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QUERCETIN IMPROVES THE ENDOCRINE FUNCTION OF RAT TESTICULAR TISSUE UNDER *IN VITRO* CONDITIONS

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SUMMARY

Compounds of natural origin are often used for their beneficial effects on the male endocrine system and the synthesis of steroid biomolecules in testicular tissue. One of such compounds is quercetin (QUE), which belongs to the flavonoid family and is found in a wide range of vegetables, fruits and plant products. The purpose of this study is to evaluate the impact of QUE on the endocrine function of rat testicular fragments under *in vitro* conditions. Testicular fragments from adult Wistar rats ($n=9$), cultured in the D-MEM medium with different concentrations of QUE (namely 1, 10 and 100 $\mu\text{mol/L}$) for 24 h at 37°C (5% CO_2), were used in the experiment conducted. Following culture, the medium was separated and the levels of cholesterol (CHOL) and male hormones were measured. CHOL values were quantified spectrophotometrically, whereas the concentrations of androstenedione (ANDRO), dehydroepiandrosterone (DHEA) and testosterone (TEST) were quantified using the enzyme-linked immunosorbent assay (ELISA) commercial kit. The results obtained indicate that 10 $\mu\text{mol/L}$ QUE significantly increased ($P<0.001$; $P<0.05$) the concentrations of all the steroid biomolecules considered (CHOL, ANDRO, DHEA and TEST) when compared to the control samples. Accordingly, our findings confirm the positive impact of QUE on the endocrine function and steroidogenesis of rat testicular tissue under *in vitro* conditions.

Key words: quercetin, rat, testes, endocrine system, steroidogenesis

Abbreviations: ANDRO – androstenedione; DHEA – dehydroepiandrosterone; D-MEM - Dulbecco's modified Eagle Medium; ELISA – enzyme-linked immunosorbent assay; QUE – quercetin; SIT – sitagliptin; TEST - testosterone

INTRODUCTION

Testicular tissue plays a specific role in the male sexual differentiation and reproduction. The testes are composed of two independently functional units: the Sertoli cells, which are responsible for the spermatogenesis, and the Leydig cells, which control androgen biosynthesis (Flück & Pandey, 2017). More than 95% of circulating androgens are produced by the testes. All steroid hormones are biosynthesized from cholesterol, which is a major compound necessary for the steroidogenesis and the proper endocrine function of testicular tissue (Miller & Auchus, 2011). A deficiency in the secretion of steroid biomolecules could cause a clinical syndrome known as hypogonadism. This condition leads to a wide range of symptoms such as spermatogenesis disruption, reduced libido, smaller testes and erectile dysfunction (Dimakopoulou et al., 2019). For the improvement of the steroidogenesis in testicular tissue, flavonoids, such as quercetin (QUE), are often used. Based on previous studies, which confirmed the beneficial effects of QUE supplementation on testicular tissue (Baltaci et al., 2016; Jahan et al., 2015; Khaki et al., 2010), we

decided to analyze the effects of QUE on the steroidogenesis and levels of androgens. Quercetin (3,3',4',5,7-pentahydroxyflavone) is a bioflavonoid complex which belongs to the flavonoid group of polyphenols, commonly found in a variety of vegetables, fruits, berries, nuts or other products of plant origin. Higher concentrations of QUE can be found in red onions or kale. QUE exhibits numerous beneficial properties such as anti-inflammatory, immunosuppressive and immune-modulating activity (Li et al., 2016). Therefore, our study focused on the *in vitro* effects of QUE on the endocrine function of rat testicular tissue.

MATERIAL AND METHODS

The experimental testicular tissue was obtained from 9 adult Wistar rats (at the age of 120 days). The animals were acquired from the Institute of Experimental Pharmacology (Slovak Academy of Sciences, Slovakia) and raised at the Slovak University of Agriculture (Nitra) in plastic cages under specific conditions (24 $\pm$ 1°C, 12 h light/12 h dark photoperiod). The standard feed and water were provided *ad libitum*. The institutional and national guidelines for the care and use of laboratory animals were followed, and all the procedures were approved by the State Veterinary and Food Institute of the Slovak Republic (no. 3398/11-221/3) and Ethic Committee.

All the animals were ethically euthanized using anesthesia injection. The animal testes were removed through an opening in the scrotum made with a scalpel. The reproductive organs were cleaned of excess tissue, washed in saline solution (Imuna Pharm, A.S., Šarišské Michaľany, Slovak republic) and cut into smaller fragments. Consequently, the testicular fragments were cultured in a culture medium supplemented with different concentrations of quercetin (namely 1, 10 and 100 μ mol/L) for 24 hours at 37°C (5% CO₂). The culture medium contained D-MEM (Dulbecco's modified Eagle Medium, Sigma-Aldrich, St. Louis, USA), a 10% fetal bovine serum (Sigma-Aldrich) and a 1% antibiotic and antimycotic (Sigma-Aldrich) solution for minimizing the risk of microbial growth. After cultivation, the medium was separated into clean test tubes and used for the evaluation of steroid biomolecules.

A cholesterol level analysis was performed using the CHOD-PAP commercial kit (DIALAB, Vienna, Austria), whereas cholesterol level measurements were carried out using the Rx Monza (Randox Laboratories, Ltd., Crumlin, united Kingdom) automatic spectrophotometer. Testosterone, androstenedione and dehydroepiandrosterone concentrations were analyzed using the ELISA commercial kits (DIASYS, Holzheim, Germany). The hormone values obtained were quantified with a combined micro-plate spectro-fluoro-luminometer (Glomax, promega, Madison, USA) at 450 nm (Jambor et al., 2017).

Statistical data were processed using the GraphPadPrism program (version 6.02 for Windows; GraphPad Software, La Jolla, California, USA, www.graphpad.com). The statistical assessment performed entailed a comparative analysis of the experimental and control groups using the Dunnett's test. This test is based on a comparison of the mean obtained and the control mean, and the evaluation of the confidence interval for the difference between the two means. The statistical significance was set at the following levels: ***P<0.001; **P<0.01; *P<0.05.

RESULTS AND DISCUSSION

CHOL is the initial source for the TEST synthesis in the Leydig cells, and its biosynthesis progresses via ANDRO and DHEA, which are the progenitors of the final TEST steroid biomolecule (Gao et al., 2018). The evaluation of CHOL concentrations in the medium (Fig. 1) indicate a significant increase (P<0.001; P<0.05) in the experimental groups supplemented with 10 and 100 μ mol/L QUE in comparison with the control group. Osawe & Farombi (2018) applied QUE as a therapeutic treatment against spermiotoxicity induced by sulphasalazine in male rats. QUE significantly preserved the organ weights and sperm motility, increasing the CHOL levels in the testis. Conversely, the studies of Padma et al. (2012) and Jahan et al. (2016) confirmed that QUE had the ability to statistically reduce the levels of CHOL and triglycerides in the blood plasma of rats. Previous research suggests that QUE decreases the level of CHOL in the blood plasma and increases the synthesis of CHOL in testicular tissue, thus elevating the concentrations of male hormones.

Figure 1. The levels of cholesterol in the medium after the co-cultivation of rat testicular tissue with different concentrations of QUE under *in vitro* conditions (***P<0.001; **P<0.01; *P<0.05)

Figure 2. The levels of androstenedione in the medium after the co-cultivation of rat testicular tissue with different concentrations of QUE under *in vitro* conditions (***P<0.001; **P<0.01; *P<0.05)

Figure 3. The levels of dehydroepiandrosterone in the medium after the co-cultivation of rat testicular tissue with different concentrations of QUE under *in vitro* conditions (***P<0.001; **P<0.01; *P<0.05)

ANDRO and DHEA are synthesized in testicular tissue, but their concentration and androgenicity are lower than TEST (Martin & Touaibia, 2020). Figure 2 indicates that the 10 µmol/L concentration of QUE statistically enhanced (P<0.001) the ANDRO concentration relative to the control sample. A significant improvement (P<0.05) was also recorded in the case of 1 and 100 µmol/L QUE. As Figure 3 shows, the concentration of DHEA was significantly higher (P<0.05) in the group with 10 µmol/L QUE than that recorded in other experimental groups. Similar impacts of the same dose of QUE were observed in the case of the TEST concentrations considered. Nowadays, there is a

limited body of information on the effects of QUE on the ANDRO or DHEA concentrations in testicular tissue. Nevertheless, Ujah et al. (2017) confirmed the benefits of QUE treatment in rats, which had cadmium chloride-induced testicular toxicity. QUE improved the androgenic activity of the damaged testicular tissue and positively affected the steroidogenesis and concentrations of male hormones in the blood plasma. QUE application increased not only the synthesis of male hormones but also the steroidogenic gene expression in the Leydig cells, which are the prime contributors of androgen synthesis and secretion in testicular tissue (Cornier et al., 2017). Both previous research and our findings indicate that QUE positively affects steroidogenesis by improving CHOL levels (Fig. 1), thus enhancing the synthesis of ANDRO (Fig. 2) and DHEA (Fig. 3) in testicular tissue. Consequently, these changes may lead to a significant increase in TEST (Fig. 4.) concentrations, supporting the proper physiological process of steroidogenesis.

Figure 4. The levels of testosterone in the medium after the co-cultivation of rat testicular tissue with different concentrations of QUE under *in vitro* conditions. ***P<0.001; **P<0.01; *P<0.05

As one of the major male androgens, TEST plays a crucial role in the male sexual differentiation and spermatogenesis. Testicular tissue is the central source of the circulating TEST and a precursor of steroids such as DHEA and ANDRO (Winters, 2020). Low TEST levels are associated with hypogonadism, which is implicated in smaller testes, low sperm count and libido (Nassar et al., 2018). Figure 4 shows a statistical improvement ($P<0.05$) in TEST levels of the group enriched with 10 µmol/L QUE compared the control group. Other concentrations (namely 1 and 100 µmol/L) did not induce any significant changes. According to Jahan et al. (2015) QUE by itself was capable to significantly increase ($P<0.001$) the levels of intra-testicular TEST concentration in the rat testicular tissue and reduce the toxic impact of arsenic. These results are consistent with the results of Abdel Aziz et al. (2018) who report that QUE administration (10 mg/kg B.w.t.) significantly restored ($P<0.05$) the concentration of TEST after atrazine-induced reproductive toxicity in sexually mature male Albino rats. Ahmed et al. (2019) argued the protective effects of QUE (in combination with sitagliptin (SIT)) on the testicular toxicity induced by doxorubicin in rats. The data obtained confirmed a significant increase ($P<0.0001$) in the levels of TEST in the groups treated with QUE/SIT combination. Moreover, 10 and 20 mg/kg of QUE positively influenced the levels of serum TEST in male Wistar rats (El Mazoudy et al., 2015). Moreover, the report of Jenkinson et al. (2012) confirms that QUE has the ability to inhibit the enzymatic activity in tissues and slow down the TEST excretion by blocking the enzyme that converts TEST into testosterone glucuronide.

CONCLUSION

Quercetin supplementation during the *in vitro* culture of rat testicular tissue improved the concentrations of all steroid biomolecules, including cholesterol, androstenedione, dehydroepiandrosterone and testosterone. These findings confirm that QUE exerts a positive impact on the endocrine function and supports the physiological progress of steroidogenesis in the testes of male Wistar rats. In future research, QUE could be used as a co-treatment in the case of hypogonadism or endocrine dysfunction.

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