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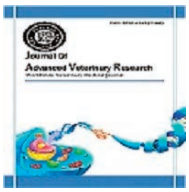

The Protective Effect of Silymarin against Ochratoxin A Induced Histopathological and Biochemical Changes in Chicks

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ABSTRACT

Protective effects of herbal feed additive Silymarin against the deleterious toxic effects of ochratoxin A (OTA) on internal organs and blood biochemistry was seen. The observed histopathological and biochemical changes were well expressed in OTA-exposed chicks without Silymarin supplementation, followed by chicks treated supplementary with Silymarin in addition to OTA treatment, whereas no pathological changes were seen in the control chicks or the chicks treated with Silymarin only. The observed increase in the serum levels of uric acid, glucose and the enzyme activities of AST and ALT in OTA treated chicks and the found decrease of the same biochemical indices in the chicks protected by Silymarin in addition to OTA treatment also supported the protective effects of this herbal additive on the kidneys and liver. The same herbal substance Silymarin could be used as a practical approach for safely utilizing of OTA-contaminated feed.

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Introduction

Many plants and their extracts have been known as good protectors against many diseases or intoxications in animals or humans since several thousands of years. Even these days, a lot of people use some target plants or their extracts for medical purpose to cure their animals or relatives (Tene *et al.,* 2007; Kaur *et al.,* 2012, 2013). In some countries such as India, more than 7000 plant species are currently used for medical purpose and treatment of various ailments (Mukherjee and Wahile, 2006). The interest towards traditional medicine, e.g. medicinal plants and plant extracts is currently increased, because the same are considered as a natural and healthy alternative to synthetic medical drugs. Therefore, any new information in this area of research, e.g. animal studies, would be very helpful to clarify various protective and/or pharmacological effects of various medicinal plants or their extracts and their possible application in the treatment of some ailments in animals/humans.

The herb *Silybum marianum* or its extract Silymarin used in this experimental study was found to have strong anti-oxidative properties and various protective effects on internal organs and biochemical functions, e.g. anti-inflamatory, immunomodulatory, membrane-stabilizing, nephroprotective, hepatoprotective and liver regenerative effects (Salmi and Sarna, 1982; Flora *et al.,* 1998; Wilasrusumee *et al.,* 2002; Katiyar, 2005; Shalan *et al.,* 2005; Pradhan and Girish, 2006; Saller *et al.,* 2007; Pradeep *et al.,* 2007a,b; Morishima *et al.,* 2010; Shaker *et al.,* 2010; Abenavoli *et al.,* 2011; Karimi *et al.,* 2011; Muhammad *et al.,* 2012; Arora *et al.,* 2014; Bahmani *et al.,* 2015; Stoev *et al.,* 2019). Shaarawy *et al.* (2009) reported significant decreases in the serum activities of aspartate-aminotransferase (AST), alanine-aminotransferase (ALT) and alkaline phosphatase (ALP) in the provoked by N-nitrosodiethylamine (NDEA) hepatotoxic damages in rats, when the same were protected by Silymarin (Shaarawy *et al.,* 2009). In another experiment, Muhammad *et al.* (2012) were also found a strong hepatoprotective effect of *Silybum marianum* by measuring the serum activities of the same enzymes in the aflatoxin intoxicated chicks. Stoev *et al.* (2019) also reported a strong hepatoprotective, nephroprotective and immunomodulating effects of *Silybum marianum* powder against ochratoxin A (OTA) intoxication in broiler chicks. A hepatorotective effect of *Silybum marianum* was also reported by Pradeep *et al.*

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(2007b) in hepatotoxic damages of rats provoked by diethylnitrosamine. The aim of this experiment was to investigate a possible practical manner for safe utilization of OTA-contaminated fodder for chicks in order to prevent its rejection and subsequent farm losses.

Materials and methods

Experimental design, birds, housing and diets

The study was conducted in the Poultry unit, Faculty of Agriculture, Trakia University, Stara Zagora, Bulgaria. The experimental poultry house and equipment's were cleaned and disinfected before starting the experiment. The completely randomised experimental design included 144 one day-old Ross 308 male broiler chicks that were obtained from a local commercial hatchery. From day 1 up to day 10 all broiler chicks were fed on the complete standard Starter ration (produced by Melchran EOOD, Stara Zagora, Bulgaria) suitable for their age without any OTA or Silymarin in it. From day 11 up to day 42 (for 32 days) the chicks were wing-banded, and assigned randomly in fourth groups of 36 birds each, with six subgroups (replicates) of 6 birds each. They were housed in separate pens into wire floor experimental cages that were placed in an environmentally controlled experimental poultry house with continuous infra-red lighting at a temperature suitable for their age. Pens were equipped with plastic feeders and drinkers. All broilers were kept under the same managerial, hygienic and environmental conditions. The rearing environment complied with the Ross breeder's recommendations (Aviagen, 2009). Water and feed were provided ad libitum throughout the experiment. The trial was terminated when the broiler chicks were 42 day of age. A three-phase feeding program was used with commercial complete standard, no medicated type corn-wheat-soybean based diets in mash form, produced by Melchran Ltd., Stara Zagora, Bulgaria: Starter (from 11th to 21st day); Grower (from 22nd to 35th day) and Finisher (from 36st to 42nd day) (Table 1). The experimental basal diet (BD) was formulated to meet broiler chick's nutritional requirements, according to the National Research Council (NRC, 1984) and Aviagen (2009) nutrient recommendations. The BD was the same for all groups.

The commercially used standard feeds did not contain detectable amounts of any known mycotoxins, e.g aflatoxins, deoxynivalenol, fumonisin B1, ochratoxin A (OTA), T-2 toxin and zearalenone. The Silymarin and OTA were periodically homogenized with the chick' ration (each week) in other to give the required levels. After supplementation of Silymarin and/or OTA, the basal diet was mixed thoroughly as a single batch to reduce diet variability of different treatments. The complete feed per experimental treatment was stored in sacks that had been appropriately and clearly labelled. During the study a total of four dietary treatments were applied:

Group 1: Basal diet (BD) with no supplementation (Negative control, NC);
Group 2: BD with 1.0 % Silymarin (Positive control, PC);
Group 3: BD with 3.0 mg/kg OTA;
Group 4: BD with 3.0 mg/kg OTA plus 1.0 % Silymarin.

Table 1. Composition and nutrient content of basal diets

Dietary ingredient. % (as-is)	Diets		
	Starter	Grower	Finisher
Corn	30	33	33.34
Wheat	29	30.4	30
Soybean meal (47% CP*)	30	23	19
Sunflower meal (37% CP)	3	5	8
Sunflower oil	3.5	4.7	6
L-Lysine HCL (56%)	0.3	0.28	0.35
DL-Methionine (free base)	0.25	0.18	0.17
L-Threonine (free base)	0.08	0.08	0.08
Phytase®	0.01	0.01	0.01
Calcium carbonate	0.61	0.5	0.28
Dicalcium phosphate	2.2	1.85	1.8
Salt ($NaCI_2$)	0.25	0.2	0.15
Sodium bicarbonate	0.3	0.3	0.32
Vitamin-Mineral Premix**	0.5	0.5	0.5
Calculated nutrient composition			
Metabolizable energy. Kcal/kg	3003	3121	3195
Crude Protein (%)	22	19.7	19
Calcium (%)	1	0.9	0.76
Available phosphorus (%)	0.46	0.4	0.39
Methionine (%)	0.58	0.49	0.48
Methionine + Cysteine (%)	0.94	0.82	0.8
Lysine (%)	1.32	1.14	1.12
Threonine (%)	0.86	0.77	0.74
Tryptophan (%)	0.25	0.22	0.21
Sodium (%)	0.2	0.18	0.16

* CP - Crude Protein

**The vitamin and mineral premix contained vitamins and trace elements to meet the requirements specified by the NRC (1994). The premix provided (units/kg diet): Retinol, 3,600 μg; Cholecalciferol, 125 μg; α-tocopherol, 34 mg; Menadione, 3 mg; Thiamine, 2 mg; Riboflavin, 7 mg; Pyridoxine, 5 mg; Cobalamin, 15μg; Nicotinic acid, 50 mg; Pantothenic acid, 15 mg; Folic acid, 1 mg; Biotin, 200 μg; Iron, 80 mg; Copper, 10 mg; Manganese, 100 mg; Cobalt, 0.5 mg; Zinc, 80 mg; Iodine, 1 mg; Selenium, 0.2 mg; Molybdenum, 0.5 mg.

Silymarin

Silymarin with molecular weight 482.44 g/mol and purity (UV 60%) used in this study was produced by Samwon International LTD., Nanjing, China. The dose of Silymarin was designed having in mind several factors such as body weight and the dose conversion factors available in the literature (WHO norms and other standard toxicological manuals) for various animals and chicks, and therefore, the same might be the nearest effective dose for alleviating the effects of toxicants, incl. mycotoxins.

Ochratoxin A (OTA) production

Aspergillus ochraceus (isolate D2306, as used by Tapia and Seawright, 1984, and Stoev *et al*., 2000b, 2019) was grown on sterilised shredded wheat (40 g) in 500 ml conical flasks, moistened by a 40% (v/w) addition of sterile water and incubated on a rotary shaker at 27C for 2 weeks. The brown granular product, which bore no obvious sign of fungal growth or sporulation, was sterilised at 80°C for 1 hour (yield 2 kg) and stored at -20°C. A sample was analysed for ochratoxins; batches typically contained OTA (about 2 mg/g) and relatively small component of the biologically-inactive deschloro-analogue ochratoxin B. No other mycotoxins were produced in this solid substrate fermentation process and the necessary dilution by approximately 10^3 when homogenised into chick ration made only a minimal addition of other components of the moulded shredded wheat substrate.

Measurements

The chicks were weighed at day 1 (b.w. was ranged between 40 and 50 g) and before the beginning of the experiment at day 10 (b.w. was ranged between 180 and 240 g) and the same were randomly assigned to different experimental or control groups that time. Blood/serum for biochemical studies and tissues samples from various internal organs for pathomorphological investigations were collected from six birds per group (two birds per replicate) at the end of the experiment at day 42 (32 days after the onset of the experiment). The blood samples were bled from wing vein (v. *subcutanea ulnaris*) and were left to clot for 1 h. Then blood sera were centrifuged at 2000g for 10 min, collected in different sterile tubes, and processed immediately for the respective biochemical investigations.

Histological examination

Materials for histological examination were taken from liver, heart, kidneys, lung, thymus, bursa Fabricii, spleen, intestine, cerebellum, brain and medulla and subsequently fixed in 10% neutral buffered formalin. The fixed tissues were processed for paraffin embedding, sectioned at 6 µm and stained with haematoxylin-eosin.

Clinical biochemistry

Blood and/or serum samples were examined for various haematological and biochemical parameters within 1-2 h of collection, immediately after separation of the serum. The blood sugar was measured by test of Boehringer Mannheim (Mannheim, Germany); the serum uric acid by EnzUric-FT-test (Labordiagnostica, Gopecke, Germany); the serum enzyme activity of AST and ALT was measured by Cormay test (Smolenskiego, Warsaw, Poland).

Ethics approval and consent to participate

The Animal Care Ethic Committees approved the study protocol and ethical clearance (No 111 from 20/11/2014) was issued for the study by the Bulgarian Agency for Food Safety. The experiments were conducted within standard ethical norms and no birds were subjected to undue stress. The chicks were housed, maintained and slaughtered in accordance with the relevant international rules and recommendations.

Statistical methods

The data were statistically processed using one-way ANOVA with Tukey's test as a post-hoc test in order to estimate significant differences between the mean values of various parameters in different groups of chicks. Results were presented as mean ± SEM. Differences among groups were considered significant at $P<0.05$.

Results

Clinical observation

Only scarce clinical features, e.g. weakness and growth depression were seen in chicks exposed to 3 ppm OTA in the diet without supplementation of Silymarin. These clinical features were seen mainly during the last days of the experiment.

Biochemical and haematological findings

The serum levels of glucose were increased in response to OTA-treatment, but Silymarin was found to protect against this increase in the chicks supplementary treated with this additive, in addition to OTA treatment. A significant increase in the serum levels of uric acid was seen in the chicks exposed to OTA when compared to the control chicks, but such increase was not observed in the group supplemented by Silymarin and simultaneously treated with OTA. The serum enzymes activities of AST and ALT were only increased in chicks exposed to OTA without Silymarin supplementation in their diet and a significant protective effect of Silymarin was seen against OTA toxicity (Table 2). There were no significant differences in the serum levels of all investigated biochemical indices between the control chicks and the chicks treated with Silymarin only.

Gross pathology

Macroscopical examination at slaughter time revealed the presence of small haemorrhages on the epicardium and duodenal mucosa in the chicks of OTA treated group without Silymarin supplementation. A slight catarrhal enteritis and

Table 2. Mean serum values of glucose, uric acid, AST and ALT in groups of chicks (n=6) given Silymarin and/or OTA

Group	Glucose (mmol/L)	Uric acid (µmol/L)	AST (U/L)	ALT (U/L)
Silymarin	11.7±0.23^a	109.0±8.8^a	217.0±6.7^a	5.8±0.79^a
3 ppm OTA	14.1±0.33^b	291.0±22.5^b	345.0±14.7^b	15.8±0.87^b
3 ppm OTA + Silymarin	12.7±0.19^a	114.0±9.5^a	235.0±9.9^a	5.2±1.13^a
Control	12.1±0.32^a	107.0±4.0^a	222.0±10.3^a	6.1±1.0^a

Different superscripts mean significant difference ($p<0.05$)

Table 3. Comparative pathomorphological changes in some internal organs of chicks

Pathomorphological lesions in kidneys	OTA	OTA+Sil	Silymarin	Control
Degenerative changes in tubular epithelium	+++	+	-	-
Congestion of peritubular capillaries or haemorrhages	++	+	-	-
Focal mononuclear infiltration in interstitium	+	-	-	-
Activation of capillary endothelium	+	-	-	-
Hypercellularity of some glomerules	+	-	-	-
Pathomorphological lesions in liver				
Granular or vaccuolar degeneration in hepatocytes	+++	+	-	-
Activation of Kupffer's cells or capillary endothelium	++	-	-	-
Hyperaemia or perivascular mononuclear infiltration	++	-	-	-
Pathomorphological lesions in myocardium				
Irregular staining or lytic changes in myofibrils	+	-	-	-
Granular degeneration	+	-	-	-
Pathomorphological lesions in lung				
Peribronchial/perivascular mononuclear infiltration	+	-	-	-
Pathomorphological lesions in the brain				
Lytic changes in neurons and glia cells	++	+	-	-
Pericapillary and/or pericellular oedema	++	+	-	-
Pathomorphological lesions in cerebellum				
Degenerative changes in Purkinje's cells	+	-	-	-
Oedema and lytic changes in white matter	+	-	-	-
Perivascular or pericellular edema	+	-	-	-
Pathomorphological lesions in medulla				
Lysis of neurons in lumbosacral region	+	-	-	-
Pathomorphological lesions in bursa of Fabricius				
Degeneration or cell depletion in lymph follicles	++	+	-	-
Interfollicular oedema	++	+	-	-
Pathomorphological lesions in thymus				
Degenerative changes or depletion of cells in cortex	++	+	-	-
Haemorrhages and telangiectasis	+	-	-	-
degenerative changes or cells depletion in medulla	+	-	-	-
Pathomorphological lesions in spleen				
Degenerative changes in germinal centres	+	-	-	-
Reduction in size or number of lymph follicles	+	-	-	-
Pathomorphological lesions in small intesines				
Degenerative changes of surface/glandular epithelium	++	+	-	-
Depletion of cells in lymph follicles of mucosa	++	+	-	-
Mononuclear infiltration in lamina propria	+	-	-	-

+: Slight damage or damage seen occasionally in few chicks
++: Slight damage in all chicks or moderate damage in less than half of the chicks
+++: Moderate damage in all chicks and/or strong damage in less than half of the chicks
OTA: Ochtatoxin A; Sil: Silymarin

hyperaemia of mucosal surface was also observed in the same chicks. Kidneys and liver in a few chicks from the group exposed to OTA only showed a slight congestion and enlargement. The gall bladder of a few chicks of the same group was distended by bile.

Histopathology

Pathomorphological investigations of internal organs showed that the strongest degenerative changes were seen in chicks treated with OTA alone, followed by those treated with OTA and Silymarin, whereas no degenerative changes were found in control chicks or in chicks given only Silymarin (Table 3).

In the kidneys of chicks exposed to OTA, a slight to moderate congestion of peritubular capillaries was seen (Fig. 1A). Well expressed degenerative changes, e.g. granular or hydropic degeneration, cloudy swelling and desquamation of epithelial cells was seen in the convoluted tubules of renal cortex of chicks treated with OTA only (Fig. 1B). Rarely, a scarce nodular infiltration of mononuclear cells was found in the interstitium of kidneys in the chicks exposed to OTA without Silymarin supplementation. In the chicks exposed simultaneously to OTA and Silymarin, only focal granular degeneration in the epithelial cells of convoluted tubules and slight congestion of peritubular capillaries was found (Fig. 1C). Slight activation of capillary endothelium and hypercellularity of some glomerules were rarely seen in the kidneys of chicks exposed to OTA without Silymarin supplementation.

Pathomorphological investigations of liver revealed a cloudy swelling and granular or vacuolar degeneration of hepatocytes in the chicks of OTA exposed groups, but the same were less pronounced in the chicks with Silymarin supplementation (Fig. 1D, Fig. 1E). A slight activation of capillary endothelium and Kupffer's cells and slight hyperaemia of sinusoidal capillaries were also found in the chicks treated with OTA without Silymarin supplementation (Fig. 1F).

In the heart, some lytic changes and irregular staining due to the increased eosinophilia of part of the myofibrils were found in the OTA exposed chicks (Fig. 2A). A slight granular degeneration was rarely seen in the myofibrils of the same chicks.

In the lung, there was only a slight perivascular or peribronchial mononuclear cell infiltration in the chicks of exposed

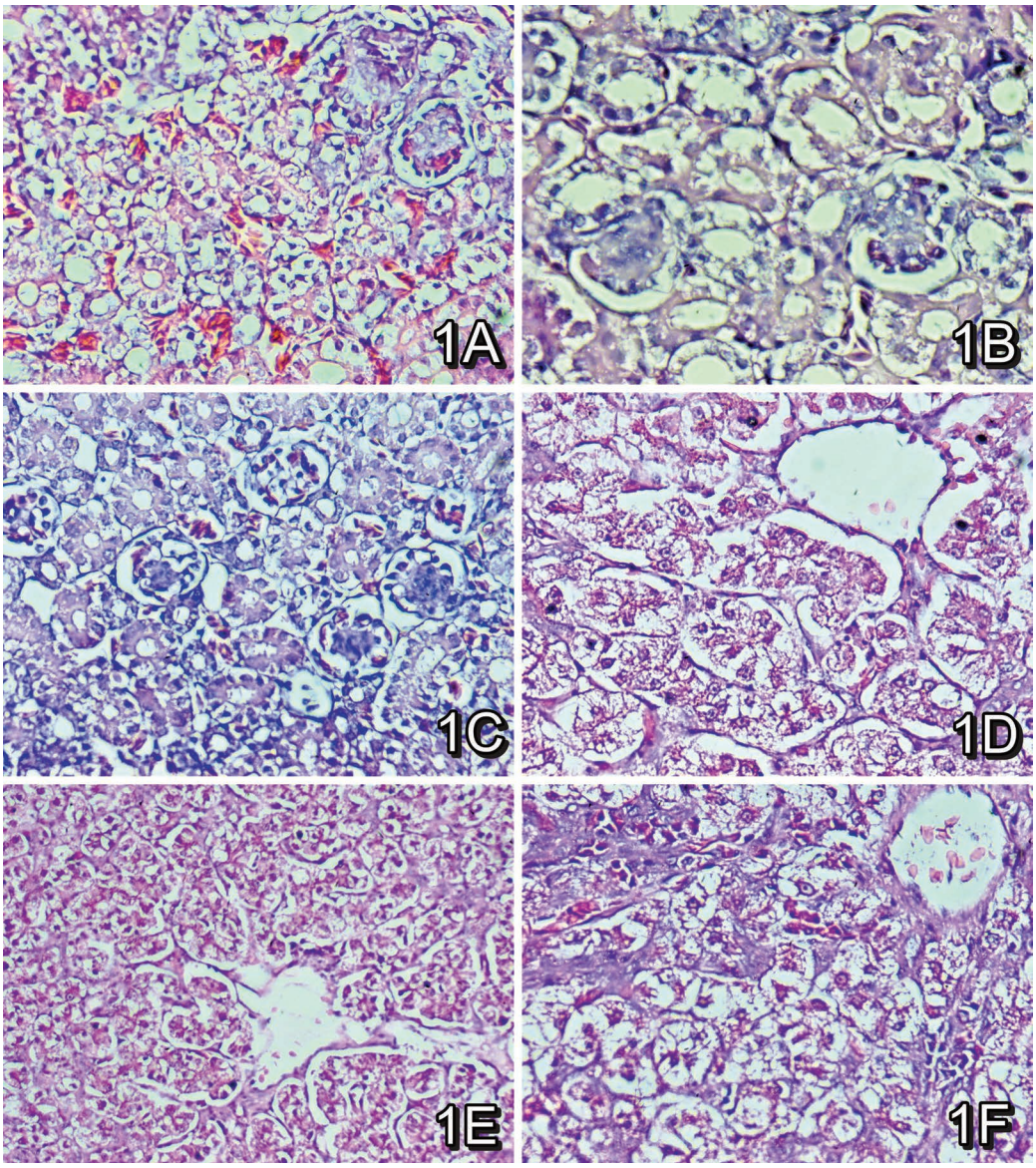

Fig. 1. 1A). Hyperaemia of peritubular capillaries in the kidney of chick treated with 3 ppm OTA for 35 days. HE. 260; 1B). Degenerative changes and desquamation in tubular epithelial cells in kidney of chick treated with 3 ppm OTA for 35 days. HE. 300; 1C). Slight degenerative changes and desquamation in tubular epithelial cells in kidney and slight hyperaemia of peritubular capillaries of chick treated with 3 ppm OTA and Silymarin for 35 days. HE. 260; 1D). A moderate granular degeneration in hepatocytes in the liver of chick treated with 3 ppm OTA for 35 days. HE. 260; 1E). A slight granular degeneration in hepatocytes in the liver of chick treated with 3 ppm OTA and Silymarin for 35 days. HE. 200; 1F). A slight hyperaemia of sinusoidal capillaries, slight activation of capillary endothelium or Kupffer's cells and granular degeneration in hepatocytes in the liver of chick treated with 3 ppm OTA for 35 days. HE. 260.

to OTA only.

In the brain, a slight pericellular or pericapillary oedema and slight lytic changes were seen in some of the neurons and glia cells in the chicks exposed to OTA being less pronounced in the chicks additionally supplemented with Silymarin.

In the cerebellum, slight degenerative changes were only found in the region of the Purkinje's cells mainly in the chicks of OTA treated group without Silymarin supplementation (Fig. 2B) in addition to a slight perivascular and/or pericellular oedema. In the white matter of the cerebellum, slight oedematous changes were seen in some of the chicks exposed only to OTA (Fig. 2C).

In the medulla, only scarce changes, e.g. lysis in some neurons in the lumbosacral region were seen in the chicks of OTA exposed group without Silymarin supplementation.

In the lymphoid organs, the main degenerative damages were found in chicks exposed to OTA only, followed by the chicks treated with OTA and Silymarin. In the bursa of Fabricius, there was a well visible depletion of lymphoid cells or slight degenerative changes in the lymph follicles, in addition to inter-follicular oedema in the chicks exposed to OTA only (Fig. 2D). These changes were slightly pronounced in the chicks exposed simultaneously to OTA and Silymarin (Fig. 2E).

In the thymus, degenerative changes and a depletion of lymphoid cells in the cortex and focal haemorrhages were seen mainly in the chicks exposed to OTA only (Fig. 2F). The range between cortex and medulla was not well defined due to the depletion of lymphoid cells in the cortex and often the cortex was very thin in the chicks treated with OTA only (Fig. 2F). In the chicks exposed additionally to Silymarin along with OTA exposure only local depletion of lymphoid cells in the cortical area was seen. Slight degenerative changes were found in the medulla only in the chicks exposed to OTA only.

In the spleen, a depletion of cells and slight degenerative changes were found in the white pulp of germinal centres only in the chicks exposed to OTA without Silymarin supplementation. The lymph follicles were slightly reduced in number and size in the same chicks.

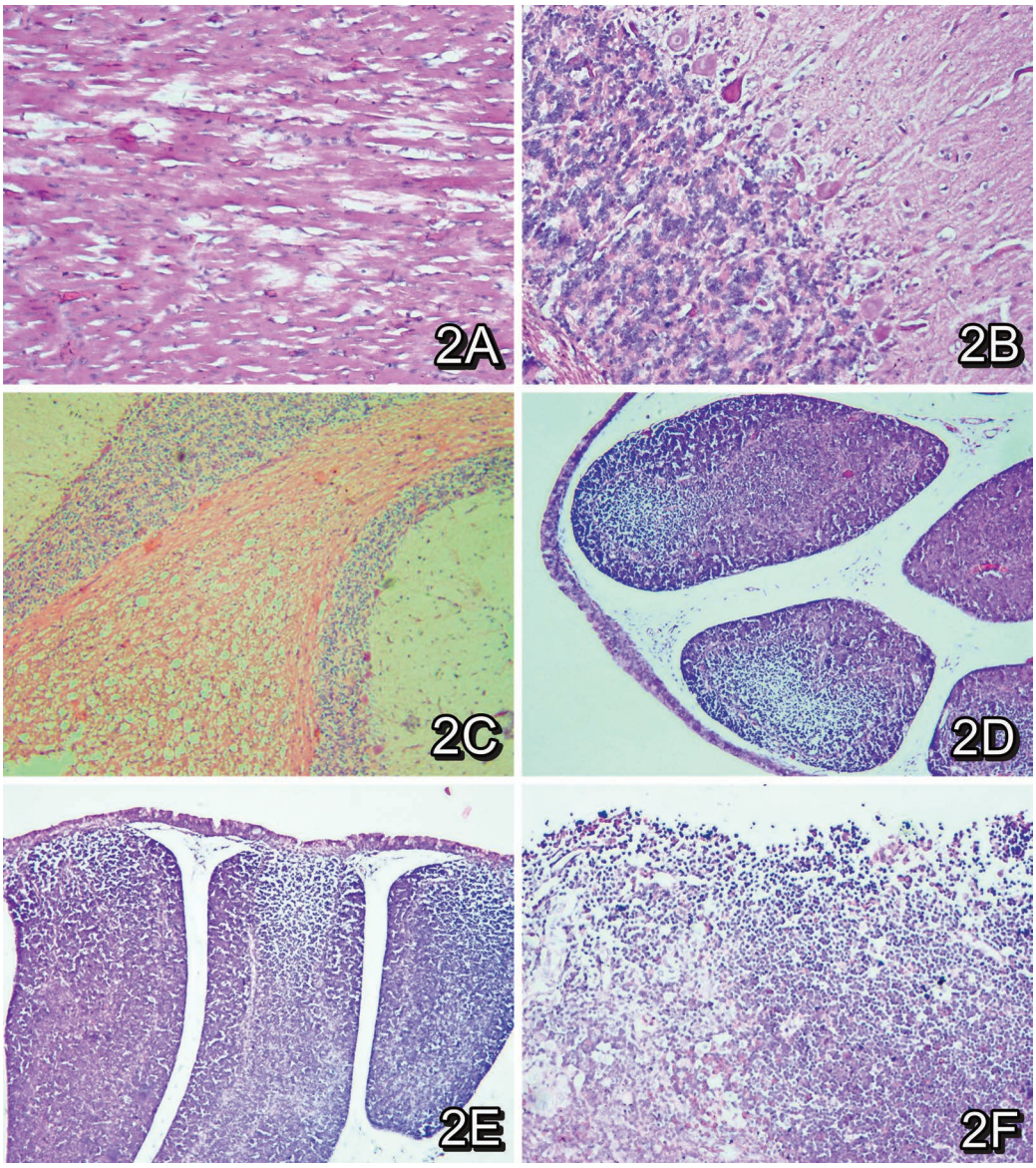

Fig. 2. 2A). Slight lytic changes and irregular staining of some myofibrils as a result of the increased eosinophilia in the myocardium of hearth in chick treated with 3 ppm OTA for 35 days. HE. 200; 2B). Slight degenerative changes in the region of the Purkinje's cells, e.g. lysis or pyknosis of tigroid substance in the cerebellum of chick treated with 3 ppm OTA for 35 days. HE. 260; 2C). Slight oedematous changes in the white matter in the cerebellum of chick treated with 3 ppm OTA for 35 days. HE. 200; 2D). Moderate degenerative changes and/or depletion of cells in the lymph follicles and interfollicular oedema in bursa of Fabricius in chick treated with 3 ppm OTA for 35 days. HE. 200; 2E). Slight degenerative changes and/or depletion of cells in the lymph follicles and slight interfollicular oedema in bursa of Fabricius in chick treated with 3 ppm OTA and Silymarin for 35 days. HE. 200; 2F). Slight degenerative changes and depletion of lymphoid cells in the cortical zone of thymus and not well defined range between medulla and cortex in chick treated with 3 ppm OTA for 35 days. HE. 260.

In the intestinal mucosa, slight degenerative changes and depletion of cells were found in the lymph follicles mainly in the chicks of OTA exposed groups and the same changes were better expressed in the group exposed to OTA without Silymarin supplementation. A slight mononuclear cell infiltration was seen in some places of lamina propria of chicks exposed to OTA only. Slight focal degenerative changes and loss of surface epithelium and slight degenerative changes in the glandular epithelium were seen mainly in the intestinal mucosa of chicks treated with OTA, being slighter expressed in the chicks exposed additionally to Silymarin.

Pathomorphological changes were not seen in internal organs in any of the chicks within the control and/or Silymarin treated groups.

Discussion

The analysis of the results reveals that the intensity of the pathomorphological and biochemical changes were most well expressed in OTA-treated chicks without Silymarin exposure, whereas slight or no changes were seen in the chicks treated with Silymarin in addition to OTA exposure. Strong hepatoprotective and nephroprotective effects were seen in OTA-exposed chicks treated additionally with Silymarin as can be seen from the biochemical and pathomorphological changes. The absence of significant increases in the serum level of uric acid and the serum enzyme activities of AST and ALT in the chicks protected with Silymarin against the toxic effect of OTA clearly suggest the strong protective effects of this herbal extract on the liver and kidneys. The absence of degenerative changes or depletion of cells in the immunocompetent organs of chicks suggests about possible immune stimulation and/or immune protection properties of Silymarin. Therefore, the same herbal extract of *Silybum marianum* could be possibly used as a practical approach for safely utilizing OTA-contaminated feed preventing the rejection of it.

A protective effect of *Silybum marianum* or its extracts was reported against NDEA-induced hepatotoxicity in rats

(Shaarawy *et al*., 2009) as well as against aflatoxin-induced liver damages in chicks (Tedesco *et al*., 2004; Kalorey *et al*., 2005; Muhammad *et al*., 2012) as seen from the decrease in serum enzyme levels of AST, ALT and ALP, which was supported in the present findings. The same enzymes have been reported as sensitive biomarkers for proving hepatic damages in chicks or animals.

A strong protective effect of Silymarin was also seen against cisplatin-induced toxicity in kidneys and the subsequent increase in the lipid peroxidation in rats (Karimi *et al*., 2005) or against kidneys damages in dogs induced by gentamicin exposure (Varzi *et al*., 2007) or against diabetic nephropathy induced by alloxan (Soto *et al*., 2010) and this protective effect on kidneys was confirmed in the present experiment with chicks exposed to OTA as can be seen from the significant decrease in the serum uric acid level and the slight degenerative damages in the renal tubular epithelium in Silymarin protected chicks.

The possible mechanism of protective effect of *Silybum marianum* and its standardized seed extract Silymarin could be possibly due to the decrease in lipid peroxidation and the increase in endogenous antioxidants supporting the integrity of the plasma membrane, thereby suppressing the leakage of some target enzymes destroying the cells (Pradeep *et al*., 2007a; Kiruthiga *et al*., 2007, 2010; Upadhyay *et al*., 2010; Karimi *et al*., 2011). It is well known that Silymarin is composed of 4 flavonoids: silybin, isosilybin, silydianin and silychristin (Pradhan and Girish, 2006). Among them, silybin is considered to have very strong biological activities, e.g. strong hepatoprotective and nephroprotective activities against various exo- or endo-toxicants (Salmi and Sarna, 1982; Shalan *et al*., 2005; Pradhan and Girish., 2006; Ludovico *et al*., 2010; Shaker *et al*., 2010). Some experiments suggest that *Silybum marianum* could be used as a feed additive, which could protect chicks against deleterious effects of mycotoxins, e.g. aflatoxins, fumonisins or ochratoxin A (Tedesco *et al.,* 2004; Kalorey *et al.,* 2005; El-Adawi *et al.,* 2011; Muhammad *et al.,* 2012).

This study suggests that Silymarin could be successfully used "in addition to" mycotoxin binders for ameliorating the adverse effects of mycotoxin contaminated feed in commercial chick farms.

The found increase in serum levels of uric acid suggests that the kidney function is strongly damaged as a result of OTA exposure. The enhanced lipid peroxidation, which is induced by OTA (Rahimtula *et al*., 1988; Stoev *et al*., 2000b, 2002) could lead to structural damages in the cellular membranes, which may subsequently induce an influx of cellular calcium and cell necrosis (Orrenius and Bellomo, 1986).

The swelling and degenerative changes of the epithelial cells of kidneys and liver could be due to the way of excretion of OTA via the kidneys or liver, e.g. enterohepatic recirculation of OTA (Fuchs *et al*., 1988; Roth *et al*., 1988), realizing the known toxic effect of OTA on the same organs (Dwivedi and Burns, 1984; Stoev *et al*., 2000a, 2002, 2004). The oedematous changes found in the cerebellum and brain, e.g. haemorrhages on various internal organs are possibly due to vascular damages provoked by OTA-toxicity (Stoev *et al*., 2000a, 2002, 2004). A disturbance in blood clotting due to a decrease in fibrinogen blood level and an increase in the prothrombin time, seen in ochratoxin A treated animal (Prior and Sisodia, 1978; Doerr *et al*., 1981), may also contribute to the same haemorrhages.

The current experiment clearly shows that Silymarin, given as feed additive to the feeds, could be used in the real practice for safely utilizing of OTA-contaminated feed avoiding the known deleterious effects, e.g. biochemical and histopathological changes provoked by mycotoxin exposure (Stoev, 2013, 2015, 2017), and the respective condemnation of such feed.

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Conflict of interest

The authors declare no competing financial interest.

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