Journal of Microbiology, Biotechnology and Food Sciences

Petruška et al. 2013 : 2 (Special issue 1) 1540-1549

REGULAR ARTICLE

EFFECT OF QUERCETIN ON HAEMATOLOGICAL PARAMETERS OF RABBITS: A GENDER COMPARISON

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ABSTRACT

The aim of the present study was to determine the effect of application of quercetin in various doses on selected haematological parameters of rabbit's blood.Adult female rabbits (n = 20) and male rabbits (n = 20) were used in experiment.Animals were divided into four groups (n=5 in each group), one control group (C) and three experimental groups (E1, E2 and E3) in both sexes. Experimental groups received quercetin in injectable form (intramuscularly) at 10 $\mu g.kg^{-1}$ in E1 group, 100 $\mu g.kg^{-1}$ in E2 group, and 1000 $\mu g.kg^{-1}$ in E3 group for 90 days 3 times a week. Control group received injection water.Higher values in WBC (total white blood cell count), GRA (granulocytes), RBC (red blood cells count) and HGB (haemoglobin) in experimental groups in comparison with the control group were found,however without significant differences (P>0.05).Increase in HCT in all experimental male groups in comparison with the control group without significant differences (P>0.05) was observed.Other haematological parameters were not influenced by this natural antioxidant.

Keywords:flavonoids, quercetin, rabbit, gender comparison

INTRODUCTION

The flavonoids are comprised of a large group of low molecular weight polyphenolic secondaryplant metabolites which are important to the health of animals and humans. Dietary exposure to flavonoids is not insignificant. For instance, the average Western diet contains approximately 1 $g.day^{-1}$ of mixed flavonoids **(Hollman and Katan, 2009)**. Ingestible flavonoids are found in fruits, vegetables, nuts, seeds, stems, flowers, roots, bark, tea, wine and coffee. They are prominent components of citrus fruits and other food sources **(Jellin *et al.*, 2003)**. Their effects on a variety of inflammatory processes have been reviewed **(Wright *et al.*, 2012)**. They are also potent antioxidants and possess significant vitamin C-sparing activity **(McCullough *et al.*, 2012)**. The flavonoids display a remarkable array of biochemical and pharmacological actions **(Middleton, 1988)**, some of which suggest that certain members of this group of compounds significantly affect the function of the immune system.

Quercetin is a polyphenolic flavonoid, classified as a flavonol and has also been reported that possesses anti-cancer activity **(Pilorget *et al.*, 2003)**, anti-allergicactivity **(Morimoto *et al.*, 2003)**, vasorelaxative effect **(Novakovic *et al.*, 2006)**, anti-ischemic activity **(Lee *et al*, 2000)**, anti-inflammatory activity **(Kim *et al.*, 2004; Orsolic *et al.*, 2004)** and the antioxidant capacity of these molecules seems to be responsible for many of their beneficial effects and confers a therapeutic potential in diseases such as gastric or duodenal ulcers and hepatic pathologies **(González-Gallego *et al.*, 2007)**. Quercetin is highly abundant in food and beverage sources that are part of the human diet such as broccoli, lettuce, apples, tomatoes, onions, tea and coffee **(Jellin *et al.*, 2003)**. Within the flavonoid family, quercetin is the most potent scavenger of reactive oxygen species (ROS) **(Heijnen *et al.*, 2002; Boots *et al.*, 2008)**.

The objective of our study was to determine the effect of chronic application of quercetin in various doses on selected haematological parameters of rabbit's blood in both genders.

MATERIAL AND METHODS

Animals and diet

Adult female rabbits (n=20) and male rabbits (n=20) of meat line M91, 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 (Table 1) composed of a pelleted concentrate.

Animals were divided into four groups (n=5 in each group), one control group (C) and three experimental groups (E1, E2 and E3) in both sexes. Experimental groups received quercetin(Sigma Aldrich, Saint Louis, USA) in injectable form (intramuscularly) at 10 $\mu g.kg^{-1}$ in E1 group, 100 $\mu g.kg^{-1}$ in E2 group, and 1000 $\mu g.kg^{-1}$ in E3 group for 90 days 3 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 the State Veterinary and Food Institute of Slovak Republic, no. 3398/11-221/3.

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

Component	
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 $MJ.kg^{-1}$

Blood sampling and analyses

Blood samples from *vena auricularis* were taken from all animals by macromethods in morning twice during the experimental period.

In whole blood, selected haematological parameters [total white blood cell count (WBC), lymphocytes count (LYM), medium size cell count (MID), granulocytes count (GRA), lymphocyte percentage (LYM%), medium size cell percentage (MID%), granulocytes percentage (GRA%), red blood cell count (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDWc), platelet count (PLT), platelet percentage (PCT), mean platelet volume (MPV) and platelet distribution width (PDWc)] were measured using haematology analyzer Abacus junior VET (Diatron®, Vienna, Austria).

Statistical analyses

The data used for statistical analyses represent means of values obtained in three blood collections performed on separate days. 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 11.0 (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 (Tab. 2 and 3).Addition of quercetin slightly influenced some haematological parameters in blood of rabbits;however differences among the groups were insignificant ($P>0.05$). We observed higher values in WBC in all experimentalmale groups in comparison with the control group, but without significant differences ($P>0.05$). In female rabbits slightly higher values of WBC in E1 and E2 group and slightly lower value in E3 group in comparison with the control group was found, but differences among the groups remained insignificant ($P>0.05$).In our previous study higher values in WBC in rabbits treated with quercetin when compared to the control was reported **(Petruška *et al.*, 2012)**. After treatment with quercetin WBC of rabbits was slightly increased as response on quercetin

application.**Adebayo *et al.* (2010)**reported thatforeign substancesin the animal body can results inWBC increase.**Kandaswamy *et al.* (2013)**found that application of higher doses (50 $mg.kg^{-1}$ of body mass) of quercetin insignificantly decreased WBC and RBC in rats. Similar results were recorded by **Parabathina *et al.* (2011)** in experiment with quercetin and vitamin E in rabbits. The discrepancies in the results are due to using of different dose and different combination of natural substances, different kinds of animals involved in the experimental interventions and length of treatment periods.

Quercetin preparation caused slight insignificant decrease (P>0.05) in LYM in all female experimental groups in comparison with the control group.Quercetin had not influenced this parameter in male rabbits. Statistical analyses showed no significant differences (P>0.05) between both genders. **Soung *et al.* (2006)** found thatdaily consumption of 60 mg of isoflavones immediate one year did not cause any changes in LYM in women.

In this study we observed insignificant (P>0.05) increase in GRA in all experimental groups*vs.* the control group, howeverno significant changes between both sexes were confirmed.On the contrary **Capcarova *et al.* (2011)**foundinsignificant decreased in GRA in study with sumac (*Rhuscoriaria*)inclusion to the feed for rabbits. The discrepancies in the results are due to using of different dose and kind of natural substances and length of treatment periods.

RBC are one of important factors affecting whole blood viscosity, since RBC accounts for almost 50% of blood volume and constitute the majority of the cellular content in blood **(Hai-Xia Li *et al.*, 2009)**.Quercetin preparation caused increase of RBC and HGB in comparison with the control group in both sexes, but differences among the groups remained insignificant (P>0.05). Similar results found **Capcarova *et al.* (2011)** in study with sumac.

Higher values of HCT in all male experimental groups in comparison with the control group were measured, but without significant differences (P>0.05). The similar results observed **Hai-Xia Li *et al.* (2009)** in study with carthamins yellow from *Carthamustinctorius*L applied to the male rats.

The values of other haematological parameters (MID, LY%, MI%, GR%, MCV, MCH, MCHC, RDWc, PLT, PCT, MPV and PDWc) were not influenced (P>0.05) after quercetin treatment.

In this study the values of parameters in all groups corresponded with normal hematological values in rabbit blood determined by **Hewitt et al. (1989)**. Treatment with phytoadditives had no significant effect on haematological parameters of male laboratory mice **(Singh and Singh, 2008)**, rats **(Babayi et al., 2007)** and rabbits **(Ram et al., 1997)**.

We have not found other evidence in literature about the effect of quercetin on haematological parameters of rabbits concerninggender comparison.

Table 2 Haematological parameters of rabbits after quercetin treatment (male groups)

Parameter	C	E1	E2	E3
WBC	9.59±0.43	10.78±1.01	10.19±1.64	11.34±2.92
LYM	5.95±1.58	5.97±2.11	5.46±2.27	5.93±1.52
MID	0.71±0.33	1.02±0.09	0.6±0.43	1.05±0.50
GRA	2.94±2.26	4.82±2.73	3.44±1.19	4.35±1.82
LY%	62.46±18.51	55.95±19.45	48.82±23.34	53.58±11.21
MI%	4.36±0.83	9.50±1.58	5.27±3.70	8.82±2.45
GR%	60.56±30.33	44.42±24.30	45.90±25.64	37.60±10.42
RBC	6.67±1.00	7.46±0.63	7.56±0.49	7.80±0.41
HGB	122.50±19.68	139.20±8.04	139.25±4.27	140.20±5.45
HCT	38.59±6.46	44.74±3.01	45.06±1.04	45.61±2.58
MCV	57.50±3.41	60.00±3.16	59.75±2.50	58.60±2.19
MCH	18.35±0.97	18.68±0.61	18.45±0.71	17.98±0.87
MCHC	317.75±3.50	311.40±9.63	308.75±2.63	307.60±9.07
RDWc	15.82±0.63	15.56±1.63	15.32±0.85	15.56±0.45
PLT	375.75±55.20	291.00±105.33	264.33±72.84	329.75±108.33
PCT	0.23±0.04	0.17±0.05	0.22±0.10	0.21±0.07
MPV	6.07±0.39	6.02±0.50	6.25±0.71	6.58±0.32
PDWc	32.35±1.28	31.16±0.89	33.57±2.78	33.80±0.69

WBC - total white blood cell count (10^9/l); LYM - lymphocytes count (10^9/l); MID - medium-size cell count; GRA - granulocytes count (10^9/l); LYM% - lymphocyte percentage; MID% - medium-size cell percentage; GRA% - granulocytes percentage; RBC - red blood cell count (10^{12}/l); HGB - haemoglobin (g/l); HCT - haematocrit (%); MCV - mean corpuscular volume (fl); MCH - mean corpuscular haemoglobin (pg); MCHC - mean corpuscular haemoglobin concentration (g/l); RDWc - red cell distribution width (%); PLT - platelet count (10^9/l); PCT - platelet percentage; MPV - mean platelet volume (fl); PDWc - platelet distribution width (%).

C – control group, E1 (10 μg.kg^{-1}), E2 (100 μg.kg^{-1}), E3 (1000 μg.kg^{-1}) – experimental groups. The values shown are the mean±SD (standard deviation).

Table 3 Haematological parameters of rabbits after quercetin treatment (female groups)

Parameter	C	E1	E2	E3
WBC	9.66±1.16	10.00±0.54	9.85±1.06	8.99±2.41
LYM	6.18±1.98	5.01±2.44	4.64±0.65	3.36±1.06
MID	0.56±0.32	0.61±0.28	0.81±0.31	0.74±0.41
GRA	3.02±1.12	3.44±1.39	4.67±1.36	5.01±2.46
LY%	67.78±20.55	53.40±18.55	47.32±6.41	38.54±12.64
MI%	6.28±3.78	6.92±2.77	6.97±2.14	8.57±4.89
GR%	33.57±13.53	46.52±5.85	47.05±9.99	54.48±18.00
RBC	6.92±0.53	6.99±0.59	7.29±0.32	7.09±0.29
HGB	131.60±6.80	133.00±12.41	135.40±4.56	131.20±4.56
HCT	42.46±2.43	42.68±3.20	43.44±2.23	43.39±1.87
MCV	61.40±2.40	61.00±1.22	59.60±1.81	61.20±1.20
MCH	19.02±0.71	19.04±0.29	18.56±0.59	18.52±0.64
MCHC	309.40±2.88	311.60±6.54	311.60±5.50	302.80±10.56
RDWc	15.58±0.66	15.18±0.46	14.88±0.71	14.84±0.43
PLT	337.50±64.40	282.20±83.26	359.20±122.30	332.00±30.38
PCT	0.20±0.03	0.19±0.02	0.21±0.07	0.19±0.02
MPV	6.04±0.43	6.00±0.41	5.98±0.44	5.72±0.49
PDWc	31.80±1.67	31.16±1.76	31.96±1.16	30.68±2.69

WBC - total white blood cell count (10^9/l); LYM - lymphocytes count (10^9/l); MID - medium-size cell count; GRA - granulocytes count (10^9/l); LYM% - lymphocyte percentage; MID% - medium-size cell percentage; GRA% - granulocytes percentage; RBC - red blood cell count (10^{12}/l); HGB - haemoglobin (g/l); HCT - haematocrit (%); MCV - mean corpuscular volume (fl); MCH - mean corpuscular haemoglobin (pg); MCHC - mean corpuscular haemoglobin concentration (g/l); RDWc - red cell distribution width (%); PLT - platelet count (10^9/l); PCT - platelet percentage; MPV - mean platelet volume (fl); PDWc - platelet distribution width (%).
C – control group, E1 (10 μg.kg^{-1}), E2 (100 μg.kg^{-1}), E3 (1000 μg.kg^{-1}) – experimental groups. The values shown are the mean±SD (standard deviation).

CONCLUSION

In this experiment the intramuscular application of quercetin resulted in slight changes in some haematological parameters of rabbits. Administration of quercetin three times a week caused insignificant changes in WBC, LYM, GRA, RBC, HGB and HCT between the control and experimental groups in both sexes. The values of other haematological parameters (MID, LY%, MI%, GR%, MCV, MCH, MCHC, RDWc, PLT, PCT, MPV and PDWc) were not influenced ($P>0.05$) after quercetin treatment. Statistically quercetin had no influence on gender of rabbits.

Further investigation with different doses of quercetin will be worthy of further investigation.

Acknowledgments: This work was financially supported by VEGA scientific grants 1/0084/12, 1/0022/13 and KEGA 030SPU-4/2012.

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