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THE EFFECTS OF CHRONIC ADMINISTRATION OF *ALLIUM URSINUM* EXTRACT ON SYSTEMIC REDOX STATUS IN RATS

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Abstract: The present study aimed to estimate the effects of chronic treatment with *Allium ursinum* extract on the redox status in rats. The administration of extract in a dose of 500 mg/kg significantly reduced the concentration of all tested pro-oxidative markers compared to the control group. Additionally, the application of extract caused a significant elevation in the antioxidant activity compared to the control group. Our results have shown that the four-week intake of the methanolic *Allium ursinum* extract significantly modulates the redox status and stands out as a powerful antioxidant.

Keywords: *Allium ursinum*, extract, oxidative stress, rat

Introduction

Allium ursinum L. (synonyms: wild garlic, ramson, or bear garlic) is a perennial herbaceous plant belonging to the Alliacee family and has been used for centuries in traditional medicine as a prophylactic and therapeutic agent. Several classes of bioactive compounds have been isolated from A. ursinum so far, among them sulfur and phenolic compounds as quantitatively the most distributed constituents, responsible for the pharmaceutical effects of the plant (Sobolewska et al., 2015). Taken into consideration that oxidative stress response to *A. ursinum* has still not been clarified, the aim of this study was to assess the effects of this extract on systemic redox status in male *Wistar albino* rats.

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Materials and methods

Plant Material and Extract Preparation

The whole plant *A. ursinnum* was collected in May on Mt Bukulja. The collected material was dried under the shade and powdered (sieve 0.75). The methanol extract was prepared by extracting 100 g of aerial part of the plant with 500 ml of methanol by heat reflux extraction, at the temperature of 90°C, in 2 h (Hijazi et al., 2015). The mixture was filtered through filter paper. The dry extract was obtained by evaporation under reduced pressure. The residue was stored in a dark glass bottle at +4°C for further processing. To feed the animals, AUE was daily dissolved in the water just before administered to experimental animals.

Animals and Experimental Design

The present study was carried out on 40 male *Wistar albino* rats. Rats were randomly divided into four groups depending on the applied treatment.

1. CTRL, rats who drank only tap water for 28 days;
2. 125 AUE, rats who drank tap water containing 125 mg/kg of methanol extract of *A. ursinum* for 28 days;
3. 250 AUE, rats who drank tap water containing 250 mg/kg of methanol extract of *A. ursinum* for 28 days;
4. 500 AUE, rats who drank tap water containing 500 mg/kg of methanol extract of *A. ursinum* for 28 days.

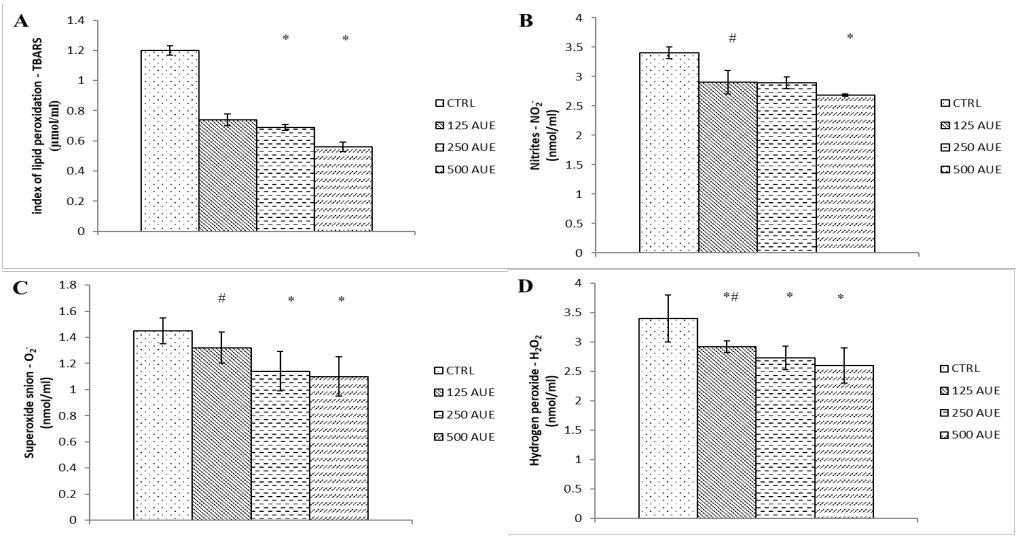
Oxidative Stress Markers and Antioxidant Enzyme Determination

After four weeks, the rats were sacrificed and the blood samples were collected from a jugular vein to test the systemic redox state. Plasma samples and erythrocytes were separated via centrifugation of heparinized venous blood. In plasma samples, we measured 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). In the lysate, we determined the activity of non-enzymatic antioxidants, including reduced glutathione (GSH) and the activity of the enzymatic defense system by evaluating the catalase (CAT) and superoxide dismutase (SOD) levels (Bradic et al., 2019). All mentioned biochemical parameters of oxidative stress were determined spectrophotometrically (Shimadzu UV-1800UV-VIS spectrophotometer, Japan).

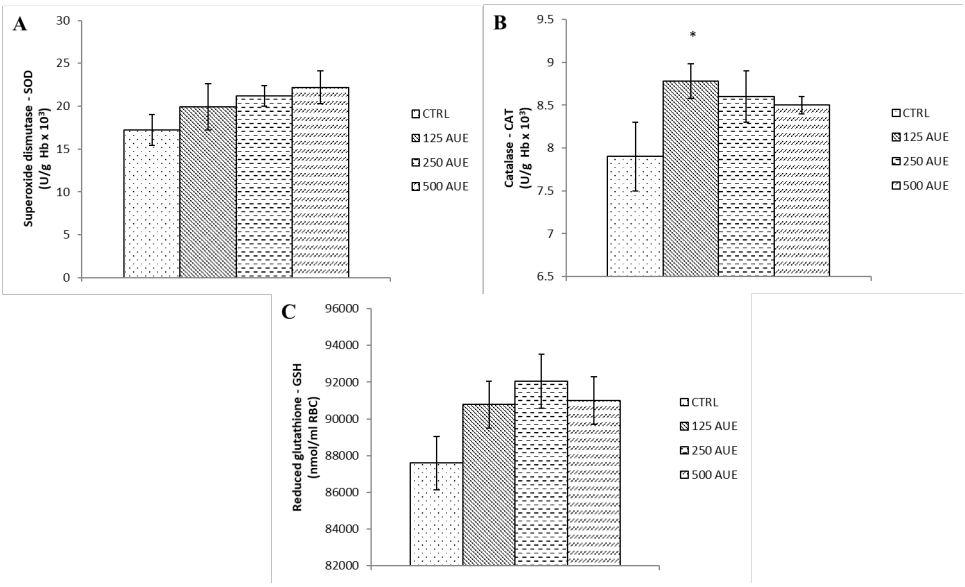
Results and discussion

All tested pro-oxidants were statistically significantly reduced in the group of rats using AUE at a dose of 500 mg/kg compared to the CTRL group. Application of the extract at a dose of 250 mg/kg reduced the values of O_2^-, H_2O_2, and TBARS, while the lowest dose led to a decrease in the concentration of TBARS and H_2O_2 compared to untreated rats. By comparing the values of pro-oxidants between the groups on the treatment with different doses, lower values of all markers are observed after the treatment with the extract at a dose of 500 mg/kg in relation to 125 mg/kg (Graph 1.).

The application of AUE caused a significant elevation in the antioxidant activity compared to CTRL. However, the highest dose of extract managed to lead to the highest SOD value compared to the control group, while the application in the lowest dose led to a significant jump in the CAT value. On the other hand, the GSH activity did not change significantly under the influence of AUE in all three doses (Graph 2).



Graph 1. Effects of consumption of increasing doses of AUE on the value of pro-oxidative markers in plasma samples: TBARS (A), NO_2^- (B), O_2^- (C), H_2O_2 (D). Values are shown as mean ± SE. *Statistically significant difference at the level of $p < 0.05$ in relation to the CTRL group; #Statistically significant difference at the level of $p < 0.05$ compared to 500AUE.



Graph 2. Effects of consumption of increasing doses of AUE on the value of SOD (A), CAT (B), and GSH (C). Values are shown as mean ± SE. *Statistically significant difference at the level of $p < 0.05$ in relation to the CTRL group

The results demonstrated a significant drop in the values of all markers after treatment with the extract compared to the CTRL group but with the most prominent changes in groups treated with the highest dose. The same trend was observed in SOD activity, while when it comes to CAT activity, the lowest dose led to the most significant increase in relation to the CTRL group. However, GSH levels did not change significantly after 4 weeks of intake of *A. ursinum* in all three doses. In a study by Masjedi et al. (2013), methanol extract of *A. ursinum* was applied in diabetic rats at doses of 60 and 120 mg/kg. The activity of SOD and CAT was significantly increased in treated animals, which is in accordance with the results, providing the ability of *A. ursinum* to reduce oxidative stress and provide beneficial systemic effects. A previously conducted study examined whether *A. ursinum* has better antioxidant properties compared to other *Allium* species. It was observed that wild garlic possesses a relatively higher concentration of antioxidants and better scavenger activity compared to the other *Allium* species from Balkan flora. The results of this study clearly indicated the ability of *Allium* species to remove free radicals and prevent oxidative stress from occurring (Stajner et al., 2008). According to a previously published study, methanolic extract was considered to be the richest in the number of phenolic

acids and flavonoids due to its strongest ability (followed by ethanol) to isolate phenolic compounds (Pavlović et al., 2017). Contrastingly, the results of the study conducted on cell lines that are previously exposed to doxorubicin treatment indicate the powerful antioxidant effect of ethanol AUE but poor the effect of the methanolic extract on the antioxidant capacity of wild garlic. The discrepancy between their results and the findings of other studies may be attributed to different study designs and measurement of the impact of *A. ursinum* on different parameters of redox status by using various tests (Pop et al., 2020).

Conclusion

In summary, our results pointed out that a four-week administration of *A. ursinum* extract leads to beneficial adaptations, manifested as decreased oxidative stress and an improved antioxidant defense system. In addition, these findings can lead to the development of strategies to prevent oxidative stress. Further investigations should be conducted to clarify the exact mechanism of the *A. ursinum* extract protective effects.

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