control and Cafeteria Diet groups. We may explaine this fact as a result of Nrf2 activation by sulforaphane from broccoli and stimulation of GST2 and GST3 synthesis to neutralize ROS. GPx2 and GPx3 activities were significantly higher in Broccoli group, similarly to GST2 and GST3. The activity of GPx2 in Cafeteria diet + Broccoli group was significantly higher in compare to Control group.

We proposed that during healthy condition and obesity bioactive compound of broccoli sulforaphane had activated Nrf2. Nrf2 in priority increased expression of *GST* and *GPx* genes as his targets for antioxidant defense and left *SOD* without expression. This hypothesis would be tested and supplemented by determination of other antioxidant enzymes and Nrf2 targets.

*Conclusions*. Cafeteria diet did not significantly affect the activity of SOD isoforms, but led to redistribution of in the activity of GST and GPx isoforms in murine liver. Feeding with broccoli spouts significantly increased the activity of 2 and 3 isoforms of GST and GPx in murine liver compared to values in control mice and mice fed with cafeteria diet. Combination of Broccoli + Cafereria diet had small activating effects on antioxidant enzyme acivity, compared with cafeteria diet.

***Key words:*** obesity; cafeteria diet; Nrf2; broccoli; antioxidant enzymes.

*Authors' Contribution*. M.V. Ivanochko – performance of experiments (maintenance and feeding of mice, electrophoresis), data analysis, visualization and wtitting of original graft; O.I. Demyanchuk — performance of experiments (electrophoresis); M.M. Bayliak — experimental design, writing — review and editing. V.I. Lushchack — conceptualization, writing — review and editing, funding acquisition.

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