Animal welfare, etológia és tartástechnológia

Animal welfare, ethology and housing systems

Volume 9 Issue 3

Különszám/Special Issue

Gödöllő

2013

HAEMATOLOGICAL CHANGES IN RABBIT'S BLOOD AFTER TWO WEEKS EXPOSURE OF PATULIN.

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Abstract

The aim of present study was to analyse haematological parameters in blood of rabbits after patulin administration during two weeks. Animals (adult female rabbits, body weight 4 ± 0.5 kg) were divided into two groups: control group (C) and experimental group (E). Animals in E group received intramuscular injection of patulin (10 μg.kg^{-1}) 4 times. After 2 weeks of exposure the blood was collected and selected haematological parameters (total white blood cell count, lymphocytes count, medium size cell count, granulocytes count, red blood cell count, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, red cell distribution width, platelet count, platelet percentage, mean platelet volume and platelet distribution width) were analysed by Abacus Junior Vet (Diatron®, Vienna, Austria). Significant decrease ($P < 0.05$) of mean corpuscular haemoglobin concentration in E group in comparison with the C group was observed. In the case of hemoglobin concentration no significant change between both groups were found. Patulin had no effect on the other analysed haematological parameters.

Keywords: patulin, haematology, rabbit

Introduction

Patulin, a genotoxic mycotoxin produced by several species of *Aspergillus, Penicillium* and *Bysochlamys* **(Puel et al., 2010; Ozsoy et al., 2008)** is the most common mycotoxin in apples and apple-derived products **(Puel et al., 2010)**. It contaminates further fruits, such as grapes, oranges, pears, oranges, pears and peaches **(Saxena et al., 2009; Ozsoy et al., 2008)**. The effects caused by this mycotoxin are based on several studies made during the past fifty years; these involve acute, chronic and cellular level effects **(González-Osnaya et al., 2007)**. Cellular effects of patulin include formation of reactive oxygen species, cell cycle arrest, cytochrome c release from mitochondria, caspase-3 activation, PARP cleavage, ATF3 expression and subseyuent apoptosis **(Kwon et al., 2012; Fribley et al., 2011; Ozsoy et al., 2008)**. Patulin may be neurotoxic, immunotoxic, immunosuppressive, genotoxic, teratogenic and carcinogenic **(González-Osnaya et al., 2007)**. *In vivo* patulin caused severe damage in several organ systems like kidney, intestinal tissue **(McKinley et al., 1982; Speijers et al., 1988)** and immune system **(Escoula et al., 1988)**, if applied at a range between 2.5 and 41 mg.kg^{-1} bw. Regarding carcinogenicity, the International Agency for Research on Cancer classified patulin in group C, since the evidence of carcinogenicity was considered limited in experimental animal **(International Agency for Research on Cancer, 1986)**. Patulin has a strong affinity for sulfhydryl groups. Patulin adducts formed with cysteine are less toxic than the unmodified compound in acute toxicity, teratogenicity, and mutagenicity studies. Its affinity for SH-groups explains its inhibition of many enzymes **(Puel et al., 2010)**. Toxic signs consistently reported in all studies were agitation, in some cases convulsions, dyspnea, pulmonary congestion, edema, and ulceration, hyperemia and distension of the gastro intestinal tract **(WHO, 1998)**. Based on the provisional maximum tolerable daily intake (400 ng.kg^{-1} bw/day), several countries have set legislations for the maximum amount of patulin in apples products. In the Europen Unioin, the limit is

set to 50 µg.kg^{-1} in apple juice and cider, 25 µg.kg^{-1} in solid apple products (*e.g.* apple sauce) and 10 µg.kg^{-1} in products for infants and young children (**Commission of the European Communities, 2003)**. The aim of present study was to analyse haematological parameters in blood of rabbits after patulin administration during two weeks.

Materials and Methods

Animals

Adult female rabbits (n = 32), 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.

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 two groups: control group (C) without addition of patulin and experimental group (E) with addition of patulin (10 µg.kg^{-1}). Animlas from experimental group received patulin through intramuscular injection twice a week for two weeks.

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 patulin administration.

In whole blood, selected haematological parameters [total white blood cell count (WBC), lymphocytes count (LYM), mediumsize cell count (MID), granulocytes count (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 blood collection. To compare the results, t-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 $P < 0.05$.

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}

Results and Discussion

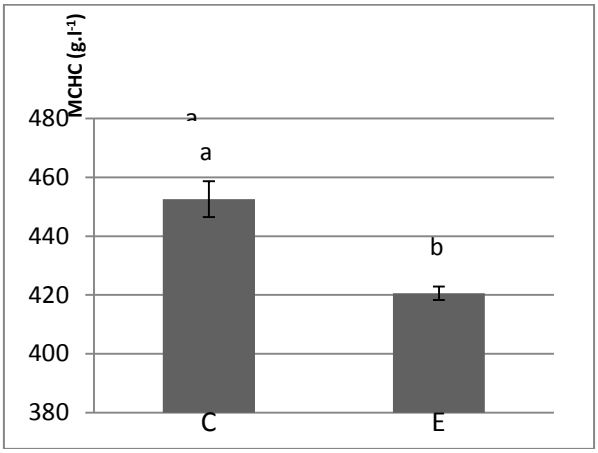
The results are presented in Table (2). Patulin had no significant influence on the most of observed parameters ($P > 0.05$). Mean Corpuscular Haemoglobin Concentration (MCHC) is the mean concentration g.l^{-1} of haemoglobin of cells, as an index that is independent of the size of the red cells and therefore, is a true expression of Hb level **(Sharma and Sinhg, 2007)**. In present study significant decrease ($P < 0.05$) of MCHC in E group in comparison with the control group (C) was observed (Figure 1). In another study **Petruška and Capcarová (2012)** observed after chronic application of quercetin and acute dose of T-2 toxin slight–decrease of MCHC in all experimental groups in comparison with the control group. **Capcarová et al. (2011)** found no effect of *Rhus coriaria* on MCHC in rabbits after dietary inclusion. Similar results were observed by **Ewuola and Egbunike (2008)** who examined haematological response of growing rabbit bucks fed dietary fumonisin B_1. In our study in the case of HGB no significant change ($P > 0.05$) in experimental group in comparison with the control group was observed, however slight decreased in E group was noted. In different study **Raju and Dewegoeda (2000)** observed similar effect of aflatoxin, ochratoxin and T2-toxin on HGB of broilers. **Ewuola and Egbunike (2008)** found that after fumonisin B_1 administration in growing rabbits haemoglobin concentration decreased, but only in concentration 10 mg.kg^{-1} of fumonisin B_1 in diet was significantly lower in comparison with other experimental groups. **Gbore and Akele (2010)** found that concentration of haemoglobin of female rabbits significantly decreased after fumonisin administration. Increase of MID and PLT in experimental group in comparison with the control group was found in this study, but without significant differences ($P > 0.05$). **Petruška and Capcarová (2012)** recorded decrease of PLT count in rabbits after quercetin and T-2 toxin treatment. **Ewuola and Egbunike (2008)** found no changes in count of PLT after treatment of fumonisin B_1 in growing rabbits. In this paper lower LYM count was found in E group when compared with the control, but without significant differences ($P > 0.05$). **Gbole and Akele (2010)** observed significant decrease of PLT after fumonisin administration in female rabbits. In another study **Petruška and Capcarová (2012)** found after chronic application of quercetin and acute dose of T-2 toxin slight decrease of PLT in rabbits. Patulin had no effect on the other analyzed parameters. Changes in haematology parameters could be related to the fact that patulin has a strong affinity for sulfhydryl groups. Its affinity for SH-groups explains its inhibition of many enzymes. It is interesting how little is known yet on the pharmacokinetic behaviour and metabolism of patulin.

Table 1

Haematological parameters of rabbits after patulin administration

Parameter	C	E
WBC	9.81±1.78	9.74±2.52
LYM	5.71±1.28	2.19±0.25
MID	0.28±0.09	0.51±0.15
GRA	3.86±0.91	4.75±1.19
RBC	6.00±0.13	5.44±0.12
HGB	142.55±4.74	128.62±8.25
HCT	32.37±2.70	30.59±1.93
MCV	54.05±5.65	56.28±3.61
MCH	23.79±1.31	23.67±1.47
MCHC	452.59±6.07^{a}	420.56±2.79^{b}
RDWc	19.70±1.05	19.37±0.65
PLT	201.52±65.73	235.30±82.55
PCT	0.11±0.04	0.14±0.05
MPV	5.42±0.27	5.79±0.34
PDWc	27.44±2.03	28.97±1.97

WBC. total white blood cell count ($10^{9}.l^{-1}$); LYM. lymphocytes count ($10^{9}.l^{-1}$); MID. medium-sized cell count; GRA. granulocytes count ($10^{9}.l^{-1}$); RBC. red blood cell count ($10^{12}.l^{-1}$); HGB. haemoglobin ($g.l^{-1}$); HCT. haematocrit (%); MCV. mean corpuscular volume (fl); MCH. mean corpuscular haemoglobin (pg); MCHC. mean corpuscular haemoglobin concentration ($g.l^{-1}$); RDWc. red cell distribution width (%); PLT. platelet count ($10^{9}.l^{-1}$); PCT. platelet percentage; MPV. mean platelet volume (fl); PDWc. platelet distribution width (%).
C - control group without addition of patulin. E - experimental group with addition of patulin (10 $\mu g.kg^{-1}$). The values shown are the mean ± SD (standard deviation). a.b - in row means significant difference (P ‹ 0.05).



Figure

Effect of patulin on MCHC in blood of rabbits

C = control group. E = experimental group

Conclusion

In this experiment the two weeks intramuscular application of patulin resulted in slight changes in haematological parameters of rabbits. Administration of patulin three times a week significantly decreased MCHC in E group in comparison with control group. Decrease in HGB was found without significant differences. We observed insignificant increase in MID and PLT in E group in comparison with the control group.

Further investigation with haematological response of animals will be worthy of further investigation.

Acknowledgments
This work was financially supported by VEGA scientific grant, 1/0084/12, and 1/0022/13.

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