

EFFECT OF LONG-TERM APPLICATION OF QUERCETIN ON CONTENT OF SERUM BILIRUBIN AND ALBUMINS OF RABBITS AND GENDER COMPARISON

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ABSTRACT

The aim of present study was to determinate the effect of quercetin on content of serum bilirubin and albumins in rabbits. Adult rabbits meat line M91 were divided into three experimental groups (E1, E2 and E3) and the control group without quercetin addition. Experimental rabbits received quercetin intramuscularly in various doses; 10 $\mu\text{g.kg}^{-1}$ in E1 group, 100 $\mu\text{g.kg}^{-1}$ in E2 group, and 1000 $\mu\text{g.kg}^{-1}$ in E3 group for 90 days, 3 times per week. Application of quercetin increased content of bilirubin and decreased content of albumins in all experimental groups compared to the control group. Gender comparison has not confirmed significant changes. In conclusion, as the bilirubin serves in organism as antioxidant with the ability to scavenge free radicals, our results could contribute to the positive effect of quercetin on antioxidant balance, however further studies are needed.

Keywords: Rabbits, quercetin, bilirubin, albumins, gender comparison



INTRODUCTION

Flavonoids are polyphenolic compounds with antioxidant properties that occur ubiquitously in foods of plant origin (Formica and Regelson, 1995). A high dietary intake of flavonols, a subclass of flavonoids, has been inversely associated with the incidence of cardiovascular disease (Hertog et al., 1993; Knekt et al., 1996). A number of different mechanisms may be involved in the biological activities exerted by the flavonols including free radical scavenging, chelation of metal ions and/or through association with different endogenous proteins (Cook and Samman, 1996; Rice-Evans et al., 1996; Kandaswami and Middleton, 1994; van Acker et al., 1995).

Flavonoids are widely distributed in the plant kingdom and are categorized as flavonol, flavanol, flavanone, flavone, anthocyanidin, and isoflavone and they are absorbed from food. Quercetin, 3,3',4,5,7-pentahydroxyflavone, is a typical flavonol-type flavonoid ubiquitously present in fruits and vegetables (Murota and Terao, 2003). Numerous *in vitro* studies have revealed diverse biological effects of quercetin, including apoptosis induction, antimutagenesis, protein kinase C (PKC) inhibition, lipoxygenase inhibition, histamine-release inhibition, superoxide dismutase (SOD)-like activity, modulation of cell cycle, angiogenesis inhibition, and inhibition of angiotensin converting enzyme II (Formica and Regelson, 1995). Quercetin intake is therefore suggested to be beneficial for human health and its antioxidant activity should, at least partly, yield such a variety of biological effects (Rice-Evans and Packer, 2010).

Bilirubin (BR) is a natural product of heme catabolic pathways, it could provide continuous protection of natural lipids against oxidative free radical damage (McDonagh, 1979). Many studies indicate the important role of BR as a natural antioxidant, at least *in vitro*. Free heme, which is toxic, is degraded via cleavage of its tetrapyrrole ring by hemoxygenase (Maines, 2005). Hemoxygenase degrades heme to biliverdin, which is then reduced by biliverdinreductase (BVR) to bilirubin. BVR mediates reduction of biliverdin to bilirubin being the much more potent antioxidant and subsequent oxidation of bilirubin by hydrogen peroxide back to biliverdin forming a catalytic antioxidant cycle that is driven by NADPH, the reducing cofactor of BVR (Sedlak and Snyder, 2004). There is also evidence that BR can protect cells (Tomaro and Batlle, 2002) and low density lipoprotein (LDL) (Wu et al., 1996) against lipid peroxidation and contribute to the antioxidant capacity of jaundiced new-born infants (Bélanger et al., 1997).

Albumin has several important physiological and pharmacological functions. It transports metals, fatty acids, cholesterol, bile pigments, and drugs. It is a key element in the regulation of osmotic pressure and distribution of fluid between different compartments (Bourdon and Blache, 2001). Among endogenous antioxidants, albumin represents a very abundant and important circulating antioxidant in plasma (Brahimi-Horn and Pouyssegur, 2007; Musante et al., 2006), a body compartment known to be exposed to continuous oxidative stress (Cha and Kim, 1996). Such albumin-bound bilirubin acts as an inhibitor of lipid peroxidation and can protect α -tocopherol from damage mediated by peroxy radicals (Neuzil and Stocker, 1993).

The aim of the present work was to determine effect of long-term application of quercetin in various doses on concentration of albumins and bilirubin in rabbit's blood and gender comparison.

MATERIAL AND METHODS

Animals and diet

Adult female rabbits (n = 20) and male rabbits (n = 20) of meat line M91, maternal albinotic line (crossbreed Newzealand white, Buskat rabbit, French silver) and paternal acromalictic line (crossbreed Nitra's rabbit, Californian rabbit, Big light silver) were used in experiment. Rabbits were healthy and their condition was judged as good at the commencement of the experiment. Water was available *ad libitum*. Groups of adult animals were balanced for age (150 days) and body weight (4 ± 0.5 kg) at the beginning of the experiment. Adult rabbits were fed diet of a 12.35 MJ.kg⁻¹ of metabolizable diet (Tab. 1) composed of a pelleted concentrate.

Animals were divided into four groups (n=10 in each group), one control group (C) and three experimental groups (E1, E2 and E3). Experimental groups received quercetin in injectable form (intramuscularly) at 10 $\mu\text{g.kg}^{-1}$ in E1 group, 100 $\mu\text{g.kg}^{-1}$ in E2 group, and 1000 $\mu\text{g.kg}^{-1}$ in E3 group for 90 days 3 times a week. Rabbits without quercetin application served as the control.

In this animal study, institutional and national guidelines for the care and use of animals were followed, and all experimental procedures involving animals were approved by the State Veterinary and Food Institute of Slovak Republic, no. 3398/11-221/3.

Blood sampling and analyses

After 3 mounts of intramuscular application of quercetin, rabbits were slaughtered and blood samples were obtained. The blood serum was separated from whole blood by centrifugation at 3000g for 30 min. The concentrations of bilirubin (BR) was determined using automatic analyzer Microlab 300 (Merck®, Germany) and concentration of albumins (ALB) was determined by spectrophotometric analysis (Genesys 10, Thermo Fisher Scientific Inc., USA).

Statistical analyses

The data used for statistical analyses represent means of values obtained in blood collection (end of the experiment). One-way ANOVA test was applied to calculate basic statistic characteristics and for determination of significant differences between the experimental and control groups. Statistical software SIGMA PLOT 11.0 (Jandel, Corte Madera, CA, USA) was used.

Table 1 Chemical composition (g.kg⁻¹) of the experimental diet

Component	Contents
Dry matter	926.26
Crude protein	192.06
Fat	36.08
Fibre	135.79
Non.nitrogen compound	483.56
Ash	78.78
Organic mater	847.49
Calcium	9.73
Phosporus	6.84
Magnesium	2.77
Sodium	1.81
Pottasium	10.94
Metabolizable energy	12.35 MJ.kg ⁻¹

RESULTS AND DISCUSSION

In this study, the effect of quercetin on antioxidant status of rabbits (BR and ALB) was measured from blood serum. The results are summarized in figures 1 and 4. Bilirubin has been reported as a member of the antioxidant family and is even known to have toxic effects at high concentration. The combined evidence from animal and human studies indicates that bilirubin is a major physiologic cytoprotectant and might alleviate oxidative stress in the blood (Sedlak and Snyder, 2004). No significant differences ($P > 0.05$) in bilirubin concentration were found between the control group and experimental groups in this work. The content of bilirubin was the highest at 100 µg.kg⁻¹ of quercetin (E2 group) and at 10 µg.kg⁻¹ of quercetin (E1 group). The increased of bilirubin content may be connected with inhibition of lipid peroxidation in blood serum and inhibition of radical oxygen formation. Dudnik et al. (2008) reported that hyperbilirubinemia in patients with primary biliary cirrhosis is accompanied by a decrease in the concentration of lipid peroxidation products and increase in antioxidant activity of blood serum.

In another studies with *Rhuscoriaria* Capcarova et al. (2011a) observed no significant differences in BR concentration in rabbits between the control group and experimental groups. Capcarova et al. (2011b) in the study on broiler chickens with addition of probiotic strains (*Lactobacillus fermentum* CCM 7158 and *Enterococcus faecium* M-74 to feed mixture found insignificant increase in BR content in all experimental groups in comparison with the control group. Based on the literature natural substances could slightly modify the serum bilirubin content and consequently influence antioxidant balance in the organism.

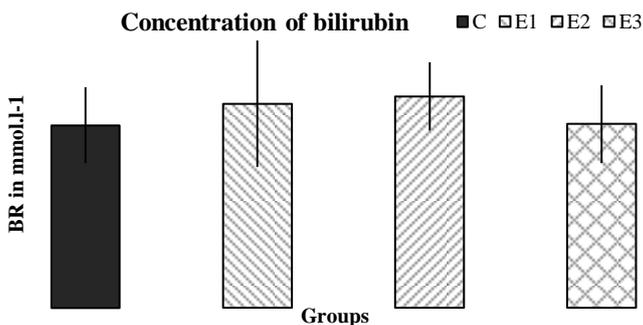


Figure 1 The content of bilirubin in rabbits blood after chronic quercetin application. C – control group, E1 - 10 µg.kg⁻¹, E2 – 100 µg.kg⁻¹, E3 - 1000 µg.kg⁻¹ of quercetin. Values are means ± SD

Gender comparison revealed that the application of quercetin resulted in decrease of BIL concentration in all experimental male groups in the comparison with the control group, however differences were not significant ($P > 0.05$). The lowest value was observed in E3 (1000 µg.kg⁻¹ of quercetin) group after quercetin treatment.

On the other hand we found increase in content of BIL in experimental female groups (E1 and E3) in comparison with the control group, but differences were not significant ($P > 0.05$). Generally, quercetin treatment increased content of BIL in female more than in male groups. Hopkins et al. (1996) found that male gender had a lower level of BIL in comparison with female gender and also male groups had higher risk of familial coronary artery disease (CAD), that supporting the potential antioxidant protective effect of bilirubin.

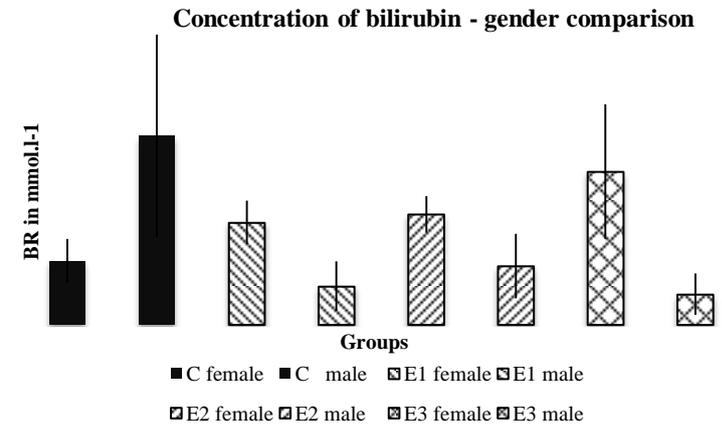


Figure 2 The content of bilirubin in rabbits blood after chronic quercetin application – gender comparison. C – control group, E1 - 10 µg.kg⁻¹, E2 - 100 µg.kg⁻¹, E3 - 1000 µg.kg⁻¹ of quercetin. Values are means ± SD

Albumins represent a very abundant and important circulating antioxidant (Roche et al., 2008). Study of Bourdon et al. (1999) confirmed and extended the idea that serum albumin is an important protein that presents direct protective effects. In this study, we found decrease in the content of serum albumins in quercetin groups (E1 - E4) vs. control group, but differences were no significant ($P > 0.05$). Similar results found Capcarova et al. (2010) in study with *L. fermentum* and *E. faecium*. In another study Capcarova et al. (2011a) found significant ($P < 0.05$) increase in content of ALB in experimental groups in comparison with the control group after addition the *Rhuscoriaria* to the feed mixture. Several lines of evidence strongly suggest that a reduced serum albumin concentration, although within the normal range, is associated with mortality risk (Bourdon et al., 1999).

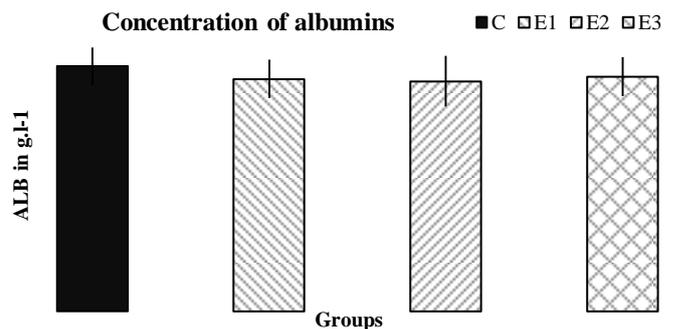


Figure 3 The content of albumins in rabbits blood after chronic quercetin application. C – control group, E1 - 10 µg.kg⁻¹, E2 - 100 µg.kg⁻¹, E3 - 1000 µg.kg⁻¹ of quercetin. Values are means ± SD

In the male groups we found slightly higher level of ALB in E3 group and slightly lower level of BIL in E1 and E2 groups in comparison with the control group, but without significant differences ($P > 0.05$).

In the female groups we observed insignificant decrease content of ALB in all groups in comparison with the control group after quercetin treatment. When we compared both genders we found almost similar level of BIL in female and male genders. In the different study Capcarova et al. (2013) found that the dose of bee pollen had no effect on albumin content in female group of chickens but it increased the value of this parameter in male group of chickens in the dose of 600 mg.kg⁻¹.

Concentration of albumins - gender comparison

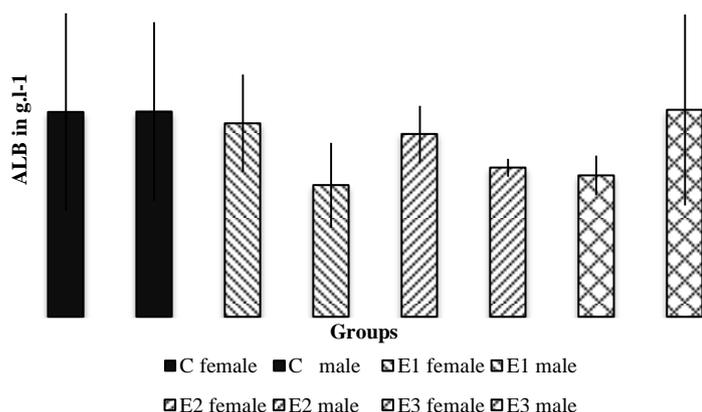


Figure 4 The content of albumin in rabbits blood after chronic quercetin application – gender comparison. C – control group, E1 - 10 $\mu\text{g.kg}^{-1}$, E2 - 100 $\mu\text{g.kg}^{-1}$, E3 - 1000 $\mu\text{g.kg}^{-1}$ of quercetin. Values are means \pm SD

CONCLUSION

The intramuscular application of the quercetin three times a week to the rabbits resulted in some changes in internal milieu of animals. Application of quercetin increased the content of bilirubin in all experimental groups in comparison with the control group. Content of serum albumins decreased in all experimental groups. Gender comparison revealed that the application of quercetin resulted in changes in level of bilirubin and albumins. In conclusion we can say that these results show a positive effect of quercetin on content of bilirubin. To our knowledge, there are not a lot of similar studies concerning the effect of intramuscular application of quercetin and its effect on antioxidant status of rabbits. Research on the field of quercetin will be worthy of further investigation.

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