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Zeetress (a polyherbal formulation of *W. somnifera*, *O. sanctum*, and *E. officinalis*) prevents salinomycin toxicity in broiler chicks

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Abstract

Previously we have evaluated the dose dependent toxicity of salinomycin and maduramycin in broiler chicks and have studied changes in serum biochemical profiles in salinomycin or maduramycin intoxicated broiler chicks. In continuation, we here evaluated the effect of salinomycin toxicity on erythrocyte antioxidant status in broiler chicks. Activities of erythrocyte glutathione peroxidase, glutathione reductase and catalase was elevated progressively and glutathione concentration was reduced significantly in salinomycin (120 ppm in feed) intoxicated birds suggesting that salinomycin toxicity could be mediated by free radical generation and depletion of glutathione levels further contributing to the oxidative damage. Hence we evaluated the effect of antioxidant (Vitamin C, Endox Dry) and Zeetress (a polyherbal formulation of quality extracts of *W. somnifera*, *O. sanctum*, and *E. officinalis*) supplementation on salinomycin toxicity in broiler chicks. Both the antioxidants and zeetress significantly protected the broiler chicks against salinomycin toxicity. There weren't any significant changes in the values observed from control or salinomycin 60-ppm fed chicks. We conclude that salinomycin toxicity is associated with oxidative stress and antioxidant from both natural and synthetic source could be valuable supplements in broiler feeds to prevent ionophore toxicity incidences.

Key words:- salinomycin, glutathione peroxidase, glutathione reductase, glutathione, catalase.

Introduction

In broiler chicks raised on deep litter, regular supplementation of anticoccodial agent in feed is indispensable to prevent losses caused by coccidiosis (1,2). Unfortunately their narrow margin of safety is cause for concern and the difficulty in ensuring an even distribution of drug due to its sedimentation and segregation in the feed adds to this problem (3,4). Salinomycin is one such polyether ionophore compound being extensively used to control coccidiosis because of its broad spectrum of activity and slow development of resistant strains (5). Though salinomycin is the least toxic of all the ionophores presently available, regular incidences of toxicity such as reduce feed intake and depressed growth in chicks is reported. Which occurs due to improper mixing of feed and as well by sedimentation and segregation of salinomycin in feed during storage resulting in its increased concentration in the bottom part of the stored feed (4). In general about 20-50 % over dosage causes first evidence of toxicity (6), which ranges from incoordination, leg weakness, diarrhea, reduced feed intake and weight depression (3). We have previously evaluated the dose dependent toxicity of salinomycin (7) and maduramycin (8) in broiler chicks and have studied changes in serum biochemical profiles in salinomycin and maduramycin intoxicated birds. Since in these studies we found the salinomycin-induced damage being ubiquitous (7), we speculated the role of oxidative stress in the process. To address this issue we designed this study to evaluate the effect of ionophore on erythrocyte antioxidant enzymes. To further confirm the role of oxidative stress in salinomycin toxicity we simultaneously supplemented the broiler feed with two different antioxidants and Zeetress (a polyherbal formulation of quality extracts of *W. somnifera*, *O. sanctum*, and *E. officinalis*). Our results for the first time provide an experimental proof for the role of oxidative stress in salinomycin toxicity.

Materials and Methods:

Animal ethics: The trial was approved by the Institutional Animal ethics Committee, ANGRAU, Hyderabad and complied with Halenski's declaration on handling of experimental animals.

Materials:

Salinomycin (Coxistac 12 % premix) was a kind gift from Pfizer Ltd.; Mumbai, NDV, IB and IBD vaccine were procured from Venkateswara Hatcheries, Hyderabad, H & E stain was procured from MS Qualigens Pvt. Ltd., Hyderabad. Vitamin C was procured from Himedia Ltd, Bombay, India. Endox Dry was kind gift from Kemin Nutritional Technologies (India). Zeetress was procured form Natural Remedies, Bangalore, India. Feed ingredients were procured form the authenticated suppliers to the poultry farm, ANGRAU, Hyderabad. All the kits for biochemical studies were procured from Bhat- Biotech India Pvt., Ltd. Bangalore.

Chicks: Sixty male one-day old broiler chicks (Venkateswara Hatcheries, Hyderabad) were randomly divided into 6 groups of 10 chicks each. The birds were vaccinated against New Castle Disease (NDV; day 7 & 21), Infectious Bronchitis (IB, day 1) and Infectious Bursal Disease (IBD, day 14 & 23) as per the schedule recommended by the Venkateswara Hatcheries, Hyderabad, India. All the birds were housed under a cage system in a well-ventilated broiler shed with ad-lib feed and water access. Chicks of group I served as control without coccidostat in the feed, groups II and III birds were fed with salinomycin @ 60 (S_{60}) and 120 ppm (S_{120}) respectively in feed. Group IV birds received salinomycin (120 ppm) and Vitamin C (300 mg/kg, a dietary antioxidant), group V birds received salinomycin (120 ppm) and Endox Dry (125mg/kg in feed, a synthetic antioxidant) and group VI birds received salinomycin (120 ppm) and Zeetress (10g/1000 birds in drinking water, an antioxidant from natural source).

Feed was prepare in the farm feed milling unit using the following ingradients:

Ingredients	Kgs	Ingredients	Kgs
Maize (Yellow)	63.16	B_E	0.01
Soya	33.28	B_{12}	0.01
Di-calcium phosphate	1.35	Choline Chloride	0.05
Lime Stone	1.4	Probiolac	0.01
DL methionine	0.15	Trace Minerals	0.10
AB_2D_3K	0.01	Coccidostat (Salinomycin)	60 or 120 ppm

Note: The feeder and water container were cleaned and changed twice daily. The day today farm activities complied with the good farm management practice.

Body weights and Feed Conversion efficiency (FCR): The growth pattern was studied by recording the weekly body weights (0-6 weeks) of all the birds in each group and the average body weight was calculated for each group. FCR was calculated using the following formula: FCR = Total feed consumed / total body weight gained (7).

Hematological studies: Hemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC) and platelet count were estimated using automated Animal Blood Counter (Sos-Icsaquoits).

Biochemical studies: Blood samples were drawn from wing vein once every fortnight using a 24-gauge needle attached to 2-ml heparinized syringe. The blood was immediately transferred to test tubes placed in a ice bath and was centrifuged within 30 seconds for 5 min at

2000 x g. The plasma separated was removed and the remaining pellet was suspended and washed twice in alcevers buffer. A drop of 0.1% saponin was added to lyse the red blood cells (RBC). A 5% red blood cell lysate was used for all the biochemical estimations. Plasma separated was used for the assay of alanine aminotransferase {ALT} (9), aspertate aminotransferase {AST} (9), alkaline phosphatase {ALP} (10), total protein (Doumas, 1978), albumin (11), globulin (11), urea (12), creatinine (13), cholesterol (14), triglycerides (15) and lactate dehydrogenase {LDH} (16) using standard kits procured from Bhat Biotech (I) Ltd., Bangalore India.

Estimation of glutathione peroxidase activity: Erythrocyte glutathione peroxidase activity was estimated as per the method of Paglia and Valentine (17), with slight modification. Briefly, the RBC lysate was prepared as mentioned above. A 5% red blood cell lysate was made in 1 ml phosphate buffer of pH 7.4 in a test tube, to which 50 µl of 50 mM reduced glutathione and 50 µl H_2O_2 was added. The tubes were incubated at 25^0c for 5 min following which the reagents mixture was transferred to quartz cuvette and 50 µl of 0.2 mM NADPH was added. Absorbance (A_{320}) was monitored at 1min interval for 5 min. Erythrocyte glutathione peroxidase activity was calculated as ΔA_{320} x 3781 units/ml (where ΔA_{320} is the average change in absorbance per min at 320 nm) and expressed as Units/mg protein. Protein content was assayed by the method of Lowry.

Estimation of glutathione reductase activity: Erythrocyte glutathione reductase activity was estimated as per the method of Paglia and Valentine (17) with slight modification. Briefly, the RBC lysate was prepared as mentioned above. A 5% red blood cell lysate was made in 1 ml phosphate buffer of pH 7.4 in a test tube, to which 50 µl of 50 mM oxidized glutathione (GSSG), 50 µl FAD and 25 µl 80 mM EDTA was added. The tubes were incubated at 37^0c for 15 min following which the reagents mixture was transferred to quartz cuvette and 50 µl of 4 mM NADPH was added. Absorbance (A_{340}) was monitored at 1min interval for 5 min. Erythrocyte glutathione reductase activity was calculated as ΔA_{340} x 3781 units/ml (where ΔA_{340} is the average change in absorbance per min at 340 nm) and expressed as Units/mg protein.

Estimation of glutathione levels: Erythrocyte glutathione levels was estimated as per the method of Patterson and Lazarow (18). Briefly, the RBC lysate was prepared as mentioned above. Protein was precipitated from the lysate by slowly adding 1 ml of 1M sulfosalicylic acid to 1 ml of lysate. The precipitate was extracted was transferred to a test tube to which 500 µl of 4 % sulfosalicylic acid, 500 µl potassium iodide and 1 drop of 1 % starch was added following which it the mixture was titrated with 1 µN potassium iodate till a sharp persistent blue color appeared. Glutathione concentration (mg/100ml) was calculated using the formula {(500 x volume of 1 µN potassium iodate)/3.26}.

Estimation of catalase activity: Erythrocyte catalase activity was estimated as per the method of Britton and Maehly (19), with slight modification. Briefly, 10 µl of the RBC lysate (prepared as mentioned above) was added to a quartz cuvette with magnetic stirrer containing 1 ml of 1:500 H_2O_2 following which absorbance was recorded at 280 nm every 30 sec for 5 min. catalase activity was estimated using the following formula.

Velocity constant (K) (sec^-) = $(2.3/t_2 - t_1) \times \log (X_1/X_2)$

Where $X_1 = A_{280}$ at t_1 and $X_2 = A_{280}$ at t_2.

Specific activity of catalase ($mole^- sec^-$) = K/e

Where e = avian erythrocyte catalase molarity constant (2.5×10^{-2} moles).

Catalase activity was expressed as U/mg protein.

Statistical analysis: All the results are expressed as mean ± SD. The data was analyzed using Jandel SigmaStat 2, statistical software. Significance of difference between two groups was evaluated using one-way analysis of variance (ANOVA) with *post-hoc* analysis using *Tukey`s test* or *Bonferroni`s test*. $P<0.05$ was considered statistically significant.

Results

Salinomycin treated (120-ppm) chicks showed symptoms of toxicity, which ranged from in-coordination, lethargy, leg weakness, diarrhea, reduced feed intake and weight loss. Birds in sternal recumbency with neck, wings and hind limbs outstretched were very characteristic and prominent after 4^{th} week of salinomycin exposure. However, the S_{60} and control group birds did not show these symptoms throughout the study.

The weekly average body weight (g) and feed conversion ratio (FCR) of salinomycin intoxicated birds was significantly poor as compared to control or S_{60} birds (Table 1) (Arun *et al.*, 2003a). Supplementation of feed with antioxidants i.e. S_{120} + V: Vitamin C (300 mg/kg, in feed), S_{120} + E: Endox Dry (125 mg/kg, in feed), S_{120} + Z: Zeetress (10g/1000 birds, in water) significantly prevented the toxic effects of S_{120} on broiler weight gain and FCR (Table 1).

S_{120} resulted in significant in all the biochemical parameters studied (our previous report) and all the three antioxidants i.e. vitamin C, Endox Dry and Zeetress improved the biochemical profiles to near normal values (Figures 1,2 & 3). Similarly event the hematological profiles were unaltered in the antioxidant supplemented salinomycin intoxicated birds (Table 2).

Erythrocyte glutathione peroxidase, glutathione reductase and catalase activities were significantly ($p<0.05$) elevated from 2^{nd} week onwards (with maximal difference at 6^{th} week) in S_{120} treated chicks, while the activities of these enzymes remained normal in control and S_{60} treated chicks. Erythrocyte glutathione concentration was significantly ($p<0.05$) reduced from 2^{nd} week onwards (with maximal difference at 6^{th} week)

Table 1. Effect of salinomycin body weights (at weekly intervals from day old) and feed conversion ratio (FCR). Values are expressed as Mean ± SD, (n = 10). # P < 0.05 Vs rest of the group. C: Control group fed on non-ionophore diet. S_{60}: Salinomycin 60 ppm fed group, S_{120}: Salinomycin 120 ppm fed group, S_{120} + V: Vitamin C (300 mg/kg, in feed) supplemented group, S_{120} + E: Endox Dry (125 mg/kg, in feed) supplemented group, S_{120} + Z: Zeetress (10g/1000 birds, in water) supplemented group.

	Weekly body weights (grams)						
Group	**1**	**2**	**3**	**4**	**5**	**6**	FCR
C	142.6 ± 5.8	324.3 ± 8.8	596.0 ± 3.9	902.4 ± 7.3	1274.4 ± 4.2	1608.2 ± 4.8	1.83 ± 0.08
S60	134.9 ± 4.6	278.4 ± 8.4	585.1 ± 4.8	826.1 ± 6.4	1236.7 ± 4.2	1575.8 ± 7.2	1.93 ± 0.09
S120	126.8 ± 9.9	259.4 ± 3.9	389.1 ± 7.8	333.3 ± 8.7	334.4 ± 9.7	393.4 ± 7.3$^{\#}$	3.10 ± 0.05$^{\#}$
S120 + V	129.3 ± 9.0	281.4 ± 6.2	491.3 ± 5.3	733.0 ± 8.6	1018.1 ± 6.8	1286.4 ± 10.8	2.12 ± 0.03
S120 + E	127.7 ± 7.9	277.6 ± 8.1	492.0 ± 6.4	713.4 ± 6.1	1054.4 ± 4.1	1334.4 ± 4.2	2.07 ± 0.06
S120 + Z	134.9 ± 4.6	278.4 ± 8.4	485.1 ± 4.8	726.1 ± 6.4	1036.7 ± 4.2	1275.8 ± 7.2	1.93 ± 0.05

Table 2. Effect of salinomycin on hematological profile. Values are expressed as Mean ± SD, (n = 10). @ P < 0.001 Vs Control and S 60 group; # P < 0.05 Vs rest of the groups. C: Control group fed on non-ionophore diet. S_{60}: Salinomycin 60 ppm fed group, S_{120}: Salinomycin 120 ppm fed group, S_{120} + V: Vitamin C (300 mg/kg, in feed) supplemented group, S_{120} + E: Endox Dry (125 mg/kg, in feed) supplemented group, S_{120} + Z: Zeetress (10g/1000 birds, in water) supplemented group. Hb: Hemoglobin (g%); TEC: Total erythrocyte count (10^6/µl); TLC: Total leukocyte count (10^3//µl); Platelets (10^4//µl); PCV: packed cell volume (%); MCV: Mean corpuscular volume (μ^3); MCH: Mean corpuscular hemoglobin (ρg); MCHC: Mean corpuscular hemoglobin concentration (g/100 ml)

	4th Week					**6th week**				
Group	**Hb**	**TEC**	**TLC**	**Platelets**	**PCV**	**Hb**	**TEC**	**TLC**	**Platelets**	**PCV**
C	5.4 ± 0.6	1.5 ± 0.6	15.2 ± 0.8	20 ± 1.5	19 ± 1.5	6.2 ± 0.4	6.4 ± 0.7	15.0 ± 0.8	18.0 ± 1.6	16 ± 1.41
S 60	8.9 ± 0.4	2.7 ± 0.5	26.1 ± 0.9	29 ± 1.6	26 ± 1.6	9 ± 0.6	3.1 ± 0.3	27.2 ± 0.9	30 ± 1.5	27 ± 1.5
S 120	1.3 ± 0.7$^{\#}$	16.5±0.7$^{\#}$	22 ± 1.6$^{\#}$	18±1.41$^{\#}$	4 ± 0.7$^{\#}$	4 ± 0.7$^{\#}$	1.3 ± 0.2$^{\#}$	14.6±0.6$^{\#}$	19 ± 1.4$^{\#}$	16 ± 1.8$^{\#}$
S_{120} + V	7.2 ± 0.4	2.8 ± 0.3	27.5 ± 0.9	30 ± 1.6	23 ± 1.6	8.5 ± 0.7	3.0 ± 0.5	28.1 ± 0.8	30 ± 1.7	28 ± 1.6
S_{120} + E	9.0 ± 0.5	2.8 ± 0.8	26.8 ± 0.9	30 ± 1.2	27 ± 1.5	9.2 ± 0.6	3.1 ± 0.4	28 ± 0.8	31 ± 1.5	27 ± 1.6
S_{120} + Z	10 ± 0.6	2.9 ± 0.8	26.9 ± 0.9	30 ± 1.7	27 ± 1.5	9.6 ± 0.5	3.0 ± 0.5	28.4 ± 0.8	30 ± 1.4	28 ± 1.6

	MCV	**MCH**	**MCHC**	**MCV**	**MCH**	**MCHC**
C	93.1 ± 1.1	34.5 ± 1.2	37 ± 1.6	93.3 ± 1.4	32 ± 1.2	34.3 ±1.1
S 60	96.3 ± 1.3	34.4 ± 1.8	35.8 ± 1.5	90 ± 1.6	30.7 ± 1.3	34.1 ± 0.9
S 120	138.5 ± 1.4$^{\#}$	40 ± 1.5$^{\#}$	28.9 ± 1.2$^{\#}$	123.1 ± 1.8$^{@}$	30.8 ± 0.9$^{\#}$	25 ± 1.3$^{\#}$
S_{120} + V	92.6 ± 1.2	33.2 ± 1.4	33.6 ± 1.3	91 ± 1.2	31.8 ± 1.2	31.1 ± 0.8
S_{120} + E	94.2 ± 1.1	33.5 ± 1.6	33.8 ± 1.2	92 ± 1.8	32.9 ± 1.1	32.2 ± 0.7
S_{120} + Z	95.4 ± 1.0	32.7 ± 1.7	34.7 ± 1.3	91 ± 1.4	34.8 ± 1.0	33.4 ± 0.9

*Table 3. Effect of salinomycin on erythrocyte antioxidant enzyme levels. Values are expressed as Mean ± SD, (n = 10). * P < 0.05, ** P < 0.01, ***P < 0.001 Vs Control and S_{60} group. C: Control group fed on non-ionophore diet. S_{60}: Salinomycin 60 ppm fed group, S_{120}: Salinomycin 120 ppm fed group, S_{120} + V: Vitamin C (300 mg/kg, in feed) supplemented group, S_{120} + E: Endox Dry (125 mg/kg, in feed) supplemented group, S_{120} + Z: Zeetress (10g/1000 birds, in water) supplemented group. GSH: Reduced glutathione; GSH-R: Glutathione reductase; GSH-PX: Glutathione peroxidase; CAT; Catalase.*

	GSH (mg/dl)	**GSH-R (U/mg protein)**	**GSH-PX (U/mg protein)**	**CATALASE (U/mg protein)**
			2nd week	
C	10.5±0.06	38.6±0.4	80.2±0.6	3.5±0.1
S_{60}	10.3±0.07	37.9±0.3	81.4±0.4	3.5±0.1
S_{120}	8.7±0.07*	63.4±0.2*	95.5±0.3*	4.35±0.1*
S_{120} + V	10.2 ± 0.06	39.9 ± 0.2	82.6 ± 0.5	3.52 ± 0.3
S_{120} + E	11.5 ± 0.06	40.1 ± 0.2	82.2 ± 0.6	3.5 ± 0.2
S_{120} + Z	10.6 ± 0.08	40.2 ± 0.3	81.1 ± 0.8	3.51 ± 0.1
			4th week	
C	10.6±0.09	38.1±0.2	81.1±0.8	3.5±0.1
S_{60}	10.8±0.08	38.0±0.3	82.2±0.8	3.5±0.1
S_{120}	5.5±0.08**	95.4±0.4***	130.4±0.8***	5.25±0.3**
S_{120} + V	13.2 ± 0.06	38.5 ± 0.2	83.5 ± 0.7	3.5 ± 0.1
S_{120} + E	12.5 ± 0.07	39.8 ± 0.3	83.5 ± 0.6	3.51 ± 0.2
S_{120} + Z	11.7 ± 0.08	40.1 ± 0.1	83.4 ± 0.7	3.52 ± 0.2
			6th week	
C	10.9±0.08	38.8±0.4	81.4±0.8	3.5±0.1
S_{60}	11.1±0.07	38.5±0.3	81.5±0.7	3.5±0.1
S_{120}	5.6±0.08***	108.5±0.6***	150.1±0.6***	6.23±0.2**
S_{120} + V	13.0 ± 0.07	38.8 ± 0.7	83.9 ± 0.5	3.52 ± 0.2
S_{120} + E	12.8 ± 0.08	39.1 ± 0.8	84.1 ± 0.4	3.5 ± 0.1
S_{120} + Z	12.5 ± 0.08	40.3 ± 0.6	84.1 ± 0.7	3.52 ± 0.2

in S_{120} treated chicks but remained normal in Control and S_{60} treated chicks (Table 3). Similar results were also observed with maduramycin, which is yet another commonly used ionophore in poultry diet (unpublished data).

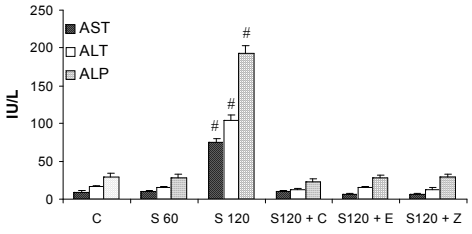

Figure 1. Effect of salinomycin on serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) at 6th week. Values are expressed as Mean ± SD, (n = 10). # P < 0.05 Vs rest of the groups. C: Control group fed on non-ionophore diet. S_{60}: Salinomycin 60 ppm fed group, S_{120}: Salinomycin 120 ppm fed group, S_{120} + V: Vitamin C (300 mg/kg, in feed) supplemented group, S_{120} + E: Endox Dry (125 mg/kg, in feed) supplemented group, S_{120} + Z: Zeetress (10g/1000 birds, in water) supplemented group.

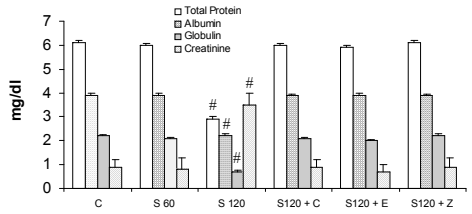

Figure 2. Effect of salinomycin on serum total proteins, albumin, globulin and creatinine levels. Values are expressed as Mean ± SD, (n = 10). # P < 0.05 Vs rest of the groups. C: Control group fed on non-ionophore diet. S_{60}: Salinomycin 60 ppm fed group, S_{120}: Salinomycin 120 ppm fed group, S_{120} + V: Vitamin C (300 mg/kg, in feed) supplemented group, S_{120} + E: Endox Dry (125 mg/kg, in feed) supplemented group, S_{120} + Z: Zeetress (10g/1000 birds, in water) supplemented group.

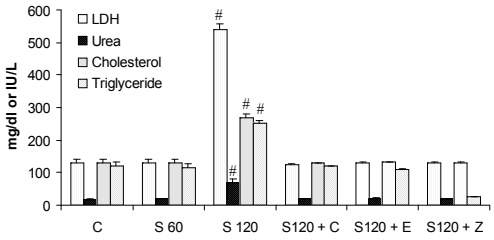

Figure 3. Effect of salinomycin on serum Lactate dehydrogenase (LDH), Urea, Cholesterol and Triglyceride levels. Values are expressed as Mean ± SD, (n = 10). # P < 0.05 Vs res t of the groups. C: Control group fed on non-ionophore diet. S_{60}: Salinomycin 60 ppm fed group, S_{120}: Salinomycin 120 ppm fed group, S_{120} + V: Vitamin C (300 mg/kg, in feed) supplemented group, S_{120} + E: Endox Dry (125 mg/kg, in feed) supplemented group, S_{120} + Z: Zeetress (10g/1000 birds, in water) supplemented group. LDH (IU/l); Urea, cholesterol and triglycerides (mg/dl).

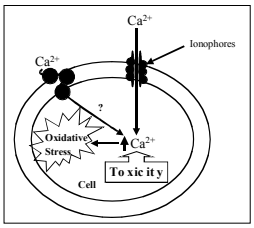

Figure 4: Schematic diagram representing the proposed model from the study. The model depicts the rise in intracellular calcium levels contributing to the oxidative stress, which could account for the salinomycin toxicity.

Discussion

In our previous report (7) we showed that S_{120} induced pathology was evident in skeletal muscle, kidneys, liver, heart, intestines, lungs and bursa with corresponding changes in the biochemical parameters. Such a ubiquitous damage made us to speculate the possible role of free radical in the salinomycin toxicity. To explore this possibility we studied some of the antioxidant enzymes, which are indicators of oxidative stress in the salinomycin intoxicated broiler chicks. We studied these antioxidant levels in the erythrocyte fraction as it gives an indication of the total body oxidative stress.

Ionophores are known to non-selectively transport mono and divalent cations in to the cells, Ca^{2+} being one among them. An elevated cytoplasmic calcium $[Ca^{2+}]_i$ can trigger many biochemical pathways responsible for cell death (20-22). Oxidative stress (enhanced free radical generation) being one such biochemical mechanism. Ionophores such as salinomycin form lipid soluble complexes with cations of which K^+, Na^+, Ca^{2+} and Mg^{2+} are most significant biologically (6). The high diffusion rate of these complexes across lipid barriers together with a high cation (Ca^{2+}) selectivity results in increased intracellular Ca^{2+} which when exceeds the ability of mitochondria to sequester Ca^{2+} effectively leads to lysosomal degranulation, releasing reactive oxygen metabolites (ROM) in the process (20). These ROM induce a series of free radical degenerations, which subsequently results in membrane damage followed by, cell death (23), this may be probable mechanism of salinomycin toxicity (Figure 4). Glutathione peroxidase, glutathione reductase, glutathione and catalase are part of antioxidant defense system, which effectively scavenge the free radicals (24). An elevation in glutathione peroxidase, glutathione reductase and catalase activities are indicative of excessive free radical production in the system (25). Reduced intracellular glutathione concentration is closely related to increase lipid peroxidation, which in turn affects cell membrane integrity (26).

In the present study erythrocyte glutathione peroxidase, glutathione reductase and catalase activities were significantly ($p<0.05$) elevated in salinomycin treated (120 ppm) group, while the erythrocyte glutathione concentration was significantly ($p<0.05$) reduced in this group, thus indicating ongoing free radical damage in the system.

Hence we tried to prevent the toxic effects of salinomycin by simultaneous supplementation of antioxidants in the feed or water. For this we selected three different class of antioxidants namely, vitamin C (a dietary antioxidant), Endox Dry (A synthetic antioxidant) and Zeetress (a poly herbal formulation antioxidant). Concurrent supplementation of the these antioxidant prevented the salinomycin toxic effects in broiler chicks as evident from the body weights, FCR,

hematological profiles, biochemical profiles and erythrocyte antioxidant enzyme profiles.

From the present results, it can be concluded that salinomycin induces toxicity at higher than the recommended dose and the toxicity is due to free radical release and/or generation and depletion of glutathione concentration. Thus, our study is the first report suggesting the role of free radicals in the salinomycin toxicity. Further we suggest that supplementing antioxidants (both from natural and synthetic sources) in the broiler feed can prevent any untoward incidences of ionophore toxicity.

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