Hepatoprotective effect of breads with extracts of plants growing in the Far East

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Abstract:
Breads with proven hepatoprotective properties can make a significant contribution to preventing liver disease. This work aimed to study hepatoprotective and antioxidant effects of breads enriched with water and ethanol extracts of polyphenol-containing viburnum (Viburnum sargentii Koehne L.), magnolia-vine (Schisandra chinensis L.), and grapes (Vitis amurensis L.). It was based on an experimental model of toxic hepatitis in mice intoxicated with carbon tetrachloride. Experimental groups of animals were fed on bread with extracts for 7 days and control groups had a bread-free diet. We analysed their body weight, liver lipid metabolism, “lipid peroxidation – antioxidant protection” system, and antiradical activity. The level of reduced glutathione and malonic dialdehyde was determined by micro-thin-layer chromatography. Superoxide dismutase, glutathione reductase, and glutathione peroxidase activity was measured to analyse the antioxidant system. The total content of common polyphenols in breads was determined by the colorimetric method with the Folin-Chocaltceu reagent. The animals on a bread-free diet showed an impaired lipid metabolism and higher activity of liver enzymes. They had a 22% increase in liver weight and a 1.9 times depletion of antiradical protection (6.65 ± 0.15 Trolox units/mg protein vs. 13.15 ± 0.21 Trolox units/mg protein in the control; P < 0.001). We also registered a 2.5 times decrease in superoxide dismutase, a key enzyme of the antioxidant defence system. The animals fed on breads with the above extracts showed a statistically significant normalization of the parameters, compared to the bread-free group. We found that those breads had hepatoprotective and antioxidant effects on the animals, stabilizing their general condition and normalizing their biochemical parameters and antioxidant system.

Keywords: Bread, plant extracts, viburnum, magnolia-vine, grapes, polyphenols, antioxidant, hepatoprotector


INTRODUCTION

Current environmental problems are increasingly exposing the world population to various stress factors such as foreign substances (xenobiotics). The liver is commonly known as the main barrier in the human body that neutralizes xenobiotics and toxic substances. Thus, preventing liver disease is highly important.

According to modern studies, free radical reactions play a significant role in the development of pathology during toxic liver damage. Reactive oxygen species activate the process of lipid peroxidation in hepatocyte cell membranes, causing liver dysfunction. Inhibiting such processes with various biologically active substances (BASs), including natural antioxidants, can have a preventative or therapeutic effect.

Foods with proven hepatoprotective and antioxidant properties can make a significant contribution to solving this problem. Today, bread is a basic food product, and breadmaking is a socially important industry [1]. Bread is part of a daily diet for the vast majority of consumers in many countries. Thus, it can serve as a basis for preventative and dietetic specialized products that can contribute to the prevention of various liver diseases [2].

Introducing plant polyphenols into the product formulation is one of the ways to develop breads with antioxidant and hepatoprotective properties. Characterized by high antioxidant activity, plant polyphenols are a source of effective hepatoprotectors. They are able to eliminate increased lipid peroxidation during toxic hepatitis and improve the antitoxic function of liver cells [3].

In contrast to synthesized polyphenols, natural polyphenols have an extremely low toxicity and do not cause adverse reactions (allergies, addiction, or
accumulation). According to studies, plant phenolic compounds display a wide range of biological (including hepatoprotective) effects. This is due to their antioxidant properties and an ability to act as traps for free radicals of various types [4, 5].

Being plant secondary metabolites, phenolic compounds are contained in traditional plant products used by humans (fruits, vegetables, berries, etc.). Thus, they are evolutionarily adapted for the human body, form complexes with transition metal ions and block lipid peroxidation processes [6–8].

However, of all the plant materials studied (plants, fruits, vegetables, cereals, seeds, bark, etc.), only 10% are sufficiently rich in polyphenolic compounds and can be used as effective natural antioxidants [9, 10]. Both foreign and Russian studies have revealed a need for enriching breads with plant polyphenols to ensure their antioxidant and hepatoprotective properties. For this, we can use such innovative ingredients as berry and fruit extracts, pectin-rich fruits, blueberry paste, and bioflavonoids from larch, vegetables (onions, beets), or ground coffee [17–19].

We evaluated sensory and physicochemical properties of breads containing various concentrations of polyphenols. The bread showed an increased antioxidant activity. Preclinical studies in animals with various diseases are vital in justifying the choice of new ingredients, their efficacy and biological effects in foods with preventative properties. However, scientific literature lacks data on experimental studies in animals that confirm the preventive efficacy of hepatoprotective ingredients in breads. This determined the aim of our study.

The Far East, particularly the Ussuri taiga with its wild plants, is a valuable source of plant raw materials rich in various BASs and used in medicine, pharmacology, animal husbandry, and food industry. BASs are often used as physiologically functional ingredients for dietetic (medicinal and preventative) foods [20].

People have to consume enormous quantities of plants to obtain the required amount of macro- and microelements, as well as other BASs, which is often impossible for many reasons. In this regard, food products are enriched with individual components of plant materials and their extracts that have high biological activity.

Water and ethanol extracts of plant materials are concentrates of biologically active substances. They are effective in small amounts and therefore can be added to products in amounts similar to dried berries [21]. Low contents of biologically active physiologically functional ingredients ensure the preservation of traditional sensory characteristics in the products, which is an important factor for the consumer. Baking technology (with temperatures up to 200–220°C) can neutralize the alcohol component of water and ethanol extracts and their specific sensory properties. This makes such extracts a promising ingredient for breads with dietetic and preventative properties.

For this study, we developed breads with three biologically active dietary supplements (BADSs): Caliphene, Eklikit, and Diprim. They are water and ethanol extracts developed at V.I. Ilyichov Pacific Oceanological Institute, Russia.

Caliphene was obtained from viburnum (Viburnum sargentii Koehne L.) processing waste with proven membrane- and hepatoprotective, antioxidant, antiradical, and other properties. The content of common polyphenols in it was 32.8 ± 2.4 g/L.

Eklikit was extracted from magnolia-vine (Schisandra chinensis L.) processing waste. Its polyphenol complex included proanthocyanidins, leucoanthocyanins, catechins, flavonols, organic acids, free amino acids, etc. The content of common polyphenols was 14.4 ± 1.7 g/L.

Diprim was made of grape (Vitis amurensis L.) stalks. Its main component was such polyphenolic compounds as catechins and their polymer forms, leucoanthocyanins, flavonoids, procyanidins, oligomeric tannins, and lignin. The content of common polyphenols was 35.4 ± 2 g/L [20].

Biomedical studies have found a multifactorial positive effect of Caliphene, Eklikit, and Diprim on the human body, both when used as a dietary supplement and as part of foods, including breads [3, 22–25].

The quality and safety evaluation of breads enriched with water and ethanol extracts showed their compliance with the regulatory documents of the Russian Federation and the Eurasian Economic Union [26, 27].

The preclinical tests of extract-containing breads in animals using standard pharmaceutical models demonstrated their stress-protective (or adaptogenic) and actoprotective effects [28].

We believe that hepatoprotective and antioxidant properties of breads containing water and ethanol extracts are determined by the extract chemical composition as reported in [29, 30], a significant content of polyphenols, and its stability in the products.

Our experimental study in animals with toxic hepatitis aimed to evaluate the hepatoprotective and antioxidant effects of breads enriched with polyphenol-containing functional ingredients, namely viburnum, magnolia-vine, and grape water and ethanol extracts (hereinafter referred to as “extracts”).

STUDY OBJECTS AND METHODS

The objects of our experimental studies were breads produced by the traditional method from wheat flour with the addition of water and ethanol extracts of viburnum, magnolia-vine, and grapes [31].

To determine the potential efficacy of introducing Caliphene, Eklikit, and Diprim, the total content of common polyphenols was measured by the colorimetric
method with the Folin-Chocalteu reagent using gallic acid as a standard [32].

We applied a standard experimental model of toxic hepatitis in mice through their intoxication with carbon tetrachloride, one of the strongest stimulants of lipid peroxidation. Carbon tetrachloride is an organ-specific toxin with a hepatotropic effect. Its toxicity is primarily associated with the prooxidant effect of free radicals that form during its metabolism, trichloromethyl and trichloromethyl peroxy. These radicals initiate lipid peroxidation followed by a chain reaction of free radical oxidation, leading to profound disruption of the functional properties of liver cell membranes, their lysis, and death [1].

The experiments were performed on white mice bred in the Pacific Institute of Bioorganic Chemistry, Russia.

For the experimental model of toxic liver damage by carbon tetrachloride, we used adult male mice with an average weight of 25.00 ± 1.56 g. The animals were kept in standard vivarium conditions in compliance with all the rules and recommendations of the European Convention for the Protection of Vertebrate Animals (Strasbourg, 1986).

The experimental model of carbon tetrachloride intoxication (toxic hepatitis) in animals followed the methodological guidelines for studying hepatoprotective activity of pharmaceutical substances [33, 34].

1.25 mL/kg of carbon tetrachloride (50% solution in olive oil) was injected into the dorsal nuchal fold of mice for 4 days. After that, the mice were given portions of breads containing Caliphene, Eklikit, and Diprim with a total complex of common polyphenols for 7 days. A grid containing bread for 7 days after the toxicant withdrawal.

The control groups included:
- group 1: intact mice fed on the normal diet;
- group 2: mice with toxic hepatitis fed on the normal diet; and
- group 3: mice with toxic hepatitis 7 days after the toxicant withdrawal.

The experimental groups included:
- group 4: mice with toxic hepatitis fed on Diprim-containing bread for 7 days after the toxicant withdrawal;
- group 5: mice with toxic hepatitis fed on Caliphene-containing bread for 7 days after the toxicant withdrawal; and
- group 6: mice with toxic hepatitis fed on Eklikit-containing bread for 7 days after the toxicant withdrawal.

The biomaterial was obtained as follows. Blood samples were taken from the cervical vein of mice in all the groups and collected in test tubes with heparin to measure antioxidant protection. Mice blood serum was used to study lipid metabolism and some biochemical parameters. Mice livers were extracted, washed in a physiological solution, and used to evaluate weight and biochemical parameters of mice.

The antioxidant system was assessed by measuring the activity of superoxide dismutase, glutathione reductase, and glutathione peroxidase, the antiradical activity of blood, as well as the level of reduced glutathione and malondialdehyde [35–37].

The “lipid peroxidation – antioxidant protection” system was studied with biochemical methods. Common lipids were extracted from liver tissue and prepared according to Folch et al. [38]. Micro-thin-layer chromatography on silica gel was used to measure the quantity of phospholipid and neutral lipid fractions. The Russian KSK silica gel was used as a sorbent.

The chromatographic distribution of neutral lipids was performed by one-dimensional thin-layer chromatography on silica gel in a solvent system of “hexane:sulfuric ether:acetic acid” in a ratio of 90:10:1 (v/v/v). Lipid stains were identified using purified preparations of Russian origin. Neutral lipid fractions were quantified according to Amenta [40]. After chromatography, standards and samples were detected with iodine vapours.

The fractions (triacylglycerols, cholesterol, free fatty acids, cholesterol esters, and fatty acid esters) were transferred from the plates to a tube with a special spatula. The tubes were filled with 2 mL of bichromate reagent and heated on a boiling water bath for 15 min. After the samples cooled, 4 mL of distilled water was added to them. They were then stirred and centrifuged at 3000 rpm for 10 min. Optical density was measured on a spectrophotometer at a wavelength of 440 nm. The results were expressed as a percentage of the sum of all fractions.

The results were processed with Instat 3.0 (GraphPad Software Inc. USA, 2005). The parametric Student’s t-test or the non-parametric Mann-Whitney U-test were used to determine the statistical significance of the differences depending on the distribution parameters. The differences were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

As shown in Table 1, the content of polyphenols in, and the antiradical activity of, the breads containing Diprim, Caliphene, and Eklikit extracts are statistically significant and higher than in the control sample (bread without additives). The antiradical activity of breads with plant extracts is determined by polyphenolic structures.

The data confirmed the efficacy of adding Diprim, Caliphene, and Eklikit as functional ingredients to breads with preventative properties in preclinical studies.
The differences are statistically significant at:

- $P < 0.001$ vs. group 3

Table 1: Antiradical activity and common polyphenols in the breads ($M \pm m$)

<table>
<thead>
<tr>
<th>Name of product</th>
<th>Antiradical activity, $\mu$mol trolox/g product</th>
<th>Common polyphenols, mg/g product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bread (control)</td>
<td>0.78 ± 0.013</td>
<td>120 ± 1.8</td>
</tr>
<tr>
<td>Bread + Diprim</td>
<td>0.97 ± 0.012$^1$</td>
<td>388 ± 2.4$^1$</td>
</tr>
<tr>
<td>Bread + Caliphene</td>
<td>0.95 ± 0.018$^1$</td>
<td>385 ± 2.6$^1$</td>
</tr>
<tr>
<td>Bread + Elikit</td>
<td>0.94 ± 0.015$^1$</td>
<td>384 ± 2.5$^1$</td>
</tr>
</tbody>
</table>

Table 2: Weight changes in carbon tetrachloride intoxicated mice and their correction with extract-containing breads ($M \pm m$)

<table>
<thead>
<tr>
<th>Mice groups</th>
<th>Mice weight, g</th>
<th>Mice liver weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 control</td>
<td>25.40 ± 0.88</td>
<td>1.99 ± 0.06</td>
</tr>
<tr>
<td>Group 2 carbon tetrachloride</td>
<td>19.43 ± 0.57$^3$</td>
<td>2.55 ± 0.12$^3$</td>
</tr>
<tr>
<td>Group 3 deprivation (toxicant withdrawal)</td>
<td>21.52 ± 0.95$^1$</td>
<td>2.39 ± 0.08$^1$</td>
</tr>
<tr>
<td>Group 4 deprivation + bread with Caliphene</td>
<td>25.87 ± 0.72$^c$</td>
<td>2.09 ± 0.06$^b$</td>
</tr>
<tr>
<td>Group 5 deprivation + bread with Diprim</td>
<td>25.29 ± 0.89$^b$</td>
<td>2.06 ± 0.08$^b$</td>
</tr>
<tr>
<td>Group 6 deprivation + bread with Elikit</td>
<td>25.18 ± 0.98$^c$</td>
<td>2.07 ± 0.09$^b$</td>
</tr>
</tbody>
</table>

The differences are statistically significant at: $1 - P < 0.05$; $2 - P < 0.01$; $3 - P < 0.001$ vs. control; $a - P < 0.05$; $b - P < 0.01$; $c - P < 0.001$ vs. group 3.

Four days of carbon tetrachloride injections resulted in the mice developing toxic hepatitis with its characteristic symptoms. Their hair became dull and stuck together. They were apathetic and had a poor appetite. Their liver metabolic reactions were impaired, manifesting in:

- weakened antioxidant defences;
- activated lipid peroxidation;
- poor membrane permeability;
- imbalanced lipid metabolism;
- intensified lipolytic processes; and
- poor cholesterol metabolism (esterifying liver function).

Table 3: Biochemical blood parameters of carbon tetrachloride intoxicated mice and their correction with extract-containing breads ($M \pm m$)

<table>
<thead>
<tr>
<th>Mice groups</th>
<th>ALT, $\mu$mol/mL/h</th>
<th>ARA, trolox units/mg protein</th>
<th>SOD, activity units/mL blood</th>
<th>MDA, $\mu$mol/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 control</td>
<td>1.72 ± 0.09</td>
<td>13.15 ± 0.21</td>
<td>678.49 ± 6.47</td>
<td>3.70 ± 0.10</td>
</tr>
<tr>
<td>Group 2 carbon tetrachloride</td>
<td>34.24 ± 1.80$^1$</td>
<td>6.65 ± 0.15$^1$</td>
<td>276.56 ± 8.64$^1$</td>
<td>6.83 ± 0.09$^3$</td>
</tr>
<tr>
<td>Group 3 deprivation (toxicant withdrawal)</td>
<td>13.38 ± 1.20$^3$</td>
<td>8.85 ± 0.65$^3$</td>
<td>435.55 ± 2.75$^3$</td>
<td>5.62 ± 0.05$^3$</td>
</tr>
<tr>
<td>Group 4 deprivation + bread with Caliphene</td>
<td>1.90 ± 0.08$^c$</td>
<td>12.80 ± 1.01$^c$</td>
<td>683.43 ± 4.48$^c$</td>
<td>3.72 ± 0.13$^c$</td>
</tr>
<tr>
<td>Group 5 deprivation + bread with Diprim</td>
<td>1.83 ± 0.07$^c$</td>
<td>12.54 ± 0.89$^c$</td>
<td>685.74 ± 4.32$^c$</td>
<td>4.03 ± 0.16$^c$</td>
</tr>
<tr>
<td>Group 6 deprivation + bread with Elikit</td>
<td>1.87 ± 0.07$^c$</td>
<td>12.02 ± 0.75$^c$</td>
<td>665.43 ± 4.78$^c$</td>
<td>3.61 ± 0.14$^c$</td>
</tr>
</tbody>
</table>

The differences are statistically significant at: $1 - P < 0.05$; $2 - P < 0.01$; $3 - P < 0.001$ vs. control; $a - P < 0.05$; $b - P < 0.01$; $c - P < 0.001$ vs. group 3. Abbreviations: ARA – antiradical activity, SOD – superoxide dismutase, MDA – malondialdehyde.

Tables 2–4 present the indicators of metabolic processes in the control groups of mice (intact and mice with toxic hepatitis), compared with those in the experimental groups of mice (fed on breads with Diprim, Caliphene, and Elikit extracts after carbon tetrachloride withdrawal).

After four days of carbon tetrachloride injections, the weight of the mice decreased to 19.43 ± 0.57 g, which was 24% ($P < 0.001$) lower than that of the intact control mice (25.40 ± 0.88 g). This confirmed the development of toxic hepatitis (Table 2).

We found a statistically significant normalization of the parameters, starting from day 4, in the mice fed on breads with plant extracts after carbon tetrachloride intoxication (groups 4–6, compared with the control). Their weight returned to the control values; their hair became smooth and fluffy; they began to eat well and move actively. Their liver weight was higher than that in the control group, but slightly lower than in the animals with toxic hepatitis. This decrease in liver weight of the animals fed on breads with Diprim, Caliphene, and Elikit indicates the stability of the extracts and their hepatoprotective role in the product.

The development of toxic hepatitis in the control mice intoxicated with carbon tetrachloride was manifested in both the impaired biochemical parameters of their blood, indicative of free-radical processes in the body, and lipid metabolism in their livers. The activity of alanine aminotransferase (ALT), an enzyme marker of liver damage, in the blood serum of mice in this group, was almost 20 times as high (34.2 ± 1.80 μmol/mL/h) as in the control group (1.72 ± 0.09 μmol/mL/h; $P < 0.001$). This was due to the release of the enzyme from the liver cells (hepatocytes) into the blood caused by impaired membrane permeability (Table 3).

Hepatocyte membranes became more permeable as a result of lipid peroxidation. This was indicated by an increase in malondialdehyde (MDA) to 6.83 ± 0.09 μmol/mL of blood plasma in the mice with toxic hepatitis, compared to 3.70 ± 0.10 μmol/mL ($P < 0.001$) in the control group of intact mice (Table 3).

Membrane permeability was impaired by trichloromethene and chlorine (CCl₃ and Cl⁻), the
radicals forming during carbon tetrachloride metabolism in the cytochrome P-450 system after intoxication [40].

The control group of mice with toxic hepatitis that did not receive bread displayed an imbalance and depletion of enzymatic antioxidant processes and antiradical protection. This was indicated by a decrease in antiradical activity to 6.65 ± 0.15 trolox units/mg protein, compared to the control group of intact mice (13.15 ± 0.21 trolox units/mg protein; P < 0.001). Moreover, the activity of superoxide dismutase, a key enzyme of the antioxidant defense system, was 2.5 times as low as in the control group (276.56 ± 8.64 units/mL of blood compared to 678.49 ± 6.47 units/mL of blood, respectively; P < 0.001), as shown in Table 3.

A study of the liver and blood antioxidant system revealed the following simultaneous changes in antioxidant indicators: a 21% decrease in glutathione peroxidase activity (GPO; P < 0.001), a 55% decrease in glutathione reductase (GR; P < 0.001), and a 33% decrease in reduced glutathione (P < 0.001), as shown in Table 4.

Such changes in the antioxidant protection system of carbon tetrachloride intoxicated mice can be regarded as evidence of its depletion.

A study of lipid metabolism in the liver of carbon tetrachloride intoxicated mice revealed a pronounced metabolic disorder. This confirmed the efficacy of the experimental model and proved the development of toxic hepatitis in the experimental animals after the toxicant administration (Table 5).

Table 5 shows an increase in triacylglycerols (TAGs) by 17% (P < 0.001), cholesterol (CS) by 9% (P < 0.001), and free fatty acids (FFAs) by 14% (P < 0.001). These disorders were caused by a breakdown of triacylglycerols in adipose tissue (chemical stress), a release of fatty acids and glycerol into the blood, and their resynthesis as triacylglycerols in the liver. Due to impaired synthesis of phospholipids from triacylglycerols, fatty acids and TAGs accumulated in hepatocytes, leading to fatty infiltration of the liver. The increase in cholesterol was due to the inhibition of mitochondrial oxidation of acetyl CoA in the Krebs cycle, whose condensation lead to cholesterol production. A lower level of fatty acid esters (FAEs) (by 9%; P < 0.05) and cholesterol esters (CSEs) (by 22%; P < 0.001) indicated an impairment of cholesterol metabolism and esterifying function of the liver. This was due to the inhibition of the ACAT enzyme (acyl-CoA: cholesterol-acyltransferase) during carbon tetrachloride intoxication. This ratio of lipid fractions confirmed the fatty degeneration of the liver caused by the impairment of its esterifying function.

Table 4 Indicators of antioxidant liver and blood system of carbon tetrachloride intoxicated mice and their correction with extract-containing breads (M ± m)

<table>
<thead>
<tr>
<th>Mice groups</th>
<th>Reduced glutathione (µmol/g liver)</th>
<th>Glutathione reductase (nmol/min/mL plasma)</th>
<th>Glutathione peroxidase (nmol/min/mL plasma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 control (intact)</td>
<td>4.70 ± 0.15</td>
<td>88.21 ± 4.26</td>
<td>139.0 ± 4.83</td>
</tr>
<tr>
<td>Group 2 carbon tetrachloride</td>
<td>3.13 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.68 ± 3.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>110.1 ± 3.43</td>
</tr>
<tr>
<td>Group 3 deprivation (toxicant withdrawal)</td>
<td>3.09 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.32 ± 1.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>105.3 ± 6.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group 4 deprivation + bread with Caliphene</td>
<td>4.87 ± 0.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>92.48 ± 2.46&lt;sup&gt;c&lt;/sup&gt;</td>
<td>138.3 ± 2.19&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group 5 deprivation + bread with Diprim</td>
<td>4.90 ± 0.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>91.75 ± 3.62&lt;sup&gt;c&lt;/sup&gt;</td>
<td>137.3 ± 2.32&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group 6 deprivation + bread with Eklikit</td>
<td>4.87 ± 0.20&lt;sup&gt;c&lt;/sup&gt;</td>
<td>90.61 ± 2.59&lt;sup&gt;c&lt;/sup&gt;</td>
<td>137.4 ± 1.53&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The differences are statistically significant at: 1 – P < 0.05; 2 – P < 0.01; 3 – P < 0.001 vs. control; a – P < 0.05; b – P < 0.01; c – P < 0.001 vs. group 3. Abbreviations: TAGs – triacylglycerols, FFAs – free fatty acids, FAEs – fatty acids esters, CS – cholesterol, CSEs – cholesterol esters.

Table 5 Neutral lipid content in carbon tetrachloride intoxicated mice liver and their correction with extract-containing breads (M ± m)

<table>
<thead>
<tr>
<th>Mice groups</th>
<th>TAGs</th>
<th>FFAs</th>
<th>FAEs</th>
<th>CS</th>
<th>CSEs</th>
<th>Residual fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 control</td>
<td>21.91 ± 0.60</td>
<td>16.24 ± 0.24</td>
<td>15.49 ± 0.50</td>
<td>14.95 ± 0.19</td>
<td>16.97 ± 0.11</td>
<td>13.65 ± 0.15</td>
</tr>
<tr>
<td>Group 2 carbon tetrachloride</td>
<td>25.54 ± 0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.47 ± 0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.15 ± 0.27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.15 ± 0.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.31 ± 0.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.37 ± 0.44</td>
</tr>
<tr>
<td>Group 3 deprivation (toxicant withdrawal)</td>
<td>26.30 ± 0.35&lt;sup&gt;c&lt;/sup&gt;</td>
<td>17.27 ± 0.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.19 ± 0.24&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15.69 ± 0.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.53 ± 0.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.51 ± 0.35</td>
</tr>
<tr>
<td>Group 4 deprivation + bread with Caliphene</td>
<td>21.89 ± 0.31&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15.85 ± 0.28&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.72 ± 0.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.44 ± 0.26&lt;sup&gt;c&lt;/sup&gt;</td>
<td>17.05 ± 0.19&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.05 ± 0.25</td>
</tr>
<tr>
<td>Group 5 deprivation + bread with Diprim</td>
<td>21.70 ± 0.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15.63 ± 0.26&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.75 ± 0.27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.44 ± 0.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>17.25 ± 0.20&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.23 ± 0.21</td>
</tr>
<tr>
<td>Group 6 deprivation + bread with Eklikit</td>
<td>21.80 ± 0.31&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.30 ± 0.34&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.74 ± 0.28&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.80 ± 0.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15.34 ± 0.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15.02 ± 0.42</td>
</tr>
</tbody>
</table>

The differences are statistically significant at: 1 – P < 0.05; 2 – P < 0.01; 3 – P < 0.001 vs. control; a – P < 0.05; b – P < 0.01; c – P < 0.001 vs. group 3. Abbreviations: TAGs – triacylglycerols, FFAs – free fatty acids, FAEs – fatty acids esters, CS – cholesterol, CSEs – cholesterol esters.
The impaired metabolic reactions in the mice liver proved the efficacy of the experimental toxic hepatitis model through carbon tetrachloride intoxication.

Seven days after the toxicant withdrawal (deprivation period) did not normalize biochemical parameters in the liver of the mice with a bread-free diet. This indicated that their bodies’ own defences were too weak to withstand pathologic development caused by intoxication with hepatotropic poison. The animals were lethargic; they had a poor appetite and dull hair.

The malondialdehyde content in the bread-free group remained 52% \((P < 0.001)\) higher than in the control group, indicating a still high level of lipid peroxidation.

Further decrease in reduced glutathione to 3.09 ± 0.11 \(\mu\)mol/g in group 3 confirmed the ongoing destabilisation of liver cell membranes, causing their imbalance and depleting their antioxidant protection. The activity of superoxide dismutase was 57% \((P < 0.001)\) higher than in group 2 (carbon tetrachloride), due to the toxicant withdrawal, but it was 36% \((P < 0.001)\) lower than in the control group. Likewise, the antiradical activity in group 3 was 35% \((P < 0.001)\) higher than in group 2 (carbon tetrachloride), but 32% \((P < 0.001)\) lower than in the control. The results indicated the continuing free-radical processes.

Those indicators were associated with a high activity of alanine aminotransferase, a marker of liver damage, which was eight times as high as in the control group \((13.38 ± 1.2 \mu\text{mol/mL/h}; P < 0.001)\).

Among neutral lipids, the content of triacylglycerol, free fatty acids, and cholesterol remained significantly high, compared to the control groups. The blood serum of the mice with a bread-free diet showed an impaired cholesterol metabolism – a significantly low content of fatty acid and cholesterol esters – indicating a suppressed esterifying function of the liver. This was consistent with high ALT activity – 8 times as high as in the control group \((13.38 ± 1.2 \mu\text{mol/mL/h}; P < 0.001)\).

Thus, the lipid spectrum of the liver of mice that did not receive bread with extracts indicated a continuing deterioration of metabolic reactions even in the absence of the toxic agent (Tables 2–5).

Feeding the carbon tetrachloride intoxicated mice with bread containing plant extracts (groups 4–6) resulted in the correction and normalization of the parameters studied. The activity of ALT, an enzyme marker of toxic hepatitis, was not significantly different from the control values. This suggested that bread treated with plant extracts had membrane-stabilizing properties.

We found a complete normalization of the membrane lipid peroxidation parameters (in particular, malonic dialdehyde) in groups 4–6 vs. the control. The antiradical and antioxidant protection systems also showed a full recovery.

The analysis of lipid metabolism in the liver of mice in groups 4–6 showed a marked decrease in triacylglycerols, free fatty acids, fatty acid esters, and cholesterol. At the same time, there was an increase in fatty acid esters and cholesterol, compared to group 3 (deprivation). This indicated the efficacy of Caliphene, Diprim, and Eklikit extracts in restoring the esterifying function of the liver and reversing fatty infiltration.

We believe that the biochemical mechanism of restoring the liver function with functional foods after carbon tetrachloride intoxication is based on the localization of polyphenols in the lipid bilayer of the hepatocyte plasma membrane, stabilizing its permeability [41, 42].

**CONCLUSION**

Thus, the experimental model of toxic hepatitis showed that breads enriched with the plant extracts of Caliphene, Diprim, and Eklikit had pronounced hepatoprotective and antioxidant properties.

Those properties were due to the effect of polyphenols contained in the extracts on the metabolism and function of the liver. In particular, polyphenols inhibited free radical reactions, increased the liver’s antiradical and antioxidant activity, and reduced the amount of toxic lipid peroxidation products. They also stabilized hepatocyte membranes, normalized the liver’s esterifying function, and restored the weight of the animals and their liver, as well as ALT activity [43–45]. Thus, breads enriched with water and ethanol extracts obtained from viburnum (Viburnum sargentii Koehne L.), magnolia-vine (Schisandra chinensis L.), and grapes (Vitis amurensis L.) can be regarded as products with preventative properties and recommended as part of a hepatoprotective diet.

**CONTRIBUTION**

Authors are equally related to the writing of the manuscript and are equally responsible for plagiarism.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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perechnya otdelʹnykh vidov sotsialʹno znachimykh prodovolʹstvennykh tovarov pervoy neobkhodimosti, v otnoshenii kotorykh mogut ustanavlivatʹsya predelʹno dopustimye roznichnye tseny, i perechnya otdelʹnykh vidov sotsialʹno znachimykh prodovolʹstvennykh tovarov, za priobretenie opredelennogo kolichestva kotorykh khvoyastvuushchemu subʼektu, osushchestvlyayushchemu torgovuyu deyatelʹnost', ne dopusкаetsya vyplata vozvazhdeniya" [The RF Government Decree No. 530 of July 15, 2010 “Approving the Rules for establishing maximum allowable retail prices for certain types of basic foodstuffs, the List of certain types of basic foodstuffs for which maximum allowable retail prices can be established, and the List of certain types of basic foodstuffs for the acquisition of a certain amount of which the retailer is not entitled to remuneration”].


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