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ORIGINAL ARTICLE

PROTECTIVE EFFECTS OF GREEN TEA AND BILBERRY AGAINST SECONDARY HYPERLIPIDEMIA IN GENTAMICIN–INDUCED NEPHROTOXICITY IN RATS

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The aim of this study was to examine whether green tea and bilberry have beneficial effects on secondary hyperlipidemia that developed due to gentamicin-induced renal impairment.

The GM group of rats was treated with gentamicin only, the GT group was given green tea only, the B group received bilberry only, while the C group was given saline only. The GT+GM group received green tea simultaneously with gentamicin, whereas the B+GM group was given bilberry with gentamicin.

The results showed that gentamicin significantly increased total cholesterol, LDL, and triglycerides, while it decreased HDL compared with the control group. However, when either green tea or bilberry was applied together with gentamicin, this secondary hyperlipidemia was significantly ameliorated, as evidenced by a significant increase in HDL and a decrease in LDL, cholesterol, and triglycerides in comparison to the GM group.

The beneficial effects of both green tea and bilberry on secondary hyperlipidemia in gentamicin-induced nephrotoxicity occurred due to their powerful antioxidant properties. They are both functional foods, widely available in nature, and can be used as cost-effective additional therapy together with gentamicin, without affecting its activity in killing bacteria.

Keywords: gentamicin, nephrotoxicity, green tea, bilberry, lipids

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INTRODUCTION

Gentamicin (GM) is an aminoglycoside antibiotic widely used in the treatment of life-threatening bacterial infections. It has a wide spectrum of activity against both Gram-positive and Gram-negative bacteria, including *P.aeruginosa* and other Gram-negative enteric bacilli. It is still a crucial antibiotic, although dose-dependent ototoxicity and nephrotoxicity limit its use. Both adverse effects are found to be caused by oxidative stress (1,2).

There is increasing concern about secondary hyperlipidemia, which occurs in gentamicin-induced nephrotoxicity. It is documented that even other aminoglycosides, such as puromycin, cause critical hyperlipidemia with an elevation in key lipoproteins (3).

Polyphenols are secondary metabolites mainly present in fruits and vegetables that are considered to be functional foods. Those are green tea, coffee, cocoa, red wine, dark chocolate, cherries, and berry fruits (such as bilberry). Nowadays, functional foods have been the focus of research due to increasing interest in healthy diets and lifestyles (4). It is documented that they have a therapeutic effect in the prevention and amelioration of chronic and lifestyle diseases induced by oxidative stress due to their antioxidant and anti-inflammatory effects (2). In our study, we used green tea (GT) and bilberry (B) as functional foods and investigated their possible protective effect in secondary hyperlipidemia caused by GM-induced nephrotoxicity that was established in our previous experiments (5,6).

METHODS

Animals (48 adult Wistar rats, both male and female, weighing 200-250g) were acclimatized for 14 days, then divided into six groups of eight animals each. They were given a standard rat chew diet and had free access to water. Green tea and bilberry extracts were prepared as described in our earlier papers (5,6).

The control (C) group was injected intraperitoneally with saline for 15 days. The green tea (GT) group was treated with green tea extract orally (150 mg/kg/day) for 15 days. The bilberry (B) group was orally given bilberry extract (100 mg/kg/day) for 15 days. Kidney damage in the GM group was caused by intraperitoneal injection of GM (100 mg/kg/day) during the last eight days of the experiment. The last two groups (GT+GM and B+GM) were treated with the same dose of GT and B extracts, respectively, over the course of all 15 days of the experiment, and were simultaneously given GM during the last eight days.

Twenty-four hours after the last treatment, the animals were sacrificed using ketamine as an anesthetic. Blood was collected from the aorta, and its samples were sent to the Clinic of Nephrology, University Clinical Center Niš, for biochemical estimation of lipid parameters. All experimental steps were done in accordance with the principles of the local Ethical Committee, by which it was approved (number 01-2625-8).

Biochemical analysis

For biochemical analysis, lipid parameters in all animals (total cholesterol, LDL, HDL, and triglycerides) were determined using standard biochemical methods on automatic analyzers in the laboratory of the Clinic of Nephrology, University Clinical Center Niš.

Statistical analysis

All data were reported as mean ± standard deviation. Statistical significance of differences between groups was computed by one-way analysis of variance (ANOVA) followed by Tukey post hoc test for multiple comparisons (Graphpad Prism version 5.03, San Diego, CA, USA). P values less than 0.05 were considered significant.

RESULTS

Rats in the GM group showed a significant increase in total cholesterol (Figure 1), LDL (Figure 2), and triglycerides (Figure 3), as well as a decrease in HDL cholesterol (Figure 4), when compared to the C group. This is a clear indication of secondary hyperlipidemia associated with rat nephrotoxicity caused by gentamicin, which was proved in our earlier experiments (5,6). Our experimental doses of both GT (150 mg/kg/day) and B (100 mg/kg/day) ameliorated and counteracted renal impairment and secondary hyperlipidemia induced by GM, making it less toxic. In groups where both GT and B were simultaneously given with GM (GT+GM and B+GM), total cholesterol, LDL, and triglyceride levels (Figures 1, 2, and 3) were significantly lower in comparison to the GM group, while HDL levels were significantly higher (Figure 4). Nevertheless, when compared to the C group, total cholesterol, LDL, and triglyceride levels (Figures 1, 2, and 3) were higher, and HDL levels (Figure 4) were lower, but without statistical significance. In addition, there was no statistical significance among the lipid parameter values mentioned above in groups that were only given plant extracts (GT and B groups) when compared to the control group.

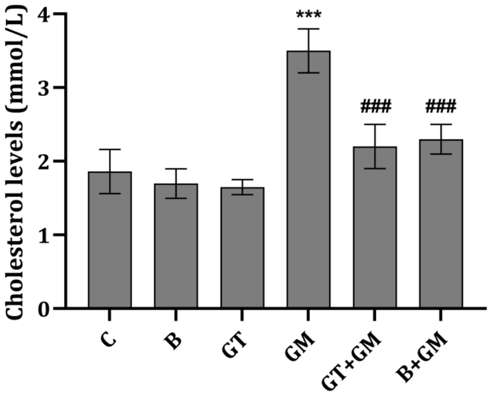

Figure 1. *Total cholesterol levels in rats*

C—control; B—bilberry; GT—green tea; GM—gentamicin
*** $p < 0.001$ vs C; ### $p < 0.001$ vs GM

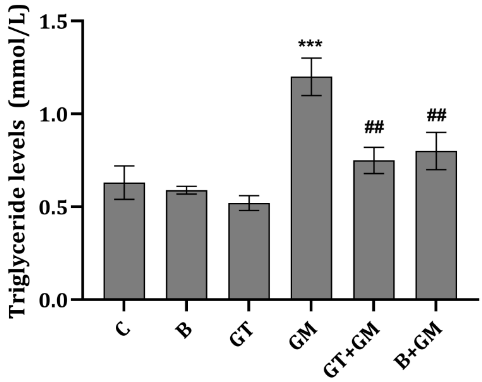

Figure 3. *Triglyceride levels in rats*

C—control; B—bilberry; GT—green tea; GM—gentamicin
*** $p < 0.001$ vs C; ## $p < 0.01$ vs GM

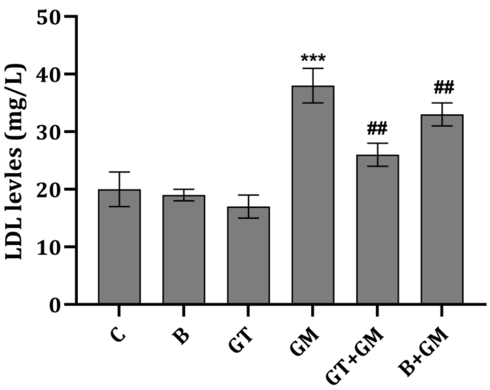

Figure 2. *LDL levels in rats*

C—control; B—bilberry; GT—green tea; GM—gentamicin
*** $p < 0.001$ vs C; ## $p < 0.01$ vs GM

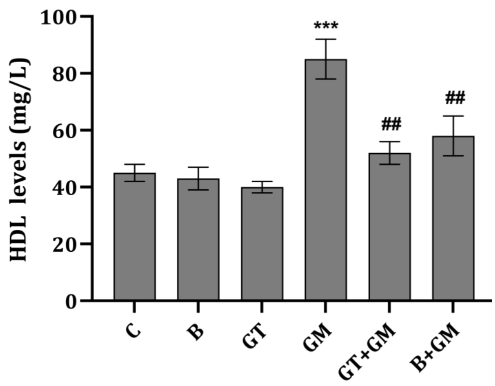

Figure 4. *HDL levels in rats*

C—control; B—bilberry; GT—green tea; GM—gentamicin
*** $p < 0.001$ vs C; ## $p < 0.01$ vs GM

DISCUSSION

The administered doses and length of treatment periods with GM, GT and B were determined according to the available literature and our earlier experiments (5,6).

Aminoglycoside antibiotics, including GM, are widely used as therapy for Gram-negative infections. One of the main adverse effects during its use is nephrotoxicity, which is responsible for 10-15% of all cases of acute renal failure. The main site for its toxicity is the proximal tubules, as GM is predominantly accumulated there (7).

In our previous studies (5,6), GM nephrotoxicity was confirmed by serum increase in urea and creatinine in rats and by histopathological changes in the kidneys, mostly in proximal tubules. The documented nephrotoxicity occurs together with secondary hyperlipidemia. The induced increase in total cholesterol that was recorded in our study was similar to the data related to hypercholesterolemia linked with puromycin-induced nephrotoxicity in rats (8).

Data found in the literature point out that the main way GM causes nephrotoxicity is through oxidative stress, by the generation of reactive oxygen species (ROS) (5,6). These oxygen metabolites cause ischemic, toxic, and inflammatory tissue impairment during GM use. The hydrogen peroxide production inside the mitochondria was boosted in a dose-dependent manner by GM treatment. It also stimulates the production of superoxide anion and hydroxyl radical in the renal cortical mitochondria. Another mechanism of its toxicity is the release of iron from mitochondria initiated by the generation of hydrogen peroxide. Since these are the ways GM induces its toxicity, research was conducted to try to use antioxidants and iron chelators as a means to reduce GM nephrotoxicity, without affecting bactericidal activity (8).

Functional foods are rich in polyphenols and are the focus of research due to increasing interest in healthy nutrition. Its daily intake has been linked with reduced risk of metabolic, cardiovascular, and lifestyle diseases, such as obesity, type II diabetes mellitus, and hypertension. Polyphenols found in green tea are catechin, epicatechin, and epigallocatechin, whereas anthocyanidins (pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and malvidin) can be found in bilberry. These polyphenols are strong antioxidants and can increase the intrinsic antioxidant defenses of an organism, thus decreasing oxidative stress (4). Applying 200 or 400 mg/kg bw/d of polyphenol extract of *A. grossedentata* via gastric gavage documented antioxidant effects with the depletion of TNF-α, IL-6, and NF-kB levels in LDLr-/- mice fed with a cholesterol-free diet (HFD) after 12 weeks, with the stronger dosage of 400mg/kg being more effective (9).

In our previous work (5,6), we proved that GM caused nephrotoxicity via oxidative stress by significantly increasing malondialdehyde (MDA) and decreasing catalase values. In this paper, we are studying the possible role of secondary hyperlipidemia in the development of renal impairment in rats induced by GM. Gentamicin caused a significant increase in total cholesterol, LDL, and triglycerides, and a significant decrease in HDL cholesterol fraction when compared to control. However, when given together with GM, both functional foods (B and GT) showed a significant hypolipemic effect, increasing HDL, but decreasing total cholesterol, LDL, and triglycerides when compared to the GM group. The antihyperlipidemic activity of both bilberry and green tea extracts is through the antioxidant effect that inhibits lipid peroxidation induced by GM. Antioxidant activity of the extracts occurs in two ways. The first mechanism is by donating a hydrogen atom. The second way is by inhibiting the lipase enzyme via the activity of the extracts, hence preventing the breakdown of lipids. Polyphenols found in functional foods are also known to decrease lipoprotein secretion through the liver and intestines and to enhance the secretion of bile acids that increase the rate of lipid excretion. By these mechanisms, they are performing their hypolipemic activity (10).

Other authors have reported that the main reason for the increase in total cholesterol of uremic patients and experimental animals is due to increased production of cholesterol in the liver and increased production of lipoproteins caused by the developed hypoalbuminemia. The hypertriglyceridemia that occurred secondary to renal damage was thought to develop because of delayed removal of triglyceride-rich lipoproteins from the circulation due to a decrease caused by lipoprotein lipase activity (11).

In our previous studies (5,6), we documented that GM caused nephrotoxicity due to oxidative stress and that both GT and B ameliorated renal impairment through their antioxidant activity. In this experiment, we tried to analyze if these same substances would have a beneficial effect in secondary hyperlipidemia caused by GM-induced nephrotoxicity. Both are considered to be functional foods.

It has been demonstrated that interest in functional food has significantly increased. Polyphenols, the strong antioxidants abundantly found in functional foods, including GT and B, are documented to have beneficial effects on hyperlipidemia through various mechanisms. Both GT and B, as sources of polyphenols, are widely available in nature, part of everyday nutrition, and are generally safe. They may offer safe and cost-effective therapy that can ameliorate not only GM-induced nephrotoxicity, but also secondary hyperlipidemia that comes with it, without affecting its antibacterial activity.

Acknowledgements

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Statement of Ethics

This study protocol was reviewed and approved by the Faculty of Medicine Ethical Committee, approval number 01-2625-8, issued on April 8, 2014.

Competing Interest

The authors declare no relevant conflicts of interest.

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