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# EFFECT OF QUERCETIN ON ANTIOXIDANT STATUS OF RABBITS AFTER ONE MONTH EXPOSURE

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**ABSTRACT**

The aim of the present study was to investigate the short-term effect of quercetin in various doses on level of antioxidant enzymes in rabbit's blood. Adult rabbits were divided into three experimental groups (E1, E2 and E3) and the control group without quercetin addition. Quercetin was applied intramuscularly in various concentrations; 10 $\mu g.kg^{-1}$ in E1 group, 100 $\mu g.kg^{-1}$ in E2 group, and 1000 $\mu g.kg^{-1}$ in E3 group for 30 days, 3 times per week. Application of quercetin insignificantly increased the level of SOD in the experimental groups in comparison with the control group. Gender comparison showed higher level of SOD in all experimental male groups in comparison with the female groups. The level of GPx activity decreased in all experimental groups but without significant differences. In gender comparison we found higher activity of GPx in female groups in comparison with the male groups. In conclusion, as the quercetin 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, superoxide dismutase, glutathione peroxidase, gender comparison

## INTRODUCTION

Oxidative stress has been shown, both in experimental and clinical studies held in recent years, to play a key role in the pathogenesis of many diseases. Oxidative stress is effective on the pathological processes of diseases like cancer, cardio-vascular diseases, rheumatoid arthritis, *diabetes mellitus*, and neurological disorders such as Alzheimer and Parkinson **(Valko *et al.*, 2007)**. The increased reactive oxygen species (ROS) in human body has various sources such as auto-oxidative glycation, activation of protein kinase C, mitochondrial respiratory chain deficiencies and increased oxidase enzyme activities **(Forbes *et al.*, 2008; Derubertis *et al.*, 1994)**. However, the body has its antioxidant system to prevent ROS production and the probable damages ROS can cause. The most important elements of the intracellular antioxidant defense are superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) enzyme activities **(Altan *et al.*, 2006)**.
Another molecule with a potent antioxidant effect is quercetin, a common flavonoid in nature. It exists in many nutrients, mostly in red onions, grapes, berries, cherries, broccoli, citrus fruits, tea (*Camelia sinensis*) and capers **(Bischoff, 2008)**. Quercetin is able to preclude oxidative stress by directly inactivating free radicals, by inhibiting xanthine oxidase and lipid peroxidation, and by affecting antioxidant pathways both *in vivo* and *in vitro* **(Hanasaki *et al.*, 1994; Plumb *et al.*, 1994; Fiorani *et al.*, 2001; Morand *et al.*, 1998)**. Quercetin, as a potent antioxidant agent, can be expected to reduce the damages in tissues caused by free radicals and by oxidative damages.
The aim of the present work was to determine effect of short-term application of quercetin in various doses on level of antioxidant enzymes in rabbit's blood.

## 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 (Table 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 $\mu g.kg^{-1}$ in E1 group, 100 $\mu g.kg^{-1}$ in E2 group, and 1000 $\mu g.kg^{-1}$ in E3 group for 30 days 3 times a week. Control group received injection water (Imuna Pharm a.s. Šarišské Michaľany, Slovak Republic).
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 1 month of intramuscular application of quercetin, blood samples from vena auricularis from all animals by macromethods were taken.
The levels of superoxide dismutase (SOD) and glutathione peroxidase (GPx) were 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 | |
|---|---|
| *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

It is known, that natural substances can cause changes in antioxidant status. In this study, the effect of short-term exposure of quercetin in various doses on selected antioxidant enzymes of rabbits was measured from blood. The results are presented in figures 1- 4. SOD is important antioxidant enzyme responsible for the elimination of superoxide radical **(Hu, *et al*., 2005)**. In our study quercetin treatment slightly increased the activity of SOD in rabbit's blood. We observed insignificant ($P > 0.05$) increase in activity of SOD in experimental groups E1 and E3 and decrease in E2 group in comparison with the control group.

**Demir *et al*. (2011)** found insignificant decrease in activity of SOD in rat after 4 week of quercetin treatment. These discrepancies in literature could be caused by using different kind of animals. In another study **Huk *et al.* (1998)** found that lower concentration of quercetin in blood increased SOD activity and higher concentration (up to 30 μmol.l$^{-1}$) of quercetin decreased SOD and declined beneficial effect. In our study the doses of quercetin used in experiment were low and similar to possible daily intake.

Gender comparison revealed that the application of quercetin resulted in decrease of SOD level in all experimental female groups in the comparison with the control group, however differences were not significant ($P > 0.05$). The lowest value was observed in E2 (100 μg.kg$^{-1}$ of quercetin) group after quercetin treatment.

On the other hand we found increase of the antioxidant enzyme in experimental male groups (E1 and E3) in comparison with the control group, but differences were not significant ($P > 0.05$). Generally, quercetin treatment increased SOD activity in male more than in female groups.

**Manach *et al*. (1997)** found higher value of SOD activity in female mice in comparison with male group. In another study **Malorni *et al*. (2008)** found higher values in SOD activity in female vascular smooth muscle cells (VSMC) in comparison with male VSMC. According to authors decrease of SOD might contribute to increased oxidative stress in the aorta.

We have not found any data in literature regarding effect of quercetin on genders in rabbits in SOD activity.

GPx catalyses the reduction of hydroperoxides using glutathione (GSH), thereby protecting mammalian cells against oxidative damage. In fact, GSH metabolism is one of the most essential antioxidative defense mechanisms **(Rikans and Hornbrook, 1997; Grazioli *et al*., 1998; Liu *et al*., 2010)**. In this study short-term application of quercetin slightly decreased the activity of GPx in first and second experimental groups in comparison with the control group and slightly increased in third experimental group. Generally we can conclude that quercetin had no effect on GPx activity and the values from all experimental groups were similar to those measured in the control group. **Wiegand and Boesch-Saadantmandi (2009)** found insignificant decrease in activity of GPx in rats after quercetin treatment.

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

In the male groups we observed insignificant increase of activity of GPx in E2 and E3 groups. The lowest value was observed in E1 (10 μg.kg$^{-1}$ of quercetin) group after quercetin treatment. When we compared both genders we found higher activity of GPx in female groups. **Panemangalore and Bebe (2009)** think that the decrease in liver glutathione peroxidase activity could be due to the greater utilization of glutathione for detoxification of electrophiles and free radicals produced by the flavonoids and their metabolites.

We have not found any data in literature regarding effect of quercetin in various doses on genders in rabbits in GPx activity.

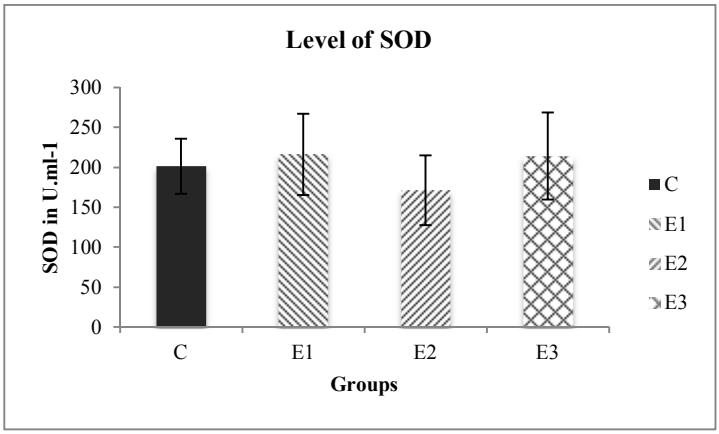


**Figure 1** The activity of SOD of rabbit's blood after short quercetin exposure in vivo. C – control group, E1 - 10 μg.kg$^{-1}$, E2 - 100 μg.kg$^{-1}$, E3 - 1000 μg.kg$^{-1}$ of quercetin. Values are means ± SD

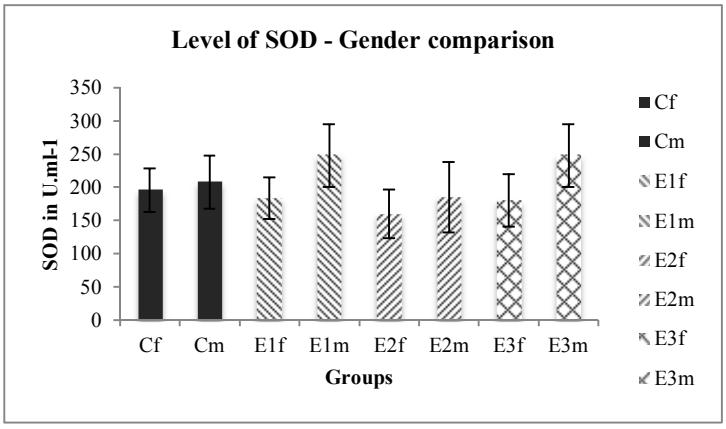


**Figure 2** The activity of SOD of rabbit's blood after short quercetin exposure in vivo, gender comparison.. Cf/m – control group, E1f/m - 10 μg.kg$^{-1}$, E2f/m - 100 μg.kg$^{-1}$, E3f/m - 1000 μg.kg$^{-1}$of quercetin, f- female; m – male. Values are means ± SD

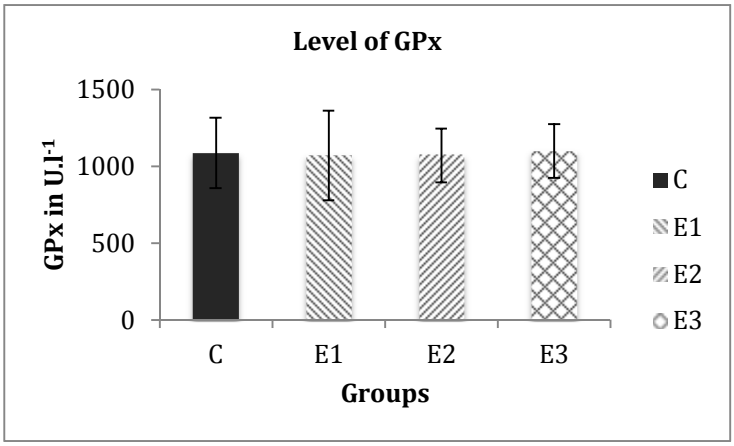


**Figure 3** The activity of SOD of rabbit's blood after short quercetin exposure in vivo. C – control group, E1 - 10 μg.kg$^{-1}$, E2 - 100 μg.kg$^{-1}$, E3 - 1000 μg.kg$^{-1}$of quercetin. Values are means ± SD

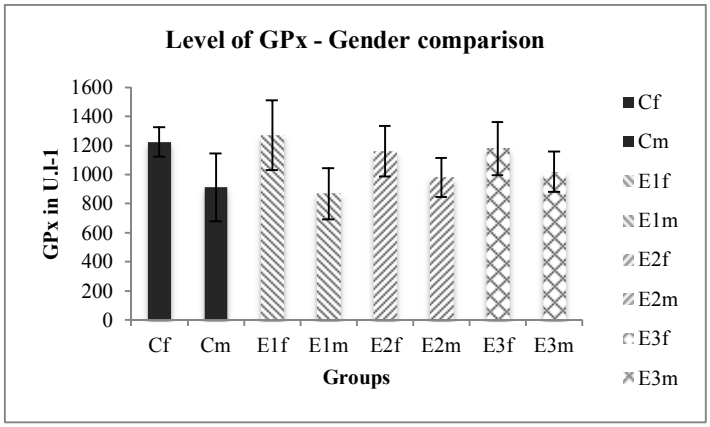


**Figure 4** The activity of GPx of rabbit's blood after short quercetin exposure in vivo, gender comparison. Cf/m – control group, E1f/m - 10 $\mu g.kg^{-1}$, E2f/m - 100 $\mu g.kg^{-1}$, E3f/m - 1000 $\mu g.kg^{-1}$of quercetin, f- female; m – male. Values are means ± SD

## CONCLUSION

The intramuscular application of the quercetin three times a week to the rabbits resulted in some changes in activity of antioxidant enzymes (SOD and GPx). Application of quercetin insignificant increased the level of SOD in selected experimental groups in comparison with the control group and we found higher level of SOD in all experimental male groups in comparison with the female groups. The level of GPx activity decreased in all experimental groups but without significant differences. In gender comparison we found higher activity of GPx in female groups in comparison with the male groups. In conclusion, our results showed a positive effect of short-term application of quercetin on level of antioxidants enzymes. To our knowledge, there are not a lot of similar studies concerning the short-term 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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## REFERENCES

ALTAN, N., DINSEEL, A.S., KOCA, C. 2006. Diabetes mellitus ve oksidatif stres. *Turkish Journal of Biochemistry,* 31, 51-56.

BISCHOFF, S. C. 2008. Quercetin: potentials in the prevention and therapy of disease. *Current Opinion in Clinical Nutrition and Metabolic Care*, 11, 733-740.

DERMIR, F., UZUN, F.G., DURAK, D., KALENDER, Y. 2011. Subacute chlorpyrifos-induced oxidative stress in rat erythrocytes and the protective effects of catechin and quercetin. *Pesticide Biochemistry and Physiology*, 99, 77-81.

DERUBERTIS, F.R., CRAVEN, P.A. 1994. Activation of protein kinase C in glomerular cells in diabetes. Mechanisms and potential links to the pathogenesis of diabetic glomerulopathy. *Diabetes*, 43, 1-8.

FIORANI, M., De SANCTIS, R., MENGHINELLO, P., CUEEHIARINI, L., CELLINI, B., DACHŔ, M. 2001. Quercetin prevents glutathione depletion induced by dehydroascorbic acid in rabbit red blood cells. *Free Radical Research*, 34, 639-648.

FORBES, J.M., COUGHLAN, M.T., COOPER, M.E. 2008. Oxidative stress as a major culprit in kidney disease in diabetes. *Diabetes*, 57, 1446-1454.

GRAZIOLI, V., SCHIAVO, R., CASARI, E., MARZATICO, F., RODRIGUEZ, R., GAETANI, P. 1998. Antioxidant enzymatic activities and lipid peroxidation in cultured human chondrocytes from vertebral plate cartilage. *FEBS Letters*, 431, 149-153.

HANASAKI, Y., OGAWA, S., FUKUI, S. 1994. The correlation between active oxygen scavenging and antioxidative effects of flavonoids. *Free Radical Biology and Medicine*, 16(6), 845-850.

HU, Y., ROSEN, D. G., ZHOU, Y., FENG, L., YANG, G., LIU, J., HUANG, P. 2005. Mitochondrial manganese –superoxide dismutase expression in ovarian cancer: role in cell proliferation and response to oxidative stress. *Journal of Biological Chemistry*, 280, 39485-39492.

HUK, I., BROVKOVYCH, V., NANOBASH, J. V., WEIGEL, G., NEUMAYER, CH., PARTYKA, L., PATTON, S., MALINSKI, T. 1998. Bioflavonoid quercetin scavenges superoxide and increases nitric oxide concentration in ischemia-reperfusion injury: an experimental study. *British Journal of Surgey*, 85, 1080-1085.

LIU, CAHN-MIN., ZHENG, YUAN-LIN., LU, UN., ZHANG, ZI-FENG., FAN, SHAO-HUA., WU, DONG-MEI., MA, JIE-QIONG. 2010. Quercetin protects rat liver against lead-induced oxidative stress and apoptosis. *Enviromental Toxicology and Pharmacology*, 29, 158-166.

MALORNI, W., STRAFACE, E., MATARRESE, P., ASCIONE, B., COINU, R., CANU, S., GALLUZZO, P., MARINO, M., FRANCONI, F. 2008. Redox state and gender differences in vascular smooth muscle cells. *FEEBS Letters*, 582, 635-642.

MORAND, C., CRESPY, V., MANACH, C., BESSON, C., DEMIGNÉ, C., RÉMÉSY, C. 1998. Plasma metabolites of quercetin and their antioxidant properties. *American Journal of Physiology*, 75, 212-219.

PLUMB, W., PRICE, K. R., WILIAMSON, G. 1999. Antioxidant properties of flavonol glycosides from green beans. *Redox Report*, 4, 123-127.

RIKANS, L.E., HORNBROOK, K.R. 1997. Lipid peroxidation, antioxidant protection and aging. *Biochemica et Biophysica Acta*, 62, 116-127.

MANACH, C., MORAND, C., DEMIGNE, C., TEXIER, O., REGERAT, F., REMESY, C. 1997. Bioavailability of rutin and quercetin in rats. *FEBS Letters*, 409, 126-137.

VALKO, M., LEIBFRITZ, D., MONCOL, J., CRONIN, M.T., MAZUR, M., TELSER, J. 2007. Free radicals and antioxidants in normal physiological functions and human disease. *Internationl Journal of Biochemistry and Cell Biology*, 39, 44-84.

WIEGAND, H., and BOESCH-SADANTMANDI, Ch., 2009. Effects of quercetin and catechin on hepatic glutathione-stransferase (GST), NAD(P)H quinone oxidoreductase 1 (NQO1), and antioxidant enzyme activity levels in rats. *Nutrition and Cancer*, 61, 717-722.