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Effects of Silymarin on blood parameters of broilers in an experimental chronic mycotoxicosis

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Abbreviation Key: Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT), Alkaline Phosphatase (ALP), aflatoxin B1 (AFB1),

Abstract:

Silymarin, the principal constituent of *Silybum marianum*, is one of the most commonly used herbal therapies as a natural antioxidant, hepatoprotector and antihepatotoxic agent, in counteracting the toxic effects of mycotoxins. To determine the efficacy of Silymarin, the levels of hematological and serum biochemical parameters of 70 Ross broilers from 35 to 49 days of age in an experimental chronic mycotoxicosis and in the treatment with Silymarin were evaluated, in seven dietary treatment groups (*Aspergillus flavus*, *Aspergillus fumigatus*, mixed, treatments with Silymarin and control). After culture for heavy sporulation, harvested spores were suspended in sterile normal saline and poured on fresh mash feed, incubated and incorporated as 10% of total feed. Hematological and biochemical values including Albumin, total protein, glucose, creatinine, uric acid, ALT, ALP, AST, Hb, PCV, RBC, total and differential WBC counts were recorded every 7 days. The analysis of results shows that Silymarin significantly helps to keep the levels of hematological and serum biochemical parameters in normal range. These findings show that Silymarin can be used effectively to reduce the toxic and suppressive effects of mycotoxicosis.

Keywords: *Aspergillus flavus*, *Aspergillus fumigatus*, broiler, ross, silymarin.

Introduction

Few fungal species are pathogens in poultry and *Aspergillus* spp. are among the 3 most common mycotoxigenic genera (Kunkle, 2003). Mycotoxins and in particular, aflatoxins, have been reported to be hepatotoxic, mutagenic, immunosuppressive, and carcinogenic. The methods used to prevent, reduce, or remediate the toxicity of *Aspergillus* are in great demand. Various mycotoxin detoxification procedures including ammoniation and the use of adsorbent compounds such as hydrated sodium calcium aluminosilicate (HSCAS) adsorbent (Harvey, 1993) and esterified glucomannan (Aravind, 2003) have been studied. Numerous herbal products have documented action in supporting immune system, reducing inflammatory response

through their antioxidant properties (Mills and Bone, 2000; Cutter, 2000). Among the common medicinal plants, *Silybum marianum* is one of the most commonly used herbal therapies (Van Erp *et al*., 2005; Verma and Thuluvath, 2007) and Silymarin, the standardized bioactive extract of *S. marianum*, shows some therapeutic potential in the treatment of alcoholic cirrhosis (Saller *et al*., 2001; Wellington and Jarvis, 2001). Moreover, Silymarin contains a mixture of flavonolignans including silibinin, isosilibinin, silidianin and silichristin (Skottova *et al*., 2003; He *et al*., 2004), and some of the flavonolignans such as isosilibinin B might possess improved potency in prostate cancer prevention and treatment (Davis-Searles *et al*., 2005). The crud form of milk thistle extract, Silymarin, and the major pure pharmacologically active flavonoid, silibinin, have been shown to be *in vivo* immune-response modifiers (Varghese *et al*., 2005).

Silymarin has the ability to preserve the phagocytic function of avian macrophages (Grizzle *et al*., 2003) and may alter indices of liver functions including alanine transaminase (ALT) and aspartate transaminase (AST) values in the serum (Wellington and Jarvis, 2001; Bean, 2002). However, results from previous reports support further studies on the use of Silymarin as an immunosupportive agent in avian production (Grizzle *et al*., 2003) and its great potential as a feed additive for supportive therapy in birds. In poultry, Silymarin has been shown to be effective against the toxic effects of aflatoxin B_1, preventing negative effects on the performance of broilers. Hematological and biochemical parameters of chickens could be useful in diagnosing the various pathological and metabolic disorders (Islam *et al*., 2004). Therefore, this study focused on the effects of silibinin, a major active component of *S. marianum* (He et al., 2004; Varghese et al., 2005; Pradhan and Girish, 2006), on hematological and serum biochemical parameters in Ross broiler chickens fed with diets contaminated with *A. flavus* and *A. fumigatus* alone and in combination.

Materials and Methods

Chickens: Seventy one-day-old Ross strain broiler chicks were raised on pen-floor, fed *ad libitum* with diet fully fit with Ross broiler strain catalogue. On day 35, the chickens were randomly divided into seven groups (A, B, C, D, E, F and G) and each group was raised in a separated pen (10 chickens/pen).

Fungi: Spores of *A. flavus* and *A. fumigatus* were inoculated on Sabouraud's Dextrose Agar (Oxoid, UK) plates and incubated aerobically at 32°C for periods enough for heavy sporulation. Spores of the fungi were harvested separately and suspended in 10 ml normal saline solution to a concentration, which made OD equal to No.3 McFarland solution in spectrophotometry. These suspensions separately (10 ml) or in combination (5 ml + 5 ml) were poured onto 500 g fresh mash feed and incubated aerobically in a 32°C shaker-incubator for 24 h, then added as 10% of the total daily fresh mash feed given to the chickens during the experiments (Aravin *et al*., 2003).

Silymarin: Silymarin containing some flavonolignans including Silydianin, Silychristin, Isosylbin A&B and Silybin A&B (Livomarin, Darou Pakhsh Pharmaceutical Mfg Co., Iran) was used at 600 mg/kg of BW as previously described (Tedesco *et al*., 2004).

Experimental groups: Chickens of group A were kept as a control group and fed with feed without fungi and Silymarin. Chickens of group B were fed with feed containing *A.*

flavus, while chickens of C were fed with feed containing *A. flavus* + Silymarin. Chickens of Group D were fed with feed containing *A. fumigatus*, while chickens of E were fed with feed containing *A. fumigatvus* + Silymarin. Chickens of group F were fed with feed containing *A. flavus* + *A. fumigatus*, while chickens of G were fed with the same feed as F + Silymarin.

Sampling: On days 35, 42 and 49, blood samples were taken from wing vein (brachial vein) for hematological and biochemical studies as previously described (Alcorn, 2002; Bermudez and Stewart-Brow, 2003) using 2 ml syringes. Blood samples were labeled according to the leg-number of chickens and day of bleeding. In order to minimize changes in biochemical parameters, sera were immediately separated and centrifuged at 2300 g for 5 min as recommended (Hrubec *et al*., 2004).

Hematological values: Whole blood samples were collected into tubes containing EDTA (ethylene diaminetetra acetic acid) as anticoagulant at a 1:10 dilution. Blood smears were prepared and stained with the classic Wright's method. Hematological parameters such as RBC, WBC, PCV, Hb, MCV, MCH, MCHC, ESR, together with absolute counts of heterophils, lymphocytes, monocytes, eosinophils, and basophils, as well as H / L ratio were determined by routine methods as previously described (Campbell, 1992).

Biochemical values: Serum biochemical values were determined using an auto-analyzer spectophotometer (Technicon RA 1000^{TM}, Hartwell, LA, USA) and different Pars-Azmoon kits in various wavelengths as follow:

Albumin: Serum albumin concentration was determined as mg/dl with the Bromcresol-Greeen method at 550 nm wavelength.

Total protein: Serum total protein concentration was determined as mg/dl with the Biuret method at 550 nm wavelength.

Glucose: Serum glucose concentration was determined as mg/dl with GOD-PAP method at 500 nm wavelength.

Creatinine: Serum creatinine concentration was determined as mg/dl with JAFFE method at 500 nm wavelength.

Urea: Serum urea concentration was determined as mg/dl with the PAP method at 500 nm wavelength.

Aspartate amino transferase (AST): Serum AST (GOT) concentration was determined as U/l with Opt. standard method, with IFCC at 340 nm wavelength.

Alanine amino transferase (ALT): Serum ALT (GPT) concentration was determined as U/l with Opt. standard method, with IFCC at 340 nm wavelength.

Alkaline phosphatase (ALP): Serum ALP concentration was determined as U/l with the DGKC method at 405 nm wavelength.

Statistical analysis: Various tests from SPSS 13 including One-Way ANOVA (for comparison of the parameters within and between groups at specific days) and Repeated Measure test (for comparison of each parameter among different age of experiment) were used for statistical analysis of the results. The relationship between age and values of biochemical parameters were ascertained by means of Pearson's correlation coefficient test.

Results and Discussion

The results of this study are summarized in Tables 1 and 2 as Mean ± Std. deviation.

Table 1. Serum biochemical parameters (Mean ± Std.deviation)

Serum biochemical parameters	Day	Groups						
		A	B	C	D	E	F	G
Albumin (mg/dL)	35	1.52±0.07	1.57±0.05	1.56±0.09	1.58±0.05	1.54±0.05	1.58±0.10	1.52±0.03
Albumin (mg/dL)	42	1.71 ±0.07	1.60±0.12	1.32±0.04	1.52±0.09	1.36±0.08	1.74±0.05	1.36±0.15
Albumin (mg/dL)	49	1.84±0.14	1.84±0.12	1.54±0.04	1.64±0.09	1.63±0.05	1.84±0.08	1.58±0.18
Total protein (g/dL)	35	2.88±0.03	2.86±0.06	2.74±0.05	2.75±0.10	2.74±0.05	2.84±0.05	2.82±0.05
Total protein (g/dL)	42	2.44 ±0.08	2.78±0.10	2.56±1.07	2.18±0.33	2.30±0.05	2.34±0.12	2.42±0.24
Total protein (g/dL)	49	2.42±0.08	2.90±0.12	2.70±0.13	2.08±0.32	2.16±0.08	2.36±0.17	2.46±0.23
Creatinine (mg/dL)	35	0.78±0.07	0.74±0.10	0.72±0.11	0.72±0.05	0.70±0.04	0.75±0.08	0.76±0.05
Creatinine (mg/dL)	42	0.96±0.06	0.92±0.08	1.16±0.04	1.08±0.03	1.00±0.07	0.94±0.10	1.24±0.08
Creatinine (mg/dL)	49	1.16±0.06	0.94±0.05	1.22±0.05	1.20±0.04	1.08±0.10	0.94±0.05	1.18±0.05
Urea (mg/dL)	35	8.10±0.22	8.14±0.21	8.32±0.06	8.28±0.09	8.66±0.04	8.20±0.23	8.54±0.27
Urea (mg/dL)	42	8.08±0.13	8.20 ±0.18	9.90±0.17	9.96±0.19	10.60±0.19	10.04±0.18	9.58±0.50
Urea (mg/dL)	49	7.18±0.15	8.22±0.09	10.68±0.21	10.10±0.27	10.84±0.16	10.08±0.13	9.80±0.51
Glucose (mg/dL)	35	218.00±2.16	218.20±1.11	217.00±0.81	214.00±4.46	214.20±1.11	214.00±1.68	218.60±0.81
Glucose (mg/dL)	42	240.00±0.06	252.60±6.06	240.40±4.13	246.40±6.20	254.00±3.36	252.80±4.97	239.80±1.40
Glucose (mg/dL)	49	257.40±8.34	251.00±3.33	231.40±4.20	252.20±6.80	264.80±4.61	265.40±6.23	258.00±3.47
AST (U/L)	35	251.00±2.70	250.60±2.15	252.60±1.20	243.80±1.38	243.20±0.96	255.00±2.34	252.00±0.70
AST (U/L)	42	296.06±0.92	245.00±1.58	241.80±1.06	242.80±2.22	245.20±1.39	299.00±1.30	292.60±2.03
AST (U/L)	49	304.60±0.92	253.80±0.86	250.00±0.83	252.00±1.76	252.00±1.44	305.60±2.15	299.00±2.66
ALT (U/L)	35	11.00±0.50	11.40±1.12	10.40±0.50	10.60±0.67	10.60±0.50	10.60±0.50	10.40±0.80
ALT (U/L)	42	9.56±0.50	8.02±0.05	7.52±0.38	5.78±0.11	5.06±0.11	4.50±0.33	4.06±0.16
ALT (U/L)	49	7.80±0.80	9.00±0.07	9.40±0.50	9.00±0.70	7.40±0.50	8.45±0.50	8.20±0.37
ALP (U/L)	35	3.43±0.07	3.46±0.04	3.56±0.06	3.49±0.09	3.57±0.05	3.51±0.06	3.48±0.07
ALP (U/L)	42	3.12±0.05	3.33±0.06	2.99±0.03	3.18±0.08	3.22±0.09	2.83±0.08	2.65±0.04
ALP (U/L)	49	3.07±0.05	3.24±0.08	2.94±34.4	3.13±0.08	3.14±0.09	2.88±0.03	2.29±0.01

A (control), B (*A. flavus*), C (*A. flavus* + Silymarin), D (*A.fumigatus*), E (*A. fumigatus* + Silymarin), F (*A. flavus* and *A. fumigatus*), and G (*A. flavus* and *A. fumigatus* + Silymarin).

Table 2. Hematology parameters (Mean ± Std. deviation)

Parameters	Day	Groups						
		A	B	C	D	E	F	G
RBC ($x10^6$/µL)	35	2.31 ±0.02	2.35±0.02	2.30±0.04	2.32±0.04	2.29±0.01	2.26±0.02	2.26±0.01
RBC ($x10^6$/µL)	42	2.87±0.02	2.23±0.04	2.24±0.03	2.22±0.02	2.21±0.03	2.16±0.03	2.18±0.01
RBC ($x10^6$/µL)	49	2.90±0.01	2.28±0.03	2.50±0.08	2.38±0.07	2.37±0.02	2.27±0.03	2.19±0.03
PCV (%)	35	26.00±0.37	26.60±0.24	27.00±0.31	26.20±0.37	26.00±0.31	25.60±0.24	25.00±0.31
PCV (%)	42	34.80±0.02	26.80±0.04	26.80±0.02	27.60±0.01	26.40±0.02	26.20±0.02	26.40±0.02
PCV (%)	49	35.60±0.20	27.20±0.70	31.00±0.90	28.60±0.90	28.60±0.50	27.00±0.40	26.40±0.20
Hb (g/dL)	35	10.70±0.13	10.74±0.14	10.92±0.04	10.6 4±0.17	10.56±0.02	10.60 ±0.09	10.64±0.02
Hb (g/dL)	42	13.86±0.01	9.00±0.01	9.04±0.08	8.98±0.04	8.92±0.04	8.74±0.05	8.84±0.05
Hb (g/dL)	49	15.54±0.08	9.82±0.10	10.92±0.30	10.20±0.30	10.04±0.07	9.72±0.10	9.40±0.10
WBC ($x10^3$/µL)	35	22.50±0.20	23.64±0.33	23.60±0.08	23.36±0.34	23.86±0.34	23.62±0.51	22.76±0.10
WBC ($x10^3$/µL)	42	23.14±0.05	23.96±0.05	23.74±0.01	24.99±0.02	23.26±0.02	25.15±0.05	25.14±0.03
WBC ($x10^3$/µL)	49	21.34±0.70	26.08±0.60	26.22±0.50	25.04±0.80	25.14±0.10	26.60±0.40	24.80±0.20
Heterophils (%)	35	51.60±0.96	52.10±1.01	53.00±0.02	49.00±0.96	54.00±1.01	51.00±0.02	48.00±0.02
Heterophils (%)	42	42.00±0.09	47.40±0.20	49.00±0.50	50.60±0.90	43.00±0.10	49.00±0.07	41.40±0.80
Heterophils (%)	49	38.60±0.10	45.00±0.10	44.2±0.30	37.80±0.80	38.60±0.70	43.40±0.70	42.60±0.60
Lymphocytes (%)	35	33.40±1.80	30.4 0±1.02	30.60±0.37	34.80±1.02	30.60±0.24	30.60±1.07	33.60±0.24
Lymphocytes (%)	42	45.00±0.50	35.80±0.70	35.00±0.12	34.20±0.90	38.00±0.60	40.00±0.10	38.80±0.40
Lymphocytes (%)	49	46.80±0.90	34.00±0.01	35.60±0.90	48.40±0.90	46.80±0.70	37.20±0.60	38.80±0.9
Ratio of H/L	35	1.57±0.34	1.68±0.03	1.70±0.04	1.41±0.13	1.77±0.01	1.69±0.31	1.47±0.32
Ratio of H/L	42	0.85±0.07	1.33±0.20	1.41±0.25	1.48±0.11	1.13±0.01	0.98±0.19	1.06±0.08
Ratio of H/L	49	0.83±0.12	1.32±0.01	1.33±0.49	0.78±0.10	0.83±0.12	1.18±0.19	1.10±0.19
Eosinophils (%)	35	4.80±0.70	5.60±0.34	4.40±0.40	5.00±0.10	4.40±0.50	6.00±0.10	5.60±0.50
Eosinophils (%)	42	4.52±0.40	5.20±0.80	4.80±0.10	4.60±0.50	6.00±0.10	6.80±0.10	6.40±0.40
Eosinophils (%)	49	5.40±0.50	7.00±0.10	6.80±0.09	5.20±0.40	5.40±0.50	6.60±0.15	6.40±0.50
Monocytes (%)	35	8.20±0.40	9.20±0.10	9.20±0.80	8.20±0.90	8.40±0.50	8.80±0.40	8.80±0.80
Monocytes (%)	42	6.20±0.40	9.70±0.10	8.80±0.60	8.60±0.50	10.00±0.10	10.80±0.90	10.40±0.50
Monocytes (%)	49	7.80±0.90	11.00±0.80	10.60±0.19	7.40±0.80	7.80±0.80	10.20±0.90	9.80±0.90
Basophils (%)	35	2.80±0.90	2.60±0.50	3.40±0.54	3.00±0.10	2.40±0.54	3.60±0.50	3.60±0.54
Basophils (%)	42	1.60±0.80	2.40±0.01	2.40±0.50	2.00±0.10	3.00±0.10	3.40±0.54	3.00±0.10
Basophils (%)	49	1.40±0.50	3.00±0.10	2.80±0.90	1.20±0.40	1.00±0.54	2.60±0.54	2.40±0.50

A (control), B (*A. flavus*), C (*A. flavus* + Silymarin), D (*A.fumigatus*), E (*A. fumigatus* + Silymarin), F (*A. flavus* and *A. fumigatus*), and G (*A. flavus* and *A. fumigatus* + Silymarin).

Serum biochemical parameters: As shown in Table 1, in the control group (Group A), the values of albumin, creatinine, glucose and AST increased, while the values of total protein, urea, ALT and ALP decreased with age of the chickens. In all fungi and fungi+Silymarin treated groups, the values of most of the biochemical parameters were fluctuated during the experimental period. Comparison of fungi treated groups with fungi+Silymarin treated groups could be summarized as follows:

Albumin. The values of albumin of

groups treated only with the fungi were nearly the same as those of control groups, while the values of fungi + Silymarin treated groups decreased at day 35 and increased slightly at day 42. The differences observed between the values of fungi treated and control groups were not significant. As regard the effects of Silymarin, a significant difference (P<0.047) was only observed between Groups F and G, indicating the detoxification effects of Silymarin in the latter group. Comparison of toxic effects of the fungi on chickens, significant differences (P<0.01) among Groups B, D and F were also observed with the most significant decrease in albumin levels of Group D at day 49.

ALP. The values of ALP in all groups decreased with age, but reductions in fungi treated groups (except group F) were less than the control group and reductions in fungi+Silymarin treated groups were more than the control group. Comparison of the ALP values of different groups at days 42 and 49 revealed significant differences among all groups (P<0.05). While comparison of the fungi treated groups with fungus+Silymarin treated groups showed that Group B differed significantly (P<0.05) from Groups C and F, but differed significantly (P<0.05) from Group G. These findings indicate that Silymarin had beneficial effects in chickens treated with *A. flavus* alone and in combination with *A. fumigatus.* Significant difference (P<0.01) of ALP values in each group at the different ages, may indicate that the ALP value is also age dependent.

ALT. Differences in the ALT values of the groups were significant (P<0.05) at day 42. In comparison of fungi treated groups with fungi + Silymarin treated groups, significant difference (P<0.009) were also observed. This result shows that Silymarin reduces the toxic effects of the fungi and improves indices of liver function as previously reported (Wellington & Jarvis, 2001).

AST. The AST values of the control group together with Groups E, F and G increased with age, but values of the other groups decreased at day 35 and increased during the last week of the experiment. In general, the AST values of Groups B, C, D and E differed significantly (P<0.03, P<0.001 and P<0.01, respectively) from values obtained for chickens in the control group.

Urea. The values of urea for control group decreased with age, but those of fungi treated groups increased at days 42 and 49 significantly (P<0.04) compared to the values of the control group. In regards to the efficacy of Silymarin, comparison of fungi treated groups and fungi+Silymarin treated groups showed significant increase in Group C compared to Group B and in Group D compared to Group E. Thus, comparisons between fungi showed that the urea levels of the *A. flavus* group were significantly less (P<0.01) than *A. fumigatus* and the mixed treated groups.

Creatinine, glucose and total protein. The difference among values of the groups at days 35, 42 and 49 were not significant, although a beneficial effect on improving the glycemic profile of Silymarin treatment in type II diabetic patients has been reported (Huseini et al., 2006).

Hematological values: Statistical analyses of the results on some hematological parameters are shown in Table 2 and are summarized as follows:

RBC. In the control group, the RBC value increased, while in the fungi treated group,

the RBC values decreased during the 1st week of the experiment and then increased very slightly. The difference between the groups at days 35 and 42 were significant (P<0.01). As shown in Table 2, the RBC values of Group C were closer to those of the control group than Group B, indicating the effects of Silymarin during the last week of the experiment.

PCV. The values of PCV in all groups increased with age, but the increasing rates of the fungi treated groups differed significantly (P<0.001) from those of the control group. As regard the effects of Silymarin, the PCV values of Group C were closer to the control group, indicating that Silymarin may have more detoxification effects on *A. flavus* than *A. fumigatus*.

Hb. The Hb values of chickens in the control group increased, while those of chickens treated with fungi alone or fungi+Silymarin Groups decreased at day 35 and increased slightly at day 42. The difference among the groups were significant (P<0.001).

WBC. WBC values of the fungi treated group increased, while those of the control group decreased during the experimental period and difference among the groups was significant (P<0.01). These findings may reflect the effects of the fungi on the defense system of chickens treated with fungi alone or fungi+Silymarin.

Hetrophils, lymphocytes and H/L ratio. Naturally, heterophils values of the chickens decreased by the age but the reduction rate was less in the fungi treated group in comparison to the control group. The lymphocyte values of chickens in all the groups increased by age but the rate of increase was less in the fungi treated groups, providing significant (P<0.001) differences between day 42 H / L ratio of fungi treated groups and that of the control group.

Monocytes. The monocyte values of all the groups fluctuated during the experiment but overall, the monocyte values of the chickens treated with fungi and fungi + Silymarin were higher than those of the control group (Table 2). Differences between the groups at days 35 and 42 were significant (P<0.05).

Eosinophils and basophils. The values of these two parameters were slightly higher in the fungi treated groups than the control group and differences between the groups at day 35 were significant (P<0.01).

In conclusion, these values in the control and treatment groups, can be used as a source of reference values for the evaluation and comparison of gained data in similar experimental studies. A study on the efficacy of Silymarin on detoxification of aflatoxin B1, has revealed no differences in any of the evaluated biochemical parameters, except for the lower level of serum ALT in aflatoxin-B_1 treated chickens with respect to the control (Hauser *et al*., 2004; Tedesco *et al*., 2004). However, results of the present study on biochemical parameters differ in some respects from previous studies. The results of this study on hematological parameters are in agreement with previous report, which revealed that feeding a naturally contaminated diet with aflatoxin was associated with significant decreases in hematocrit values (Aravin *et al*., 2003).

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