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STUDY OF NATURAL *ARONIA MELANOCARPA* FRUIT JUICE FOR ANTIBACTERIAL AND ANTIVIRAL ACTIVITY

S. Valcheva-Kuzmanova, V. Russev[1], K. Bojkova[1], A. Belcheva

Department of Preclinical and Clinical Pharmacology and Biochemistry, [1]Department of Microbiology and Virology, Prof. Paraskev Soyanov Medical University of Varna

ABSTRACT

Natural *Aronia melanocarpa* fruit juice (AMFJ) is rich in polyphenols most of which are anthocyanins. These compounds and other natural plant products are recently evaluated for their anti-infectious activity. The aim of the present study was to investigate the antibacterial and antiviral activity of AMFJ. AMFJ bacteriostatic activity on referent strains of *S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 was assessed by counting the number of colonies after 24h incubation at 37°C using the microbial number method. AMFJ was not bacteriostatic for *P. aeruginosa*. The microbial number for *E. coli* was 0,36x10^2 CFU/ml AMFJ and for *S. aureus* 0,87x10^8 CFU/ml AMFJ. Gram-positive bacteria should mainly be used as a test for further investigation of the bacteriostatic activity of AMFJ. AMFJ antiviral activity directed to the reproduction in ovo of *influenza virus* type A (H3N2) was investigated. AMFJ inhibited the reproduction of *influenza virus* in its initial stages, most probably, due to the formation of complex compounds between the virion, on one hand, and the polyphenols, on the other hand, which influenced the adsorption of the *influenza virus* on the cell surface.

Key words: *Aronia melanocarpa, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, influenza virus*

INTRODUCTION

Aronia melanocarpa is a bush grown in the woody regions of the Eastern part of North America from where it was carried to East Europe and Russia.

The fruits are rich in cyanidin and its glycosides (cyanidin-3-galactoside, cyanidin-3-arabinoside, cyanidin-3-xyloside and cyanidin-3-glucoside) (13). Cyanidin is a flavonoid from the athocyanin subclass (Fig. 1).

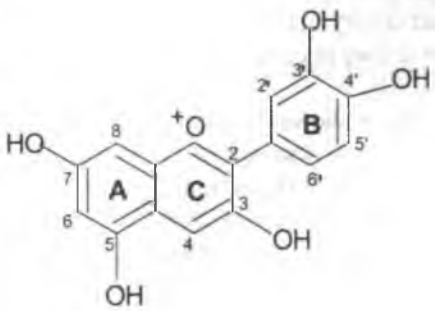

Fig. 1. Cyanidin

Address for correspondence:
S. Valcheva-Kuzmanova, Dept. of Preclinical and Clinical Pharmacology and Biochemistry, Prof. P. Stoyanov Medical University of Varna, 55 Marin Drinov St, BG-9002 Varna, BULGARIA
E-mail: pharmacol@asclep.muvar.acad.bg

The flavonoids have long been recognized to possess antiinflammatory, antioxidative, antiallergic, antiviral, hepatoprotective, antithrombotic and anticancerogenic activities (8).

Since the flavonoids are known to be synthesized in plants in response to microbial infection it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide variety of microorganisms (4). Such microorganisms are *Staphylococcus aureus, Corynebacterium diphtheriae, Pseudomonas aeruginosa, Streptococcus pneumoniae, Neisseria meningitides, Vibrio cholerae* 01, *Streptococcus mutans, Shigella, Bordetella pertussis* and *Mycoplasma pneumoniae* (3). Plant extracts rich in the flavonoids apigenin and luteolin which proved to be effective against methicillin-resistant S. aureus (15,17) are an example of new substances that could be effectively applied in infections nowadays difficult to treat.

It was demonstrated that the pomegranate peel extract rich in anthocyanins has a strong antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans* (10).

Naturally occurring flavonoids with antiviral activity have been known since the 1940s (16). Some viruses against which flavonoids exhibit inhibitory effects are the herpes simplex virus, respiratory syncytial virus, para*influenza virus* and adenoviruses, picornaviruses along with the vesicu-

lar stomatitis virus, polioviruses, influenza A and B viruses, and human immunodeficiency virus (HIV) (3).

Most antiviral studies were carried out in vitro and little is known about the antiviral effects of the flavonoids in vivo. Antiviral activity was demonstrated for the anthocyanin-rich extract from black currants (*Ribes nigrum L.*) against influenza A and B viruses (6). Anthocyanins in the fruits of elderberry (*Sambacus nigra*) inhibit the replication of the human *influenza virus* (19).

The aim of the present study was to test the natural *Aronia melanocarpa* fruit juice (AMFJ) for bacteriostatic activity in vitro and for antiviral activity *in ovo*.

MATERIAL AND MATHODS

Bacteriostatic activity

The bacteriostatic activity was determined after the serial dilution method (5). Referent strains of microorganisms were used: 24h broth cultures of *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27583 with a concentration of 10^9 colonies forming units/ml (CFU/ml). AMFJ was diluted from 10^{-1} до 10^{-10} in a sterile broth medium. One ml of the bacterial suspension was added to each tube with the diluted AMFJ. After 2h cultivation at 37°C 0,005 ml from each tube were plated to three plates with solid meat-peptone agar. The plates were cultivated for 24h at 37°C. The number of colonies was counted (CFU/ml). The mean value from three dilutions was calculated and was multiplied times the corresponding reciprocal value. The lowest concentration that still inhibited the bacterial growth was accepted for bacteriostatic.

Antiviral activity

Two virulent *influenza virus* strains were used: subtype A /Varna/3/003 and A/Varna/5/003 with an antigen formula H3N2 with an infectious activity 5,00-6,00 $lgLD_{50}$. Sterile AMJF was used. AMFJ virusocidal activity was measured on 9-11-days old chicken embryos. One ml of AMFJ was added to 1 ml virus containing suspension (5,00 lgLD50 with a hem agglutination titer 1:256). The so prepared suspension (pH 7,0) was incubated at 37°C for 1-3h. Before the embryos were infected the hem agglutination titer of the virus strains was checked. The titration of the suspension was made on chicken embryos. Embryos infected with the corresponding viruses and with AMFJ were used as controls.

The results were presented as mean values from three experiments carried out with both virus strains.

RESULTS AND DISCUSSION

Bacteriostatic activity

For the *Escherichia coli* ATCC 25922 strain it was established that CFU were still observed in dilutions from 10^{-1} до 10^{-3} and the mean value related to the dilution of 10^{-2} was 36. The microbial number for the bacterium was $0,36x10^2$.

For the *Staphylococcus aureus* ATCC 25923 strain the counted CFU were in dilutions of AMFJ from 10^{-7} to 10^{-9} and the mean value related to the dilution of 10^{-8} was 87. The microbial number for the bacterium was 0,87x108.

For the *Pseudomonas aeruginosa* ATCC 27583 strain no bacteriostatic activity of AMFJ was detected.

It is difficult to outline the probable mechanism of the flavonoid action because of controversial data concerning the structure activity relationships of flavonoids. There are data that the flavonoids without a hydroxyl group in their B rings are more effective against microorganisms than those with OH groups (2); this finding supports the idea that the membrane is the target of the flavonoid action in the microorganisms. The more lipophylic substances should be more devastating for this structure. Other authors, however, have established the opposite effect, i.e. the greater hydroxylation the greater antimicrobial activity (14).

Some authors have found a correlation between the antimicrobial and antioxidant activity of the plant products. Osato *et al.* (12) have established that the seeds and the fruits of the tropical medicinal plant *Carica papaya Linn.* are bacteriostatic for several enteric pathogens: *Bacillus subtilis*, *Enterobacter cloacae*, *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. The same parts of the plant scavenge free radicals - hydroxyl and superoxide, which are probably a part of the cellular metabolism of these bacteria.

Probably, similar mechanisms underlie AMFJ inhibitory activity on *Staphylococcus aureus* and *Escherichia coli* which was demonstrated in the present investigation.

Escherichia coli possesses adhesins which prominate on the bacterial cell surface like hairs and thus allow the bacteria to adhere on the walls of the urinary tract and other internal organs. It was shown that the extract from blueberry (*Vaccinium*) containing cyanidin-3-galactoside, cyanidin-3-arabinoside, and cyanidin-3-glucoside possesses an antiadhesin activity (11). It is similar to other findings (7) that the blueberry anthocyanins can effectively cure and prevent light chronic and recurrent infections of the urinary bladder and urinary tract; they prevent the bacterial adhesion on the urinary tract walls.

Antiviral activity

The present study showed that the hem agglutination activity of both *influenza virus* subtypes decreased - from 1:256 to 1:16 for A /Varna/3/003 and to 1:32 for A/Varna/5/003.

The studies of AMFJ antiviral activity *in ovo* showed that the infectivity of both strains of *influenza viruses* decreased to $0,lgLD_{50}$. The structural basis for the antiviral activity of naturally occurring flavonoids was studied (18). It was demonstrated that the hydroxylation in positions 3', 4', 3, 5 and 7 was linked with the highest inhibition of HSV-1 replication. According to Selway (16), the hydroxylation in position 3 was predisposing for the antiviral activity. According to Bae *et al.* (1), the flavonoids in their glycone form have a greater inhibitory activity on the infectivity of rotaviruses than in their aglycone form.

AMFJ contains cyanidin mainly in a glycone form (13). All anthocyanins have an OH-group in position 3 of the C ring, and cyanidin has hydroxyl groups in position 3', 4' of the B ring. These structural characteristics explain the good antiviral activity of AMFJ. In our study, AMFJ inhibited the reproduction of *influenza virus* in its initial stages most probably due to the formation of complexes between the virion and the phenolic substances as a result of which the adsorption of *influenza virus* on the cell surface was prevented.

Similar mechanisms of the antiviral effect of flavonids are discussed in the literature. The green tea flavonoids inhibit the infectivity of the *Influenza* A and B *viruses*. They agglutinate the viruses by binding to hem agglutinine and thus inhibit their adsorption on the host cells (9).

Knox *et al.* (6) have investigated the antiviral activity of anthocynanin containing extract from black currant (*Ribes nigrum L.*) against *Influenza* A and B *viruses*. According to them. the anthocyanins do not directly inactivate the *influenza virus*es but inhibit their adsorption on the cells and their release from the infected cell. There are data from clinical investigations that anthocyanins alleviate the symptoms of the influenza infection and lead to a faster improvement of patients in comparison with placebo (19). The beneficial effect of the anthocyanins in vivo can be explained with their ability not only to inhibit the viral replication but also to stimulate the immune system via the induction of cytokine production either in healthy individuals, or in patients with influenza (19) and in immune-suppressed patients such as those with cancer and HIV.

CONCLUSION

AMFJ is bacteriostatic against *Staphylococcus aureus* and *Escherichia coli* in vitro and has no inhibitory activity against *Pseudomonas aeruginosa*. The bacteriostatic activity against *Staphylococcus aureus* is greater than that against *Escherichia coli*. Gram-positive bacteria should mainly be used as a test for a further investigation of AMFJ.

AMFJ inhibits *in ovo* the reproduction of *Influenza virus* type A in its initial stages, most probably, due to inhibition of the virus adsorption on the cell surface. In viral infections even better effect might be expected because of AMFJ stimulation of the immune system.

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