Antioxidant Effect of Nonalcoholic Syrups Based on Wild-Growing Herbal Raw Material

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Abstract

In the experiment, the rat was used to estimate the antioxidant effect of syrups based on vegetable raw materials. Simulated stress, determines the relative weight of the liver, adrenal glands, spleen, thymus, counted the number of ulcerations in the gastric mucosa was studied lipid peroxidation was determined by cumulative antiradical activity [1]. Discovered and proved the antioxidant effect.

Keywords: Rats; Hepatotoxic; Herbal syrups

Introduction

Solving the problem of adaptation of humans and animals to inadequate environmental conditions can only be based on a thorough understanding of the mechanisms of resistance to adverse environmental factors and, in particular, the factors that enhance the stability of membrane lipids to the damaging effects of oxygen radicals [2]. It is known that drugs (group of adaptogens) increasing the body’s resistance to a wide range of adverse factors in the external and internal environment and having an antioxidant effect help stabilize the lipid peroxidation of biomembranes in conditions of prolonged cold stress, administration of carbon tetrachloride, etc. [4]. To date, numerous studies have proven antioxidant activity of polyphenolic compounds of plant origin, the most important of which in this respect are the flavonoids and anthocyanins [7-10]. The possibility of wide application of polyphenolic compounds as antioxidants determines the relevance of the search for new, inexpensive, and available raw material sources. The harmful effects of free radicals in the case of oxidative stress can be reduced by regular consumption of certain foods and beverages, pharmaceuticals, and biologically active additives (BAA) with antioxidant activity.

Methods

Studies were performed on 30th August on rats of both sexes weighing 220-290 gm contained in natural light regime and fed standard vivarium diet; the vivarium contained five to six individuals in special plastic cages on wood chips bedding with free access to food and water. We developed a range of syrups with increased physiological values of native raw materials with low sugar content [15], with the following assortment: recipe No. 1 (Altai bouquet balm). The composition was as follows [16,17,18]: plant extracts of St. John’s work herb, licorice root, root Hedysarum tea, rose hips, grass meadow geranium, chaga mushroom, sugar syrup, citric acid (acidifier), potassium sorbate (preservative), and sodium benzoate (preservative) [19,20,21].

The composition of recipe No. 2 was as follows: goji berry (powder), carrageenan (thickener), citric acid (acidifier), sugar syrup, potassium sorbate (preservative), and sodium benzoate (preservative).

The following animal groups were formed: the experimental group No. 1 received the recipe No. 1 (test solution); experimental group No. 2 got syrup formulation No. 2 (test solution); control group No. 1 received no active substances—control solution with sugar syrup, citric acid (acidifier), potassium sorbate (preservative), and sodium benzoate (preservative); control group No. 2 received active ingredients—carrageenan control solution (thickener), citric acid (acidifier), sugar syrup, potassium sorbate (preservative), and sodium benzoate (preservative); the intact group was fed the standard vivarium diet.

Under the influence of unfavorable factors, such as foreign chemicals, the concentration of oxo radicals increases sharply and under certain conditions may exceed the ability of the protective antioxidant system to withstand the ravages of these reactive molecules [22-24]. Carbon tetrachloride (CCl₄) easily metabolizes in the active trichloromethyl radical (CCL) and initiates a cascade of radical reaction initiators. For this purpose, rats were injected 4 ml/kg of carbon tetrachloride in a 50% solution in olive oil once by gavage (3). Animals received syrups by gavage, prophylactically five days prior to the injection of hepatotoxin and free days after intoxication within ten days of the experimental rats were injected syrups. The recommended dose of syrups is 2.8 ml/kg per day [25,26]. The test substances were injected 1 h prior to the injection of CCl₄.

Work was executed in compliance with all regulations and international recommendations of the European Convention for the Protection of Vertebrate Animals used for experimental studies. One day after the last injection of hepatotoxin, animals were excluded from the experiment with the use of light ether anesthesia. The effectiveness of the therapy drug under study was judged by the following tests:

1. Specific liver weight (liver weight in mg in relation to body weight per g), which characterizes the degree of inflammation in the body [3].
2. Study of lipid peroxidation (LPO) liver malondialdehyde (MDA)—a product of oxidative degradation of fatty acids by a color reaction with 2-thiobarbituric acid [13]. Measurements were performed with a spectrophotometer Shimadzu UV-1650PC.
3. Assessment of integral antiradical activity (IAA) of antioxidants carried by their ability to inhibit the oxidation of ABTS
by peroxy and alkoxyl radicals formed by thermal (378°C) decomposition of ABAP (2. 29-azobis-(2-aminopropane) hydrochloride) [12]. Measurements were performed on a spectrophotometer Shimadzu UV-2550 with a thermostated cell.

4. Determination of the stress protective activity of syrups by six-point scale [5].

The results obtained were subjected to statistical analysis using Student’s t test.

Results and Discussion

Under the influence of unfavorable factors, such as foreign chemicals, the concentration of oxyradicals increases sharply and under certain conditions may exceed the ability of the protective antioxidant system to withstand the ravages of these reactive molecules. Carbon tetrachloride (CCl4) is readily metabolized into the active trichloromethyl radical (CCl3) and triggers a cascade of radical-initiating reactions. As it is known, carbon tetrachloride that is converted with the cytochrome P-450 in free radicals and electrophilic intermediates is aggressive hepatotoxin.

On the injection of CCl4, the liver of animals reacted by increasing the relative weight—one of the first informative indicators of intoxication. The animals of the control groups showed a statistically significant increase in liver weights at 28-29% as compared to intact rats. In the experimental group, the relative liver weight compared with that of the control group decreased by 11%, indicating a lower severity of hepatitis (Table 1).

In acute CCl4 hepatitis, the severity of antioxidant protection reduced and lipid peroxidation progressed in parallel. In the lipid extracts of liver homogenates, the amount of MDA increased. Indicator of antiradical protection (IAA) decreased by 55% in the control group number 2 and 23% in the control group number 1. In the experimental group treated by syrup number 1 after toxin injection IAA decreased by 19%; in the test group number 2, by 18%. The level of MDA in control group number 2 compared to the intact group was increased by three times, and in control group number 1, no strong changes were recorded—peroxidation product content increased by 1.8 times. In the groups of animals treated with syrups, similar changes occurred in the scorecard “total antiradical system,” but did not reach the physiological values (Table 1).

Syrup injection before and after exposure to carbon tetrachloride has a protective effect, which manifests itself in preventing partial involution lymphoid organs, reducing by half the number of hemorrhages and ulcerations in the gastric mucosa. In the group of animals treated with the formulation number 1, stress-protective activity was three points higher than the corresponding activity in the control group. In the group of animals treated with the formulation number 2, this difference was four points (Table 2).

The drug is considered active if the difference in the scoring points in the control and experimental groups is more than two points [5].

Under the action of the control formulations without herbs, hepatotoxic after injection showed a significant decrease in IAA and increase in the concentration of MDA—the product of oxidative degradation of fatty acids, and the reduction of antioxidant activity was greater in the control number 2, the formulation of which included the thickening agent carrageenan. Carrageenan (E407) is a natural gelling agent obtained during the processing of red seaweed by extraction and followed by purification from impurities [10].

Syrup 1 exerts a significant number of stress-protective effects that appear by reducing the size of the adrenal glands and the intensity of the involution of the thymus and spleen. In total, stressing of the body decreased from eleven to eight points (Table 2).

Complex composition of the syrup number 1 is making it difficult to analyze the activity of each component (lack of syrups with complex composition).

Syrup number 2, containing powdered goji berries, significantly reduced the level of peroxidation products (MDA) compared with the control number 1 (Table 1); adrenal hypertrophy was 25% relative to the control and 42% relative to the norm; involutions of the thymus, 80 and 46%, respectively. Stressing of the body decreased from sixteen to twelve points (Table 2).

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**Table 1:** Effect of syrups on the relative weight of liver, MDA content, and antiradical activity of the integrated index (IAA) in homogenates of rat liver in experimental hepatitis (M ± m)

| Numbers | Group of animals | Relative liver weight (mg/g body weight) | MDA (mmol/g wet weight) | IAA (mmol/g wet weight) |
|---------|-----------------|-----------------------------------------|--------------------------|-------------------------|
| 1       | Intact          | 22.7 ± 2.0                              | 7.97 ± 0.96              | 4.50 ± 0.43             |
| 2       | Control No. 1   | 29.1 ± 2.5*                             | 14.15 ± 0.84*            | 3.49 ± 0.31             |
| 3       | Control No. 2   | 29.4 ± 2.6*                             | 23.30 ± 0.71*            | 2.54 ± 0.17*            |
| 4       | Experience No. 1| 26.0 ± 2.1                              | 13.08 ± 0.39*            | 3.67 ± 0.22             |
| 5       | Experience No. 2| 25.8 ± 2.3                              | 10.83 ± 0.54*            | 3.69 ± 0.13             |

* p < 0.05* compared to intact group.

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**Table 2:** Effect of syrups on the change in mass of organs (adrenal, thymus, spleen) and the number of ulcerations of the gastric mucosa of rats under the action of CCl4, M ± m

| Animal groups | Adrenal gland | Thymus | Spleen | Stomach ulcers |
|---------------|--------------|--------|--------|----------------|
|               | Weight mg/g body weight | % | Point | Weight mg/g body weight | % | Point | Weight mg/g body weight | % | Point | Number | Point | Score |
| Intact        | 0.12 ± 0.006  | 100    | 0      | 0.92 ± 0.08  | 100    | 0      | 3.6 ± 0.2     | 100    | 0      | 3     | 0     |
| Control No. 1 | 0.16 ± 0.01  | 133    | 3      | 0.77 ± 0.06   | 84     | 2      | 3.3 ± 0.5     | 92     | 1      | 7     | 5     | 11    |
| Control No. 2 | 0.17 ± 0.01  | 142    | 4      | 0.42 ± 0.03   | 46     | 5      | 2.7 ± 0.3     | 75     | 2      | 8     | 15    | 16    |
| Experience No. 1 | 0.13 ± 0.02 | 108    | 1      | 0.77 ± 0.07   | 84     | 2      | 3.2 ± 0.3     | 89     | 1      | 4     | 1    | 8     |
| Experience No. 2 | 0.15 ± 0.02 | 125    | 2      | 0.74 ± 0.07   | 80     | 2      | 2.6 ± 0.4     | 72     | 3      | 5     | 5     | 12    |
Conclusions

Thus, by studying the state of lipid peroxidation and IAA in the liver of animals exposed to a hepatotoxic agent—carbon tetrachloride, the presence of antioxidant activity and a pronounced stress-protective action in syrups No. 1 and No. 2 were found. The experimental results show a decrease in the intensity of free-radical oxidation with the injection of herbal remedies in the form of syrups, which is associated with the presence of a complex of biologically active substances that are part of the test plants. In particular, goji berries (Lycium barbarum) contain essential polysaccharides LBP-1, LBP-2, LBP-3, and LBP-4 that are no longer present in any food product, vitamins, 18 amino acids, and 21 minerals (including iron, zinc, iodine). Vitamin C in the berries is more than in oranges [27-30].

Present in plants, flavonoids inhibit the generation of excess free radicals, reduce their concentration in membranes from peroxidation, and the protecting molecules exhibit hepatoprotective action [31-33]. Tannins initiate repair processes and improve the performance of antioxidant systems. In addition, licorice contains carotenoids that embed into the phospholipid-protein membrane structures exhibiting antioxidant activity. It is believed that the biological activity of bioflavonoids is due to their ability to inhibit the oxidation of ascorbic acid catalyzed by heavy metal ions, which form chelates with bioflavonoids. It is also believed that the bioflavonoids are able to inhibit cleavage of lipid peroxidation. Exceptionally important anthocyanins due to the charge on the oxygen atom in the ring of anthocyanins easily penetrate cell membranes [8,34].

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