

Can a three-week administration of methanol extract of wild garlic modulate systemic redox state in hypertensive rats?

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Abstract (150 - 450 words): Wild garlic (*Allium ursinum*) is a widespread perennial herbaceous plant that has wide therapeutic applications and it is used as well as food. Natural preparations based on wild garlic have been used for gastrointestinal tract disorders, as antioxidants, antihypertensive, hypolipidemic agents, etc. Nevertheless, the data related to the effects of chronic wild garlic extract consumption on systemic redox state in hypertensive animals is yet to be understood. Therefore, the main goal of this study was to examine the effects of a three-week application of ethanolic extract from wild garlic on oxidative stress markers in spontaneously hypertensive rats. This was an experimental study conducted in 20 spontaneously hypertensive Wistar Kyoto rats divided into two groups: CTRL group - control untreated rats and wild garlic group - rats treated for three weeks with wild garlic ethanol extract once daily per os (100 mg/kg). After the accomplishment of the treatment protocol, animals were sacrificed, and blood samples were taken for determination of the levels of pro-oxidants and the activity of antioxidant enzymes. Our results indicate that there was a significant reduction in almost all of the measured pro-oxidants and an increase in antioxidant enzyme activity in rats treated with wild garlic extract compared to untreated rats. Ethanolic extract from wild garlic showed promising antioxidant potential in hypertensive conditions, however, future studies are necessary in order to reveal the full therapeutic potential of this plant species in various cardiovascular disorders.

Keywords: wild garlic, oxidative stress, rats

1. Introduction

Wild garlic (*Allium ursinum* L, ramson, or bear garlic, belonging to Alliaceae family) has been widely used as a dietary supplement and food. This plant species has been

traditionally used due to protective effects on the cardiovascular system, mainly due to antioxidants, blood pressure lowering potential, ability to decrease cholesterol levels, etc. Sulfur and phenolic compounds are the main constituents of wild garlic that are responsible for the pharmacological effects [1, 2]. Despite the use of this plant species for centuries in the prevention and treatment of cardiovascular disorders, the influence of chronic consumption on markers of redox state in the presence of hypertension has not been fully clarified. Therefore, the aim of this study was to reveal the impact of three-week wild garlic ethanol extract intake on pro-oxidant and antioxidant parameters in hypertensive rats.

2. Material and methods

This investigation was conducted in the Center for Preclinical and Functional investigations, Faculty of Medical Sciences, University of Kragujevac, Serbia. The study protocol was approved by the Ethical Committee for the welfare of experimental animals of the Faculty of Medical Sciences, University of Kragujevac, Serbia. All experiments were performed according to EU Directive for the welfare of laboratory animals (86/609/EEC) and principles of Good Laboratory Practice (GLP).

2.1 Wild garlic ethanol extract preparation

The ethanol extract of wild garlic was prepared by extracting 100 g of plant leaves with 500 ml of ethanol by heat reflux extraction. The dry extract for animal treatment was obtained by evaporation under reduced pressure and was daily dissolved in the water immediately before application to animals [1].

2.2 Experimental design

The research included 20 spontaneously hypertensive Wistar kyoto rats randomly divided into two groups: CTRL group - control untreated rats and WG group - rats treated for three weeks with wild garlic ethanol extract once daily per os (100 mg/kg). After the accomplishment of the treatment protocol, animals were sacrificed, and blood samples were taken for determination of the level of pro-oxidants and the activity of antioxidant enzymes. Plasma samples and erythrocytes were separated via centrifugation of heparinized venous blood. The concentration of pro-oxidative markers such as the index of lipid peroxidation, measured as thiobarbituric acid-reactive substances (TBARS), nitrites (NO_2^-), superoxide anion radical (O_2^-), and hydrogen peroxide (H_2O_2) was determined spectrophotometrically in plasma samples. On the other hand, in the lysate samples, the activity of non-enzymatic antioxidants such as reduced glutathione (GSH) and the activity of the enzymes such as catalase (CAT) and superoxide dismutase (SOD) was determined [3].

3. Results and Discussion

The levels of most of the measured pro-oxidants such as O_2^- , H_2O_2 and TBARS were significantly decreased in rats treated with WG compared to untreated spontaneously hypertensive rats. On the other hand, the level of NO_2^- had similar values in both groups

(Table 1). Moreover, the activity of SOD and CAT as well as the level of GSH were significantly elevated in the WG group (Table 2).

Table 1. The impact of WG on the level of pro-oxidant markers in plasma samples

Group	O ₂ ⁻ (nmol/ml)	H ₂ O ₂ (nmol/ml)	NO ₂ ⁻ (nmol/ml)	TBARS (μmol/ml)
CTRL	1.6 ± 0.3	4.6 ± 0.2	3.6 ± 0.15	2.2 ± 0.14
WG	1.1 ± 0.12*	3.7 ± 0.17*	3.2 ± 0.1	1.5 4.6 ± 0.12*

p<0.05 statistical significance at the level p<0.05 compared to CTRL group.

Table 2. The impact of WG on the level of antioxidant markers in erythrocyte samples.

Group	SOD (U/g Hb × 10 ³)	CAT (U/g Hb × 10 ³)	GSH (nmol/ml red blood cells)
CTRL	10 ± 0.21	8.3 ± 0.14	86 100 ± 245
WG	14 ± 0.18*	12 ± 0.19*	114 245 ± 311*

p<0.05 statistical significance at the level p<0.05 compared to CTRL group.

It is believed that the antioxidant activity of *Allium* species is due to the presence of various sulfur-containing compounds, polyphenols, dietary fibers and various microelements [4]. Our previous research confirmed the presence of various polyphenols in WG extract such as kempferol 3-O-Glc, p-coumarin acid, ferulic acid, kaempferol and we might assume that the observed benefits in hypertensive rats in the current study are due to the presence of those biomolecules [5]. In brief, an increase in the activity of SOD which acts as the first line of cellular defense against oxidative injury is in accordance with the drop in O₂ level since SOD catalyzes the conversion of O₂⁻ to H₂O₂. Additionally, a reduction in H₂O₂ level may be explained by the higher activity of CAT [3]. Most of previously conducted research confirmed *in vitro* antioxidant activity of wild garlic, however *in vivo* studies are limited. Recently conducted research in our laboratory also showed the antioxidant potential of wild garlic extract in ischemia-reperfusion injury model in rats [1]. Taking into consideration that oxidative stress strongly contributes to the pathogenesis of hypertension, it might be concluded that wild garlic extract can provide a significant contribution to hypertension management due to its potential to regulate redox homeostasis in conditions of elevated blood pressure levels.

4. Conclusions

Three-week application of the ethanol extract of wild garlic led to an improvement in the redox state of hypertensive rats via reduction in the production of pro-oxidants and

enhancement in antioxidant defence system capacity. Promising antioxidant potential in our study can be a basis for future clinical research before implementation of this plant species extract as an adjuvant in hypertension management.

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