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ORIGINAL CONTRIBUTION

Cloudy apple juice protects against chemical-induced oxidative stress in rat

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Abstract
Background Apples abundant in phenolic compounds show a variety of biological activities that may contribute to beneficial effects against some chronic diseases.
Purpose The aim of our study was to assess the protective effect of cloudy apple juice against chemical-induced oxidative stress in rats.
Methods Male Wistar rats were treated with apple juice per os, 10 mL/kg/day for 28 days and with a single dose of N-nitrosodiethylamine (NDEA), 150 mg/kg or carbon tetrachloride (CCl_4), 2 mL/kg, 24 h before killing. Two groups of rats not pretreated with juice were administered each of the xenobiotics alone.
Results Microsomal lipid peroxidation in the liver was decreased in rats pretreated with juice by 52–87% when compared to animals given NDEA or CCl_4 alone. Pretreatment with juice protected antioxidant enzymes: catalase, glutathione peroxidase and glutathione reductase but not superoxide dismutase. Their activity was recovered by 49–173% when compared to that in rats given either toxicant alone. The plasma activity of paraoxonase1 was reduced by both toxicants and was increased by 23% in the apple/CCl_4 group. A rise in plasma protein carbonyls caused by the xenobiotics was reduced by 20% only in apple/NDEA-treated rats. Also, in this group of animals, a 9% decrease in DNA damage in blood leukocytes was observed.
Conclusion Phytochemicals in commonly consumed apple juice may protect some macromolecules against oxidative insult induced by xenobiotics.

Keywords Apple · Lipid peroxidation · Antioxidant enzymes · Comet assay · Protein carbonyls

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Introduction

Epidemiological studies suggest that the consumption of vegetables and fruits inversely correlates with the risk of some chronic diseases. Much of this protective effect has been attributed to the biological activity of the non-nutrient secondary plant metabolites such as diverse phenolic compounds [1].

Apples, the most consumed fruits of temperate climate countries are a considerable source of phenolic compounds in human diet. Polymeric procyanidins are the dominant class of apple phenolic chemicals [2]. Several lines of evidence suggest that apples show a wide variety of biological activities that may contribute to health beneficial effects against cardiovascular diseases, asthma and pulmonary dysfunction, diabetes, obesity and cancer. As oxidative stress is thought to play an important role in the pathogenesis of numerous degenerative and chronic diseases, antioxidant activity of apple products has been extensively investigated. Radical-scavenging activity of apple juice, extracts and individual constituents as well as protection of macromolecules, mainly lipids and DNA, against oxidative damage was demonstrated in a number of

assays. Interactions of apple phytochemicals with cancer-specific mechanisms were also described, e.g. inhibition of CYP1A, cyclooxygenase-1 and aromatase or suppression of transcription factor NF-kappa B [3]. In a number of studies in animal models, chemopreventive efficacy of apple juice and extracts has been shown. Human intervention studies based on the modulation of biomarkers as well as epidemiological studies have also provided evidence of potential health-promoting or cancer-preventive activity of apples [3].

Apart from beneficial phytochemicals, human diet contains also harmful chemical species resulting from food processing and/or environmental pollution. Nitrosamines and particularly N-nitrosodiethylamine (NDEA) are examples of the environment- and food-derived carcinogens, found in processed meat and fish, cheese, tobacco smoke and alcoholic beverages. Nitrosamines are also endogenously formed on the basis of nitrite/nitrate precursors found in fertilizers and traces of pesticides and other food pollutants in stomach acidic conditions [4]. The International Agency for Research on Cancer (IARC) classified NDEA as 2A chemical, a possible human carcinogen [5]. Carcinogenic and toxic effects of NDEA are associated with metabolic activation by hepatic microsomal cytochrome P450, mainly CYP2E1. As a result of NDEA deethylation, the electrophilic ethylcarbonium ion capable of DNA adducts formation is generated [4]. Moreover, Yamada et al. [6] using the electron spin resonance technique in vivo revealed the formation of lipid-derived free radicals, which contribute to the lipid peroxidation in the liver of rats administered NDEA. In rats, NDEA caused cancer in liver and, to a lesser extent, in other organs [7].

Carbon tetrachloride (CCl_4) is a well-known model hepatotoxicant, commonly used for the screening of hepatoprotective activity of natural compounds. Although carbon tetrachloride is not directly mutagenic, it can exert genotoxic effects through reactions catalyzed by CYP2E1 that yield reactive free radicals capable of initiating direct oxidative damage to lipids, proteins and DNA. Subsequently, aldehyde-type lipid peroxidation products form bulky adducts with DNA whose mutagenic/carcinogenic potential has been corroborated. Due to these direct and indirect effects, CCl_4 is considered hepatocarcinogenic in rodents [8]. IARC has classified CCl_4 as a potential human carcinogen [9].

As NDEA and CCl_4 were proved to induce oxidative damage to macromolecules and cells, these two xenobiotics were selected for the experimental protocol of the current study. The aim of our study was to find out whether antioxidant phytochemicals present in apple juice could counteract the effects of chemically induced oxidative stress in rats.

Materials and methods

Chemicals and apple juice

Natural cloudy apple juice was produced using press pack at the Research Institute of Pomology and Floriculture, Skierniewice, Poland [10]. The content of active compounds was determined by HPLC [10] (Table 1). Antioxidant activity of juice measured in the ABTS radical cation decolorization assay [11] was 3.6 mMol Trolox equivalents/L.

The reagent kit for protein carbonyls assay was purchased from BioCell Corp. LTD (New Zealand). Agarose (normal melting point) was purchased from Prona, USA, and all other chemicals were from Sigma–Aldrich (St Louis, USA) or from local chemical suppliers.

Experimental design

Forty-eight male Wistar rats (250 ± 15 g) bred at the Department of Toxicology, Poznań University of Medical Sciences were assigned to six different treatment groups of eight animals each. The rats were kept in a 12-h light and 12-h dark cycle at an average temperature and humidity of 21 °C and 50%, respectively and fed ISO 9001-certified laboratory feed (Labofeed H). Groups II, V and VI were given by gavage apple juice, 10 mL/kg b.w./day for 28 consecutive days. Groups I (controls), III and IV were administered the same volume of distilled water for the same period. On 27th day of the experiment, rats were given intraperitoneally a single dose of the carcinogens: groups III and V, NDEA, 150 mg/kg b.w., groups IV and VI, CCl_4, 2 mL/kg b. w. After 24 h, the rats were anesthetized by ketamine, and blood was withdrawn from the heart to heparin-containing tubes. A portion of whole blood was left for the comet assay, the remaining blood was centrifuged (3,000 rpm, +4 °C), and the separated plasma was stored in −80 °C until use. Livers were removed, perfused with ice-cold 1.15% KCl and homogenized in

Table 1 Content of phenolic compounds in cloudy apple juice (mg/L)

Compounds	
(+) Catechin	11.6
(−) Epicatechin	56.3
Procyanidins	74.4
Phloretin xyloglucoside	10.0
Phloridzin	13.0
Chlorogenic acid	48.5
p-Coumaroylquinic acid	9.9
Quercetin glycosides	6.4
Sum	230.1

buffered Tris/sucrose solution (pH 7.55). Microsomal and cytosol fractions were prepared by differential centrifugation according to the standard procedure. Protein concentration in the fractions was determined using the Folin-Ciocalteu reagent. Liver homogenate for glutathione determination was prepared in phosphate buffer, pH 7.4.

The experimental protocol was approved by the Local Animal Ethics Committee guidelines for animal experimentation.

Biochemical assays

Uninduced and Fe^{2+}/ascorbate-stimulated *lipid peroxidation* was assayed in the liver microsomes and measured as the thiobarbituric acid-reactive substances (TBARS) concentration. The results were expressed in nmol malondialdehyde per mg protein [12]. Reduced glutathione concentration was assessed in the liver homogenate with Ellman's reagent [13].

Antioxidant enzymes were assayed in the liver cytosol. Superoxide dismutase (SOD) assay was based on its ability to inhibit spontaneous epinephrine oxidation [14]. Catalase (CAT) activity was determined by monitoring the rate of H_2O_2 decomposition [14]. Glutathione peroxidase (GPx) activity was determined according to Mohandas et al. [15] with H_2O_2 as a substrate. The extent of the NADPH disappearance recorded at 340 nm was a measure of the enzyme activity. Glutathione reductase (GR) activity was assayed by measuring NADPH oxidation at 340 nm using oxidized glutathione as a substrate [15].

Paraoxonase-1 (PON1) activity in plasma was measured in an arylesterase assay using phenyl acetate as a substrate. The rate of phenol generation was a measure of the enzyme activity [16].

Protein carbonyl group concentration in plasma was determined by an ELISA method according to the producer instruction. The method was based on the dinitrophenylhydrazone formation followed by the reaction with specific antibody.

Alkaline comet assay in whole blood leukocytes was performed according to the method of Hartmann et al. [17]. Heparinized blood was processed immediately after the heart puncture. Three slides were prepared for each blood sample. After the steps of cell lysis, DNA unwinding, electrophoresis and neutralization, the slides were dehydrated in absolute ethanol, dried, stored at room temperature, and protected from light. Before evaluation, the slides were rehydrated and stained with ethidium bromide. Images of comets from a Zeiss fluorescence microscope (magnification 400×) were recorded with a digital camera. One hundred cells were scored in each slide. The comets were divided into 5 groups according to the degree of DNA damage and graded from 0 (no damage) to 4 (maximal damage) [18]. A total damage score for the slide was derived by multiplying the number of cells assigned to each grade of damage by the numeric value of the grade and summing over all grades. This system of scoring gives values ranging from 0 when all 100 cells are graded "0" to maximally 400 when all 100 cells are graded "4".

Statistical analysis

The data were expressed as mean ± SD. One-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls test for multiple comparisons were used. $p < 0.05$ was considered to be the limit of significance.

Results

The level of uninduced microsomal lipid peroxidation in the liver of rats administered NDEA or CCl_4 alone was raised by 77 and 87%, respectively, when compared to the control rats. As a result of the juice pretreatment, the TBARS level in rats administered either toxicant was decreased by 52 and 76%, respectively. Fe^{2+}/ascorbate-stimulated lipid peroxidation enhanced in rats dosed with both toxicants was attenuated by the juice pretreatment to reach control level. Apple juice alone did not affect microsomal lipid peroxidation (Table 2).

A slight increase in the hepatic GSH concentration (by 16%) was observed in rats treated with NDEA, while CCl_4 injection caused 18% decrease in the GSH level. Apple juice pretreatment did not affect GSH in NDEA-treated animals but increased GSH concentration in rats injected with CCl_4 up to the level observed in controls (Table 2).

The activity of all antioxidant enzymes assayed was decreased in the liver of rats treated with both toxicants by 21–46% when compared to controls (Table 3). A decrease in the glutathione reductase (GR) activity was slight and statistically insignificant. Pretreatment with juice protected antioxidant enzymes except for superoxide dismutase (SOD). Activities of all antioxidant enzymes assayed were recovered by 49–173% when compared to that in rats treated with the toxicants alone. As a result, the activities of GPx, CAT and GR in rats pretreated with juice and administered toxicants exceeded those in control animals. The only enzyme affected by juice alone was SOD whose activity was reduced by 54% (Table 3).

The plasma activity of paraoxonase-1 was reduced in animals dosed with NDEA or CCl_4 by 22 and 25%, respectively. Protective effect of juice was observed only in the apple/CCl_4 -treated group in which 23% increase in the enzyme activity was found (Table 4).

Protein carbonyl concentration in plasma of rats dosed with NDEA and CCl_4 was increased by 30 and 37%,

Table 2 Effect of apple juice pretreatment on microsomal lipid peroxidation and reduced glutathione in the liver of rats given NDEA or CCl_4

Group	Treatment	Microsomal lipid peroxidation		GSH [μmol/g tissue]
		Uninduced [nmol TBARS/min/mg protein]	Fe^{2+}/ascorbate induced [nmol TBARS/min/mg protein]	
I	Controls	1.46 ± 0.14	21.41 ± 2.75	2.87 ± 0.25
II	Apple juice	1.37 ± 0.18	20.48 ± 1.82	2.83 ± 0.20
		–	–	–
III	NDEA	2.58 ± 1.23[a,b]	28.75 ± 5.90[a,b]	3.33 ± 0.27[a]
		[↑77%]	[↑34%]	[↑16%]
IV	CCl_4	2.74 ± 0.64[a,c]	29.90 ± 5.47[a,c]	2.35 ± 0.15[a,c]
		[↑87%]	[↑40%]	[↓18%]
V	Apple juice + NDEA	1.25 ± 0.19[b]	18.53 ± 5.40[b]	3.44 ± 0.24
		[↓52%]	[↓36%]	–
VI	Apple juice + CCl_4	0.66 ± 0.11[c]	19.74 ± 1.36[c]	2.90 ± 0.40[c]
		[↓76%]	[↓34%]	[↑24%]

Results are mean ± SD, $n = 8$. Control rats were administered water

[a] Controls are compared with juice only- and toxicant-treated groups, $p < 0.05$

[b] The NDEA-treated group is compared with the juice + NDEA-treated group, $p < 0.05$

[c] The CCl_4-treated group is compared with the juice + CCl_4 –treated group, $p < 0.05$

Values in brackets express % of change

Table 3 Effect of apple juice pretreatment on hepatic antioxidant enzymes in rats given NDEA or CCl_4

Group	Treatment	SOD [U/mg]	CAT [U/mg]	GPx [nmol NADPH/min/mg protein]	GR [nmol NADPH/min/mg protein]
I	Controls	6.78 ± 1.64	6.07 ± 1.04	1,474 ± 115	96.8 ± 9.1
II	Apple juice	3.09 ± 0.59[a]	6.15 ± 0.74	1,269 ± 144	94.1 ± 16.5
		[↓54%]	–	–	–
III	NDEA	4.42 ± 0.88[a]	3.55 ± 0.63[a,b]	879 ± 112[a,b]	86.6 ± 9.7[b]
		[↓35%]	[↓42%]	[↓40%]	–
IV	CCl_4	5.37 ± 0.98[a]	3.30 ± 0.60[a,c]	896 ± 123[a,c]	84.7 ± 11.6[c]
		[↓21%]	[↓46%]	[↓39%]	–
V	Apple juice + NDEA	4.62 ± 0.8	6.38 ± 0.77[b]	2,034 ± 296[b]	140.2 ± 17.4[b]
		–	[↑80%]	[↑131%]	[↑62%]
VI	Apple juice + CCl_4	5.19 ± 0.51	6.54 ± 0.68[c]	2,449 ± 218[c]	125.8 ± 16.3[c]
		–	[↑98%]	[↑173%]	[↑49%]

Results are mean ± SD, $n = 8$. Control rats were administered water

[a] Controls are compared with juice only- and toxicant-treated groups, $p < 0.05$

[b] The NDEA-treated group is compared with the juice + NDEA-treated group, $p < 0.05$

[c] The CCl_4-treated group is compared with the juice + CCl_4 –treated group, $p < 0.05$

Values in brackets express % of change

respectively. Pretreatment with juice resulted in 20% decrease in this parameter in NDEA-treated rats. No effect of juice on protein carbonyl level in rats administered CCl_4 was observed (Table 4).

DNA damage measured in the whole blood leukocytes was significantly increased in rats after the NDEA and CCl_4 dosing by 57 and 36%, respectively. Pretreatment of rats with apple juice did not reduce the extent of DNA damage in CCl_4-injected animals; however, in the apple/NDEA group, a slight (by 9%) but statistically significant reduction was measured (Table 4).

Discussion

Diverse natural antioxidants consumed with fresh fruit and fruit products provide beneficial effects, which are superior to results from dietary supplements of purified

Table 4 Effect of apple juice pretreatment on markers of oxidative damage assayed in the blood of rats given NDEA or CCl_4

Group	Treatment	Paraoxonase activity in plasma [U/mL]	Protein carbonyls in plasma [nmol/mg protein]	DNA damage in leukocytes (arbitrary points)
I	Controls	53.3 ± 2.1	0.27 ± 0.02	78.5 ± 7.4
II	Apple juice	52.6 ± 2.5	0.24 ± 0.03	71.0 ± 4.5
		–	–	–
III	NDEA	43.7 ± 2.3[a]	0.35 ± 0.05[a,b]	123.5 ± 7.0[a,b]
		[↓22%]	[↑30%]	[↑57%]
IV	CCl_4	39.8 ± 7.8[a,c]	0.37 ± 0.04[a]	106.5 ± 5.5[a]
		[↓25%]	[↑37%]	[↑36%]
V	Apple juice + NDEA	45.8 ± 5.6	0.28 ± 0.03[b]	112.5 ± 9.9[b]
		–	[↓20%]	[↓9%]
VI	Apple juice + CCl_4	48.9 ± 3.3[c]	0.37 ± 0.03	105.0 ± 8.6
		[↑23%]	–	–

Results are mean ± SD, $n = 8$, Control rats were administered water

[a] Controls are compared with the juice only- and toxicant-treated groups, $p < 0.05$

[b] The NDEA-treated group is compared with the juice + NDEA-treated group, $p < 0.05$

[c] The CCl_4-treated group is compared with the juice + CCl_4-treated group, $p < 0.05$

Values in brackets express % of change

phytochemicals [1]. For this reason, and taking into regard, the report of Oszmiański et al. [2] who found markedly higher content of procyanidins and pectins in cloudy juices, whose presence was associated with higher radical-scavenging and antioxidant capacity, we have chosen to study cloudy apple juice. Additionally, Barth et al. [1, 19] demonstrated higher cancer-preventive efficacy of cloudy apple juice in comparison with clear juice. The authors suggested that this effect was due to the distinct diversity of phenolic compounds that could modulate biochemical pathways by antagonistic, additive and/or synergistic mechanisms.

Although apple products have been shown to exert positive effects in numerous pathologies [3], their preventive activity against chemically induced oxidative damage has not been explored. In the present experiment, we have demonstrated that pretreatment with cloudy apple juice markedly attenuated hepatic microsomal lipid peroxidation induced with model prooxidant carcinogens, NDEA and CCl_4, which is consistent with well-documented in vitro free radical-scavenging ability and antioxidant activity of apple products [3]. Only very few publications report on decreasing lipid peroxidation by apple products in animal models. Pajk et al. [20] found that apples added to feed of pigs in which oxidative stress was induced by a high dietary content of polyunsaturated fatty acids caused about 30% decrease in plasma concentration of lipid peroxidation marker, malondialdehyde when compared with animals not treated with apples. Long-term (12 weeks) consumption of apple juice increased antioxidant status in hamsters fed an atherogenic diet leading to twofold decrease in thiobarbituric acid-reactive substances (TBARS) in the liver in comparison with animals given atherogenic diet alone [21].

Our experiment confirmed the common finding concerning the depletion of reduced glutathione in the liver of animals administered CCl_4.It is known that GSH is utilized in the process of detoxification of free radicals produced in the course of CCl_4 biotransformation [8]. Apple juice pretreatment prevented the depletion of GSH hepatic level. One of the possible explanations might be a decrease in the amount of CCl_4-derived free radicals due to their scavenging by apple juice components. Although NDEA biotransformation also results in free-radical production, the response of hepatic GSH to NDEA was the opposite. There is some discrepancy in reports concerning changes in GSH concentration in animals treated with NDEA; some authors observed the depletion of GSH [22, 23], others demonstrated an increase in GSH level [24, 25]. Our experiment confirmed the latter findings. Apple juice pretreatment did not alter the increased concentration of GSH in NDEA-dosed rats, which could be considered a beneficial effect.

Cloudy apple juice exerted very distinct protective effect on hepatic antioxidant enzymes. The decrease in their activity following NDEA or CCl_4 administration was counteracted by juice pretreatment. As a result, the activities of GPx, CAT and GR were higher than those in control animals. Apple juice pretreatment failed to prevent the decrease in SOD activity caused by injection of both toxicants. Moreover, it was the only enzyme whose activity was suppressed by apple juice administration alone.

A similar degree of hepatic SOD activity inhibition was observed in our previous study in rats treated for 4 weeks with chokeberry juice (unpublished data). It is known that CuZnSOD is inhibited by Cu chelators such as diethyldithiocarbamate [26]. As various polyphenols are able to chelate transition metals [27], it could be suggested that some phenolic compounds present in great concentrations in apple and chokeberry juice can act as SOD inhibitors.

No information about the effects of apple products on antioxidant enzymes in animal models of chemically induced oxidative stress was found in the available literature; however, potentials of other natural sources of phenolic compounds with respect to these enzymes were reported, e.g. black tea partially prevented a decrease in antioxidant enzymes activity in rats challenged with ethanol [28], grape pomace was found to restore the activity of SOD, CAT and GPx in rats treated with CCl_4 [29] and silymarin prevented NDEA-induced decrease in antioxidant enzymes activity in rats [23].

Apart from oxidant status markers in the liver, we assayed also three parameters in blood: the activity of paraoxonase-1 (PON1) and the content of protein carbonyl groups (POCs) in plasma, and the level of DNA damage in blood leukocytes.

Paraoxonase-1 (PON1) is a protein capable of protecting both high-density lipoproteins (HDL) and low-density lipoproteins (LDL) in plasma against lipid peroxidation. Suppressing oxidation of HDL by enzymatic hydrolysis of lipid peroxides, hydroperoxides and hydrogen peroxide helps preserve the antiatherogenic activity of HDL in reverse cholesterol transport to liver. PON-1 is highly susceptible to inactivation by oxidation; hence, consumption of antioxidants like quercetin, glabridin, pomegranate juice or red wine was shown to preserve the enzyme activity by reducing oxidative stress [30]. Although apple juice pretreatment markedly prevented hepatic lipid peroxidation induced by NDEA and CCl_4, PON-1 activity in plasma was moderately protected only in CCl_4 administered rats. It could be explained by different nature of oxidative damage evoked by each xenobiotic.

Protein carbonyl content is by far the most common marker of protein oxidation. Carbonyl groups are relatively difficult to induce compared to other products of protein oxidation. Thus, they are reflective of more severe cases of oxidative stress [31]. In rats dosed with both carcinogens used here, the concentration of serum protein carbonyls was distinctly increased. The protective effect of cloudy apple juice was demonstrated only in NDEA-treated rats but not in animals dosed with CCl_4. It could be suggested that despite the pro-oxidant nature of both NDEA and CCl_4, their damaging effects on plasma proteins differ in magnitude and quality; thus, protection afforded by juice pretreatment is not equally efficient.

The single-cell gel electrophoresis (comet assay) is a simple and sensitive way of showing DNA damage in cell nucleus. In the alkaline (pH > 13) procedure, single and double-strand breaks and alkali-labile sites are detected. These lesions appear upon oxidative stress and by action of active metabolites on the DNA molecule [32]. Thus, DNA damage observed in whole blood leukocytes of rats treated with NDEA or CCl_4 results from the balance between the action of xenobiotic metabolites and the power of numerous possible counteracting systems, such as endogenous antioxidant defense and repair of the occurring lesions. Hepatic DNA of animals exposed to NDEA revealed various forms of alkylated bases, mainly ethylguanines and ethylthymidines. These modified bases possessed diverse mispairing properties and susceptibility to enzymatic repair [33]. It was shown that the lesion-specific repair mechanism depended on the transfer of the alkyl group onto the protein acceptor, O^6-alkylguanine-DNA alkyltransferase followed by degradation of the enzyme protein. High doses of alkylating agents caused a depletion of this repair mechanism [34]. In our current experiment, the applied high dose of NDEA (150 mg/kg b.w.) supposedly attenuated this particular system of DNA repair. The observed increase in DNA breaks might result from degradation of the NDEA-induced alkylated DNA bases to alkali-labile sites and/or from the associated inflammatory reactions. Ueno et al. postulated that ROS appearing as by-products of the NDEA treatment stimulated the NFκB-dependent pathways of neutrophil activation and enhanced release of pro-inflammatory cytokines and nitric oxide. Stimulated neutrophils were the source of abundant reactive oxygen and nitrogen species that enhanced the oxidative damage to macromolecules [35]. Thus, it could be hypothesized that suppression of the neutrophil pro-inflammatory action by any antioxidant might reduce the oxidizing effect of NDEA.

The protective effect of apples on lymphocyte DNA damage *ex vivo* was confirmed by Maffei et al. [36] in a human intervention study. The authors found that a single dose of homogenized unpeeled apples enhanced the resistance of lymphocyte nuclear DNA to oxidative damage caused by exogenous hydrogen peroxide.

In our current experiment, we demonstrated that the intraperitoneal dosing of NDEA to rats caused a significant rise in the DNA damage observed in the whole blood leukocytes. This effect could be probably ascribed to the reactive metabolites passing from the liver to the circulation and/or to the enhanced oxidative metabolism of stimulated neutrophils or macrophages. The reduction of DNA damage observed in rats receiving cloudy apple juice prior to the NDEA injection may result from the antioxidant action of apple phytochemicals.

The examination of the in vivo action of CCl_4 confirms hepatic DNA damage mainly due to the reactions of

trichloromethyl and/or trichloromethyl peroxy radicals directly or indirectly via peroxidation products, though in vitro results are inconsistent [8]. Enhancement in CCl_4-induced DNA strand breaks was measured in rat livers and the extent of damage was diminished when the animals were pretreated with dietary antioxidants: lignan-rich flaxseed extract [37] or a carotenoid-producing alga [38]. In our current study, a significant increase in the degree of DNA damage was found in rats dosed with CCl_4, although Kadiiska et al. [39] did not observe DNA strand breaks in rat blood leukocytes after CCl_4 treatment (1,200 mg/kg b.w.).

We did not find any effect of apple juice on DNA damage induced by CCl_4; however, the extent of DNA damage evoked by NDEA was slightly attenuated by apple juice pretreatment. Various mechanisms of DNA lesion induction, described in the Introduction, may be responsible for these differences.

The protective effect of apple juice on the parameters assayed in blood, namely PON1 activity, protein carbonyls content and leukocyte DNA damage was less prominent than beneficial alterations observed in hepatic parameters. The basic mechanism of CCl_4 and NDEA action is oxidative damage focused in the liver. It cannot be excluded that acute tissue-specific oxidative insult is not reflected by a systemic stress whose markers can easily be measured in blood [38].

In summary, the present study has shown the potential protective action of apple phytochemicals by preventing damages of essential cellular macromolecules in the conditions of chemically induced oxidative stress in rat.

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Conflict of interests statement None.

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