Model study to investigate the toxic interaction between glyphosate herbicide and lead acetate on chicken embryos

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Abstract: The aim of this study was to determine the individual and combined toxic effects of Glialka Star herbicide (360 g/l) and lead acetate on the development of chicken embryos. The eggs were injected by 0.1 ml of lead acetate solution (0.01%; 0.1%) and/or by 0.1 ml of Glialka Star (glyphosate, 360 g/l; 2%). The treatments were performed on day 0 of incubation, and the embryos were examined on day 19 by the followings: rate of mortality of embryo, body mass, type of developmental anomalies by macroscopic examination. The body weight was evaluated statistically by the one-way ANOVA with Tukey and Dunnett post-test, the mortality of embryo and the developmental anomalies was analysed by Fisher test. Single treatment of both items and their combination increased the mortality of embryo but the single treatment of herbicide caused significant difference. The combination of Glialka Star and lead acetate significantly reduced the body weight of embryos but no significant difference was observed due to the single administration of the herbicide. Developmental abnormalities were observed sporadically either single or concomitant treatment. Based on the results there is a possibly additive toxic interaction between the lead acetate and Glialka Star that can highly reduce the viability of the embryos or can lead to extinction of wild birds.

Keywords: chicken embryo, lead acetate, toxic interaction, developmental abnormality, glyphosate

Introduction

The chemical plant protecting process is one of the most important polluting activities in the agricultural production. The ecosystem of a given habitat can be contaminated simultaneously by sprayed pesticides and other xenobiotics, e.g. heavy metals due to the agricultural activities during the plant protecting processes. Therefore, the chemical load can be occurred as a complex problem, so the combined toxic effect, i.e. toxic interaction of at least two substances can expected and the components can modify the effect of each other. Recently, the examination of the combination of heavy metals and other chemicals gained significant ground in both avian (Fejes et al., 2001; Kertész, 2001) and mammalian (Institóris et al., 2001; Pecze et al., 2001) toxicology research studies. Furthermore, the interaction effects are examined not only in the field of ecotoxicology, but also in all other areas that deal with health care and chemical safety issues (Oskarsson, 1983; Danielsson et al., 1984; Speijers and Speijers, 2004).

The different agricultural areas offer sources of food, shelter and breeding places to wild birds, therefore the sprayed pesticide and other chemical substances can contaminate not only the adults, but the embryos developing in egg, as well. The eggs of the wild birds may be exposed to different chemicals on the cultivated lands at the same time and their toxic effects may appear in embryo mortality and developmental anomalies.

Teratological tests carried out on avian embryos provide useful data for environmental protection and facilitate the development of environmental-friendly chemical plant protection techniques (Várnagy et al., 1996). The aim of our study was to examine the toxic effect and interaction of lead acetate and a glyphosate containing herbicide (Glialka Star) on chicken embryos after administration of single compounds and simultaneously by injection technique.

Materials and methods

Farm chicken eggs with good fertile potential (Goldavis Ltd., Hungary) were used in the experiment. The eggs based on their size and weight were divided into six homogenous groups (40 eggs in each), and were incubated in Ragus type table incubator (Vienna, Austria) ensuring the required temperature (37–38ºC), the relative humidity (65–70%) and the daily rotation.

The eggs were treated with a final volume of 0.1 ml solution or emulsion of the test items, directly into the air-chamber with a pipette on the first day of incubation. The egg-shell was bored through before the injection, and it was sealed with paraffin after treatment (Clegg, 1964). During the single and simultaneous administration lead acetate (Reanal-Ker Ltd., Budapest) with a concentration of 0.01% or 0.1% and 2% of Glialka Star glyphosate containing herbicide (Monsanto Hungary Ltd., 360 g/l) corresponding to that used in plant protection practice were applied. The control group was treated with avian physiological saline solution (NaCl 0.75%). The details of the experimental design are presented in *Table 1*.

All eggs and embryos were examined and processed on day 19 of incubation. During the processing rate of embryo mortality, body mass of embryos and type of developmental anomalies were registered.

The distribution of body weight of the live embryos was controlled by Comparison-Quantile Plot and was analysed statistically by One-Way ANOVA. Data of groups were compared by Tukey and Dunnett tests. The statistical analysis of the results of embryo mortality and developmental abnormalities were performed by Fisher's exact test (Baráth et al., 1996).

Table 1. Experimental design

Group	No of egg	Treatment (concentration)	
		Lead acetate	Glialka Star
I (control)	40	-	-
II	40	0.01%	-
III	40	0.1%	-
IV	40	-	2%
V	40	0.01%	2%
VI	40	0.1%	2%

Results and discussion

The average body weight of the embryos was 19.26±1.37 g and 18.31±1.71 g in Group II and Group III that was significantly lower as compared to the control group (22.58±1.98 g, p=0.001). Due to glyphosate treatment (2%) the body weight was 21.98±1.92 g that was significantly lower than the control. The simultaneous administration of lead acetate (0.01%) and glyphosate resulted significant decrease (p=0.05) of average body weight (Group V: 20.48±1.28 g) as compared to the control (p=0.05) (*Figure 1*). The combination of 0.1% concentration of lead acetate and 2% glyphosate caused 97.50% mortality of the embryos.The results of the embryo mortality is presented in *Table 2*.

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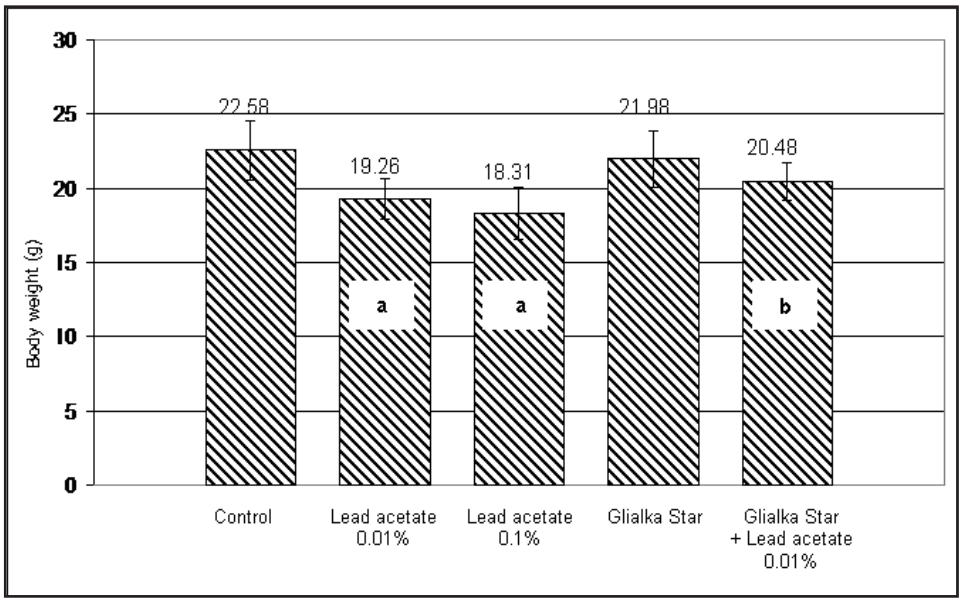
Figure 1. Body weight (g) of the chicken embryos on day 19 of incubation from teratogenicity test on Glialka Star and lead acetate after single and simultaneous administration



a: Significance decrease as compared to the control(p=0.001)
b: Significance decrease as compared to the control(p=0.05)

There were two died embryos in the control group (5%). The single administration of lead acetate increased the mortality up to 20% and 27.50% in Group II and Group III, respectively. The changes were not statistically different as compared to the Group I (control). The application of 2% Glialka Star caused 57.50% of mortality in the treated embryos in Group IV that was significant as compared to the control (p=0.001).

Table 2. Embryonic death and developmental anomalies from teratogenicity test of lead acetate and Glialka Star in chicken embryos after single and combined administration

Group	Treatment	No of embryos showing abnormality/No of live embryos	Death No/ Total eggs	Rate of developmental anomalies (%)	Mortality (%)
I	Control	0/38	2/40	0	5.00
II	Lead acetate 0.01%	1/32	8/40	3.12	20.00
III	Lead acetate 0.1%	2/29	11/40	6.89	27.50
IV	GLIALKA STAR	0/17	23/40[a]	0	57.50
V	GLIALKA STAR + Lead acetate 0.01%	0/32	8/40	0	20.00
VI	GLIALKA STAR + Lead acetate 0.1%	1/1	39/40	100.00	97.50

a: Significant difference as compared to the control (p=0.001)

Due to the simultaneous administration of 0.01% lead acetate and 2% glyphosate (Group V) induced not significant increase of embryo mortality (20%), and simultaneous administration of 0.1% lead acetate and 2% Glialka Star (Group VI) the rate of mortality increased up to 97.50%.

Developmental abnormalities were not recorded in the control group (*Table 2*). The 0.1 and 0.01% lead acetate induced leg deformation and open abdomen (Group II: 1, Group III: 2 embryos) without statistical difference as compared to Group I. Teratogenic malformations were not registered due to the single administration of Gialka Star (Group IV) and its application with 0.01% lead acetate (Group V). However, the higher concentration of lead acetate (0.1%) and 2% glyphosate containing product induced leg deformation, growth retardation and beak malformation in the survivor embryos (Group VI).

The results of the individual teratogenicity studies on lead acetate in chicken are in accordance with results of toxicity studies in other species. Depending on the dose, lead has embryotoxic potential and may cause developmental anomalies (Ferm and Carpenter, 1967; Várnagy and Budai, 1995). Similar results were found in chicken embryos treated with 0.01% lead acetate (lower body weight, higher rate of embryo mortality) but the developmental anomalies were not significant versus the control group (Juhász, 2009).

Glyphosate containing RoundUp herbicide was examined by other researchers in Wistar rats. Dams were treated orally with 500, 750 and 1000 mg/kg glyphosate via drinking water. The results showed 50% mortality rate of dams treated with 1000 mg/kg glyphosate. Skeletal alterations were observed in foetuses of the dams in Groups treated with 500, 750 or 1000 mg/kg. Based on these data can be concluded that the glyphosate containing RoundUp is toxic to dams and induces developmental retardation of the foetal skeleton (Dallegrave et al., 2003).

Generally, the simultaneous application of heavy metals and pesticides may cause significant increase of their toxic effect in comparison with the individual toxicity of the applied components. It was particularly distinct due to the combined administration of Glialka Star herbicide.

According to the published literature the toxicity of many pesticide combinations is at least additive. In some cases pesticide mixtures, if they particularly contain insecticide component, have been shown to be synergistic effects, with reported increase in toxicity up to 100-fold. However, these effects are species, time and dose dependent, therefore difficult to predict it routinely (Thompson, 1996).

Conclusions

The single treatment of lead acetate with 0.1 and 0.01% concentration induced embryotoxic effect in chicken embryo which manifested in significant decrease of body weight and elevated rate of embryo mortality.

The glyphosate containing Glialka Star plant protection product with herbicidal action was also embryotoxic on chicken embryos and resulted not significant reduce of body weight and statistically significant increase of mortality. Due to the simultaneous application of lead acetate and Glialka Star the embryo mortality was statistically higher than the individual effect.

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Developmental abnormalities were sporadically observed due to the single and concomitant administration (leg and beak deformation, growth retardation, open abdomen).

Based on the results, there are presumably addition-type toxic interaction between lead acetate and Glialka Star herbicide, that highly reduce the viability of the embryos.

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