Original Article

COMPARATIVE STUDY OF THE PROTECTIVE EFFECT OF *ARONIA MELANOCARPA* FRUIT JUICE AND QUERCETIN IN A MODEL OF PARACETAMOL-INDUCED HEPATOTOXICITY IN RATS

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Summary

Aronia melanocarpa fruit juice (AMFJ) is very rich in polyphenolic compounds. Quercetin is a naturally occurring flavonoid, one of AMFJ polyphenols. The aim of the present study was to investigate the effect of AMFJ in comparison with quercetin in a model of paracetamol-induced hepatotoxicity in rats. AMFJ at doses of 2.5 and 5.0 ml/kg and quercetin at doses of 50 and 100 mg/kg were administered daily orally from day 1 to day 7 to different animal groups. Paracetamol was applied intraperitoneally (1.0 g/kg) on day 5. Blood and liver ware taken for biochemical investigations on day 7. Liver toxicity was estimated by the serum activities of the enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Oxidative stress was estimated by the levels of thiobarbituric acid reactive substances (TBARS) in liver homogenate and serum. Paracetamol caused a significant elevation of serum AST and ALT, and induced lipid peroxidation as measured by the significant increase of TBARS in serum and liver. In animals pretreated either with AMFJ or quercetin, liver enzyme activities did not differ significantly from the control levels. Both AMFJ and quercetin prevented the elevation of TBARS in the liver at the two applied doses and in the serum only at the higher of the tested doses. In the present model of paracetamol-induced hepatotoxicity, the protective effect of AMFJ was comparable to that of quercetin.

Key words: *Aronia melanocarpa* fruit juice, quercetin, paracetamol, hepatotoxicity, rats

Introduction

The liver is a subject to acute and potentially lethal injury by several substances including some drugs such as paracetamol. Paracetamol is a commonly used antipyretic and analgesic which can lead to liver damage if taken in overdose. At therapeutic doses, the drug is converted by conjugation to water-soluble metabolites which are excreted in the urine. In overdose, the normal conjugative pathways of metabolism become saturated. Then excess paracetamol is oxidized via the P450 system to a toxic metabolite N-acetyl-P-benzoquinoneimine (NAPQI). NAPQI is rapidly conjugated with glutathione to a non-toxic conjugate. When excessive quantities of NAPQI are formed they

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cannot be fully conjugated with glutathione. Under these conditions, NAPQI covalently binds to vital proteins and plasma lipids of hepatocytes. The result is centrilobular liver necrosis and cellular death [1]. The hepatic cell injuries cause the leaking of cellular enzymes into the blood stream and thus can be measured in the serum. The well established effects of a paracetamol overdose on mitochondria include inhibition of mitochondrial respiration and enhanced formation of reactive oxygen species (ROS) and peroxynitrite [2]. The products of lipid peroxidation (thiobarbituric acid reactive substances, TBARS) in paracetamol toxicity serve as a marker of oxidative stress in the tissue [2].

Quercetin is one of the most widely distributed flavonoids. It is one of the components of AMFJ. Quercetin was shown to possess a protective effect against paracetamol-induced liver toxicity which was comparable to the effect of N-acetylcysteine, the antidote approved in paracetamol poisoning [3, 4].

Aronia melanocarpa [Michx.] Elliot (black chokeberry) fruits are extremely rich in phenolic compounds: procyanidins, flavonoids (mainly from the subclass of anthocyanins) and phenolic acids (chlorogenic and neochlorogenic) [5]. Our previous studies [6, 7], as well as the studies of other authors [8,9] have investigated the antioxidant properties of *Aronia* juice, *Aronia* extract or its phenolic constituents using different well established assays. Fresh *Aronia* berries possess the highest antioxidant capacity among berries and other fruits investigated so far [8, 9]. A previous study of ours has demonstrated a pronounced protective effect of AMFJ in a model of carbon tetrachloride-induced hepatotoxicity in rats [10]. Up to date, there are no investigations on the effect of AMFJ in a model of paracetamol-induced hepatotoxicity.

The aim of the present study was to investigate the effect of *Aronia melanocarpa* fruit juice (AMFJ) in comparison with quercetin on liver toxicity and oxidative stress in a model of paracetamol-induced hepatotoxicity in rats.

Materials and Methods

Experimental animals

Male Wistar rats (200-250 g) were used in the experiment. The animals were kept under the standard conditions of the animal house with 12-h light-dark cycle (light 700-1900) at a temperature 23-25°C. They had free access to food and drinking water with the exception of the period of 42 hours before paracetamol administration when all experimental groups were deprived of food. All procedures concerning animal treatment and experimentation were in accordance with the Principles of Laboratory Animal Care (NIH publication № 85-23, revised in 1985), with the European Communities Council Directives 86/609/EEC and the National regulations.

Experimental substances

Paracetamol (acetaminophen) and quercetin were from Sigma-Aldrich Chemie GmbH (Germany). Standart test kids of BioSystems S.A., Barcelona, Spain were used for the measurement of liver enzyme activities. Tween 80, dimethyl sulfoxide (DMSO) and all other chemicals for the biochemical analyses were of analytical grade and were obtained from Merck (Germany).

AMFJ was prepared from *Aronia melanocarpa* fruits which were handpicked, crushed and squeezed. The juice was filtered, pasteurized at 80 °C for 10 min and stored at room temperature till the experiment. The contents of phenolic substances in 100 mL AMFJ were: total phenolics, 709.3 ± 28.1 mg as gallic acid equivalents, determined spectrophotometrically according to the Folin-Ciocalteu procedure [11]; total flavonoids, 189.4 ± 8.6 mg as catechin equivalents, measured by a colorimetric assay developed by Zhishen et al. [12]; total anthocyanins, 106.8 ± 6.2 mg as cyanidin-3-glucoside equivalents, determined by a pH-differential spectrophotometry at pH 1.0 and pH 4.5 [13]; quercetin, 11.8 mg, measured by a high-performance liquid chromatography method [14]. The values were the mean of duplicate determinations of three samples.

Experimental procedure

The animals were randomly divided in five experimental groups each of 10 rats and were treated according to the experimental procedure in Table 1. AMFJ doses were diluted with distilled water to a total volume of 10 ml/kg. Quercetin as a solution in 5% DMSO was administered at a volume of 10 ml/kg. Paracetamol was applied as a suspension with 2% Tween 80 at a volume of 4.0 ml/kg.

Table 1. Experimental procedure

Group (n=10)	Orally by stomach intubation From day 1 to day 7	Itraperitoneally On day 5, 2 hours after the oral treatment
Control (C)	Distilled water (10 ml/kg)	2% Tween 80 (4.0 ml/kg)
Paracetamol (P)	Distilled water (10 ml/kg)	Paracetamol 1.0 g/kg
$AMFJ_{2.5}$ + P	AMFJ 2.5 ml/kg	Paracetamol 1.0 g/kg
$AMFJ_5$ + P	AMFJ 5.0 ml/kg	Paracetamol 1.0 g/kg
Q_{50} + P	Quercetin 50 ml/kg	Paracetamol 1.0 g/kg
Q_{100} + P	Quercetin 100 ml/kg	Paracetamol 1.0 g/kg

Serum and liver homogenate preparation

On day 7, 48 hours after paracetamol administration, the animals were anaesthetized with diethyl ether 2 hours after the last treatment with distilled water, AMFJ or quercetin. Blood was collected from the sublingual veins. It was centrifuged at 2000 rpm for 10 min and serum was obtained for the biochemical analyses.

Samples of liver tissue were homogenized with ice cold Tris/HCl, 50 mM, pH 7.4 (1:10). The homogenate was centrifuged (2000 rpm, 10 min, 4 °C) and the supernatant was used for the biochemical investigations.

Biochemical analyses

Serum activities of liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined spectrophotometrically (Aurius 2021 UV-VIS, Cecil Instruments Ltd, UK) using the standard test kids.

Lipid peroxidation levels were estimated by the thiobarbituric acid (TBA) reaction using the method of Ohkawa et al. [15]. The method measures spectrophotometrically the color produced by the reaction of TBA with lipid peroxides (thiobarbituric acid reactive substances, TBARS) at 532 nm. Malondialdehyde, the major reactive aldehyde resulting from the peroxidation of biological membrane polyunsaturated fatty acids, was used as a standard.

Statistical analysis

Data were analysed statistically by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison post test. A value of $p<0.05$ was considered as statistically significant. Data are expressed as mean ± SEM. GraphPad Prism statistical software was used.

Results

Liver enzymes

The liver enzyme activities are presented on Figure 1. The administration of paracetamol caused a significant elevation of serum AST ($p<0.01$ vs. Control) and ALT ($p<0.05$ vs. Control). AMFJ as well as quercetin at the tested doses prevented the increase of these enzyme activities. Thus, in rats belonging to groups $AMFJ_{2.5}$ + P, $AMFJ_5$ + P, Q_{50} + P and Q_{100} + P the levels of AST and ALT did not differ significantly from the control value (Figure 1).

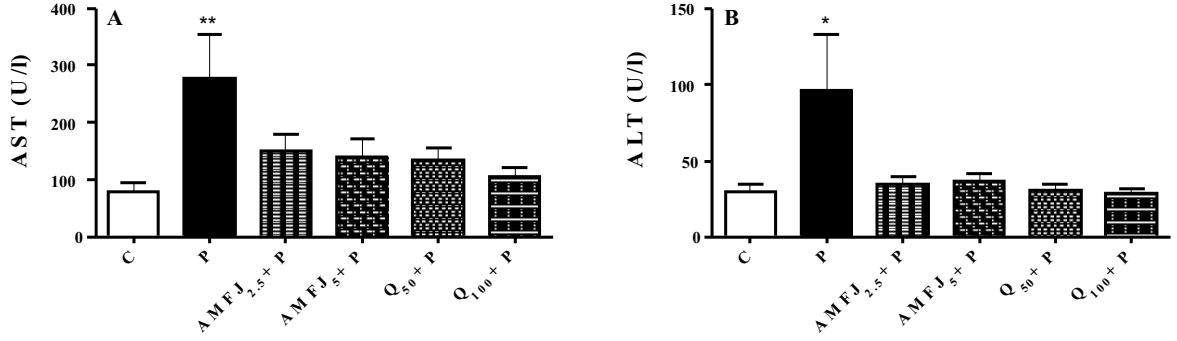

Figure 1. Effect of *Aronia melanocarpa* fruit juice (AMFJ) applied at doses of 2.5 and 5 ml/kg and quercetin (Q) at doses of 50 and 100 mg/kg on the activities of AST (panel A) and ALT (panel B) in a model of paracetamol (P)-induced hepatotoxicity in rats. Values are mean ± SEM; n = 10; *p<0.05, **p<0.01 vs. Control (C)

Thiobarbituric acid reactive substances

Paracetamol induced lipid peroxidation as measured by the significant increase of TBARS in the liver ($p<0.05$ vs. Control) and serum ($p<0.01$ vs. Control) (Figure 2). As is obvious from Figure 2, the TBARS in the livers of rats belonging to groups $AMFJ_{2.5}$ + P, $AMFJ_5$ + P, Q_{50} + P and Q_{100} + P did not differ significantly from the control level. In rat serum, there was a dose-dependent effect of AMFJ and quercetin on the concentration of TBARS. In rats treated with AMFJ at the dose of 2.5 ml/kg as well as with quercetin at the dose of 50 mg/kg the serum concentrations of TBARS were significantly higher ($p<0.05$) than the control level. In rats treated with the higher doses of AMFJ (5 ml/kg) and quercetin (100 mg/kg) the concentrations of TBARS in serum were not significantly different from the control value (Figure 2).

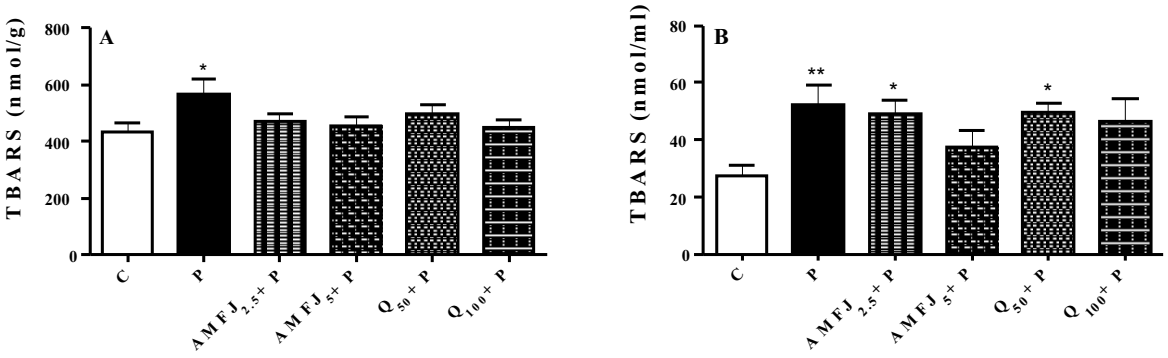

Figure 2. Effect of *Aronia melanocarpa* fruit juice (AMFJ) applied at doses of 2.5 and 5 ml/kg and quercetin (Q) at doses of 50 and 100 mg/kg on the concentration of TBARS in liver (panel A) and serum (panel B) in a model of paracetamol (P)-induced hepatotoxicity in rats. Values are mean ± SEM; n = 10; *$p<0.05$, **$p<0.01$ vs. Control (C)

Discussion

The paracetamol-induced toxicity model is commonly used to study the potential hepatoprotective activity of compounds [16]. In the present investigation, the toxic dose of paracetamol (1.0 g/kg) resulted in a significant elevation of liver enzymes as markers of the liver damage and TBARS as markers of oxidative stress. The paracetamol reactive metabolite NAPQI as described previously is responsible for the development of the hepatotoxicity. Literature data suggest that the NAPQI binds to the mitochondrial proteins and triggers a mitochondrial oxidant stress [16] which finally causes activation of apoptosis [17] and cellular necrosis [18]. Therefore, it is hypothesized that compounds with free radical scavenging and/or antioxidant activities could be protective against the paracetamol-induced liver toxicity. CYP2E1 and CYP1A2 are the major isoenzymes of paracetamol bioactivation [19]. CYP2E1 is an important isoform that participates in the generation of ROS such as superoxide and hydrogen peroxide. ROS may mediate the toxic effects of xenobiotics. Thus, in CYP2E1-knockout mice paracetamol has been found to be considerably less toxic than in wild-type animals [20].

Phytochemical screening of AMFJ has demonstrated the presence of polyphenol substances which have been reported to exert antioxidant, anti-inflammatory and hepatoprotective activities. In the present study, AMFJ at the two applied doses decreased the biochemical indices of liver toxicity and oxidative stress in rats. At least two mechanisms might account for that effect of AMFJ. One of them is the extremely high antioxidant activity of the juice and the capacity to scavenge ROS reviewed by Denev et al. [5]. The other mechanism might be the effect of the juice on the activity of liver enzymes engaged in the metabolism of paracetamol. The study of Krajka-Kuźniak et al. [21] showed that the forced feeding with chokeberry juice alone decreased the activities of cytochrome CYP1A1 and CYP 1A2, and the pretreatment with the juice further reduced the activity of CYP2E1 which was decreased by N-nitrosodiethylamine. These data suggest that AMFJ might inhibit the generation of the toxic metabolite of paracetamol NAPQI.

Quercetin is a flavonoid that has been very intensively studied. In recent years, there are investigations with quercetin in models of paracetamol-induced liver toxicity, which show a very good protective effect of the flavonoid [3, 4, 22]. In these studies, the hepatoprotective effect of quercetin was accompanied by a decrease in oxidative stress and an increase in the antioxidant protection of the animals. Guzy et al. [22] investigated the effect of quercetin on paracetamol-induced mitochondrial dysfunction and found that paracetamol caused significant changes in mitochodrial respiratory chain and mitochondrial ATPase while quercetin counteracted these effects due to its antioxidant potential.

The doses of 2.5 and 5 ml/kg of AMFJ contained respectively about 17.5 and 35 mg/kg of total polyphenols. In the present experiment, the effects of these AMFJ doses were comparable to those of quercetin at doses of 50 and 100 mg/kg. Based on previous data [23, 24], the effects of AMFJ possibly involve the synergistic interaction between flavonoids and other polyphenolic substances in the juice.

Conclusions

This study showed that AMFJ had a protective effect against paracetamol-induced hepatotoxicity in rats. The effect was comparable to that of the flavonoid quercetin and was probably due to antioxidant activity and inhibition of enzymes participating in the generation of the toxic paracetamol metabolite.

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