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# EVALUATION OF SOME BIOCHEMICAL PARAMETERS IN RABBITS BLOOD AFTER TWO WEEKS EXPOSURE OF EPICATECHIN

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## ABSTRACT

The aim of present study was to analyse biochemical parameters in blood of rabbits after epicatechin administration during two weeks. Animals (adult female rabbits, body weight 4 ± 0.5 kg) were divided into four groups: control group (C) and experimental groups (E1 – E3). Experimental groups received epicatechin in injectable form at 10 µg.kg$^{-1}$ in E1, 100 µg.kg$^{-1}$ in E2 and 1000 µg.kg$^{-1}$ in E3 for 14 days three times a week. After two weeks of exposure the blood was collected, in blood serum selected biochemical parameters [(glucose, urea, bilirubin, cholesterol, triglycerides, total proteins, calcium ($Ca^{2+}$), magnesium ($Mg^{2+}$), phosphorus (P)] were analysed by automatic analyzer Microlab 300 (Merck®, Germany). Potassium ($K^+$), sodium ($Na^+$), chlorides ($Cl^-$) were analysed by EasyLyte Plus (**Medica Corporation**, USA). Epicatechin had no significant influence on the observed parameters (*P* > 0.05). The present study has shown that the serum cholesterol level and triglycerides levels in the E2 and E3 groups were higher than in the control group, however without significant differences (*P* > 0.05). Two weeks exposure of epicatehin caused also insignificant increase of glucose in all experimental groups in comparison with the control group. Epicatechin had no effect on the others analysed biochemical parameters.

**Keywords:** epicatechin, biochemistry, rabbit

## INTRODUCTION

Epicatechin is a member of a group of polyphenolic compounds collectively known as catechins, belonging to flavonoid family. It is a constituent of grape seeds and grape skin tannins, tea tannins, cocoa flavonoids, cola nuts, strawberries and red wine **(Darlin and Raghu, 2005)**. Several epidemiological investigations and dietary interventions in humans using flavanol-containing foods indicate an inverse relationship between flavanol intake and the risk of cardiovascular disease **(Buijsse et al., 2006; Ding et al., 2006)**. A very wide range of biological actions of flavanol-rich foods supports potential protective effects including the improvement of vasodilation and endothelial function **(Schrolter et al., 2006; Widlansky et al., 2005; Heis et al., 2003; Fisher et al., 2003)**, blood pressure **(Taubert et al., 2007)**, and insulin resistance and glucose tolerance **(Grassi et al., 2005)**, the attenuation of platelet reactivity **(Holt et al., 2002)**, and the improvement of immune responses and antioxidant defense system **(Sies et al., 2005)**. A recent study has clearly revealed that polyphenols, mainly catechins, have anti-oxidative properties by the inhibition of Low-Density Lipoproteins (LDL) oxidation **(Simonetti et al., 2002; Shi et al., 2003)**. Furthermore, epicatechin also protects endothelial cells against oxidized LDL by scavenging free radicals and maintaining nitric oxide synthase **(Steffen et al., 2005)**. These results imply that flavanols can affect cellular signaling pathways, modulate cell membrane characteristics and receptor functions, alter the cellular redox environment, and influence gene expression, protein activity, and the metabolic competence of various cell types in culture **(Scalbert et al., 2005; Middleton et al., 2000)**.
However, little is known about the molecular mechanisms of flavanol-mediated bioactivities in both humans and animals.
The aim of present study was to analyse biochemical parameters in blood of rabbits after epicatechin administration during two weeks.

## MATERIAL AND METHODS

### Animals

Adult female rabbits (n = 16), 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 at any time from automatic drinking troughs. 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 composed of a pelleted concentrate.
Animals were divided into four groups: control group (C) and experimental groups (E1 – E3). Experimental groups received epicatechin in injectable form intramuscularly at 10 µg.kg$^{-1}$ in E1, 100 µg.kg$^{-1}$ in E2 and 1000 µg.kg$^{-1}$ in E3 for 14 days three times a week.
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 ethical committee.

### Blood sampling and analyses

Blood samples from *vena auricularis* were taken from all animals by macromethods after two weeks of epicatechin administration. In blood serum, selected biochemical parameters [(glucose, urea, bilirubin, cholesterol, triglycerides, total proteins, calcium ($Ca^{2+}$), magnesium ($Mg^{2+}$), phosphorus (P)] were analysed by automatic analyzer Microlab 300 (Merck®, Germany). Potassium ($K^+$), sodium ($Na^+$), chlorides ($Cl^-$) were analysed by EasyLyte Plus (Medica Medica Corporation, USA).

**Statistical analyses**

The data used for statistical analyses represent means of values obtained in blood collection. To compare the results, one-way ANOVA test was applied to calculate basic statistic characteristics and to determine significant differences among the experimental and control groups. Statistical software SIGMA PLOT 12.0 (Jandel, Corte Madera, CA, USA) was used. Differences were compared for statistical significance at the level $P< 0.05$.

## RESULTS AND DISCUSSION

The results are presented in Table (1). Epicatechin had no significant influence on the observed parameters ($P > 0.05$). The present study has shown that the serum cholesterol level and triglycerides level in the E2 and E3 groups was higher than in the control group but without significant differences ($P > 0.05$). Similar results were observed by **Tuzcu et al. (2009)** who examined effects of (+)-catechin supplementation on the some biochemical parameters in sera and erythrocytes of ovariectomized wistar rats induced by the carcinogen potassium bromate. Oxidized cholesterol has various deleterious actions such as cytotoxicity, carcinogenity, atherogenicity, inhibition of DNA synthesis, suppression of immune function and modulation of lipid metabolism both *in vivo* and *in vitro*. It has been reported that catechin inhibits cholesterol oxidation in lipoproteins through its radical scavenging action **(Loest et al., 2001)**. On the other hand **Zhon get al. (2011)** found that dietary tea catechin caused decrease of cholesterol level in blood plasma in goats, similarly as study of **Muramatsu et al. (1986)**, who studied effect of green tea catechins on plasma cholesterol level in rats. Catechins are reported to inhibit the absorption of cholesterol and to promote cholesterol excretion **(Ikeda et al., 1992)**. Epidemiological studies in humans have demonstrated that a higher content of plant polyphenols in the diet decreases the occurrence of coronary heart disease by decreasing blood lipid content, especially triglycerides and cholesterol **(Hara, 2001)**. In our study level of cholesterol and triglycerides in blood serum slightly decreased in the E1 group when compare with the control group but without significant differences. Given our results it would be necessary determine level of LDL and HDL cholesterol in blood plasma after administration of epicatechin in rabbits. Generally speaking, higher HDL and lower LDL cholesterol concentrations in plasma are beneficial in decreasing the total cholesterol level in blood **(Fielding and Fielding, 1995)**. Human studies also reported that oral administration of green tea can decrease plasma total cholesterol and triglycerides **(Raederstoff et al., 2003; Sabu et al., 2002)**.

Two weeks exposure of epicatehin caused insignificantly increase of glucose in all experimental groups in comparison with the control group; however the measured values of glucose are still in physiological range of blood glucose in rabbits. The highest content of glucose was measured in the E3 group. In the another study, **Zhon get al. (2011)** found that dietary tea catechin caused increase of glucose in blood plasma in goats. In contrast to our findings, **Khan et al. (2007)** reported that dietary green tea supplementation decreased the serum glucose concentration of rats but it enhanced the activities of carbohydrate metabolism enzymes in rats. The reason for this difference might be that different animal species show different responses to administration of flavonoids in green tea or different responses to administration only one compound - epicatechin.

Epicatechin had no effect on other measured parameters. Small slight changes in some parameters occurred and the values of these parameters were still in physiological range of blood in rabbits.

**Table 1** Biochemical parameters of rabbits after epicatechin administration

| Parameter | C | E1 | E2 | E3 |
|---|---|---|---|---|
| Glucose ($mmol.l^{-1}$) | 5.88± 0.40 | 6.60± 0.78 | 6.27± 0.76 | 6.85± 0.77 |
| Urea ($mmol.l^{-1}$) | 6.25± 0.24 | 7.38± 0.75 | 6.83± 0.75 | 7.65± 1.09 |
| Bilirubin ($\mu mol.l^{-1}$) | 5.42± 0.75 | 4.08± 0.54 | 4.46± 1.49 | 5.66± 1.95 |
| Cholesterol ($mmol.l^{-1}$) | 2.22± 0.26 | 2.12± 0.34 | 3.66± 1.04 | 3.14± 1.21 |
| Triglycerides ($mmol.l^{-1}$) | 0.98± 0.26 | 0.97± 0.26 | 1.10± 0.31 | 1.21± 0.32 |
| Total proteins ($g.dl^{-1}$) | 57.95± 3.08 | 58.49± 3.46 | 62.64± 6.36 | 61.02± 4.20 |
| Calcium ($mmol.l^{-1}$) | 3.61± 0.39 | 3.25± 0.20 | 3.12± 0.54 | 3.41± 0.34 |
| Magnesium ($mmol.l^{-1}$) | 0.57± 0.079 | 0.55± 0.048 | 0.46± 0.024 | 0.54± 0.046 |
| Phosphorus ($mmol.l^{-1}$) | 1.42± 0.36 | 1.69± 0.29 | 0.40± 0.18 | 1.91± 0.38 |
| Sodium ($mmol.l^{-1}$) | 134.15± 4.40 | 141.35± 5.14 | 139.80± 2.44 | 138.83± 1.52 |
| Chlorides ($mmol.l^{-1}$) | 105.00± 0.59 | 103.85± 6.07 | 106.60± 1.57 | 110.80± 2.32 |
| Potassium ($mmol.l^{-1}$) | 3.77± 0.26 | 3.71± 0.49 | 3.70± 0.10 | 3.74± 0.34 |

C - control group without addition of epicatechin. E1 - E3 – experimental groups with addition of epicatechin (10 $\mu g.kg^{-1}$ in E1, 100 $\mu g.kg^{-1}$ in E2 and 1000 $\mu g.kg^{-1}$ in E3 group). The values shown are the mean ± SD (standard deviation), the differences were not significant (P>0.05).

## CONCLUSION

In this experiment the two week intramuscular application of epicatechin at various doses resulted in slight changes in biochemical parameters of rabbits without significant differences. We observed small changes in content of cholesterol and triglycerides in blood serum of rabbits in groups with higher dose of epicatechin. We also observed slightly increase of glucose in blood serum. All values of these parameters were still in physiological range of blood in rabbits.

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