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Concurrent effects of experimental *Escherichia coli* infection and alpha-cypermethrin intoxication on biochemical parameters of broiler chicken

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Abstract
The poultry industry in India is a leading enterprise in the agriculture sector. On one hand where poultry farming can draw large benefit to the firms, conversely the farmers also have to deal with the bacterial and viral infections prevalent among the birds. *Escherichia coli* (*E. coli*) infection is a major bacterial cause of many localised and systemic infection in poultry. With the advent of newer ways of farming, pesticides have also gained much importance in the field. The leaching of these pesticides into the environment poses a great threat to public health as well as the animals. These pesticide residues synergise with the pathogenic effects of bacteria and potentiates in breaching the host immunity. The present study was undertaken with an aim to study the effects of experimentally alpha-cypermethrin intoxicated broiler chicken, co-infected with collibacillosis, on the biochemical parameters. A total number of one hundred and fifty day old chicks were taken for the experimental study. They were orally administered with 1/5th of maximal tolerated dose (i.e. 63.94 mg/kg. body weight) of alpha-cypermethrin through drops and concurrently infected with 10^7 CFU of *E. coli* in 1.0 ml normal saline solution via intra-peritoneal route. The serum of the birds was collected during slaughter for assessing the biochemical parameters. The biochemistry of the serum reflects the soundness of different organs in the body. Biochemical studies of the serum revealed a significant reduction in mean total protein and albumin concentration of both infected groups and alpha-cypermethrin intoxicated group as compared to control. However, serum AST, ALT, ALP and LDH showed significant increase in both infected groups and alpha-cypermethrin intoxicated group. Thus, alpha-cypermethrin toxicity (1/5th maximum tolerated dose) and *E. coli* infection significantly alters the serum biochemical profile.

Keywords: Alpha- cypermethrin, *E. coli*, haematological parameters, serum, maximum tolerated dose (MTD)

Introduction
Poultry sector share a huge chunk towards the economy and is a major source of earning for the farmers. The poultry is also struck harshly with a wide range of infections that poses a serious threat to this sector and may turn the table towards losses. Although observed in all age group, avian collibacillosis is a prevalent infectious disease that causes severe infection in young chick up-to three weeks of age [1]. Colibacillosis is one of the prime infection that incurs heavy loses to the farmers majorly due to mortality and decrease in productivity of the affected birds [2]. The acute form of colibacillosis is characterised by septicaemia resulting in death while subacute form by peri-carditis, air-sacculitis and peri-hepatitis [3]. Poultry culture is emerging globally for fulfilling the rising demand and to elevate the productivity, agrochemicals and pesticides are being used intensively to manage the coinciding elevated feed demand [4]. One of the most preferred insecticides is alpha-cypermethrin which falls under the synthetic pyrethroid category of insecticide. It possesses the ability of bio-magnification and thus tend to be a great risk for public health and animals. The exposure to cypermethrin for long term or in high dosage may cause teratogenicity, reproductive toxicity and genotoxicity in some of the non-targeted species [4]. Most of the disease problems including colibacillosis in the poultry today are usually caused by the interaction of many factors where immunosuppression plays an important key role. Moreover, pesticides like cypermethrin are also being reported to cause immunosuppression that contributes towards synergistic ill effect with infections in poultry [5]. There is paucity of literature in concurrent effects of alpha-cypermethrin toxicity and *E. coli* infection on biochemical status of broiler chicken. Considering the above points, the present study was adopted to evaluate the biochemical alteration in serum of broiler chicken experimentally intoxicated with alpha-cypermethrin and *E. coli* infection.

Materials and Methods

The study was conducted on a total number of one hundred and fifty, day old broiler chicks after the approval of the Institutional Animal Ethics Committee (VCC/IAEC/20191930-1953).

Experimental design and sample collection

The present study was undertaken in total one hundred and fifty, day old broiler chicks for evaluating the effects of alpha-cypermethrin toxicity and its interaction with *E. coli* infection on biochemical parameters. Maximum tolerated dose (MTD) of alpha-cypermethrin used in the present study for broiler chicken was calculated to be 319.69 mg/kg body weight (b. wt.). At the age of seven days, the chicks were randomly divided into two groups i.e. group A and group B containing 70 and 80 chicks, respectively. All the birds of group B were orally given 1/5th of MTD of alpha-cypermethrin (i. e. 63.94 mg/kg b. wt.) through drops and the group A was kept as control. At the age of 14 days the birds of group B were divided into two subgroups i.e. B_1 and B_2 consisting of 40 birds each. Similarly, group A was also divided into two subgroups i.e. A_1 and A_2 containing 30 and 40 birds, respectively. Thereafter, each bird of subgroups A_2 and B_2 were intraperitoneally injected with 10^7CFU *E. coli* in 1.0 ml normal saline solution (NSS). The experiment was terminated on forty second day. The serum samples were collected directly from heart of six birds from each subgroup on 0, 3rd, 11th, 21st and 28th days post infection (dpi) of *E. coli* in sterile serum collection vials for biochemical studies. A brief tabulated form of experimental design is given in Table 1.

Table 1: Experimental design and sample collection

Group	Sub-group	Treatment given	Serum collection post infection (days)
A	A1	Negative control group	0, 3, 11, 21 and 28
	A2	10^7 CFU of *E. coli*/ml, intraperitoneally	0, 3, 11, 21 and 28
B	B1	1/5th MTD of alpha-cypermethrin, orally	0, 3, 11, 21 and 28
	B2	10^7 CFU of *E. coli*/ml + alpha-cypermethrin	0, 3, 11, 21 and 28

Biochemical examination

The serum from the birds was aseptically collected in fresh serum collection vials for further biochemical examination by using semi- automatic biochemistry analyser (Erba Mannheim Chem-5 Plus, Transasia) and different associated kits.

Total serum protein concentration

Total serum protein concentration was analysed as per the method of Tietz [6] using single step reagent kits by semi-automatic biochemistry analyser.

Serum albumin concentration

Serum albumin concentration was analysed as per the method of Doumas *et al.* [7] using single step reagent kits by semiautomatic biochemistry analyser.

Serum aspartate amino transaminase (AST)/ Serum glutamic oxaloacetic transaminase (SGOT)

Serum aspartate transaminase activity was estimated by the standard methods of International Federation of Clinical Chemistry using single step reagent kits employing semiautomatic biochemistry analyser [6].

Serum alanine amino transaminase (ALT)/ Serum glutamic pyruvic transaminase (SGPT)

Serum alanine transaminase activity was estimated by the standard methods of International Federation of Clinical Chemistry using single step reagent kits employing semiautomatic biochemistry analyser [8].

Serum lactate dehydrogenase (LDH)

Serum lactate dehydrogenase activity was estimated by using single step reagent kits employing semiautomatic biochemistry analyser [6].

Serum alkaline phosphatase (ALP)

Serum alkaline phosphatase activity was estimated by using single step reagent kits employing semiautomatic biochemistry analyser [6].

Statistical analysis

The data for various parameters were subjected to statistical analysis by using Duncan Multiple Range Test as modified by Krammer [9] at 5.0% level of significance using SPSS 16.0 version software. Individual means were compared for statistical significance using least significance difference.

Results and Discussion

The present study was conducted with an objective to detail the concurrent effects of alpha-cypermethrin and *E. coli* infection on serum biochemical profile of broiler chicken. The biochemical analysis of the serum aids in contemplating the stability of organs and the effects of infection on these organs. Serum biochemical studies revealed that mean total serum protein concentration in group B_1 and B2 was significantly ($P \leq 0.05$) lower from 21 days post infection (DPI)/28 days post cypermethrin treatment (DPCT) as compared to control group (A_1). The Mean ± S.E. total serum protein concentration in groups A_1, B_1, A_2 and B_2 ranged from $3.59^a \pm 0.33$ to $3.89^b \pm 0.19$, $3.06^a \pm 0.14$ to $2.79^a \pm 0.19$, $3.19^a \pm 0.13$ to $3.59^b \pm 0.33$, and $2.96^a \pm 0.19$ to $2.63^a \pm 0.21$, respectively and are depicted in Fig.1. Similar to the present study, Yousef *et al.* [10] reported a significant decrease in total protein in rabbits treated with cypermethrin and isoflavones. Garg *et al.* [11] also recorded a similar decrease in total serum protein in broiler chicks intoxicated with a synthetic pyrethroid, organophosphate and chlorinated pesticides. Grewal *et al.* [12] reported cypermethrin toxicity in rats, by administering cypermethrin @ 14.5 mg/kg b. wt. once daily for 30 days, caused a significant decrease in the level of serum proteins. Narayani [13] reported sub-acute exposure of alphamethrin/alpha-cypermethrin in broiler chicks leads to a significant decrease in total serum protein concentration. Mean total serum protein concentrations in all infected groups (A_2 and B_2) were lower as compared to control group (A_1) throughout the experiment though this decrease was significant ($P \leq 0.05$) from 11 DPI/ 18 DPCT onwards when compared to control group (A_1). The total serum protein concentration in the birds of group B_2 as compared to group A_2 was significantly ($P \leq 0.05$) decreased on 3 DPI/10 DPCT, 21 DPI/28 DPCT and 28 DPI/35 DPCT. These observations were in consonance with work of earlier authors on *E. coli* infection [14, 15, 16, 17].

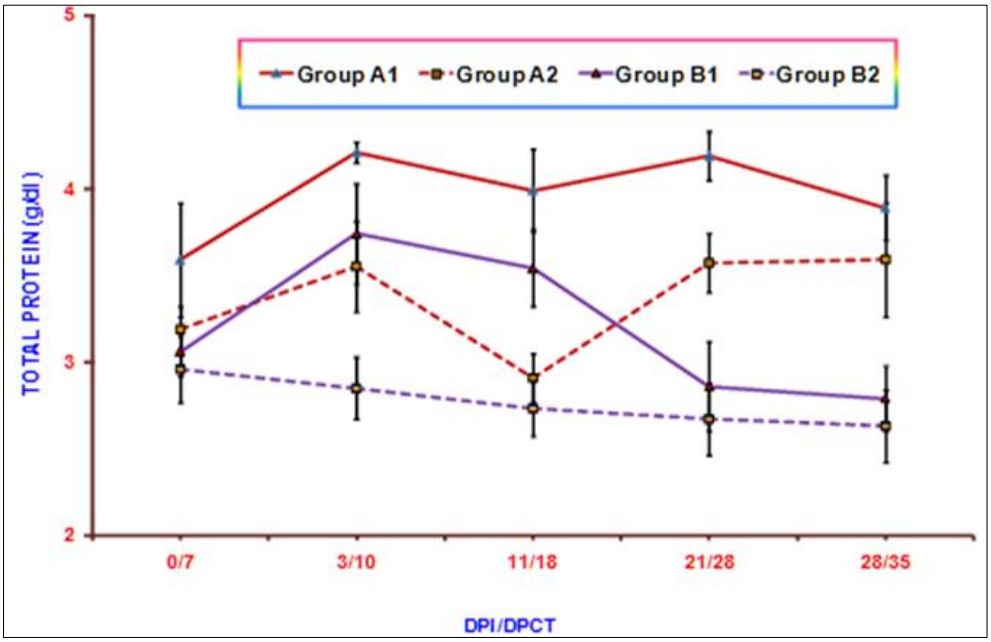

Fig 1: Total serum protein concentration (g/dl) of broiler chicks in different experimental groups at different time intervals (Mean ± S.E.)

The Mean ± S.E. serum albumin concentration in groups A_1, B_1, A_2 and B_2 ranged from $2.55^{a}\pm0.34$ to $2.57^{b}\pm0.14$, $2.22^{a}\pm0.06$ to $1.59^{a}\pm0.18$, $2.38^{a}\pm0.05$ to $2.47^{b}\pm0.08$and $2.16^{a}\pm0.07$ to $1.84^{a}\pm0.21$, respectively and are presented in Fig. 2. Mean serum albumin concentrations in both the *E. coli* infected groups (A_2 and B_2) were lower as compared to control group (A_1) throughout the experiment. Similar findings were reported by other workers in *E. coli* infection [17, 18]. According to Blood *et al.* [19], hypoproteinemic conditions due to damage to kidneys and liver might also contribute to reduced albumin and total protein synthesis which has also been observed in the present study.

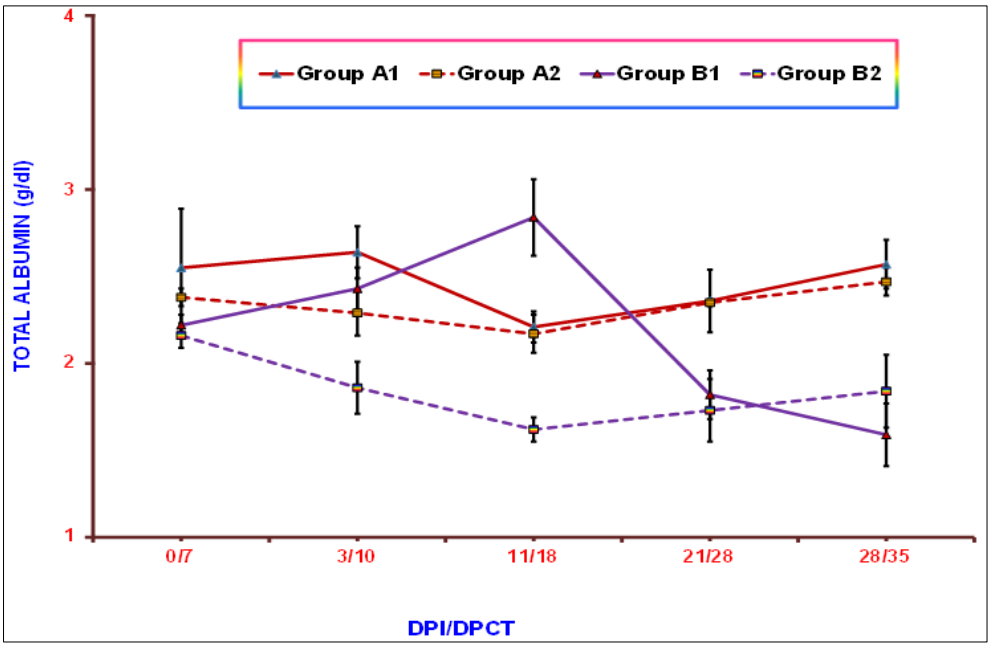

Fig 2: Serum albumin concentration (g/dl) of broiler chicks in different experimental groups at different time intervals (Mean ± S.E.)

The Mean ± S.E. serum aspartate transaminase activity in groups A_1, B_1, A_2 and B_2, ranged from $197.73^{a}\pm1.61$ to $196.36^{a}\pm2.68$, $201.45^{a}\pm7.89$ to $256.68^{b}\pm14.02$, $211.2^{a}\pm7.03$ to $211.77^{a}\pm10.87$, and $206.34^{a}\pm11.62$ to $253.96^{b}\pm13.33$, respectively while serum alanine transaminase activity in groups A_1, B_1, A_2 and B_2, ranged from $11.36^{a}\pm0.55$ to $11.62^{a}\pm0.47$, $13.53^{a}\pm1.50$ to $18.32^{a}\pm4.19$, $13.67^{a}\pm1.32$ to $26.65^{b}\pm4.46$, and $15.00^{a}\pm1.64$ to $28.15^{b}\pm3.81$, respectively, as depicted in Fig. 3 and 4. The mean values of AST activity were found increased in the birds of group B_1 throughout the experiment as compared to control group (A_1) and this increase was significant ($P\leq0.05$) from 28 DPCT onwards. Birds of group B_1 also showed an increase in serum ALT activity as compared to control group (A_1) throughout the experiment although this was significant ($P\leq0.05$) from 18 DPCT onwards. Mean serum aspartate transaminase (AST) and alanine transaminase (ALT) activities were combinedly found to be significantly ($P\leq0.05$) increased in all the *E. coli* infected groups from 3 DPI/10 DPCT onwards. The increased serum ALT and AST activity due to *E. coli* infection in broiler chicken was also reported by earlier authors [17, 20, 21, 22, 23]. A similar increase in these enzymes was also reported in

the serum of rats intoxicated with deltamethrin [24]. The increased serum AST activity indicates cellular injury to hepatic cells and cardiac muscles while elevated serum ALT is mainly due to hepatic injuries. ALT is a liver specific enzyme that is a reliable indicator of hepatic cell injury or damage [25]. Enzymes are localized in cytoplasm and thus there might be chances of their secretion and release into systemic blood circulation after structural and cellular injury to hepatic parenchyma and this can be indicated by an elevation of their level in serum.

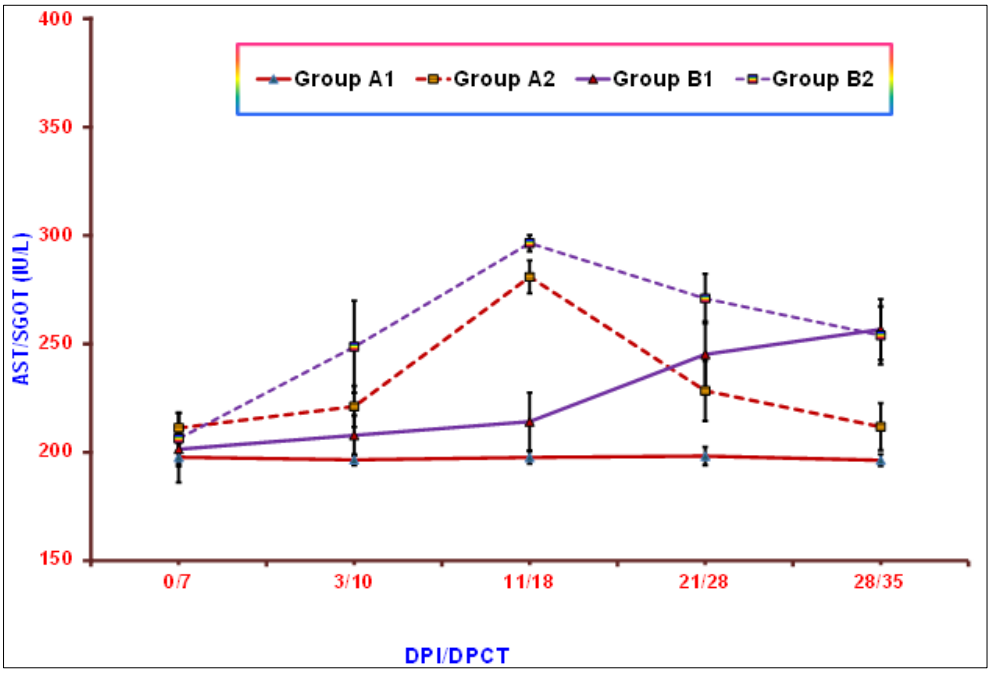

Fig 3: Serum aspartate transaminase activity (AST/SGOT, IU/L) of broiler chicks in different groups at different time intervals (Mean ± S.E.).

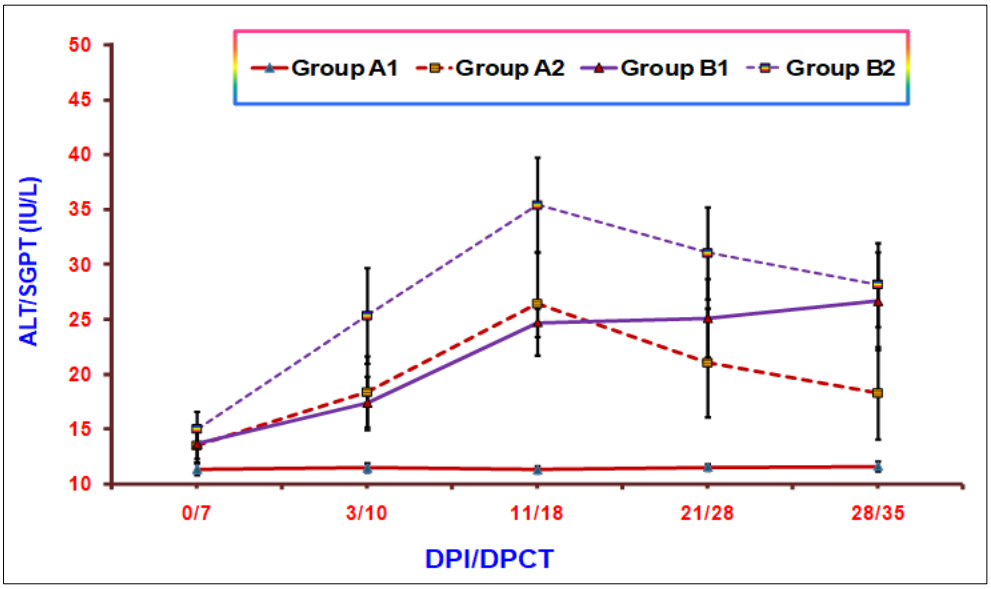

Fig 4: Serum alanine transaminase activity (ALT/SGPT, IU/L) of broiler chicks in different groups at different time intervals (Mean ± S.E.).

The Mean ± S.E. serum lactate dehydrogenase activity in groups A_1, B_1, A_2 and B_2 ranged from 333.87^{a}±12.60 to 342.90^{a}±5.88, 388.20^{b}±10.29 to 422.32^{c}±9.93, 211.20^{a}±7.03 to 211.77^{a}±10.87and 362.35^{ab}±11.17 to 498.15^{d}±13.59, respectively as illustrated in Fig. 5. In the present study, mean values of LDH were found significantly (P≤0.05) increased in the birds of group B_1 from 3 DPCT onwards as compared to the birds of control group (A_1). Level of LDH can elevate in many toxicopathological conditions like hepatocellular necrosis, renal necrosis, myocardial damage, pancreatic necrosis and hemolysis. Similar findings were also reported by authors earlier [12, 26]. They found significant increase in LDH following administration of deltamethrin in poultry birds. Mean values of lactate dehydrogenase (LDH) were also significantly (P≤0.05) higher in the birds of both the *E. coli* infected groups (A_2 and B_2) as compared to the birds of control group (A_1) throughout the experiment. This elevation in LDH, in the birds of group B_1 was observed from 3 DPCT onwards. Similar findings were also documented by Singh [27] following concurrent infection of Salmonella and chlorpyrifos in poultry.

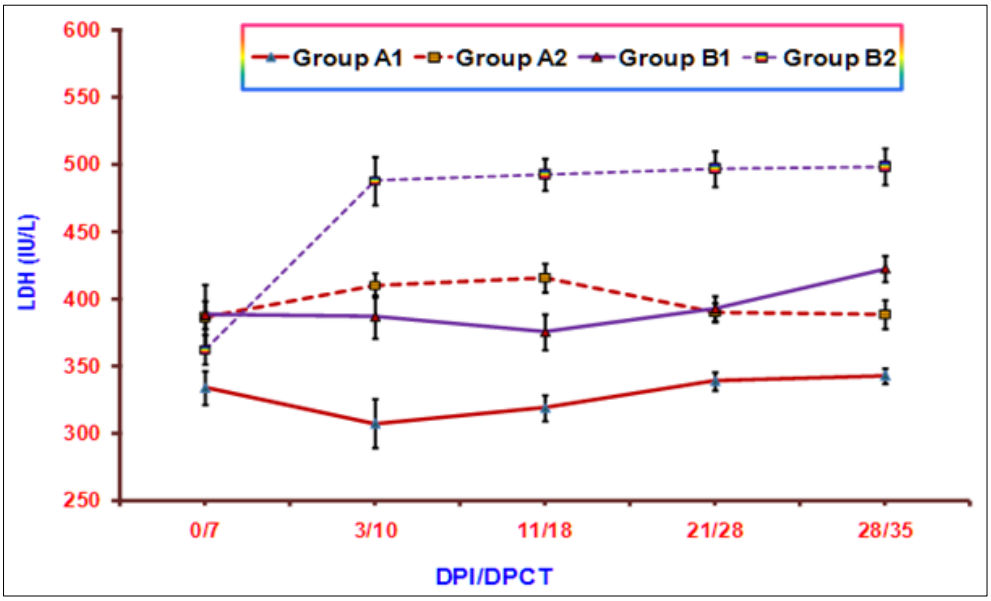

Fig 5: Serum lactate dehydrogenase (LDH, IU/L) of broiler chicks in different experimental groups at different time intervals (Mean ± S.E.).

The Mean ± S.E. serum alkaline phosphatase activity in groups A_1, B_1, A_2 and B_2 ranged from 391.33^{a}±5.14 to 427.90^{a}±15.57, 393.91^{a}±3.12 to 552.37^{bc}±21.79, 384.92^{a}±4.41 to 505.45^{b}±16.81 and 389.04^{a}±2.44 to 592.58^{c}±20.26, respectively and is graphically represented in in Fig. 6. In the present study, the mean values of serum ALP activity were found to be increased in the birds of group B_1 as compared to the control group (A_1) throughout the experiment and this increase was significant (P≤0.05) on 21 DPI/28 DPCT and 28 DPI/35 DPCT. Mean value of alkaline phosphatase (ALP) activity was higher in the birds of both the *E. coli* infected groups (A_2 and B_2) that was found to be significantly (P≤0.05) increased from 11 DPI/18 DPCT onwards. Tiwari [28] also reported the similar findings of elevated ALP in *E. coli* infected broiler chicken with deltamethrin toxicity. The findings akin to the present study were also reported in rats intoxicated with alpha-cypermethrin [29]. Alkaline phosphatase enzyme catalyzes several reactions in the body and helps in active transport of phosphate, synthesis of proteins and DNA turnover in the nucleus. According to Kaplan and Righetti [30], if cellular damage occurs in the body of an animal then phosphatase is released into the circulation and results in higher level of ALP in the blood.

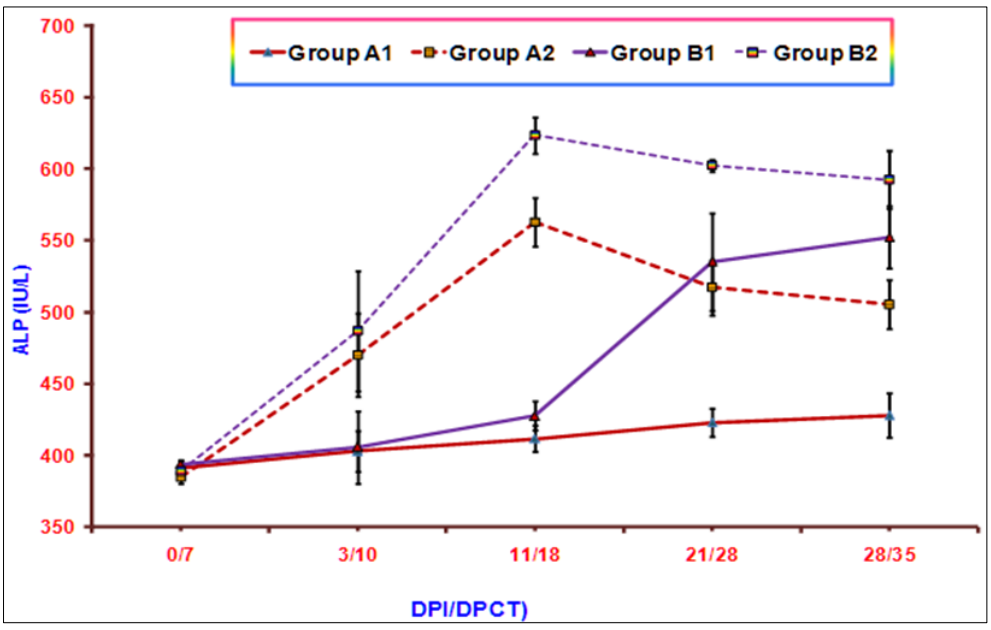

Fig 6: Serum alkaline phosphatase activity (ALP, IU/L) of broiler chicks in different experimental groups at different time intervals (Mean ± S.E.).

Conclusion

In the present study, attempts were to evaluate the effects of alpha-cypermethrin toxicity in broiler chicken co-infected with *E. coli*, on the biochemistry of serum. Based on 42 day long experimental trial, it can be concluded that *E. coli* infection can be aggravated by alpha cypermethrin toxicity. The *E. coli* infection can further deteriorate the organ function with alpha-cypermethrin toxicity, as reflected by the biochemical profile in serum. The total protein and serum albumin level were significantly decreased while a significant spike was reported in the levels of AST, ALT, LDH and ALP. The biochemical analysis of serum indicated towards the

damaged and compromised function of the organs.

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References

1. Kabir, S.M. Avian colibacillosis and salmonellosis: a closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns. International journal of environmental research and public health. 2010;7(1):89-114.
2. Otaki Y. Poultry disease control programme in Japan. Asian Livestock (FAO), 1995.
3. Saif YM, Barnes HJ, Glisson JR, Fadly AM, McDougald LR, Swayne DE. Disease of Poultry. 11th Edn., Iowa State Press, Iowa, 2003, 452-514.
4. Kaushik D, Sharma RK, Sharma S. Attenuating effects of ascorbic acid on cypermethrin induced histological and biochemical changes in developing brain of *Gallus domesticus*. Journal of Pharmacognosy and Phytochemistry. 2018;7(6):1108-1112.
5. Rehman H, Aziz AT, Saggu SH, Abbas ZK, Mohan A, Ansari AA. Systematic review on pyrethroid toxicity with special reference to deltamethrin. Journal of Entomology and Zoology Studies. 2014;2(6):60-70.
6. Tietz NW. Fundamentals of Clinical Chemistry. W.B. Saunders Co., Philadelphia, 1986.
7. Doumas BT, Arends RL, Pinto PVC. Standard Methods of Clinical Chemistry, Academic Press, Chicago. 1972;7:175-189.
8. Wroblewski F, La Due JS. Serum glutamic pyruvic transaminase in cardiac and hepatic disease. Proceedings of the Society for Experimental Biology and Medicine. 1956;91:569-571.
9. Krammer CY. Extension of multiple range tests to group correlated adjusted means. Biometrics. 1957;13(1):13-18.
10. Yousef MI, El-Demerdash FM, Kamel KI, Al-Salhen KS. Changes in some hematological and biochemical indices of rabbits induced by isoflavones and cypermethrin. Toxicology. 2003;189(3):223-234.
11. Garg UK, Pal AK, Jha GJ, Jadhao SB. Pathophysiological effects of chronic toxicity with synthetic pyrethroid, organophosphate and chlorinated pesticides on bone health of broiler chicks. Toxicologic Pathology. 2004;32(3):364-369.
12. Grewal G, Verma PK, Dhar VJ, Srivastava AK. Toxicity of subacute oral administration of cypermethrin in rats with special reference to histopathological changes. International Journal of Green Pharmacy. 2010;3(4):293-299.
13. Narayani. Toxicopathological studies on alphamethrin in broiler chicks. M.V.Sc Thesis submitted to Indira Gandhi Krishi Vishwavidyalaya, Raipur, India, 2010.
14. Saini V. Studies on the effect of neem (Azadirachta indica) leaf extract on the pathology and pathogenesis of E. coli infection in broiler chicks. M.V.Sc. thesis submitted to Chaudhary Charan Singh Haryana Agricultural University, Hisar, 2004.
15. Arshad N, Neubauer C, Hasnain S, Hess M. Peganum harmala can minimize *Escherichia coli* Infection in Poultry, but long-term feeding may induce side effects. Poult. Science. 2007;87(2):240–249.
16. Zaki MS, Fawzy O, Osfor MH. Effect of *E. coli* OH157 on baladi broiler chicken and some biochemical studies. Life Science Journal. 2012;9(1):91-94.
17. Kumari M, Gupta RP. Sequential pathological studies of experimental *Escherichia coli* infection in broiler chickens. Veterinary Practitioner. 2014;15(2):299-302.
18. Chandra N, Jain NK, Singh, RP. Effect of alpha-tocopherol on hemato-biochemical profiles of broilers in deltamethrin induced toxicity. Journal of Cell & Tissue Research. 2016;16(3):5863-5868.
19. Blood DC, Radostis OM, Gay CC, Arundel CH, Ikede BO, Mckenzie RA, *et al*. Veterinary Medicine 8th edn. The English Language Book Society and Bailliere Tindall, Eastbourne, 1994.
20. Eleiwa NZH, Sayed EME, Nazim AA. Prophylactic and therapeutic evaluation of the phytobiotic (Orego-stim) in chicken experimentally infected with *E. coli*. Journal of American Science. 2011;7(8):91-102.
21. Zaki MS, Fawzy O, Osfor MH. Effect of *E. coli* OH157 on baladi broiler chicken and some biochemical studies. Life Science Journal. 2012;9(1):91-94.
22. Abd El-Ghany WA, Ismail M. Tackling experimental colisepticaemia in broiler chickens using phytobiotic essential oils and antibiotic alone or in combination. Iranian Journal of Veterinary Research. 2014;15(2):110-115.
23. Sharma V, Jakhar KK, Dahiya S. Immuno-pathological studies on broiler chicken experimentally infected with Escherichia coli and supplemented with neem (Azadirachta indica) leaf extract. Veterinary World. 2016;9(7):735-741.
24. Tewari A, Banga HS, Gill JPS. Sub lethal chronic effects of oral dietary exposure to deltamethrin in Swiss albino mice. Toxicology and Industrial Health. 2018;34(6):423-432.
25. Ozer JM, Ratner M, Shaw W, Bailey, Schomaker, S. The current state of serum biomarkers of hepatotoxicity. Toxicology. 2008;245(3):194-205.
26. Yousef MI, Awad TI, Mohamed EH. Deltamethrin-induced oxidative damage and biochemical alterations in rat and its attenuation by Vitamin E. Toxicology. 2006;227(3):240–247.
27. Singh R. Pathobiological and immunological studies on thiacloprid toxicity and its interaction with *Salmonella* Gallinarum infection in broiler chickens. PhD. Thesis submitted to Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, 2017.
28. Tiwari S. Pathobiological and immunological studies on deltamethrin toxicity and its interaction with *Escherichia coli* infection in broiler chicken. Ph.D. Thesis submitted to Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, 2018.
29. Hocine L, Merzouk H, Merzouk SA, Ghorzi H, Youbi M, Narce M. The effects of alpha-cypermethrin exposure on biochemical and redox parameters in pregnant rats and their newborns. Pesticide biochemistry and physiology. 2016;134(10):49-54.
30. Kaplan MM, Righetti A. Induction of rat liver alkaline phosphatase: the mechanism of the serum elevation in bile duct obstruction. Journal of Clinical Investigation.s 1970;49(3):508-516.