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IMPACT OF THE T-2 TOXIN AND QUERCETIN ON RABBIT PLASMA LEVELS OF THYROTROPIN AND THYROXINE *IN VIVO*

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ABSTRACT

The aim of this article was to study the effect of quercetin combination with T-2 toxin to secretion of thyrotropin and thyroxine *in vivo*. The rabbits were divided into the control group (without quercetin and T-2 toxin), and four experimental groups with different doses of quercetin. The thyrotropin (TSH) secretion was not significantly affected by intramuscular application of quercetin and T2-toxin against control group. The secretion of TSH was non-significaty decreased by addition of T2-toxin (group 1), but it was increased after application of quercetin with T2-toxin (groups 2, 3, 4). Significant differences between control group and experimental groups were not observed in thyroxine (T_4) secretion. The highest concentration of T_4 was observed in group 3 and the lowest in group 2. Our results suggested protective effect of quercetin on TSH secretion.

Keywords: Quercetin, T-2 toxin, thyrotropin, thyroxine

INTRODUCTION

Quercetin is most widely distributed flavonoid present in fruits and vegetables **(Erlund *et al.*, 2006; Manach *et al.*, 2005)**. For example is the major component of medicinal plants such as *Ginkgo biloba*, *Hypericum perforatum* and *Sambucus canadensis* **(Häkkinen *et al.*,1999; Williamson and Manach, 2005)**, and also in onion and shallot **(Wiczkowski *et al.*, 2008)**. Earlier studies in the 1970s recognized quercetin as genotoxic, however, quercetin's in vitro mutagenicity was not confirmed by *in vivo* tests in animal models **(Harwood *et* al., 2007)**. Several studies have shown that quercetin possess many biological effects such as antioxidant, anti-carcinogenic, anti-inflammatory, bacteriostatic, cardioprotective and cytoprotective effects **(Arts and Hollman, 2005; Bonavida, 2008; Caltagirones *et al.*, 2000; Fresco *et al.*, 2006; Middleton *et* al.,2000; Piantelli *et al.*, 2006; Alasalvar and Shahidi, 2012)**.

T-2 toxin is considered to be common trichothecene mycotoxin and is produced by *Fusarium sporotrichioides* and *F. langsethiae* **(Kokkonen *et al.*, 2010)**. Mycotoxins that are contaminants of animal feed can impair growth and reproductive efficiency. Several studies confirmed toxicity of T-2 toxin, and can cause serious consequences, such as genotoxicity, cytotoxicity and neurotoxicity **(Sudakin, 2003),** induce lesions in various tissues as hematopoietic, lymphoid and gastrointestinal tissues **(IARC, 1993)**.

Thyroid-stimulating hormone (thyrotropin, TSH) is a pituitary hormone that stimulates the thyroid gland to produce thyroxine (T_4), and then triiodothyronine (T_3) which stimulates the metabolism of almost every tissue in the body. It is a glycoprotein hormone synthesized and secreted by thyrotrope cells in the anterior pituitary gland, which regulates the endocrine function of the thyroid. Thyroid hormones are synthesized, stored and secreted by the thyroid gland under control of the hypothalamus–pituitary–periphery–feedback system dependent on the supply of two essential trace elements - iodine and selenium **(Köhrle, 1999)**.

Flavonoids can affect in many enzymatic systems, involving thyroid hormones **(Middleton *et al.*, 2000)**.

Therefore, the main goal of this article was to study the effect of quercetin combination with T-2 toxin to secretion of thyrotropin and thyroxine *in vivo*.

MATERIAL AND METHODS

Adult female rabbits (n=25) at age 120 days (weighing 4.00 ± 0.2 kg) from experimental farm of the Research Institute for Animal Production Nitra (Slovak Republic) were used. Rabbits were housed in individual wire cages under constant photoperiod of 12 hours of daylight at 20-24°C temperature.

The rabbits were divided into the control group (without quercetin and T-2 toxin), and four experimental groups (table 1). Three experimental groups (2, 3, 4) received intramuscular injected quercetin (Sigma-Aldrich, Germany) for 90 days, 3 times per week. T-2 toxin (Romer Labs Division Holding GmbH, Tulln, Austria) was applied only once 72 hours before the end of the experiment. The chosen doses of quercetin and T-2 toxin were based on literature data **(Knab *et al.*, 2011; Petruška and Capcarova, 2012)**.

After application of the quercetin (90 days) and T-2 toxin, were collected blood samples into tubes with EDTA (anticoagulant) and transferred to the laboratory for analysis. In blood plasma we analyzed the levels of the thyrotropin (TSH) and thyroxin (T4) using ELISA method. Institutional and national guidelines for care and use of animals were followed, and all experimental processes were approved by State Veterinary and Food Institute of Slovak Republic, no. 3398/11-221/3 and ethics committee.

Results were analysed by one-way ANOVA; the data are presented as means ± standard deviation (SD).

Table 1 Groups of animals with quantities of quercetin and T-2 toxin

Groups	Control	1	2	3	4
Quercetin	-	-	10 μg.kg^{-1} BW	100 μg.kg^{-1} BW	1000 μg.kg^{-1} BW
T-2 toxin	-	0.08 mg.kg^{-1} BW	0.08 mg.kg^{-1} BW	0.08 mg.kg^{-1} BW	0.08 mg.kg^{-1} BW

BW – body weight

RESULTS AND DISCUSSION

Thyroid hormones are highly hydrophobic, phenolic amino acid derivatives. The literature describes studies on flavonoid action that prove direct influence by changing TSH, T_4 levels, and on the other hand some experiments show interference of flavonoids with the periphery, but without changes on serum levels **(Hamann *et al.*, 2006)**. After TSH secretion from the thyroid gland more than 99% of thyroid hormones bind to the three major thyroid hormone binding proteins transthyretin, thyroxine-binding globulin and albumin. Under normal conditions free T_4 serum levels are very low **(Cody *et al.*, 1986)**.
The thyrotropin (TSH) secretion was not significantly affected by intramuscular application of quercetin and T2-toxin against control group. The secretion of TSH was non-significaty decreased by addition of T2-toxin (group 1), but it was increased after application of quercetin with T2-toxin (groups 2, 3, 4) (Figure 1). These results point to protective effect of quercetin on TSH secretion.

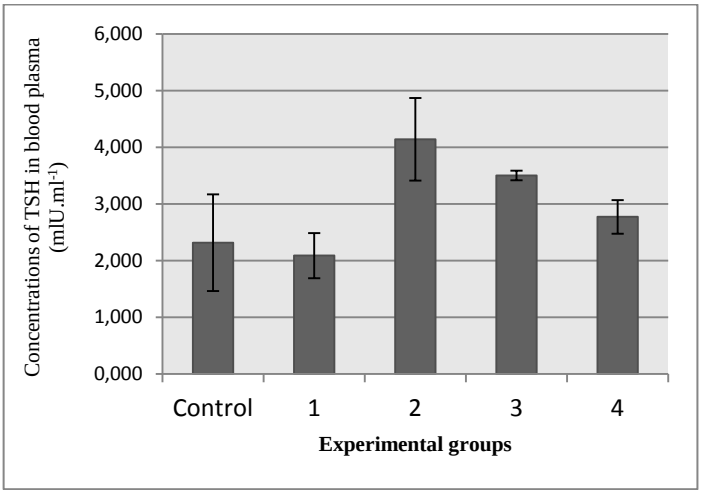

Figure 1 Effect of quercetin and T-2 toxin on concentrations of TSH in blood plasma. Each value represents the means ± SD

Significant differences between control group and experimental groups were not observed in thyroxine (T_4) secretion. The highest concentration of T_4 was observed in group 3 and the lowest in group 2 (Figure 2).

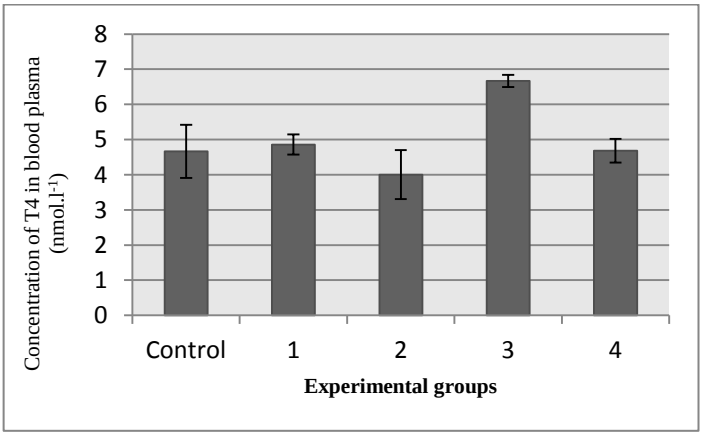

Figure 2 Effect of quercetin and T-2 toxin on concentrations of T4 in blood plasma. Each value represents the means ± SD

The concentration of thyroid hormone (T_4) in the blood regulates the pituitary release of TSH; when T_3 and T_4concentrations are low, the production of TSH is increased, and, conversely, when T_3 and T_4 concentrations are high, TSH production is decreased **(Köhrle, 1999)**,that wasn´t confirmed in our work.
Rotter *et al.* (1994) tested influence of *Fusarium* mycotoxins on T_4 levels. Serum T_4 (thyroxine) levels increased quadratically after 7 and 28 days of exposure compared to control animals. This change coincided with an increase in albumin levels, a decrease in α-globulin levels, and an overall increase in albumin/globulin ratio as the level of contamination increased.
Davis *et al.* (1983) reported that quercetin suppressed thyroxine stimulation of human red blood cell Ca2+-ATPase activity in vitro and interfered with the binding of the hormone to red blood cell membranes. In contrast, however, quercetin stimulated Ca2+-ATPase activity at low concentrations and inhibited the ATPase at 50 μM in the absence of any thyroid hormone. The effects of quercetin at the low concentrations (stimulation of Ca2+-ATPase and inhibition of membrane binding of thyroid hormone) mimicked those of thyroxine. The results were considered consistent with the thyroxine-like structure of quercetin **(Middleton *et al*., 2000)**. Several other flavonoids, including fisetin, hesperetin, tangeretin, and chalcone, were also shown to reduce the sensitivity of membrane Ca2+-ATPase to hormonal stimulation. **Richardson and Twente (1987)** showed that quercetin was capable of inhibiting in vitro and in vivo the stimulated secretion of rat pituitary growth hormone.

CONCLUSION

In summary our results have not confirmed toxic impact of T-2 toxin, however suggested protective effect of quercetin on TSH secretion, and opposite effect on T_4 secretion in blood plasma. To clarify the mechanism of T-2 toxin and quercetin action on the thyroid hormones and their stimulator (TSH), further experiments are necessary.

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