

# **Studies on biological activities of low dose of phenethylamine from hot water extract of *Chlorella pyrenoidosa***

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## **Background and objective**

*Chlorella pyrenoidosa* is a fresh water unicellular green alga. *C. pyrenoidosa* and its water extract have a long history as food supplement. Animal experiments and human trials have demonstrated that oral administration of *C. pyrenoidosa* exerts beneficial biological activities such as anti-dyslipidemia and immunomodulatory activities. There is, however, limited information on the compounds responsible for these biological activities. The objective of the present study was to identify active compounds responsible for the beneficial effects in water extract of *C. pyrenoidosa* and elucidate the mechanism of the biological activities of active compounds.

## **Phenethylamine in hot water extract of *Chlorella pyrenoidosa* expands lifespan of SOD1 mutant adults of *Drosophila melanogaster* at very low dose**

Hot water extract of *C. pyrenoidosa* (WEC) increased the lifespan of superoxide dismutase (SOD)1 mutant adults of *Drosophila melanogaster* in a dose dependent manner in the range of 200–800 µg/g of diet. Compounds in WEC were successively fractionated by solid phase extraction using a Sep-Pak C18 cartridge and size exclusion chromatography (SEC). Amino compounds in active SEC fractions were derivatized with 6-aminoquinolyl-*N*-hydroxylsuccinimidyl carbamate (AQC) and analyzed by reversed phased-liquid chromatography-tandem mass spectrometry (LC-MS/MS). Phenylalanine, phenethylamine, isopentylamine, and 2-methylbutylamine were identified in the active SEC fraction. Phenethylamine, at very low doses (6–60 ng/g of diet) that approximately corresponded to those of phenethylamine in WEC (200–800 µg/g), increased the lifespan of the *D. melanogaster* adults, while isopentylamine did not exert the lifespan elongation activity in the range of 8.6–86 ng/g of diet. Since phenethylamine did not show *in vitro* SOD-like activity, it did not increase lifespan by direct antioxidant activity.

## **Phenethylamine, found in water extract of *Chlorella pyrenoidosa*, attenuates high fat diet-induced oxidative stress in mice liver by oral administration at very low dose**

Effects of oral administration of water extract of *C. pyrenoidosa* (100 mg/kg body weight, WEC) and phenethylamine (10 and 100 µg/kg body weight/day, PL and PH, respectively) on high fat diet (HFD)-induced liver damage of mice were examined. HFD feeding for 12 weeks increased plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), and low-density lipoprotein cholesterol (LDL-C) levels and increased liver triglyceride (TG), thiobarbituric acid reactive substances (TBARS) and decreased SOD-1 in protein level, which indicate that the HFD feeding increased oxidative stress in liver and consequently damaged liver function. The HFD-induced liver oxidative stress and liver damages were attenuated in PL group. In addition, SOD-like activity and SOD1 protein level were significantly increased in PL group than those in HFD group. The HFD-induced oxidative stress in liver and increase of plasma LDL were also attenuated in WEC and PH groups in spite of no significant effect on plasma AST and ALT levels. These facts suggest that oral administration of low dose of phenethylamine attenuates the HFD-induced liver damage via improving redox status in the liver, while high dose of phenethylamine may exert some negative effects on liver function.

## **Contents of phenethylamine in some unicellular algae and fermented foods used in Japan**

Phenethylamine has been demonstrated to be distributed in some food items, particularly in fermented food such as cheese and wine. However, there are limited data on the content of phenethylamine in fermented foods frequently used in Japanese diet. LC-MS/MS analysis revealed that presence of phenethylamine in rice wines (3–18 µg/mL), soy sauces (5–16 µg/mL), soybean pastes (3–41 µg/g), yogurts (2–3 µg/g), a beer (3 µg/g), water extract of *C. pyrenoidosa* (10 µg/g of dry matter) and dried euglena (0.03 µg/g). Interestingly, a soybean paste contains 41 µg/g of phenethylamine. One serve (12 g) of the soybean paste contains approximately 500 µg of phenethylamine, which is equivalent to or somewhat lower than the dose in the above-mentioned animal experiment (10 µg/kg). It has been assumed that fermented food used in Japan exert beneficial effects on human health. The presence of phenethylamine in fermented food used in Japan may exerts some beneficial effects.

## Conclusion

Phenethylamine is identified in *C. pyrenoidosa* as an active compound, which is responsible for life span elongation activity toward SOD-1 mutant adults of *D. melanogaster* and attenuating HFD-induced liver damage via improving redox status in mice. Phenethylamine exerts these activities at very low dose (60 ng/g diet for *D. melanogaster* and 10 µg/kg body weight of mouse). However, higher dose decreases the beneficial activities. Previous and present studies show that phenethylamine is contained in many fermented foods, such as rice wines, soy pastes and soy sauces in addition to water extract of *C. pyrenoidosa*. Among them, a soybean paste has high level of phenethylamine. One serve of this soybean paste corresponds to the animal dose showing the beneficial activity. This soybean paste has long history of food. Therefore, such dose of phenethylamine does not exert adverse effects on human health. Thus, phenethylamine exerts beneficial effects on human health by consumption of some foods such as chlorella extract and some fermented foods. The present study may shed light on the function of trace monoamine in food.