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EVALUATION OF SOME HAEMATOLOGICAL PARAMETERS IN PORCINE BLOOD AFTER EXPOSURE OF PATULIN *IN VITRO**

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SUMMARY: Patulin, a genotoxic mycotoxin produced by several species of Aspergillus, Penicillium and Bysochlamys is the most common mycotoxin in apples and apple-derived products. Patulin may be neurotoxic, immunotoxic, immunosuppressive, genotoxic, teratogenic and carcinogenic. The aim of the present study was to investigate the effect of patulin on chosen haematological parameters (red blood cells - RBC, white blood cells - WBC, platelets - PLT, haematocrit - HCT, haemoglobin - HGB) in porcine blood in vitro. Samples with patulin were incubated with patulin: 10 ng.ml^{-1} in E1 group, 100 ng.ml^{-1} in E2 group, and 1000 ng.ml^{-1} in E3 group for 4 hours at 37°C. The group without any addition served as the control. Patulin caused significant decrease of WBC in all experimental groups when compared to the control. In case of RBC patulin significantly decreased the number of RBC in porcine blood followed by increase of hemolysis and decrease of HGB and HCT in all experimental groups when compared with the control group. The count of PLT was significantly decreased in all experimental groups against the control. Results of this study provide a foundation for further analysis and researches on mycotoxins impact on living cells and the system of possible protection against its effects as well as evaluation of various dose dependencies on haematological parameters.

Keywords: *patulin, porcine blood, haematology.*

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INTRODUCTION

Mycotoxins are secondary metabolites of molds that have adverse effects on humans, animals, and crops that result in illnesses and economic losses (Zain, 2011). Contamination of food and agricultural commodities by various types of toxigenic molds (fungi) is a serious and widely neglected problem (Bhat *et al.,* 2010). This contamination presented a hazard to human and animal health for decades (Gbore and Akele, 2010). 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). 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. 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). 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 Union, the limit is set to 50 $\mu g.kg^{-1}$ in apple juice and cider, 25 $\mu g.kg^{-1}$ in solid apple products (*e.g.* apple sauce) and 10 $\mu g.kg^{-1}$ in products for infants and young children (Commission of the European Communities, 2003). The aim of this study was to analyse the effect of patulin on haematological parameters of porcine blood *in vitro*.

MATERIAL AND METHODS

Animals and experimental design in vitro. Slovakian White gilts (n=24) at the age of 100-120 days were kept under standard conditions at the Experimental Station of the Animal Production Research Centre Nitra. Conditions of their care, manipulations and use corresponded to the instruction of EC no. 178/2002 and related EC documents, and they were approved by local ethics commission. Animals were slaughtered and blood samples were obtained.

Blood sampling and patulin treatment. Blood was collected into EDTA-treated tubes. Patulin (Sigma Aldrich, Saint Louis, USA) was added to blood samples at doses 10, 100 and 1000 $ng.ml^{-1}$ (Table 1). The blood samples without addition of patulin served as control group (C). The blood was incubated for 4 hours at 37°C.

Table 1: Application of patulin in to blood *in vitro*

Group	Patulin ($ng.ml^{-1}$)
C	0
E1	10
E2	100
E3	1000

n=5 in each group; C- control group, E_1 - E_3 – experimental groups with various doses of patulin

Analysis of parameters. Haematological parameters (red blood cells - RBC, white blood cells - WBC, platelets - PLT, haemoglobin - HGB, haematocrit - HCT) were measured using haematology analyzer Abacus junior VET (Diatron®, Vienna, Austria).

Statistical analysis. Sigma Plot 11.0 (Jandel, Corte Madera, USA) was used to conduct statistical analyses. One-way ANOVA was used to calculate basic statistic characteristics and to determine significant differences among the experimental and the control groups. Data presented are given as mean and standard deviation (SD). Differences were compared for statistical significance at the level $P<0.05$.

RESULTS

In this study selected haematological parameters were measured in blood samples after exposure of patulin *in vitro* for 4 hours at 37 °C. The results are shown in Figures 1-5. WBC count (Fig. 1) was significantly decreased ($P<0.05$) in all experimental groups when compared with the control group. We did not observed any significant differences ($P>0.05$) among the experimental groups. The values of WBC were similar in all experimental groups.

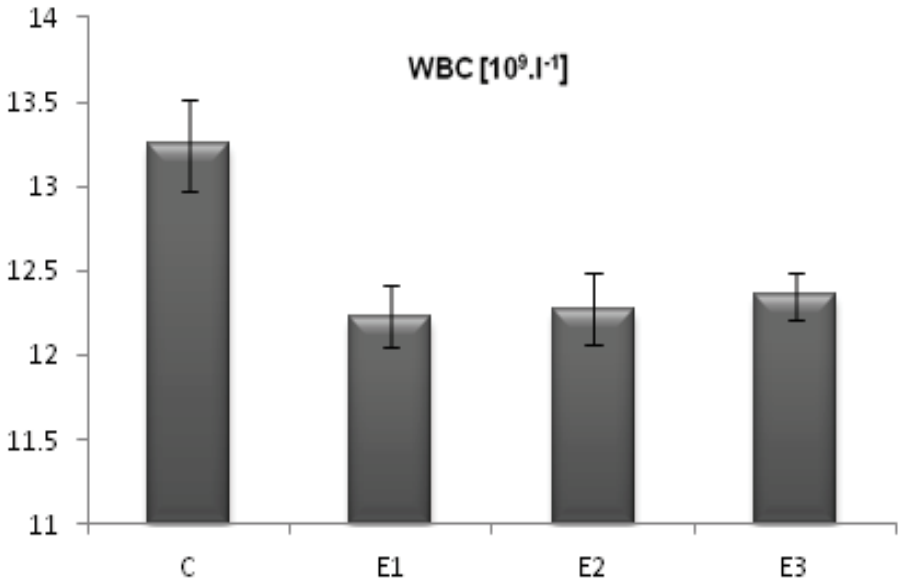

Figure 1. The effect of patulin on WBC in porcine blood *in vitro*
C - control, E1 – 10 ng.ml^{-1}, E2 100 ng.ml^{-1}, E3 - 1000 ng.ml^{-1}, WBC – white blood cells count, a,b – means significant differences ($P<0.05$), one-way ANOVA

In case of RBC count (Fig 2), value of HGB (Fig 3) and HCT (Fig 4) we observed significant decrease ($P<0.05$) of these parameters in all experimental groups when compared to the control. The lowest value of this three parameters were observed in E2 experimental group (100 ng.ml^{-1}of patulin).

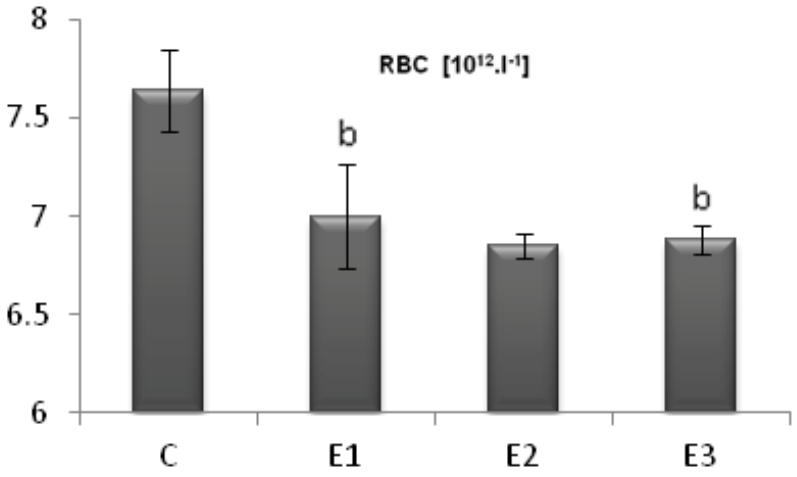

Figure 2. The effect of patulin on RBC in porcine blood *in vitro*
C - control, E1 – 10 ng.ml^{-1}, E2 100 ng.ml^{-1}, E3 - 1000 ng.ml^{-1}, WBC – white blood cells count, a,b – means significant differences (P<0.05), one-way ANOVA

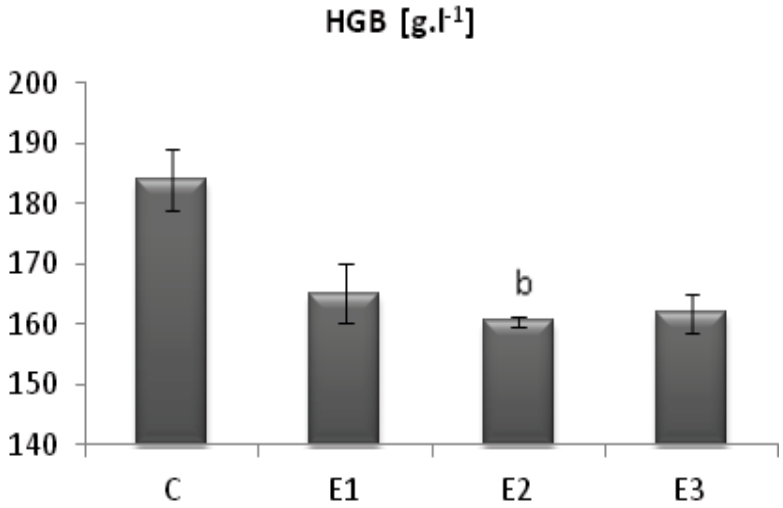

Figure 3. The effect of patulin on HGB in porcine blood *in vitro*
C - control, E1 – 10 ng.ml^{-1}, E2 100 ng.ml^{-1}, E3 - 1000 ng.ml^{-1}, WBC – white blood cells count, a,b – means significant differences (P<0.05), one-way ANOVA

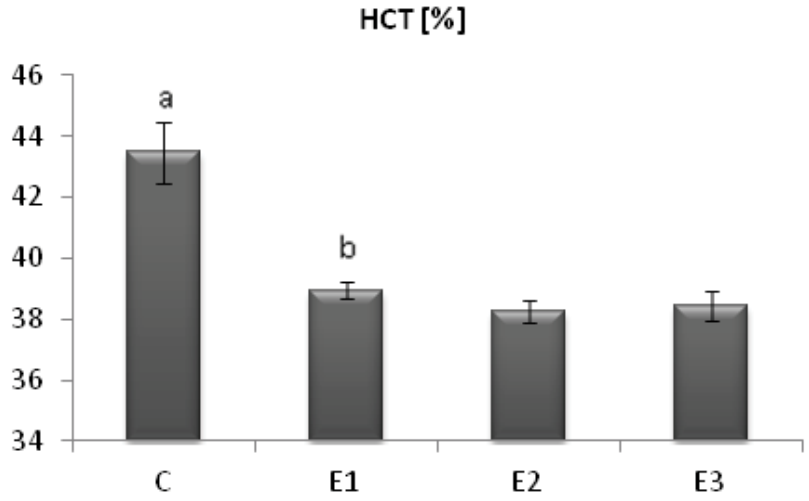

Figure 4. The effect of patulin on HCT in porcine blood *in vitro*
C - control, E1 – 10 ng.ml^{-1}, E2 100 ng.ml^{-1}, E3 - 1000 ng.ml^{-1}, WBC – white blood cells count, a,b – means significant differences (P<0.05), one-way ANOVA

Significant decrease (P<0.05) of PLT count was found in all experimental groups aginst the control. The lowest count of PLT was observed in E1 group (the lowest amount of patulin).

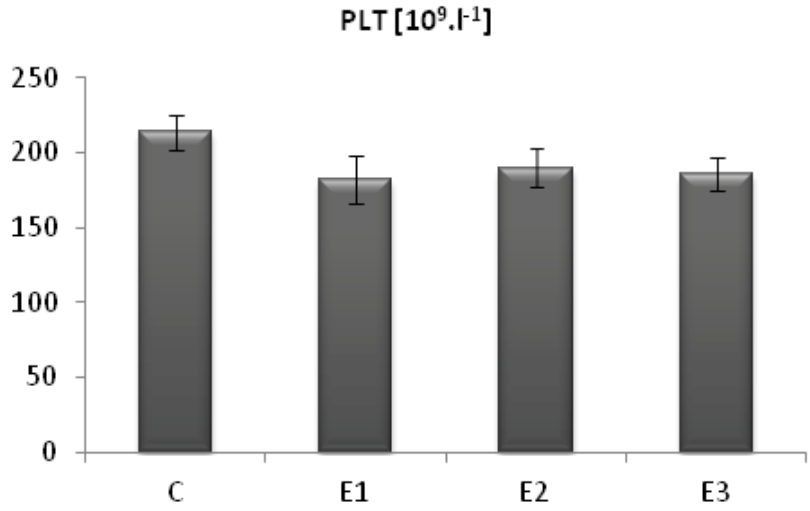

Figure 5. The effect of patulin on PLT in porcine blood *in vitro*
C - control, E1 – 10 ng.ml^{-1}, E2 100 ng.ml^{-1}, E3 - 1000 ng.ml^{-1}, WBC – white blood cells count, a,b – means significant differences (P<0.05), one-way ANOVA

DISCUSSION

Current research was aimed at understanding the role of mycotoxin patulin on haematological parameters of porcine blood *in vitro*. Under normal physiological conditions, cells interact each other to synchronize their metabolic activity, gene expression, and other basic cellular processes (Capcarova *et* al., 2013). 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). This can be the reason why patulin affected each blood parameter in this study. Thus cells were not able to maintain the basic cellular processes. Decrease of observed parameters in our study may be caused also by the fact that it is *in vitro* study and patulin was not metabolised in organism.In another *in vitro* study, we observed slight decrease of WBC in porcine blood after treatment with deoxynivalenol (Zbyňovská *et al*., 2013a). Significant decrease in WBC count was observed after exposure of zearalenone on porcine blood *in vitro* (Capcarova *et al.,* 2014). In *in vivo* study we observed insignificant ($P>0.05$) decrease of WBC after exposure of patulin in rabbits blood (Zbyňovská *et al*., 2013b).After exposure of patulin *in vitro* we observed significant decrease of RBC count followed by decrease of HGB and HCT. It may be caused by haemolysis in samples of blood after short term exposure of this mycotoxin. Similar results observed Capcarova et al. (2014) in *in vitro* study after treatment with ZEN in porcine blood. In our study all doses of patulin caused intensive haemolysis. In the study with human erythrocytes (Jilani and Lang, 2013), the percentage of haemolysed erythrocytes increased slightly but significantly following exposure of erythrocytes for 48 h to zearalenone. Lupescu *et al.* (2013) observed similar effect adding mycotoxin patulin to human erythrocytes. Patulin stimulated Ca(2+) entry into erythrocytes, an effect triggering suicidal erythrocyte death or eryptosis. Suicidal erythrocyte death what can lead to haemolysis of blood was observed after ochratoxin A (Jilani *et al*., 2012) and zearalenone exposure (Jilani and Lang, 2013), characterized by cell membrane scrambling and cell shrinkage (Lang *et al*., 2008). Circulating eryptotic erythrocytes are cleared from the blood, so after stimulation of eryptosis the percentage of eryptotic erythrocytes remains low *invivo*. The accelerated loss of eryptotic erythrocytes following *in vivo* stimulation of erythrocytes may lead to anaemia (Lang *et al*., 2008). Mycotoxins like DON also affected RBC *in vitro* in porcine blood. Their count was decreased in all experimental groups (Zbynovska *et al.*, 2013a). In *in vivo* study we observed insignificant decrease ($P>0.05$) of RBC in rabbits blood after two weeks exposure of patulin (Zbyňovská *et al*., 2013b). In different study Raju and Dewegoeda (2000) observed similar effect of aflatoxin, ochratoxin and T2-toxin alone or in combination on HGB. Ewuola and Egbunike (2008) found that after fumonisin B_1 administration in concentration 10 $mg.kg^{-1}$ in diet for growing rabbits haemoglobin concentration decreased, Gbole and Akele (2010) found that concentration of haemoglobin of female rabbits significantly decreased after fumonisin administration. In our *in vivo* study with intramuscular patulin administration to rabbits insignificant increase of PLT was observed (Zbyňovská *et al*., 2013b). On the other hand, in present *in vitro* study significant decrease ($P<0.05$) of PLT in all experimental groups was found against the control. It is possible that in organism patulin activates certain mechanisms which helps cells defend from adverse effect of patulin. In *in vitro* study with ZEN on porcine blood,

Capcarova et al. (2014) found that exposure of this mycotoxin caused significant decrease ($P<0.05$) of PLT. Results from our *in vitro* study with patulin are very similar with results of *in vitro* study with ZEA exposure (Capcarova et al., 2014). Similarly in another *in vitro* study significant decrease ($P<0.05$) of PLT in porcine blood after exposure of DON was measured (Zbynovska et al., 2013a). In general, mycotoxins are able to modulate haematological parameters *in vitro*.

CONCLUSION

In the present study patulin treatment significantly affected haematological parameters. Results of this study provide a foundation for further analysis and researches on mycotoxins impact on living cells and the system of possible protection against its effects as well as evaluation of various dose dependencies on haematological parameters.

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UTVRĐIVANJE NEKIH HEMATOLOŠKIH PARAMETARA U KRVI SVINJA POSLE TRETMANA PATULINOM *IN VITRO*

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Izvod

Patulin, genotoksični mikotoksin, proizvod nekoliko vrsta roda *Aspergillus, Penicillium*, i *Bysochlamys* je mikotoksin koji se naj češće nalazi u jabukama i proizvodima od jabuka. Patulin može bioti neurotoksičan, imunosupresivan, gernotoksičan, teratogen i karcinogen. Cilj ovog rada je da ispita uticaj patulina na neke hematološke parameter (eritrociti - EC, leukociti – LC, trombociti – TC, hematokrit – HTK i haemoglobin – HGB) u krvi svinja *in vitro*. Grupa koja nije primal patulin, služula je kao kontrola. Patulin je izazvao značajno smanjenje broja EC u svim grupama, u odnosu na kontrolu. Patulin je, takođe, izzavao značajno smanjenje broja EC, što je praćeno povećanjem hemolize i padom HGB i HTK, u svim tretmanskim grupama, u poređenju sa kontrolnom grupom. Ustanovljen je i značajan pad broja TC. Dobijeni rezultati čine dobru osnovu za dalja istraživanja i analize u vezi sa uticajm mikotoksina na žive ćelije i sisteme, kao i za iznalaženje mogućih načina zaštite od njihovog uticaja, kao i evaluaciju uticaja različitih doza mikotiksina na hematološke parameter.

Ključne reči: *patulin, krv svinje, hematologija.*

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