Prooxidant activity as a criterion for the hazard class of biogenic preparations

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Abstract. The presence of various pathogens in municipal wastewaters and the prevention of large-scale invasions is one of the acute problems of modern ecology. Reducing the load on the ecosystem and capital costs during the disinfection of wastewater, their sediments and soil from various pathogens (for example, helminth eggs) is one of the most important tasks of modern ecology. The most successful solution is the use of recycling technologies to obtain reagents that do not require preparation of disinfection objects and subsequent additional treatment. The production of biogenic preparations from agricultural waste (potato seedlings Solanum tuberosum) is low-cost and does not require complex equipment. However, the effect on various links of metabolism, in particular, the effect on free radical processes and lipid peroxidation in the cell, has not been studied enough, which does not allow us to speak about the toxicity of the drug and its effect on the ecosystem of the disinfected object. The study of the prooxidant activity of raw materials for the production of biogenic preparations in standard model systems of biochemical studies (human erythrocytes) will allow to establish the hazard class.

1 Introduction
Wastewater and food waste from municipal sources are the primary contributors of organic waste from cities and Natural sources, such as soil and wind-erosion dust, biomass open burning, sea salt spray and biogenic source (BIO), are major contributors to atmospheric emissions of trace elements globally. [1, 3].

Today, the problem of using non-toxic substances in the disinfection of environmental objects (wastewater, their sediments and soil) from pathogens is one of the most urgent. Because degradation and decomposition of the soil cover as a uniform system due to soil mechanical removal and marked chemical contamination create a situation, when most of fertile forest soils cannot be rehabilitated to generate forest vegetation [4]. Social and economic development is strongly dependent on the availability of water: it is estimated that demand for water may increase by 30% by 2040. The situation is similar with energy consumption: even a 40% increase is predicted within that time. Research on water-energy nexus is necessary to take into account all the interdependencies of the two most important areas [5].

Moreover, the effect of biogenic preparations on redox processes both in the ecosystem and in a living cell at various levels of organization has not been fully studied.

Wastewater contains important resources that should be recovered in wastewater treatment plants to generate value-added products such as renewable energy, biofertilizers and water for different purposes [6]. The recycling of resources through innovative recovery processes is only a recent objective in wastewater treatment systems. Especially when processing plant raw materials, make it possible to
obtain a wide variety of substances that suppress the growth and development of pathogens, or cause their death.

Moreover, their effectiveness in tests both in vitro and in vivo can reach more than 90%. According to its chemical composition, the active substances of such raw materials, as a rule, is of non-protein nature and can be partially or completely decomposed by the microflora of the disinfection object.

Most of these compounds act on the membranes of the pathogen and on the membrane (or membrane-like, if we are talking about a prokaryotic cell) structures of the organelles of the cell, changing its permeability. For example, steroidal glycoalkaloids of the juice of potato (Solanum tuberosum) are natural detergents with a cyclopentaphenanthrene (steroid) core [10]. In particular, solanine causes hemolysis of erythrocytes.

Potato sprouts juice has a high (more than 80%) efficiency against intestinal pathogenic protozoan cysts and a very high (more than 95%) efficiency against helminth eggs, causing their death.

The absence of prooxidant (and even a small presence of antioxidant) properties in the model system of lipoproteins of the yolk of chicken eggs was proved.

The aim of this work was to study the prooxidant activity of the juice of the nightshade tuberosum (Solanum tuberosum) in the model system of human erythrocytes. In order to gain insight on its toxic effects, intact red blood cells (RBC), human erythrocyte membranes and molecular models were used [2].

2 Materials and methods

The juice of the potato (Solanum tuberosum) was obtained from seedlings 5-8 cm tall, not containing chlorophyll, by mechanical grinding. We investigated the prooxidant activity of potato juice mixed with saline and sodium benzoate in a proportion of 0.1 ml of juice + 4.9 saline + 28 Microliters of sodium benzoate = bioeffective concentration Cmax; whole juice of Solanum tuberosum (0.02 ml per ml erythrocyte mass) – THE JUICE, a mixture of bioeffective concentration and ferric (II) -ions (0.1 C max. per Milliliter erythrocytes + 1 Millimole ferric (II)-ions per Liter). FeSO4 (conc.) (1 Millimole per Liter) was used as a source of ferric (II)-ions (molecular prooxidant).

The studies were performed in two model systems: washed erythrocytes and unwashed erythrocytes. The level of malondialdehyde (MDA), as a secondary product of lipid peroxidation (LPO), was chosen as an indicator.

A 10% suspension of erythrocytes was prepared, the test substance was added, incubated for a day at 37 ° C, MDA (nmol/ml of erythrocyte mass) was determined in it, the MDA content was recalculated per whole erythrocyte mass, taking into account dilution (x10) [11].

3 Results and discussion

When studying the prooxidant properties of Solanum tuberosum juice in a model system of washed human erythrocytes, it was found that the most significant changes in the MDA level were observed when ferric (II)-ions were introduced into the system: the MDA level increased by 15.3% and amounted to 120.2 ± 6.8 nanomole per Liter (p <0.05). When injected into the system of washed human erythrocytes 10-3Cmax. in Solanum tuberosum juice, the MDA level decreased insignificantly by 7.6% and amounted to 92.4 ± 3.0 nanomole per Liter.

When introduced into the system of bioeffective concentration Cmax. and whole juice (THE JUICE) of Solanum tuberosum, the change in the MDA level was also insignificant: in the case of Cmax. - insignificantly decreased compared to the control by 11.1% and amounted to 92.4 ± 3.0 nanomole per Liter, in the case of Solanum tuberosum whole juice - it increased insignificantly by 1.1% compared to the control and amounted to 105.2 ± 6.3 nanomole per Liter. When using a mixture of ferric (II)-ions+Cmax. the MDA level insignificantly decreased by 2.1% compared to the control and amounted to 101.8 ± 1.9 nanomole per Liter, in the case of the ferric (II)-ions+ THE JUICE mixture - an insignificant decrease by 8.5%, and amounted to 95.1 ± 3.2 nanomole per Liter (Table 1).

When studying the MDA level in the model system of unwashed human erythrocytes, it was found that the most significant changes were achieved when potato whole juice was introduced into the model system, as well as the ferric (II) -ions + THE JUICE mixture: when whole juice was introduced, the
MDA level increased by 60.3% compared with the control, and amounted to 162.2 ± 8.0 nanomole per Liter (p <0.05); in the case of the ferric (II-)ions + THE JUICE mixture, the MDA level was 146.7 ± 10.4 (p <0.05), which is 44.9% higher than the control. When ferric (II)-ions were introduced into the model system of unwashed erythrocytes, the MDA level was 181.5 ± 11.5 nanomole per Liter (p <0.05), which is 79.3% higher than the control and 64% higher than the same indicator in the case of model system of washed erythrocytes (Table 2).

**Table 1.** MDA level (nanomole per Liter) in the model system of washed erythrocytes under the influence of various concentrations of Solanum tuberosum juice

| C                      | M±μ      | %    |
|------------------------|----------|------|
| 10^{-3} C_{max.}       | 96,1±3,9 | 7,6  |
| C_{max.}               | 92,4±3,0 | 11,1 |
| THE JUICE              | 105,2±6,3| 1,1  |
| Fe^{+2} + C_{max.}     | 101,8±1,9| 2,1  |
| Fe^{+2} + THE JUICE    | 95,1±3,2 | 8,5  |
| Fe^{+2}                | 120,2±6,8| 15,3*|
| Control                | 104,0    |      |

Significance of differences in relation to control: * - p <0.05; ** - p <0.01; *** - p <0.001

This nature of the changes can be explained by the fact that the membrane of erythrocytes, not washed from blood plasma, is less resistant to exposure, due to its aggregation with plasma proteins.

This may explain the significant change in the MDA level in the model system of unwashed erythrocytes when exposed to both bioeffective concentration C_{max.} and Solanum tuberosum whole juice, while the level of MDA in the model system of washed erythrocytes when exposed to bioeffective concentration C_{max.} and whole juice did not differ significantly from the control. In both model systems, the introduction of only ferric (II)-ions was accompanied by significant changes in the MDA level [13]. However, in the model system of unwashed erythrocytes, the level of MDA recorded after the introduction of a molecular prooxidant was 5.18 times higher than that recorded in the model system of washed erythrocytes. This can also be explained by the destabilization of the erythrocyte membranes by blood plasma proteins [14].

**Table 2.** MDA level (nanomole per Liter) in the model system of unwashed erythrocytes exposed to various concentrations of *Solanum tuberosum* juice

| C                      | M±μ      | %    |
|------------------------|----------|------|
| 10^{-3} C_{max.}       | 107,2±3,9| 5,9  |
| C_{max.}               | 124,5±9,9| 23,0*|
| THE JUICE              | 162,2±8,0| 60,3*|
| Fe+2 + C_{max.}        | 106,7±10,4| 5,4  |
| Fe^{+2} + THE JUICE    | 146,7±10,4| 44,9*|
| Fe^{+2}                | 181,5±11,5| 79,3*|
| Control                | 101,2    |      |

Significance of differences in relation to control: * - p <0.05; ** - p <0.01; *** - p <0.001
Thus, in both model systems, the absence of pronounced prooxidant properties of the juice of seedlings of Solanum tuberosum was shown. In the model system of washed erythrocytes, the ability of the active substances of Solanum tuberosum juice to influence the LPO process has not been reliably confirmed; In the model system of unwashed erythrocytes, a significant increase in the MDA level was recorded when introducing whole juice, practically the same as when introducing ferric (II)-ions, at the same time, as 10-3 dilution of the bioeffective concentration C_{max} did not cause significant changes in the MDA level compared to the control, which does not allow us to speak about the prooxidant properties of Solanum tuberosum juice. Solanums also accumulate an array of biologically active steroid derivatives of cholesterol, predominated by a diverse family of steroidal glycoalkaloids (SGAs). SGAs are generally regarded as defense compounds against herbivorous insects and phytopathogens, but also impart a bitter taste and are antinutrients that can be toxic to humans [7, 8]. Significant changes in the MDA level in the model system of unwashed erythrocytes can be caused by the fact that steroid glycoalkaloids (solanine, solanidine, etc.) are similar in their mechanism of action to saponins and are also detergents [9].

4 Conclusions

In the study of the prooxidant properties of the juice of the nightshade tuberosum (Solanum tuberosum) in the model system of washed human erythrocytes, it was found that the most significant change in the MDA level occurred when the actual molecular prooxidant, ferric (II)-ions, was introduced into the system: the change was 120.2 ± 6.8 nanomole per Liter (p <0.05), which is 15.3% higher than the control. When using a different range of juice concentrations (10^{-3} - dilution of C_{max}, C_{max}, THE JUICE), as well as their mixture with ferric (II)-ions, changes in the MDA level were insignificant.

When studying the prooxidant properties of Solanum tuberosum juice in the model system of unwashed human erythrocytes, it was found that the most significant changes in the MDA level were observed when whole juice, a mixture of whole juice and ferric (II)-ions were introduced into the system: for whole juice, the change in MDA level was 162.2 ± 8.0 nanomole per Liter (p <0.05), which is 60.3% higher than the control, for a mixture of whole juice and ferric (II)-ions - 146.7 ± 10.4 (p <0.05), which is 44.9% higher than the benchmarks.

Unreliability of changes in the MDA level in the model system of unwashed human erythrocytes when using 10-3 dilutions of the bioeffective concentration C_{max}. Solanum tuberosum juice does not allow us to speak about the presence of prooxidant properties but we can associate biogenic preparations from potato sprouts to the IV hazard class (low-hazardous substances).

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