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Infusion of beta vulgaris seeds: protective action in experiment in vivo

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Abstract

This paper shows the positive effect of the infusion of Beta Vulgaris seeds in the experiments in vivo. Due to the significant contamination with the copper compounds of some industrial regions and their accumulation in living systems, methods of health improvement are necessary, in this regard, the use of plant-based food preparations as detoxifiers is promising. In the experiment on rats, the high efficiency of prescribing the infusion of common beetroot seeds was shown in subacute and chronic poisoning of rats with copper acetate in high concentrations. It was found that the use of the infusion of Beta Vulgaris seeds in combination with copper acetate poisoning leads to a significant decrease in copper accumulation in parenchymal organs (liver, kidneys and spleen). Simultaneous biochemical analysis of rat blood showed a decrease in the activity of liver enzymes ALT and AST, a decrease in bilirubin, cholesterol. Therefore, the infusion of Beta vulgaris seeds of the family Amaranthaceae can be recommended for development as an effective means for the health improvement.

Keywords: infusion of Beta Vulgaris seeds, hepato- and nephroprotective effect, copper acetate, chronic poisoning, accumulation of copper

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INTRODUCTION

Currently, there are intensified searches for herbal remedies that could reduce the negative impact on the human body of the environment, especially in industrial regions. There are regions in which environmental objects are significantly contaminated with heavy metals, in particular, copper compounds. Copper is an important trace element for all life forms. However, in copper ore regions there is a significant accumulation of copper in living systems due to the consumption of low-quality food (Tarasenko, Konkabayeva and Ishmuratova 2016). In this regard, it is important to search and study new effective and harmless preparations that could be used by the population, both for the purpose of prevention and with the aim of removing from the body already accumulated compounds of harmful substances.

Recently, researchers have been paying great attention to herbal preparations, including food purpose, which can be used as detoxifiers. Since ancient times, plants have been used by traditional medicine to treat various ailments. With the development of technology, researchers have learned to identify various active substances from plants. To date, most of these proteins have been identified, and many of them on the surface of the seed coat (Chiji et al. 1984, Lipkin et al. 2005, Oksana and Artur 2019). In particular, the representative of the family Amaranthaceae Beta vulgaris is characterized by a variety of secondary metabolites, which have been attributed a positive effect on the metabolic, cardiovascular, and gastrointestinal health of a person (Arulselvan and Subramanian 2008, Broekaert et al. 1992, Clifford et al. 2015, Pavlov et al. 2005). One of the important and well-documented uses of herbal products is their use as a hepatoprotective agent (Esatbeyoglu et al. 2014, Lee et al. 2005, Wettasinghe et al. 2002). Many medicinal properties were found in roots, leaves and stems of Amaranthaceae: antioxidant, anti-inflammatory, anti-cancer, antimicrobial (Azeredo 2009, Čanadanović-Brunet et al. 2011, Kapadia et al. 1996, Kim et al. 2003). Betanin, a beetroot pigment, can also inhibit lipid peroxidation in the hepatocyte liposome

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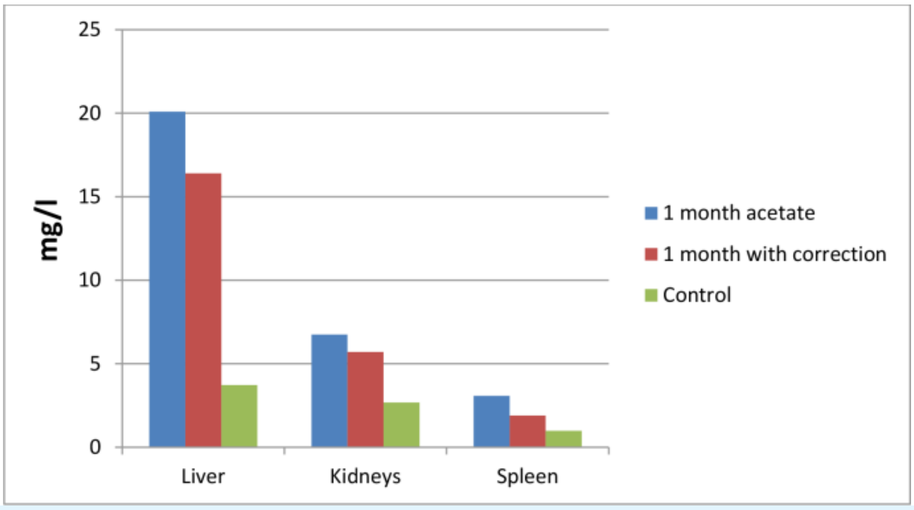

Fig. 1. The copper content in the organs of animals poisoned with copper acetate for one month after correction with infusion of Beta vulgaris seeds

system (Vidal et al. 2014), and Beta vulgaris extract was able to inhibit protein oxidation caused by the release of hypochlorous acid from active neutrophils (Pietrzkowski et al. 2010, Reddy et al. 2005). Along with this, the therapeutic effect of the seeds of the plant Beta vulgaris, belonging to the family Amaranthaceae, has not been sufficiently studied yet. In this regard, the aim of our study was to explore the protective properties of seeds in modelling subacute and chronic intoxication of laboratory animals with copper acetate.

MATERIALS AND METHODS

Preparation of Infusion of Beta Vulgaris Seeds

Seeds for study were obtained from the collection of Michurin agricultural town and were identified by the experts of the Department of Botany of the Faculty of Biology of E.A. Buketov Karaganda State University. 3.5 g of dried seeds were taken, they were infused in 200 mL of water at a temperature of 80-90 degrees for 1 hour and filtered. Then the freshly prepared filtrate was used in the study for five hours.

Animals and Tissue Preparation

This study was conducted in accordance with the ethical principles set forth in the European Community Directive (86/609EC) and the requirements of the World Animal Protection (WSPA).

In the experiment, a total of 50 male non-linear rats were used. The rats were housed five per cage and had free access to food and water. They were exposed to a 14-10-h light-dark cycle the room temperature was controlled at 22 ± 3 C. Modelling of copper intoxication of rats was performed using copper acetate, which was daily administered at a dose of 0.49 mg per rat orally. Animals were exposed to copper acetate when they weighed 200 ± 30 g. Experiments were performed during 4 and 16 weeks. The 50 non-linear rats were divided into four groups according to:

G1: Rats exposed to Cu (in the form of copper acetate, 1 mL per rat) for 4 weeks.

G2: This group received copper acetate (1 mL per rat) + seeds infusion (2 mL per rat during first 5 hours of the light part of the day) for 4 weeks.

G3: Rats exposed to Cu (in the form of copper acetate, 1 mL per rat) for 16 weeks.

G4: This group received copper acetate (1 mL per rat) + seeds infusion (2 mL per rat during first 5 hours of the light part of the day) for 16 weeks.

G5: Rats (n=10) received water during 16 weeks.

Animals were sacrificed by cervical decapitation under ether anesthesia. Organs (liver, kidneys, spleen) were removed, washed with normal saline and weighed. Organs were kept at ice-cooled conditions until the moment of research. Copper accumulation in parenchymal organs was determined by the GF AAS method SpectrAA140, Varian, Australia.

At the same time, rat blood was collected for biochemical analysis. The liver enzymes ALT, AST, cholesterol, bilirubin, glucose, urea, creatinine and total protein were determined in blood serum samples on the automatic biochemical analyzer Bs-200 (Mindray).

Statistics

The mean +- SEM values were calculated for each group to determine the significance of the intergroup difference. To determine the difference between groups, Student's "t"-test was used. $P<0.05$ were considered to be significant.

RESULTS AND DISCUSSION

The results of determining the copper content in parenchymal organs after 1 month of the experiment allowed us to find that significant accumulation of metal occurred in the organs under study, exceeding the control values, especially in the liver (**Fig. 1**).

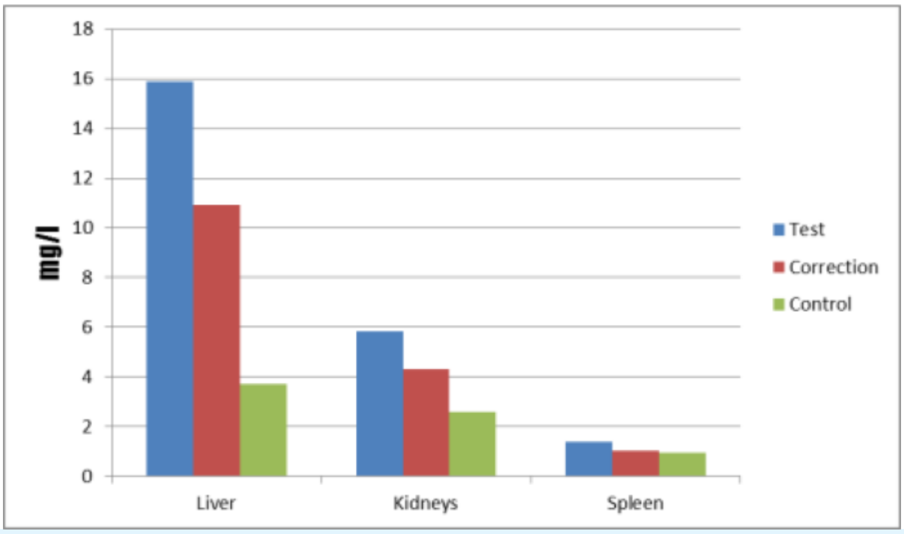

Fig. 2. The copper content in the organs of animals poisoned with copper acetate for 4 month after correction with infusion of Beta Vulgaris seeds

The liver of animals exposed to copper for one month contained 5.4 times (P≤0.001) higher metal concentration than control animals. At the same time, the kidneys and spleen contained 2.7 (P≤0.05) and 3.1 times (P≤0.01) more copper, respectively. The highest concentration of copper is in the liver, which is more likely due to liver function.

With the combined administration of copper acetate and infusion of common beetroot seeds to rats for 1 month, a lower accumulation of copper was found in all organs under study, however, the differences from the tissues of animals that were injected only with copper acetate were not significant, with the exception of spleen tissue (P≤0.05).

After four months, the copper concentration in the animal studied organs became lower: the liver had a 4.2 times higher copper content than the control; kidneys 2.2 more than control; spleen 1.4 times more than control. These figures are probably due to the adaptation properties of the organism of rats (**Fig. 2**).

In rats that received correction in the form of an infusion of Beta vulgaris seeds with copper acetate for 4 months, a lower copper content was observed compared with the experimental group.

The animals that received the correction had a 1.5 times lower concentration of copper in the liver, at the same time, they still exceeded the control data by 2.9 times. At the same time, the kidneys of this group had a 1.3 times lower concentration of copper, compared to animals exposed to the metal, and also contained 1.6 times more copper than the control animals. The spleen of animals with correction, in turn, had 1.3 times less copper than animals without correction, but 7% more copper than the control group. This result shows the detoxifying properties of the infusion of Beta vulgaris seeds.

Table 1. The content of protein, urea, creatinine, glucose and cholesterol in the blood serum of rats with the combined administration of copper acetate and infusion of beetroot seeds

Group	Protein g/L	Urea µmol/L	Creatinine µmol/L	Glucose mmol/L	Cholesterol mmol/L
1-poisoning 1 month	73.60±7.17	6.48±0.82	46.12±2.62*	5.64±0.39	4.96±0.45
2-poisoning 1 month + correction	76.80±4.04	5.52±1.15	72.40±6.95	4.88±0.44	4.50±0.74
3-poisoning 4 months	74.6±4.54	5.70±0.40	52.62±3.57*	5.12±0.57	5.68±0.64*
4-poisoning 4 months + correction	73.00±5.44	6.92±0.45	62.00±5.22	5.30±0.49	3.85±0.42*
5-control group	62.00±4.12	7.01±0.64	73.01±5.73*	4.4±0.32	3.94±0.18*

Note: *P≤0.05

The body's defences during intoxication in animals depend on the functional state of many systems, but mainly the detoxification function of the liver and excretory function of the kidneys. The biochemical blood analysis shown in **Table 1** is a recognized informative test that reflects the general condition of animals, and allows us to judge the reactivity of the body.

Considering the indices of the liver enzymes ALT and AST as biomarkers reflecting the functional activity of the liver, we note that these indices in rats in the 1-month experiment were 213.2 ± 45.05 and 239 ± 35.54 µkat/L, respectively, which is significantly higher than control (P≤0.001). In animals treated with copper acetate for 4 months, the level of enzymes was also high and reached, respectively: ALT-229.8 ± 32.4 and AST -243± 41.14 µkat/L (**Fig. 3**).

The level of bilirubin at the end of 1 month after the intake of copper acetate was 21.96 ± 6.33 µmol/L, and after 4 months of taking copper acetate, the bilirubin content increased to 24.92 ± 4.71 µmol/L. These results were 2 and 2.5 times higher than the control values, respectively (P≤0.05). The obtained data indicate the

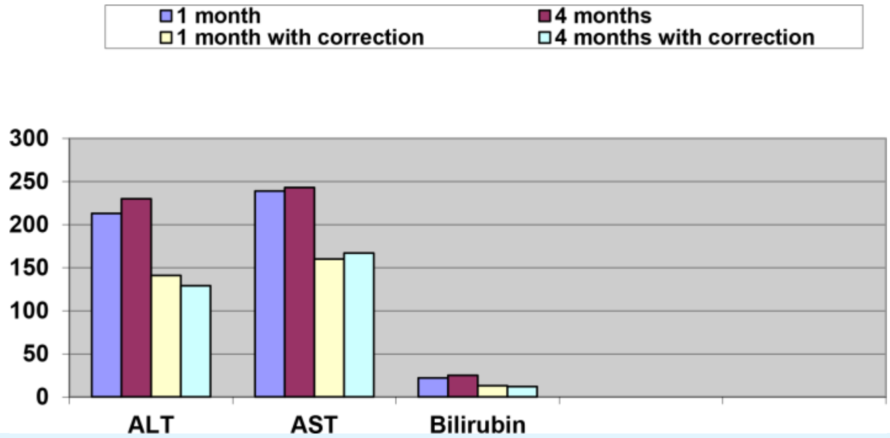

Fig. 3. Biochemical parameters of rat blood serum with combined administration of copper acetate and infusion of common beetroot seeds

ongoing destructive processes in the liver tissue under the influence of copper acetate.

The intake of copper acetate in combination with the correction of the infusion of beetroot seeds showed a positive dynamic. So, in a 1-month experiment, when rats took copper acetate in combination with seed infusion, the content of liver enzymes was lower than in the group without correction: ALT decreased 1.5 times and AST 1.8 times, making respectively 141.4 ± 2.34 and 159.8 ± 11.58 μkat/L. The level of bilirubin was also 1.7 times lower than in the group without correction (12.78 ± 2.92 μmol/L). No significant differences were found in cholesterol content.

In animals that took copper acetate with infusion of seeds for 4 months, the ALT level was 1.8 times lower than in the group of animals that did not receive the infusion. The bilirubin content decreased by 2 times (**Fig. 3**). The level of cholesterol decreased significantly in comparison to control and correction ($P \leq 0.05$).

All biochemical parameters presented in **Table 1** also reflect, to one degree or another, the functional state of the organs under study, however, we did not find significant differences in the groups, except for the content of creatinine and cholesterol (**Table 1**). Reduced indicators of creatinine in a biochemical blood analysis during poisoning with copper acetate for one and 4 months can be caused by impairment of liver functional properties in animals. In the groups that received correction in the form of infusion of seeds, the indicators were within the control value.

We found that in rats treated with copper acetate without correction, liver aminotransferases are significantly activated and bilirubin level increase. This is evidence of structural damage to hepatocytes, apparently associated with the activation of lipid peroxidation, which was recorded in a number of works (Agarwala et al. 2006, Arulselvan et al. 2013). Along with this, there is evidence that long-term feeding with red beetroot juice when liver is damaged by N-nitrosodiethylamine (NDEA), DNA damage is reduced, as well as other biomarkers of liver damage (Koubaier et al. 2014). The pigments giving red colour to the beetroots are bioactive compounds and provide antioxidative activity for human health (Gliszczyńska-Świgło et al. 2006). In addition, it has been proven that the root system of beetroot in the process of growth also has a powerful antioxidant effect against various oxidative systems in vitro (Babagil et al. 2018). Thus, our obtained results and literature indicate the antioxidant effect of various parts of the Beta vulgaris plant: both leaves, roots, and presumably seeds that have not been previously studied. The results of biochemical parameters and the nature of the cumulation of copper acetate in parenchymal organs confirm the protective effect of the infusion of beetroot seeds when prescribed together with a toxicant.

CONCLUSION

Although our obtained results are preliminary, they clearly indicate the healing properties of the infusion of Beta vulgaris seeds of the family Amaranthaceae. When prescribing the infusion of beetroot seeds in a certain concentration, a positive result is observed, which manifested itself in a decrease in the accumulation of copper in parenchymal organs and an improvement in biochemical parameters, which are markers of liver and kidney function.

For this reason, the infusion of Beta Vulgaris seeds can be recommended for the development of a prophylactic preparation that reduces environmental risk factors for human health.

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