

toxicity could be probably due to oxidative damage by free radical generation (5) and such damage can be prevented by the supplementation of antioxidants in feed (6). Though the ionophore toxicity is attributed to oxidative damage, the mechanisms and extent of toxicity caused by salinomycin has not yet been established completely. Besides, though salinomycin has been graded as a powerful coccidiostat, it has been used with great limitation. Therefore, study of detailed mechanism of toxicity due to the oxidative stress would be beneficial to evolve a suitable remedy to prevent and treat salinomycin toxicity to facilitate the utilization of this coccidiostat without any adversities in the poultry industry. The role of zinc in protecting the erythrocytic membrane from oxidative free radicals has been reported (7). Keeping these facts in view, an experimental study was planned in broilers to ascertain the mechanism and extent of toxicity caused by salinomycin (60 and 120 mg/kg feed) and to evaluate the antioxidant and curative role of zinc against salinomycin induced toxicity in broilers.

METHODS

The study was conducted on male broiler chicks, procured from Venkateswara Hatcheries, Hyderabad, acclimatized for a week. The chicks of 1 week old were randomly divided into 7 groups of 6 birds in each and fed with basal diet and water was provided *ad libitum*. Group 1 was fed on basal diet as control without any coccidiostat, group 2 on salinomycin therapeutic dose (60 mg/kg feed), group 3 on salinomycin toxic dose (120 mg/kg feed), group 4 on salinomycin (60 mg/kg feed) +

zinc (80 mg/kg feed), group 5 on salinomycin (120 mg/kg feed) + zinc (80 mg/kg feed) for 6 wk duration.

Group 6 and 7 were fed on feed containing salinomycin at the rate of 120 mg/kg for the first 4 weeks of experimental study. Thereafter, for the next 2 weeks, group 6 was maintained on zinc (80 mg/kg feed) with out salinomycin and group 7 on salinomycin (120 mg/kg feed) along with zinc (80 mg/kg feed).

Salinomycin was procured from Pfizer Ltd., as Coxistac (12% premix) and zinc from Ranbaxy Laboratories Ltd. as zinc oxide (74% premix). The total duration of experiment was 6 weeks. The University Technical Committee and Institutional Animal Ethics Committee approved the experimental protocol. Birds of all the groups were vaccinated for New castle disease on 4th and 21st day, fowl pox vaccine on 10th day and infections bursal disease vaccine on 14th day.

Body weights

The body weight gains were recorded at weekly intervals to determine growth pattern.

Antioxidant enzymes

The blood samples were collected from wing vein in sterilized vials using dipotassium salt of EDTA as anticoagulant for the assay of antioxidant enzymes in blood such as glutathione peroxidase (GSH-Px) (8), glutathione reductase (9), catalase (10) and glutathione (GSH) (11).

Sero-biochemical parameters

The serum separated from coagulated blood was analyzed for alanine aminotransferase (ALT, aspartate aminotransferase (AST), total proteins, albumin, globulin, total cholesterol, high density lipoproteins (HDL), triglycerides, low density lipoproteins (LDL), urea, creatinine and blood urea nitrogen (BUN) using commercially available diagnostic kits (Sigma Diagnostics Pvt. Ltd., Baroda).

Statistical analysis

The data were analyzed by two-way analysis of variance (12). $P < 0.01$ was considered to be statistically significant.

RESULTS

The body weight gain in toxic control

(723 ± 119) was significantly ($P < 0.01$) decreased as compared to group 1 (2046 ± 94), 2 (1877 ± 58), 4 (2072 ± 105) and 5 (1865 ± 60). The body weight gain in group 6 (1659 ± 62) and 7 (1470 ± 95) revealed a significant ($P < 0.01$) increase on supplementation with zinc during the last 2 weeks.

The antioxidant enzymes in blood such as GSH-Px, glutathione reductase and catalase were significantly ($P < 0.01$) elevated, while GSH level was significantly ($P < 0.01$) lowered in toxic controls (III, VI and VII) than group I, II, IV and V. On supplementation of zinc in group VI and VII as a therapy, the antioxidant enzyme level in blood was revived to normal at 7th week. The GSH level in group VII was significantly ($P < 0.01$) lower than group VI at 7th week (Fig. 1-4).

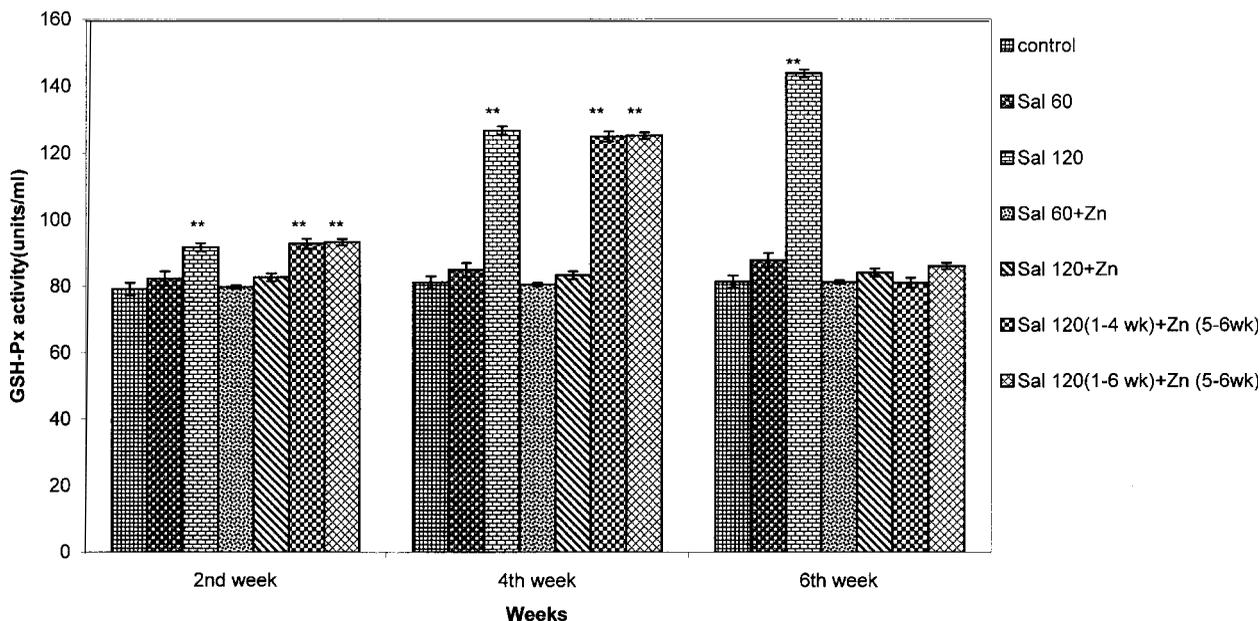


Fig. 1: GSH-Px activity of different groups of broiler chicks at the end of 2nd, 4th and 6th weeks. Values are expressed as mean \pm SE of 6 observations. ** $P < 0.01$ 2 way ANOVA.

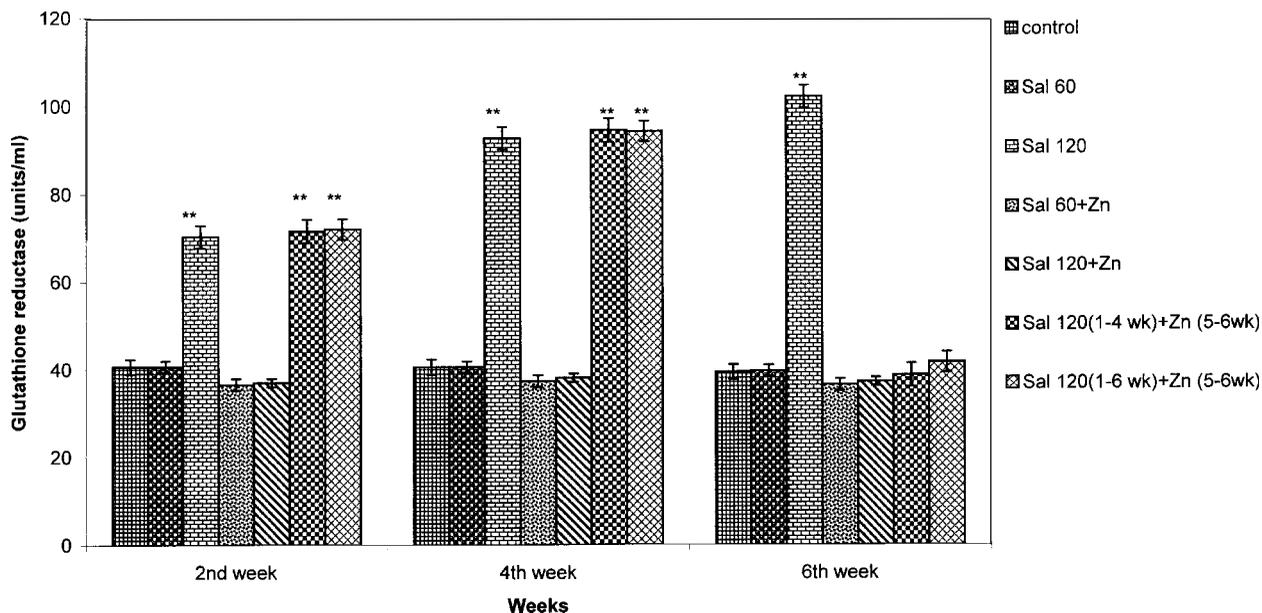


Fig. 2: Glutathione reductase activity of different groups of broiler chicks at the end of 2nd, 4th and 6th week. Values are expressed as mean \pm SE of 6 observations. **P<0.01 2 way ANOVA.

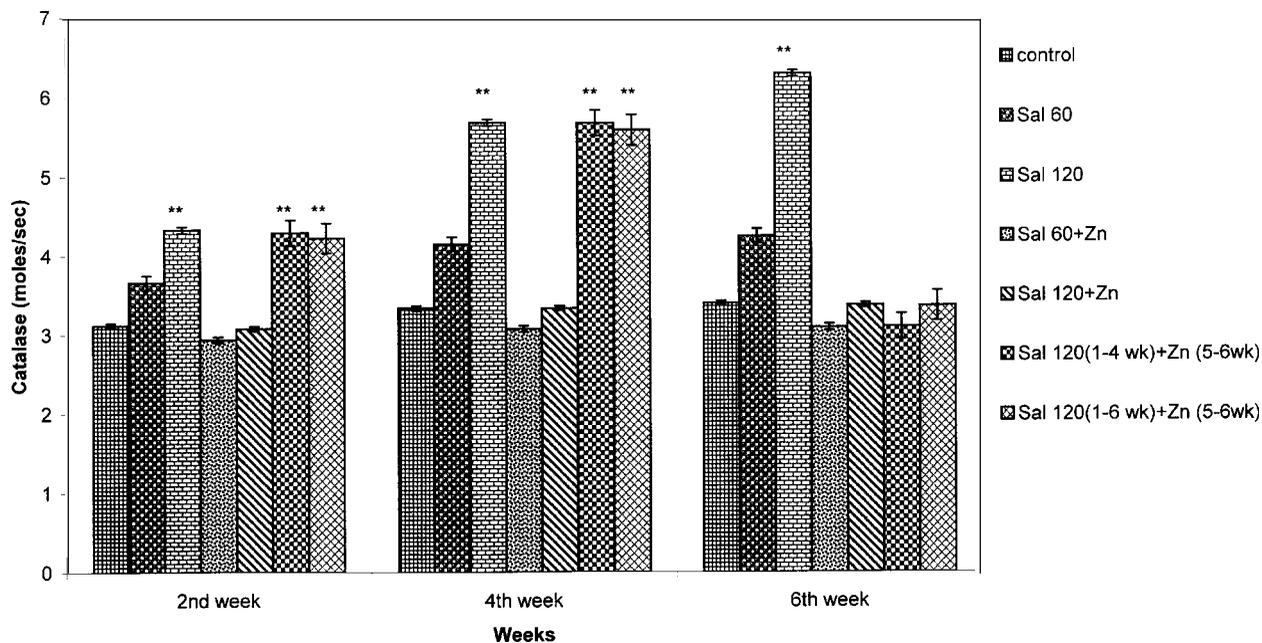


Fig. 3: Catalase activity of different groups of broiler chicks at the end of 2nd, 4th and 6th week. Values are expressed as mean \pm SE of 6 observations. **P<0.01 2 way ANOVA.

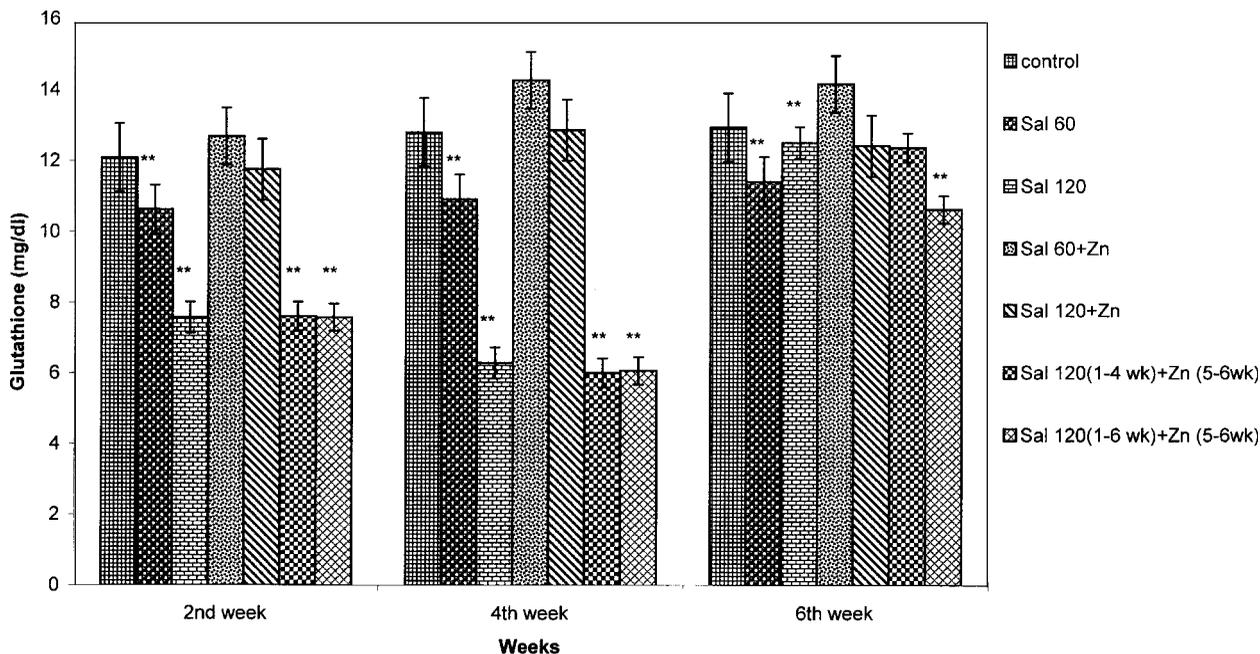


Fig. 4: Glutathione concentration of different groups of broiler chicks at the end of 2nd, 4th and 6th week. Values are expressed as mean \pm SE of 6 observations. ** $P < 0.01$ 2 way ANOVA.

The values of AST (units/ml) and ALT (units/ml) in group 3 were 85.66 ± 8.53 and 108.67 ± 6.10 , respectively at the end of 6th wk against the values of the control group 1 (15.02 ± 1.35 and 20.67 ± 1.90 , respectively). At the end of the experiment, the activities of AST and ALT in group 2, 4, 5, 6 and 7 were comparable to the values of group 1 with out any significant difference.

Total protein concentration (g/dl) in group 3 at the end of 6th wk was 3.42 ± 0.07 , which was significantly ($P < 0.01$) lower as compared to group 1 (6.09 ± 0.06) and there was no significant difference in the total protein concentration of group 2, 4, 5, 6 and 7 as compared to group 1 at the end of 6th wk.

Total cholesterol (mg/dl), triglycerides (mg/dl) and LDL (mg/dl) in group 3 were 270.01 ± 7.60 , 242.12 ± 7.57 and 202.36 ± 9.28 ,

respectively at the end of 6th wk, which were significantly ($P < 0.01$) higher as compared to those of group 1 (125.92 ± 3.02 , 103.90 ± 3.61 and 71.82 ± 2.11 , respectively). The values of the above variables of group 2, 4, 5, 6 and 7 were comparable to those of group 1 with out any significant difference. The HDL concentration (mg/dl) was significantly ($P < 0.01$) lower in group 3 at the end of 6th wk as compared to that of control (333.32 ± 0.89) and the remaining groups.

Renal profile biomarkers such as serum urea (mg/dl), serum creatinine (mg/dl) and BUN (mg/dl) were significantly ($P < 0.01$) higher (69.27 ± 3.90 , 3.54 ± 0.15 and 32.35 ± 1.83 , respectively) in group 3 as compared to those of group 1 (19.27 ± 1.00 , 0.69 ± 0.08 and 8.99 ± 0.48 , respectively) at the end of 6th wk. The values of group 2, 4, 5, 6 and 7 did not differ significantly as compared to group 1 at the end of 6th wk.

All the parameters in study revealed that the values in group 6 and 7 were comparable to the values of group 3 giving treatment at the end of 4th wk. The parameters were revived to normal following treatment with zinc in group 6 and 7, and the values were comparable to those of group 1 at the end of 6th wk of experiment.

DISCUSSION

In the present study, significantly lower body weight in toxic controls (3, 6 and 7) was in agreement with the previous reports wherein the adverse effects of salinomycin at higher dose on body weight gain were recorded. In poultry feed, excessive multiplication of saprophytic bacteria results in release of various metabolites that might enhance the toxicity of salinomycin (13) in terms of depressing growth rate. The anorectic properties of ionophores accompanied with poor feed conversion efficiency resulted in depressed body weight gain. Zinc supplementation in group 4 and 5 throughout the study and during the therapy in 6 and 7 improved the body weight gain.

The activities of GSH-Px, glutathione reductase and catalase enzymes and also the GSH levels were determined as they from the components of antioxidant defense system, which effectively scavenges the organic form of hydroperoxide free radicals (14). In toxic controls, the activities of antioxidant enzymes such as GSH-Px, glutathione reductase and catalase were significantly increased, with a significant decrease in GSH level. These findings indicate an ongoing free radical induced damage in the living system (14). This was

supported by the earlier reports (5), where an increase in erythrocyte GSH-Px and catalase activity was observed in chicks fed on toxic dose of monensin. An elevated activity of catalase with decreased GSH levels and GSH-Px activity was reported in chicks treated with 140 mg/kg feed of salinomycin (15). In control and antioxidant supplemented groups, the erythrocyte antioxidant activity remained normal throughout the study. The supplementation of zinc in group 6 and 7 at 6th week resulted in the revival of antioxidant enzyme activity to normal which can be attributed to the antioxidant effect of zinc (7).

The serobiochemical parameters were used as biomarker indices for assessing the extent of tissue damage. Elevated levels of AST and ALT were recorded in toxic controls, which might be due to oxidative damage by free radicals generated by salinomycin resulting in hepatocellular injury. These results are in accordance with certain other reports (16, 17, 18). In group 6 and 7, following zinc supplementation, the enzymatic activity was revived to normal, which could be attributed to the antioxidant property of zinc in feed. As the liver is the exclusive site of protein synthesis (19), salinomycin resulted in a significant hypoproteinaemia, in toxic dose, due to oxidative damage on hepatic cells, whereas in control and group 2, 4 and 5, all the protein variables were normal. In group 6 and 7, following zinc supplementation, the protein variable was revived to normal.

In the present study, the lipid profile such as total cholesterol, triglycerides and LDL were significantly elevated with a significant decrease in HDL in the serum

of toxic controls was recorded in comparison to the control and group 2, 4 and 5. This might serve as a biomarker for cardiac damage as well as renal failure (19), which could be probably due to free radical, induced oxidative damage. The results are in confirmity with Bartov and Jensen (20) who reported a significant increase in plasma cholesterol level in monenein toxicity. In group 6 and 7, zinc supplementation revealed a significant alteration in lipid profile to normal. This could be attributed to the antioxidant property of zinc (7). The free-radical induced

oxidative damage by salinomycin on kidney resulted in significant increase in urea, creatinine and BUN in toxic control. However, the renal index remained normal in group 1, 2, 4 and 5. On zinc supplementation, the values were revived to normal in group 6 and 7.

From this study it is concluded that salinomycin induces toxicity by generating free radicals by disturbing the antioxidant defence, which could be effectively prevented and countered by the use of zinc as an antioxidant.

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