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RAPID COMMUNICATION

Ellagitannins – Compounds From Pomegranate as Possible Effector in Steroidogenesis of Rabbit Ovaries

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Summary

This study has observed possible effect of ellagitannins – compounds from pomegranate on process of steroidogenesis in ovaries. The aim of the study was to investigate the possible effect of punicalagin on secretion of steroid hormones – progesterone, androstenedione, testosterone and 17β-estradiol by ovarian fragments of rabbits *in vitro*. Ovarian fragments from sexually mature female New Zealand white rabbits (n=20) were incubated without (control group) or with punicalagin at various doses 1, 10 and 100 μg.ml^{-1} for 24 h. Hormones were evaluated by ELISA (The Enzyme-Linked Immunosorbent Assay). Data showed that progesterone and 17β-estradiol (but not androstenedione and testosterone) release by rabbit ovarian fragments was significantly affected by punicalagin addition at various doses. Punicalagin (at 100 μg.ml^{-1}) significantly *(P<0.05)* increased progesterone secretion. On the other hand, the release of 17β-estradiol was significantly (*P<0.005*) decreased by punicalagin addition (at 10 μg.ml^{-1}). Our results suggest that punicalagin could have dose-dependent impact on secretion of steroid hormones progesterone and 17β-estradiol by rabbit ovarian fragments and it may be effector in process of ovarian steroidogenesis.

Key words

Ellagitannins • 17β-estradiol • Punicalagin • Pomegranate • Steroidogenesis

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Ellagitannins (ETs) are bioactive polyphenols abundant in some fruits (Landete 2011). ETs are included within the so-called hydrolysable tannins that can be hydrolyzed producing ellagic acid *via* spontaneous lactonization of hexahydroxydiphenic acid (Larrosa *et al.* 2006). Ellagic acid is a part of polyphenol – punicalagin, which is included and responsible for antioxidant activity in pomegranate. Pomegranate is a well-known source of many valuable substances, except for hydrolysable tannins (punicalagins and punicalins) (Gil *et al.* 2000), contains condensed tannins (proancyanidins) (Poyrazoglu *et al.* 2002), anthocyanins (Hernández *et al.* 1999), phenolic acids (ellagic acid, gallic acid) (Mousavinejad *et al.* 2009). Papoutsi *et al.* (2005) investigated the influence of ellagic acid on the activity of the estrogen receptor subtypes α and β in HeLa cells. The aim of our study was to examine the secretion of steroid hormones – progesterone, androstenedione, testosterone and 17β-estradiol by rabbit ovarian fragments after punicalagin addition as main ellagitannin of pomegranate. Sexually mature female New Zealand white rabbits (n=20) from an experimental farm of the Animal Production Research Centre Nitra, Slovak Republic were used. Rabbits (age 150 days, weight 4.00±0.5 kg) were housed in individual flat-deck wire cages under a constant photoperiod of 12 h of day-light, the temperature 20-24 °C and humidity 55±10 %. Institutional and national guidelines for the care and use of animals were followed, and all experimental procedures were approved by the State Veterinary and Food Institute of Slovak Republic (no. 3398/11-221/3) and Ethics committee.

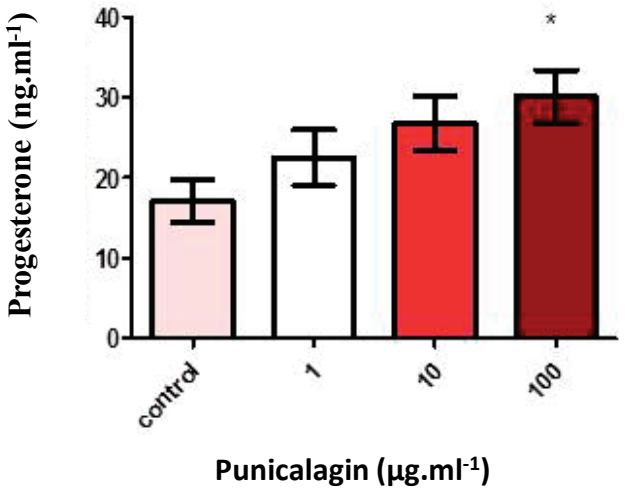

Fig. 1. Dose-dependent effect of punicalagin on progesterone (ng.ml^{-1}) secretion by rabbit ovarian fragments. Significant (P<0.05) differences between control and experimental groups. The results were evaluated by One Way ANOVA. ELISA.

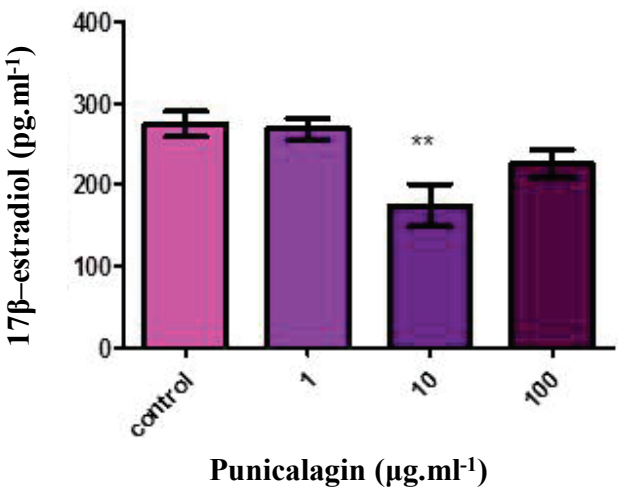

Fig. 2. Dose-dependent effect of punicalagin on 17β-estradiol (pg.ml^{-1}) secretion by rabbit ovarian fragments. Significant (P<0.005) differences between control and experimental groups. The results were evaluated by One Way ANOVA. ELISA.

Ovaries were collected and transported to the laboratory at the ambient temperature in a glass container within 30 min of slaughter. Thereafter ovaries were washed in sterile physiological solution and dissected using a blade knife to 8 approximately equal parts (weight 4.8-5.6 mg). These ovarian fragments were washed again two times in sterile physiological solution and cultured in 1 ml of medium supplemented with 10 % fetal bovine serum and 1 % antibiotic-antimycotic solution and without (control group) or with punicalagin (SigmaAldrich, St. Louis, MO, USA) addition at various concentrations – 1, 10, 100 µg.ml^{-1} for 24 h. Progesterone, androstenedione, testosterone and 17β-estradiol concentrations in culture medium were evaluated by ELISA (The Enzyme-Linked Immunosorbent Assay, Dialab, Wiener Neudorf, Austria). The absorbance was determined at a wave length 450 nm on the microplate ELISA reader (Thermo Scientific Multiskan FC, Vantaa, Finland). Each experimental group was represented by four culture wells of ovarian fragments (each dose = 4 replicates, biological parallels). Assays of hormone levels in the culture media were performed in duplicate. The data are presented as means of values obtained from three separate experiments performed on separate days using separate pools of ovaries from 20 animals. Significance of differences between the control and experimental groups were evaluated by one-way ANOVA using the statistical software GraphPad Prism 5 Demo (GraphPad Software Incorporated, San Diego, California, USA). The data are expressed as means ± SEM. Differences were compared for statistical significance at $P<0.05$.

Punicalagin influenced the secretion of steroid hormones progesterone and 17β-estradiol by rabbit ovarian fragments (Figs 1, 2). Firstly, punicalagin at concentration 100 µg.ml^{-1} significantly *(P<0.05)* increased the release of progesterone (Fig. 1). Secondly, values of androstenedione were very low and the concentration in each case was under measurable limits. Thirdly, similarly testosterone levels were low. Fourthly, punicalagin at concentration 10 µg.ml^{-1} significantly (*P<0.005*) decreased the release of 17β-estradiol.

Influence of punicalagin on the release of progesterone could indicate that the backdoor pathway of steroid biosynthesis might be under the impression of punicalagin. Recently, Ming *et al.* (2014) used pomegranate extraction the prostate cancer cells to suggest that steroid biosynthesis might favour the backdoor pathway over the classical Δ4 and Δ5 pathways. They described the effect of pomegranate extract on androgen biosynthesis pathways specifically using two prostate cancer cell lines and prostate cancer mouse model. Ming *et al.* (2014) described androstenedione in downtrend. In our study levels of androstenedione were very low and in most of the samples were under the measurable spectrum. Ming *et al.* (2014) also presented most of the steroids (testosterone and dihydrotestosterone) in very low concentrations in prostate cancer cells and culture media, while dehydroepiandrosterone and progesterone were used as precursor substrates to help boost cell production of steroids, but testosterone was significantly decreased in samples treated with pomegranate extracts. In our study

the levels of testosterone in culture medium were low. Punicalagin, as main ellagitannin of pomegranate, had influence on secretion of 17β-estradiol as final product of the pathway. At the concentration 10 $\mu g.ml^{-1}$ significant ($P<0.005$) decrease was noted in rabbit ovarian fragments. Using lyophilized fresh pomegranate juice, Kim *et al.* (2002) reported that phenols from pomegranate inhibit 55 % estrogen activity by normal human breast epithelial cells. Punicalagin, which was used in the present study or its derivatives (ellagic acid etc.) might have a possible effect on certain steps in process of steroidogenesis. Similarly, the effect of other antioxidant from red grape – resveratrol, as possible effector in the process of steroidogenesis, was shown in previous study (Kolesarova *et al.* 2012).

Our results suggest that punicalagin could have dose-dependent impact on secretion of steroid hormones progesterone and 17β-estradiol by rabbit ovarian fragments and it may be effector in process of ovarian steroidogenesis.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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