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INFLUENCE OF FRUIT JUICE FROM *ARONIA MELANOCARPA* ON THE PROCESS OF LIPID PEROXIDATION IN A MODEL OF CARBON TETRACHLORIDE-INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

The fruit juice from *Aronia melanocarpa* (FJAM) is rich in anthocyanins. We studied the effect of FJAM on the process of lipid peroxidation in a model of carbon tetrachloride (CCl_4)-induced hepatotoxicity in rats. Malondialdehyde (MDA) levels were measured in plasma and liver homogenate. CCl_4 induced a significant elevation of MDA levels in the plasma ($p<0,05$) and in the homogenate ($p<0,01$) in comparison with distilled water-treated controls. FJAM applied alone did not significantly influence on the MDA levels. The pretreatment of the rats with FJAM before their treatment with CCl_4 lead dose-dependently to MDA levels in the plasma and homogenate which did not differ in most animal groups from the controls and were significantly lower from those of the CCl_4-treated rats.

Key words: *Aronia melanocarpa*, carbon tetrachloride, malondialdehyde, lipid peroxidation, rats

INTRODUCTION

A lot of *in vitro* and *in vivo* studies have convincingly shown that free radicals are able to cause a direct reversible and irreversible damage of each biomolecule liable to oxidation and, in this way, they participate in the cellular and tissue damage practically in every disease (9).

Polyunsaturated fatty acids in the membrane bilayers are the main target for attack by free radicals (5) which initiate in the membranes a process of lipid peroxidation. Once initiated the lipid peroxidation continues to generate as a chain reaction lipid peroxides and aldehydes. The accumulation of hydroperoxides in the cellular membrane can exert a profound effect on its permeability and selectivity and, as a result, cause a change of the cellular homeostasis and metabolism (3).

Carbon tetrachloride is metabolized in the organism by cytochrome P450 and as a result highly reactive free radicals are obtained which initiate lipid peroxidation, apoptosis and cellular death (1).

Aronia melanocarpa fruits are of a high content of flavonoids, mainly anthocyanins (13). The flavonoids are natural substances with a phenolic structure and demonstrate anti-inflammatory, antiallergic, anti-viral, and cancer preventing properties (11). Many of their effects are based on the antioxidant activity. They are powerful antioxidants scavenging free radicals and inhibiting the lipid peroxidation (11).

The aim of the present study was to investigate the influence of the FJAM on the process of lipid peroxidation in a model of CCl_4-induced hepatotoxicity in rats.

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MATERIAL AND METHODS

Animals

Male Wistar rats (200-250g) were used for the experiment. The animals were kept under the standard conditions of the animal house with 12h light-dark cycle (light 7 00-19 00) at a temperature 23-25°C. They had free access to food and water.

Experimental substances

FJAM was prepared from *Aronia melanocarpa* fruits which were handpicked, crushed and squeezed. The juice was filtered, pasteurized and stored at 0°C.

CCl_4 was from Sigma Chemical Co (St. Louis, MO). All chemicals used for the biochemical analyses and histopathological examination were of analytical grade and were obtained from Merck (Germany).

Experimental procedure

The experimental animals were randomly divided in eight experimental groups of 7 rats each. The rats were orally treated for two days by a direct stomach intubation in accordance with the experimental procedure shown in Table 1.

Table 1. Experimental setting

Group	Number of rats	Treatment	Dose
I (Control group)	7	Distilled water Sunflower oil	10 ml/kg 2 ml/kg
II (CCl_4-group)	7	Distilled water 10% sol. of CCl_4 in sunflower oil	10 ml/kg 2 ml/kg
III (FJAM 5)	7	FJAM Sunflower oil	5 ml/kg 2 ml/kg
IV (FJAM 10)	7	FJAM Sunflower oil	10 ml/kg 2 ml/kg
V (FJAM 20)	7	FJAM Sunflower oil	20 ml/kg 2 ml/kg
VI (FJAM 5 + CCl_4)	7	FJAM 10% sol. of CCl_4 in sunflower oil	5 ml/kg 2 ml/kg
VII (FJAM 10 + CCl_4)	7	FJAM 10% sol. of CCl_4 in sunflower oil	10 ml/kg 2 ml/kg
VIII (FJAM 20 + CCl_4)	7	FJAM 10% sol. of CCl4 in sunflower oil	20 ml/kg 2 ml/kg

CCl_4 (0,2ml/kg) was applied as a 10% solution in sunflower oil in a total volume of 2ml/kg b. w. The interval between the pretreatment (distilled water or FJAM) and the application of CCl_4 or sunflower oil was 2 hours.

Plasma and homogenate preparation

The animals were anaesthetized with diethylether 24h after the last treatment. Blood was collected from the sublingual veins. It was centrifuged at 2000xg for 10min and plasma was obtained for the measurement of malondialdehyde (MDA) content.

After the dacapitation of the animals parts of rat liver were minced in a beaker with a pair of scissors. Samples of liver tissue were homogenized with ice cold 0,9% solution of NaCl (1:10) and MDA content was determined in the liver homogenates.

All procedures concerning animal treatment and experimentation were in accordance with the International Guiding Principles for Animal Research adopted by the local Ethical Commission of Prof. Paraskev Stoyanov Medical University of Varna.

MDA measurement

MDA was measured by its thiobarbituric acid reactivity (TBA) according to Porter *et al.* (14). The method is based on the ability of MDA to form a complex with TBA in an acid medium (pH about 3) and at a high temperature (96°C). MDA values in nmol/ml plasma and nmol/g liver were determined using the extinction coefficient of the MDA-TBA complex at 532 nm = $1,56x10^{-5}$ cm^{-1} M^{-1} solution.

Statistical analysis

The results were assessed by means of one-way analysis of variance (one-way ANOVA) followed by Dunnett's Multiple Comparison Test. A value of $p<0,05$ was considered statistically significant. Results were presented as mean ± standard error. GraphPad Prism statistical software was used.

RESULTS AND DISCUSSION

MDA in rat plasma

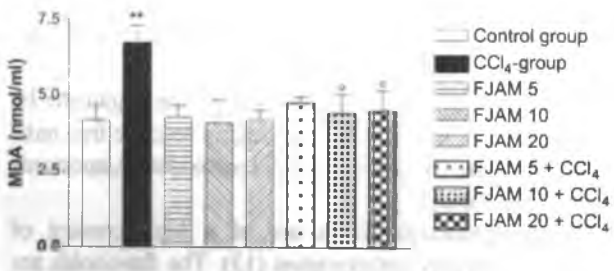

*Fig. 1. Influence of FJAM on MDA plasma levels of rats in a model of CCl_4-induced hepatotoxicity. Statistical significance: **p<0,01 vs control group, °p<0,05 vs CCl_4-group.*

MDA values in the plasma of the animals after the application of FJAM (5ml/kg, 10ml/kg and 20ml/kg) and sunflower oil did not differ from those of the controls. The

treatment with CCl_4 resulted in a significant ($p<0,01$) elevation of plasma MDA in comparison with the controls. Pretreatment with FJAM before the CCl_4-treatment resulted in plasma MDA levels similar to control ones. Plasma MDA concentrations of the groups pretreated with FJAM (10 ml/kg and 20 ml/kg) were significantly lower ($p<0,05$) than those of the CCl_4-group (Fig. 1).

MDA in liver homogenate

FJAM application in the three examined doses did not cause any significant changes in MDA levels in the liver homogenate in comparison with the control group. CCl_4 induced a significant ($p<0,01$) elevation of MDA levels in the liver homogenate. MDA content in liver homogenates of the animals pretreated with FJAM (10ml/kg and 20ml/kg) before CCl_4 did not differ significantly from the control values and were significantly ($p<0,01$) lower in comparison with those of the CCl_4-group. MDA levels of the animals pretreated with the lowest dose of FJAM (5ml/kg) did not differ significantly from the CCl_4-group and was significantly ($p<0,05$) higher in comparison with the control level (Fig. 2).

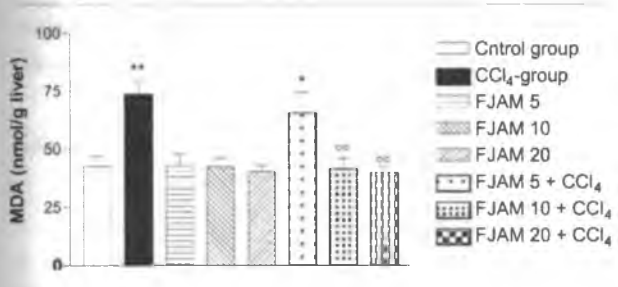

*Fig. 2. Influence of FJAM on MDA levels in rat liver in a model of CCl_4-induced hepatotoxicity Statistical significance: *$p<0,05$ vs control group, **$p<0,01$ vs control group, °$p<0,05$ vs CCl_4-group.*

There are data that the CCl_4-induced hepatocellular damage is due to its reactive metabolites. CCl_4 is metabolized by cytochrome P450 to the highly reactive trichlormethyl radical (CCl_3). The covalent binding of CCl_3· to the cell components initiates the inhibition of lipoprotein secretion and thus steatosis whereas reaction with oxygen to form CCl_3-00 initiates lipid peroxidation. The latter process results in loss of calcium homeostasis and, ultimately, apoptosis and cell death (1).

In the present investigation FJAM prevented the CCl_4-induced lipid peroxidation process assessed by MDA level in plasma and liver. The mechanism of this effect is, probably, similar to that proposed by Letteron *et al.* (10) for the explanation of the protective effect of the flavonoid silymarin. These authors concluded that silymarin prevented the CCl_4-induced lipid peroxidation by a dual mechanism: by decreasing the metabolic activation of CCl_4 by cytochrome P450 into free radicals as well as by scavenging free radicals. The ability of anthocyanins to scavenge free radicals has been demonstrated in other studies, too (4,12,17,18,20). The intoxication with CCl_4 is a model of tissue damage by reactive metabolites. They are obtained in the organism from oxygen. About 4% of the oxygen metabolized in the mitochondria suffers only one-electron reduction and is released as a superoxide anion from which after transformations other reactive oxygen species (ROS) are obtained. The organism has mechanisms for the neutralization of a certain quantity of ROS. When the cellular antioxidant mechanisms become insufficient for the maintenance of the concentration of the accumulating radicals at a low, 'safe' level oxidative damage of the membrane lipids, nucleic acids, carbohydrates and proteins occurs (7,16). The lipid peroxides are potentially toxic and capable of damaging most cell kinds (6-8). The lipid peroxidation products arising from the dying cells can exert a cancerogenic effect. Lipid peroxidation contributes to the development of atherosclerosis, stroke and myocardial infarction (6), takes part in different pathologic conditions including ageing, hepatotoxicity, hemolysis, cancer promotion, and inflammation (2,15).

CONCLUSION

A protective effect of the anthocyanins from *Aronia melanocarpa* fruits was demonstrated in a model of CCl_4-hepatotoxicity. Tissue damage by CCl_4 is due to its reactive metabolites. Oxygen-derived ROS act in a similar way. The anthocyanins inhibit the lipoxygenase reaction either by scavenging free radicals, or by chelating transition metal ions (19) involved in ROS generation. This makes the anthocyanins in the FJAM very powerful antioxidants appropriate for the prophylaxis of many diseases in the pathogenesis of which products of the lipid peroxidation take part.

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