Effectiveness of food concentrate phenolic compounds of apples in experimental membrane pathologies


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Apple fruits are an available source of phenolic compounds that exhibit a wide range of biological activities (antioxidant, anti-inflammatory, membrane stabilizing, etc.). The antioxidant properties of food concentrate phenolic compounds of apples (Concentrate) were studied in vitro in models of spontaneous and ascorbate induced lipid peroxidation (LPO) in rat liver homogenate, and acute carbon tetrachloromethane hepatitis was chosen as in vivo model in rats. Membrane stabilizing activity was evaluated by the degree of hemolysis in blood samples from the tail vein. The effect of Concentrate on vascular permeability was studied considering the time of animal skin papules staining at the site of injection of phlogogenic substances. Hepatoprotective activity in the model of acute carbon tetrachloromethane hepatitis was assessed by changes in prooxidant-antioxidant status in liver homogenate and liver enzymes activity in serum. Significant antioxidant effect of Concentrate was fixed in models of spontaneous and ascorbate induced LPO (TBA reactors’ content was 3.12 times and 2.25 times lower than control for spontaneous LPO and ascorbate induced LPO, respectively) and under tetrachlorohydrocarbons hepatitis. The membrane-protective activity of the studied Concentrate was also high and reached 50.1%. Also, Concentrate demonstrated capillary-strengthening properties, reducing the permeability of the vascular wall, which was caused by three different cholorogens, most notably by zymosan (Concentrate significantly delayed the stain utilization from the bloodstream by 2.14 times compared to control). Newly developed concentrate showed complex hepatoprotective activity, improving the indices of antioxidant-protective status and activity of liver cytoxins enzymes in rats with tetrachlorohydrocarbons hepatitis. The transparent corrective effects of Concentrate are the result of synergism and additivity of its multiple components and indicate the prospects of its further research in order to develop medications for the prophylaxis and treatment of diseases associated with membrane damage.

Keywords: plant polyphenols; antioxidant activity; membrane stabilizing effect; anti-inflammatory effects; capillary-strengthening properties.
It is known that polyphenolic extracts from apple fruit lower cholesterol (Sormellla et al., 2019), increase the high-density lipoproteins content (Zhu et al., 2021) and prevent obesity and type 2 diabetes (Han et al., 2021), and as a consequence, reduce the risk of cardiovascular disease. The antiapoptotic (Liu et al., 2021) and antiinflammatory (Martino et al., 2019) activities of apple fruit concentrates have also been demonstrated. In addition, prophylactic administration of apple polyphenols has been shown to reduce pathological changes caused by cerebral ischemia (Yousfi-Manesh et al., 2021), can prevent lung disease development and progression (Birru et al., 2021) and liver fatty infiltration (Xu et al., 2019).

In most cases, the positive effect was accompanied by prevention or reduction of oxidative stress and some anti-inflammatory effects. It is believed that the powerful antioxidant activity of apple fruits is mediated by various polyphenolic compounds of apple fruit, represented by chrologeanic acid, flosrin, proanthocyanidin B4, epicatechin, catechin, rutin, etc., which apple pulp contains in large quantities (from 0.01% to 1% of fresh fruit weight) (Sure et al., 2017).

The composition and, accordingly, the level and spectrum of activity of polyphenolic extracts of apple varies greatly depending on the variety and place where fruit matures, the part of apple fruit from which they are obtained as well as the method of extraction (Shafi et al., 2019; Zhang et al., 2020). In addition, the molecular mechanisms of the effects of polyphenols on the membrane composition are not fully understood.

Thus, the aim of this study was to investigate the antioxidant, membrane stabilizing and hepatoprotective activities of concentrate of apple polyphenolic compounds in models in vitro and in vivo.

Materials and methods

The experiments were conducted in compliance with “General ethical principles of animal research” (Ukraine, 2001), harmonized with the “European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes” (Strasbourg, 1986). Rats were fed a standard diet, free access to water, and kept in vivarium standard environmental conditions (temperature 20–24 °C, humidity 50–65%).

The filmar vein was exposed on the right hind paw and 1% trypan blue solution in a dose of 2 mL/kg body weight, which was prepared on physiological solution, was injected into it with the help of a tuberculin syringe. After 10 minutes of intravenous injection of the dye, phlogogenic substances were administered intradermally in a volume of 0.02 mL undiluted egg white, 0.1% zymosan suspension or 0.1% histamine solution. Concentrate at a dose calculated by total polyphenol content of 9 mg/100 g of body weight and the reference medication – classic medication with capillary-strengthening effect – quercetin at a dose of 50 mg/kg body weight was administered intraestrally 40 min before the experiment. The effect of Concentrate on vascular permeability by the method of P. P. Golikov (Stefanov, 2001). For this purpose, rats weighing 180–220 g were fixed on the operating table belly up, hair on the abdomen was removed. The femoral vein was exposed on the right hind paw and 1% trypan blue solution in a dose of 2 mL/kg body weight, which was prepared on physiological solution, was injected into it with the help of a tuberculin syringe. After 10 minutes of intravenous injection of the dye, phlogogenic substances were administered intradermally in a volume of 0.02 mL undiluted egg white, 0.1% zymosan suspension or 0.1% histamine solution. Concentrate at a dose calculated by total polyphenol content of 9 mg/100 g of body weight and the reference medication – classic medication with capillary-strengthening effect – quercetin at a dose of 50 mg/kg body weight was administered intraestrally 40 min before the experiment. The effect of Concentrate on r vascular permeability was assessed by the time of papule staining (animal skin at the site of phlogogenic substances injection) in seconds. The next phlogogenic substances were used: egg albumin (protein), zymosan and histamine.

The study of Concentrate hepatoprotective properties was done in a model of acute carbon tetrachloride hepatitis in rats. Animals were divided into four groups: group 1 – intact animals administered placebo; group 2 – control pathology (single intragastric administration of 50% carbon tetrachloride oil solution at a dose of 1 mL/100 g body weight); group 3 – animals treated with Concentrate at a dose of 9 mg/100 g of body weight; group 4 – animals that administered “Silibor” at a dose of 25 mg/kg body weight. As reference medication in the evaluation of hepatoprotective properties of Concentrate hepatoprotector “Silibor” was chosen, which contains bioflavonoids from the Spotted Milk thistle fruits (produced by PhC “Zdrovye”, Kharkiv) at a dose of 25 mg/kg body weight – ED95 for hepatoprotective effect. Concentrate and reference medication were administered intragastrostrally 1 hour before and 2 hours after tetrachloromethane injection. The next day the animals were decapitated under light ether anesthesia. Then blood samples were used to make serum and liver was removed and weighed to calculate liver mass and prepare the homogenate.

The liver mass coefficient (LMC) of the studied animals was calculated as the ratio of liver mass to animal weight in percent (Stefanov, 2001). The intensity of LPO processes was determined by the content of TBA reactants in the liver homogenate (Yoshida et al., 2013). The content of reduced glutathione (GSH) was investigated spectrophotometrically by the optical density of the complex with allsous (Prohorova, 1982). Cata
dase activity was determined spectrophotometrically by reducing the hydroxorgen peroxide uptake and expressed in μmol H2O2/min per mg of protein.
(Koroljuk et al., 1988). The activity of alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma-glutamyltranspeptidase (γ-GTP) was determined in blood serum using standard sets of reagents ("Philist Diagnostics", Dnipro, Ukraine).

Statistical processing of the data obtained was performed using the program SPSS 22.0 software (IBM, USA). Experimental data are presented as x ± SD (mean ± standard deviation). The data was analyzed by descriptive statistics tools. The significance of intergroup comparisons was assessed by ANOVA. The Tukey HSD test was used for multiple comparisons in post-hoc analysis, for pairwise comparison t-test was used. The difference was considered significant at P < 0.05.

**Results**

During liver homogenate incubation in buffer solution at a temperature of 37 ºC, a significant increase in TBA reactants' content was observed (Fig. 1). Herewith, this increase in the TBA reactants' content was observed during the first 15 minutes of incubation. After 15 minutes, the TBA reactants' content did not change. The TBA reactants' accumulation was more pronounced when ascorbate, a potent inductor of non-enzymatic LPO, was added to the incubation medium. Thus, the rate of TBA reactants' accumulation in the first 15 minutes of incubation was 0.41 nmol/g of tissue per minute in spontaneous LPO and 0.51 nmol/g of tissue per minute in ascorbate induced LPO.

**Fig. 1.** Dynamics of TBA reactants’ accumulation during incubation of rat liver homogenate at t = 37 ºC in the absence of inductors and in the presence of ascorbate (x ± SD, n = 7): *** – the difference is significant compared to spontaneous LPO (P < 0.001) in the same time points of measurement

At the same time, growing of the TBA reactants’ level after 5 minutes of incubation was not observed under conditions of both spontaneous and ascorbate induced LPO.

When α-tocopherol was added to the incubation medium, the TBA reactants' accumulation was also less pronounced in comparison with the control group, but more pronounced in comparison with the Concentrate addition (Fig. 2 and 3). Thus, in the case of spontaneous LPO, the TBA reactants' content in the study time with α-tocopherol in the incubation medium was on average 2.49 times higher, and in ascorbate induced LPO 1.47 times higher compared to samples which the Concentrate was added.

**Fig. 3.** Effect of Concentrate (apple polyphenols) and α-tocopherol on the development of ascorbate induced LPO during incubation of rat liver homogenate at t = 37 ºC in the presence of ascorbate (x ± SD, n = 7): **** – the difference is significant compared to control (P < 0.001) in the same time points of measurement, Tukey HSD test; # and ### – the difference is significant compared to α-tocopherol (# – P < 0.05, ### – P < 0.001) in the same time points of measurement

In the further series of experiments, we studied Concentrate antioxidant properties in a model of acute tetrachloromethane induced hepatitis. According to the data obtained (Table 1), the injection of tetrachloromethane in rats leads to a 2.50-fold increase in the content of the final products of LPO – TBA reactants in the liver homogenate compared to control. There was also growth in the intensity of spontaneous (2.96 times) and ascorbate induced (2.64 times) LPO in the liver homogenate of animals injected with carbon tetrachloride (Table 1).

When different rat experimental groups were fed Concentrate and α-tocopherol while subject to liver damage with carbon tetrachloride, the content of LPO products was significantly lower compared with control group. The Concentrate antioxidant activity was 47.8% and was comparable to α-tocopherol. Administration of the studied Concentrate to rats, as well as α-tocopherol, leads to decrease in the intensity of spontaneous and ascorbate induced LPO in the rats’ liver under Control pathology (Table 1), which indicates the same ability to stabilize membranes.

The analysis and evaluation of experimental results of the membrane-protective Concentrate activity showed that in the Control group of animals the degree of hemolysis was 36.2% (Table 2). Previous administration of the Concentrate and reference medications to animals significantly affected this index. When rats were fed Concentrate, the degree of hemolysis was significantly reduced compared to the control group of animals twofold. The membrane-protective activity of the Concentrate was quite high and reached 50.1%, which is probably due to the polyphenolic composition of the Concentrate. The degree of hemolysis under the Concentrate impact was also significantly lower than in the group of animals administered reference medications (1.38 times vs α-tocopherol and 1.57 times vs quercetin). Thus, considering membrane protection activity, the studied Concentrate was comparable to α-tocopherol and exceeded the similar activity of quercetin.

The study of the capillary-strengthening effect of Concentrate showed that in the control group of animals faster staining of skin papules was observed provoked by zymosan (85.7 s), slower – histamine (172.8 s) and slowest – protein (268.5 s, Table 3). The results of the experiment revealed...
Concentrate membrane-stabilizing properties, which were most pronounced under increased permeability of the rats’ vascular wall by zymosan. Concentrate significantly delayed the utilization of the dye from the bloodstream by 2.14 times compared to the control pathology. Under quercetin administration, there was also considerable and significant 1.84 times change in the papule staining time compared with the control group.

Table 1

<table>
<thead>
<tr>
<th>Table 1</th>
<th>The effect of Concentrate (apple polyphenols) and α-tocopherol on the TBA reactants’ content, antioxidant activity and development of spontaneous and ascorbate induced LPO in the liver of rats under carbon tetrachloride induced hepatitis (x ± SD, n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indices / animal groups</td>
<td>Control</td>
</tr>
<tr>
<td>TBARS, nmol/g tissue</td>
<td>1.80 ± 0.17</td>
</tr>
<tr>
<td>Spontaneous LPO, nmol TBARS/g tissue</td>
<td>3.54 ± 0.48</td>
</tr>
<tr>
<td>Ascorbate induced LPO, nmol TBARS/g tissue</td>
<td>5.19 ± 0.59</td>
</tr>
</tbody>
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Notes: * and *** – the difference is significant compared to the control (* – P < 0.050, *** – P < 0.001), Tukey HSD test; ## – the difference is significant compared to Phlogogen+Quercetin (P < 0.001), Tukey HSD test; *** – the difference is significant compared to Hepatitis (P < 0.001), Tukey HSD test.

Table 2

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Membrane-protective activity of Concentrate (apple polyphenols), α-tocopherol and quercetin in a model of spontaneous hemolysis in rats (x ± SD, n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indices / animal groups</td>
<td>Control</td>
</tr>
<tr>
<td>Degree of hemolysis, %</td>
<td>36.20 ± 7.9</td>
</tr>
<tr>
<td>Membrane-stabilizing activity, %</td>
<td>3.82 ± 0.69</td>
</tr>
</tbody>
</table>

Notes: *** – the difference is significant compared to the Control (P < 0.001), Tukey HSD test; ***/### – the difference is significant compared to Phlogogen + Quercetin (P < 0.001), Tukey HSD test.

Table 3

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Capillary-strengthening action of Concentrate (apple polyphenols) and quercetin in rats (x ± SD, n = 7)</th>
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</thead>
<tbody>
<tr>
<td>Velocity of papules coloring under phlogogenic substances injection (c)/ Animal groups</td>
<td>Control</td>
</tr>
<tr>
<td>Zymosan</td>
<td>85.7 ± 8.6</td>
</tr>
<tr>
<td>Histamine</td>
<td>172.8 ± 17.5</td>
</tr>
<tr>
<td>Protein</td>
<td>326.5 ± 28.0</td>
</tr>
</tbody>
</table>

Notes: *** – the difference is significant compared to the Control (P < 0.001), Tukey HSD test; *** – the difference is significant compared to Phlogogen+Quercetin (P < 0.001), Tukey HSD test.

Table 4

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Influence of Concentrate and “Stilbor” on the course of acute toxic hepatitis caused by the introduction of carbon tetrachloride in rats (hepatitis) (x ± SD, n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analyzed biosample</td>
<td>Indices / animal groups</td>
</tr>
<tr>
<td>Liver</td>
<td>LMC, %</td>
</tr>
<tr>
<td>Liver homogenate</td>
<td>TBARS, nmol/g tissue</td>
</tr>
<tr>
<td></td>
<td>GSH, µmol/g</td>
</tr>
<tr>
<td></td>
<td>Catalase, µmol H2O2/min/g protein</td>
</tr>
<tr>
<td>Blood serum</td>
<td>ALT, IU/L</td>
</tr>
<tr>
<td></td>
<td>ACP, µcat/L</td>
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<tr>
<td></td>
<td>γ-GTP, µcat/L</td>
</tr>
</tbody>
</table>

Notes: * and *** – the difference is significant compared to the Control (* – P < 0.05, *** – P < 0.001), Tukey HSD test; *** – the difference is significant compared to Hepatitis (P < 0.001), Tukey HSD test; LMC – liver mass ratio, GSH – reduced glutathione, ALT – alanine aminotransferase, ACP – alkaline phosphatase, γ-GTP – gamma-glutamyltranspeptidase.

Concentrate administration to animals in both prophylaxis and treatment regimen stimulated the restore clinical and biochemical parameters and reduced hyperlipoperoxidation under experimental pathology. Thus, the studied Concentrate significantly reduced the TBA reactants content by 1.91 times (Table 4). The antioxidant status of animals treated with apple phenolic compounds also improved antioxidant defense. In particular, the GSH level in the liver homogenate was restored almost to normal, and the catalase activity was 1.53 times higher than in the control pathology group. Along with positive changes in the indicators of LPO and antioxidant system in the liver tissue, in the serum there was a decrease in the activity of cytolysis enzymes (Table 4). Thus, the activity of ALT was 1.45 times, and the activity of enzymes ACP and γ-GTP 1.18 and 1.22 times, respectively, significantly lower than in rats of the control pathology group. In the group of animals injected with “Stilbor”, significant reduction in LMC by 1.32 times was observed, as well as with the introduction of Concentrate. The content of LPO products in the liver homogenate also decreased by 1.57 times compared with the control group (Table 4). From the antioxidant system there was an increase in the GSH content by 1.75 times and the activity of the antioxidant enzyme catalase by 1.69 times (Table 4).

Discussion

Thus, the obtained data show that the studied Concentrate is able to effectively block both spontaneous and ascorbate induced activation of LPO processes in vitro and in vivo, which indicates its pronounced antioxidant properties. The biological activity of the new Concentrate is determined, primarily, by gallic, caffeic, chlorogenic, ursolic acids; quercetin, epicatechin, leucanthocyanidins and ascorbic acid (Zagoryko...
It is known that these compounds are able to “capture” directly free radicals and reactive oxygen species, chelate metals with variable valence, inhibit enzymes involved in the formation of reactive oxygen species, and restore antioxidant activity (Barnamoto & Kopustinskaie, 2018; Yahf, 2018).

The results obtained in the experiment correspond with the literature data that the polyphenolic compounds contained in the studied concentrate are able to normalize the antioxidant-prooxidant balance, resulting in LPO inhibition. Thus, marked suppression of oxidative stress (decrease in the content of reactive oxygen species and nitrogen and TBA reactants, as well as increased activity and expression of antioxidant enzyme genes) in various tissues with different pathologies is accompanied by prooxidant-antioxidant balance (Xiang et al., 2017; Sedlak et al., 2018; Chang et al., 2021; Yousefi-Manesh et al., 2021). The ability of Concentrate to inhibit ascorbate induced LPO may be related to the binding of iron ions by polyphenols, which are required for induction of LPO by ascorbate.

Powerful antioxidant properties of polyphenols prevent lipid oxidation and, thus, prevent the membrane destruction, which was demonstrated in our study by reducing the degree of hemolysis. Membrane-stabilizing activity of polyphenols from various plant extracts was also shown in publications (Ydyrys et al., 2021). Procyanthocyanidins from pine bark also had a significant membrane-protective effect on erythrocytes in uncontrolled type 2 diabetes mellitus (Visser et al., 2017).

The capillary-strengthening effects of apple polyphenols, which were found in our experiment, are the consequence of antioxidant, membrane-stabilizing and anti-inflammatory effects of the studied Concentrate. The results of the analysis of 22 randomized controlled investigations also indicate pronounced vasoconstrictive properties of polyphenols (Martini et al., 2020). The vascular protective mechanism of chlorogenic acid mediate by many effects: reduction of oxidative damage to endothelial cells, reduction of proinflammatory cytokines, inhibition of E-selectin and adhesion molecule expression, inhibition of angiotsin-converting enzyme and proliferation of cells, etc.

Carbon tetrachloride induced liver damage is a classic model of so-called free radical pathologies, which is used to study the antioxidant properties of biologically active compounds. Significant hepatotoxicity of carbon tetrachloride is associated with its high solubility in lipids and accumulation in the hydrophobic layer of biomembranes, as well as membrane structure damage due to activation of LPO processes by trichloromethyl radical, a product of transformation of this xenobiotic (McGill & Jaesch). Thus, our data on the LPO activation processes and reduction of antioxidant activity in acute tetrachlorothelene induced hepatitis in the liver are consistent with the literature data on the key role of free radical processes in hepatotoxicity of carbon tetrachloride.

The pathophysiological mechanisms of tetrachloromethane induced hepatitis are quite similar to the development of steatosis in humans. Intoxication of animals with carbon tetrachloride is to some extent a standard of liver damage with characteristic manifestations of organ dysfunction and pathomorphological changes in the hepatobiliary system. The necrogenic effect of carbon tetrachloride is triggered by damage to lysosomes, mitochondria and other cell membrane structures due to direct alkylation and activation of LPO induced by the formation of free radical metabolites of carbon tetrachloride (Clemens et al., 2019). The results obtained indicate edema of the organ and circulatory disorders (increased LMC, the cytolytic syndrome development (increased ALT activity) and impaired liver function (increased activity of ALP and γ-GTP).

Administration of Concentrate to rats under acute carbon tetrachloride poisoning significantly improves the antioxidant-prooxidant status of the liver by reducing the LPO development and stabilization of cell membranes, and improving antioxidant protection. Significant reduction of the TBA reactants’ content and the restoration of the functioning of the disturbed components of antioxidant protection (GSH level and catalase activity) of the liver is realized, probably due to the participation of the hydrogen atom polyphenols. In addition to antioxidant and membrane-stabilizing effects, polyphenols are also likely to have anti-inflammatory and anti-inflammatory effects caused by carbon tetrachloride (Liu et al., 2018). Gallic acid and its esters are known to have potent anti-inflammatory activity that can inhibit NF-κB activation by inhibiting the production of interleukin 1 and tumour necrosis factor (TNF) (BenSaid et al., 2017). Another apple polyphenol, f loretin, inhibited the expression of the inflammatory mediator interleukin 6 and the chemokine receptor IL8 and CCL20 lymphocytes, reducing inflammation in chronic obstructive pulmonary disease (Birru et al., 2021).

The results of other researchers, who studied effects of blueberry polyphenolic extract (Liu et al., 2019), green tea (Wang et al., 2019) and cherry fruit polyphenols (Sobehet et al., 2020) in the model of acute carbon tetrachloride hepatitis, showed a similar protective effect: decrease in the TBA reactant content and increase in the activity of superoxide dismutase / glutathione peroxidase and the GSH content, decrease in the activity of transaminases, as well as decrease in the levels of proinflammatory cytokines, total bilirubin and lipids in blood plasma. Hepatoprotective activity was found for some components of the studied apple Concentrate. Thus, gallic acid has been shown to exert its protective effect by enhancing the expression of antioxidant enzyme genes and inhibiting the expression of interleukins (IL1), IL6, cyclooxygenase-2 and TNFα (Ojebuwa & Otaikh, 2021). Quercetin also normalized prooxidant-antioxidant status and reduced serum markers of liver damage (Huang et al., 2018; Liu et al., 2020). Epicatechin (Alkini et al., 2021) and anthocyanins (Popovic et al., 2019) had significant antioxidant and hepatoprotective efficacy.

Conclusions

The data obtained indicated a pronounced antioxidant effect of food concentrate of apple phenolic compounds in in vitro and in vivo models of spontaneous and ascorbate induced lipid peroxidation and carbon tetrachloride hepatitis respectively. The studied Concentrate showed a significant membrane-stabilizing activity, demonstrated capillary-strengthening properties, revealed a complex hepatoprotective effect that was comparable to reference medications.

The discovered corrective pharmacological effects of food concentrate of phenolic compounds of apples indicate the prospects of its further study in order to develop medications for prophylaxis and complex therapy for diseases that are caused by membrane damage.

References


