JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

ASSESSMENT OF SELECTED ANTIOXIDANT PARAMETERS IN RABBIT BLOOD EXPOSED TO EPICATECHIN *IN VIVO* – FOUR WEEKS EXPOSURE

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doi: 10.15414/jmbfs.2016.5.special1.7-9

ARTICLE INFO

Received 16. 12. 2015
Revised 21. 1. 2016
Accepted 26. 1. 2016
Published 8. 2. 2016

Regular article

OPEN ACCESS

ABSTRACT

The aim of present study was to analyse selected antioxidant parameters in blood of rabbits after epicatechin administration during four 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 in doses 10 $\mu g.kg^{-1}$ in E1, 100 $\mu g.kg^{-1}$ in E2 and 1000 $\mu g.kg^{-1}$ in E3 for four weeks three times a week. At the end of experiment the blood was collected, selected antioxidant parameters (catalase - CAT, glutathione peroxidase - GPx, superoxide dismutase - SOD, uric acid - UA, bilirubine and albumin) were analysed by Genesys 10 spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) using the commercial kits. The present study has shown that the activity of SOD and activity of CAT was lower in all experimental groups when compare with the control group after four week exposure of epicatechin but without significant differences. Activity of GPx was higher in all experimental groups against the control group but also without significant differences. The highest concentration of UA in rabbit serum was observed in E1 experimental group with the lowest concentration of epicatechin when compared with the other experimental groups and with the control group but without significant differences. Concentration of bilirubine in rabbit serum after administration of epicatechin was insignificantly lowest in all experimental groups in comparison with the control group. Content of albumin was not affected by epicatechin. Further research needs to be focused on the generation of data dealing with antioxidant effects, in both human and animals.

Keywords: Antioxidant parameters, epicatechin, rabbit blood

INTRODUCTION

Oxidative damage to important biomolecules, including lipoprotein and DNA, is considered to accompany arteriosclerosis, carcinogenesis and acceleration of aging. This oxidative damage may be inhibited by daily intake of antioxidants **(Ames, 1983)**. Natural sources for these compounds include fruits and vegetables such as grapes, raspberries, onions, tomatoes, red wine, tea, etc. **(Kähkönen *et al.*, 1999)**. Their antioxidant properties are well defined by *in vitro* experiments **(Rice-Evans *et al.*, 1997; Nastume *et al.*, 2004)** and there are indications of their beneficial effects in the prevention of diseases, when they are part of the diet **(Ortega, 2006)**. The antioxidant activity of dietary polyphenols is considered to be much greater than that of the essential vitamins, therefore contributing significantly to the health benefits of fruit **(Tsao and Yang, 2003)**. The term antioxidant refers to free radical scavengers, inhibitor of lipid peroxidation and chelating agents **(Lee *et al.*, 2003)**. 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 **(Quine and Raghu, 2005)**. Several epidemiological investigations and dietary interventions in humans using flavanol-containing foods indicate an inverse relationship between flavanol intake and the improvement of immune responses and antioxidant defense system **(Sies et al., 2005)**. Specifically, in the last 10 years, a strong interest has been raised in the use of flavonoids and their derivatives for the therapeutic use, such as anti-inflammatory, anticancer, anti-ischemic, and antithrombotic components. Besides from presenting potent antioxidant properties *in vitro*, these compounds have also the ability to modulate the activity of the antioxidant defense enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) **(Chan *et al.*, 2002; Ying *et al.*, 2004; Li *et al.*, 2007)**. SOD converts superoxide to hydrogen peroxide, which is then removed by GPx and CAT **(Afonso *et al.*, 2007)**. Against oxidative stress, the human organism deploys an interactive network of antioxidants. The first line of defense consists of preventive antioxidants, which suppress the formation of free radicals (e.g. antioxidant enzymes). The another line of defense consists of antioxidants that scavenge free radicals by suppressing chain initiation and/or stopping multiple chain reactions (e.g. uric acid, albumin, bilirubin) **(Simos *et al*, 2012)**. Uric acid is a powerful antioxidant and is a scavenger of singlet oxygen and radicals **(Ames *et al.*, 1981)**. Bilirubin is a bile pigment and has an important role as an antioxidant. Bilirubin, through a hydrogen donation mechanism, participates as a scavenger of secondary oxidants formed in the oxidative process and thereby might alleviate oxidant stress in the blood. It might primarily protect cells against lipid peroxidation **(Sedlak and Snyder, 2004)**. Albumin may represent the major and predominant circulating antioxidant in plasma **(Cha and Kim, 1996)**. Albumin represents the quantitatively most important source of thiol in plasma, and this circulating store may be altered in situations where antioxidants become limiting, resulting in changes in the redox status **(Durand *et al.*, 1997)**. 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 selected antioxidant parameters in blood of rabbits after epicatechin administration during four 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 (table 1).

Animals 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 4 weeks 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 macromethod after four weeks of epicatechin administration. Catalase - CAT, glutathione peroxidase - GPx, superoxide dismutase - SOD, uric acid - UA, bilirubine and albumin were measured using the Genesys 10 spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Activity of CAT was performed according to **Beers and Sizer (1952)** monitoring the decrease of H_2O_2 at 240 nm in blood plasma. The calculation of CAT activity was based on the rate of decomposition of H_2O_2, which was proportional to the reduction of the absorbance during 1 min. **(Tvrda *et al.*, 2013)**. SOD and GPx activity and UA content were analyzed with the RANDOX assay kits (Randox Laboratories, Crumlin, UK) according to the manufacturer´s instructions. Albumin concentration was measured using the ALB BioLa Test (PLIVA-Lachema, Brno, Czech Republic) commercial kit. Concentration of bilirubine was measured by photometric test using the commercial kit DiaSys (Diagnostic Systems GmbH, Germany).

Table 1 Chemical composition (g.kg^{-1}) of the experimental diet.

Component	Content (g.kg^{-1})
Dry matter	926.26
Crude protein	192.06
Fat	36.08
Fibre	135.79
Non-nitrogen compounds	483.56
Ash	78.78
Organic matter	847.49
Calcium	9.73
Phosphorus	6.84
Magnesium	2.77
Sodium	1.81
Potassium	10.94
Metabolizable energy	12.35 MJ.kg^{-1}

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

Polyphenols have various important biological properties in both plants and animals that can be divided into two main categories, with antioxidant and nonantioxidant function **(Shay *et al,* 2015).** Regarding antioxidant action, it is noteworthy that polyphenols are the most abundant antioxidants in the diet with a total daily intake as high as 1 gram, exceeding the intake of vitamin C by about 10-fold and that of vitamin E and carotenoids by about 100-fold **(Scalbert *et al,* 2008)**. In our study, we used 10, 100 and 1000 μg per kg of body weight of epicatechin and analysed selected antioxidant parameters in rabbit blood. The results are presented in Table (2). Epicatechin in these concentrations had no significant influence on the observed parameters ($P > 0.05$). The present study has shown that the activity of SOD and activity of CAT was lower in all experimental groups when compare with the control group after four week exposure of epicatechin but without significant differences ($P > 0.05$). Activity of GPx was higher in all experimental groups against the control group but also without significant differences ($P > 0.05$). It has been reported that catechins has a strong anti-superoxide formation effect, by scavenging superoxide anion **(Ho *et al.* 1999; Reddy *et al*. 2004).** Catalase, present in phagocytes is effective only at high concentration of hydrogen peroxide **(Halliwell and Gutteridge 1989).** Base on this, we can suppose that in this case if there has been an increase of oxidative stress, epicatechin was able to scavenge reactive oxygen species and concentrations of antioxidant enzymes in blood did not rise. In the other hand, the decrease in the activity of SOD may be attributed to the saturation of SOD during the process of converting O_2• to H_2O_2 (**Eraslan *et al*., 2007**). The major function of GPx, which uses glutathione (GSH) as a substrate, is to reduce soluble H_2O_2 and alkyl peroxides (**Bebe and Panemangalore, 2003**). GPx also can decompose H_2O_2 to water (**Tian *et al.*, 1998**). It may be reason why the activity of GPx was slightly increased.

Table 2 Selected antioxidant parameters in rabbit blood after four weeks of exposure of epicatechin

Parameter	SOD	CAT	GPx	UA	Bilirubine	Albumin
Group	[U.ml^{-1}]	[U.mg protein^{-1}]	[U.l^{-1}]	[μmol.l^{-1}]	[μmol.l^{-1}]	[g.l^{-1}]
C	94.75±6.24	0.35±0.12	1205.02±189.49	82.78±11.79	1.85±0.48	35.56±1.23
E1	86.25±7.81	0.28±0.06	1447.92±301.20	109.33±16.46	3.13±0.97	33.58±0.53
E2	89.50±4.20	0.22±0.07	1302.79±41.00	76.53±13.53	2.82±0.34	33.06±1.58
E3	90.00±3.60	0.28±0.07	1235.51±85.19	78.49±9.66	2.2±0.70	36.37±0.38

C - control group without addition of epicatechin. E1 - E3 – experimental groups with addition of epicatechin (10 μg.kg^{-1} in E1, 100 μg.kg^{-1} in E2 and 1000 μg.kg^{-1} in E3 group). The values shown are the mean ± SD (standard deviation).

Uric acid (UA) is involved in a complex reaction with several oxidants and may have some protective effects under certain conditions. On the other hand, uric acid cannot scavenge all radicals, with superoxide as an example. Uric acid is an antioxidant only in the hydrophilic
environment, which is probably a major limitation of the antioxidant function of uric acid **(Sautin and Johnson, 2008)**. In our study, the highest concentration of UA in rabbit serum was observed in E1 experimental group with the lowest concentration of epicatechin (10 μg.kg^{-1}) when compared with the other experimental groups and with the control group but without significant differences ($P > 0.05$). Administration of (+)- catechin hydrate increased levels of uric acid which may be one of the reasons for protection against diet induced oxidative stress **(Mehra *et al*., 2007)**. Administration of EGCG in healthy human individuals increased plasma antioxidant activity which was not due to changes in EGCG concentration but due to changes in plasma urate concentrations, which might have interfered with the effect of EGCG to promote antioxidant activity **(Susanne *et al.*, 2005)**. In the other hand, high plasma uric acid (UA) is a prerequisite for gout and is also associated with the Metabolic Syndrome and risk factors for cardiovascular diseases **(Kim *et al.*, 2009)**. In our study, the concentration of UA in blood serum of rabbits was lower in groups with higher concentration of epicatechin (100 μg.kg^{-1} in E2 and 1000 μg.kg^{-1} in E3 group) against the control group however without significant differences ($P > 0.05$). There is still no consensus if UA is a protective or a risk factor, however, it seems that the quantity and the duration of the concentration of the uric acid in the blood is essential for this answer **(Oliveira and Burini, 2012)**.
Bilirubin has been reported as a member of the antioxidant family and is even known to have toxic effects at high concentration. The combined evidence from animal and human studies indicates that bilirubin is a major physiologic cytoprotectant and might alleviate oxidative stress in the blood **(Sedlak and Synder, 2004)**. Our results showed that concentration of bilirubine in rabbit serum after administration of epicatechin was insignificantly ($P > 0.05$) lowest in all experimental groups in comparison with the control group. In the study of authors **Petruška *et al.* (2013)** that long-term application of quercetin caused the increase of concentration of bilirubine in rabbit serum. Findings of **Loprinzi and Mahoney (2015)** suggested an association between flavonoid-rich fruit and vegetable consumption and bilirubin levels. If confirmed by prospective and experimental studies, then regular consumption of flavonoid-rich fruits and vegetables should be promoted to increase levels of bilirubin.
Albumin represents a very abundant and important circulating antioxidant **(Roche *et al*., 2008)**. Study of **Bourdon *et al*. (1999)** confirmed and extended the idea that serum albumin is an important protein that presents direct protective

effects. In our study, concentration of serum albumin was very similar among all groups. Thus, we can say that any used concentration of epicatechin have effect on this parameter in rabbits. In study of **Petruška *et al.* (2013)** they observed slight decrease in the content of serum albumin in quercetin groups vs. control group of rabbits. Several lines of evidence strongly suggest that a reduced serum albumin concentration, although within the normal range, is associated with mortality risk **(Bourdon *et al*., 1999).**

CONCLUSION

Selected antioxidant parameters in rabbit blood after four weeks exposure of epicatechin in this study were assessed. Four weeks of intramuscular application of epicatechin at various doses resulted in slight changes in selected antioxidant parameters of rabbits without significant differences. Catechins when compared with other classes of flavonoids are found to be very active in reducing the amount of strand breakage and residual base damage by mechanism other than directly scavenging of hydroxyl radicals before they react with DNA. To determine whether epicatechin act as effective antioxidant *in vivo*, future studies in animals and humans should employ sensitive and specific biomarkers of oxidative damage to DNA, proteins and lipids.

Acknowledgments: This work was financially supported by VEGA scientific grant 1/0760/15. This work was co-funded by European Community under project no 26220220180: Building Research Centre "AgroBioTech".

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