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Biochemical Studies in Experimentally *Escherichia Coli* Infected Roiler Chicken Supplemented with Amla (*Emblica officinalis*) Extract

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ABSTRACT

The present study was undertaken in broiler chickens to study the effect of dry fruit extract of amla supplementation on biochemical changes in relation to the severity of colibacillosis. Experimental colibacillosis could be produced in broiler chicks by intraperitoneal inoculation of *E. coli* O120 after 12 hours of the infection as evidenced by clinical signs. One hundred and sixty-eight day old healthy broiler chicks were procured from a local hatchery. These chicks were divided into two groups (A and B) containing eighty-four birds each. Diet of all the chicks of group A was supplemented with grinded dry fruit extract of Amla at the rate of 10g/kg of feed whereas; chicks of group B were given normal feed and water. At the age of 7 days chicks of group A1 and B1 were injected intraperitoneally with standard infective dose of pathogenic *E. coli*. (i.e. at the rate of 10^7 CFU of *E. coli*/0.5 ml). During the experiment blood was collected from six chicks from each group at days 0, 3, 7, 14, 21, 28 post-infection for biochemical studies. Serum samples collected for biochemical studies revealed significant increase in serum alanine transaminase, aspartate transaminase, lactate dehydrogenase activities and decrease in creatine phosphokinase activity. Decrease in total protein, albumin concentrations in both the infected groups was also noticed but this was non-significant. On the basis of results of the present study it is concluded that 10g/kg dry fruit extract of amla supplementation significantly reduced the severity and recovery period of colibacillosis in chicks as evidenced by biochemical parameters.

Keywords: Amla, biochemical parameters, broiler chicken; *Escherichia coli* infection

India has a population of around 1.23 billion and that number is growing rapidly every year. Poultry industry in India has become one of the fastest growing industries and a leading industry in the field of agriculture. India has become third largest egg producer in the world with a production of around 84 billion eggs every year and fourth largest broiler producer after China, Brazil and USA. India's poultry industry has shifted itself from traditional mere backyard poultry farming to the recent Integration farming system. According to the 19th Livestock Census 2012, the total number of poultry population in India was 7, 29,209 thousands including fowl, ducks, turkeys and others. Though there is a huge improvement in poultry sector, the health status of the birds at still at risk. Poultry birds are affected by various diseases that affect their production and growth. Amongst these, the conditions affecting the gastro-intestinal tract are quite common and include salmonellosis, colibacillosis, ranikhet disease, coccidiosis, necrotic enteritis etc. Importance of these diseases can be judged from the fact that incidence of coccidiosis was found to be 15.5% followed by *Escherichia coli* (*E. coli*) infections (14%), fowl typhoid, fatty liver syndrome and Ranikhet disease (Suresh *et al*., 1990). These infections lead to pathological changes in different systems of the poultry, therefore it is pertinent to study these changes, aiding to diagnosis. Infection with *E. coli*, a Gram-negative bacterium, is frequently diagnosed in poultry, where they usually start as a respiratory infection. Avian colibacillosis is a bacterial disease of birds caused by *E. coli*, which is considered as one of the

principal causes of morbidity and mortality, associated with heavy economic losses to the poultry industry as it is associated with various disease conditions, either as primary pathogen or as a secondary pathogen (Kabir, 2010). *E. coli* in poultry, referred to as avian pathogenic *E. coli* (APEC), adhere to mannose sugars located on the epithelial cells of the trachea by means of their F1 pili. After this initial colonisation, the bacteria spread to the lungs and air sacs, giving rise to air-sacculitis. By efficient evasion of the host's immune defences, *E. coli* are able to pass into the bloodstream, leading to infection of the internal organs, for example, the pericardium (pericarditis), liver (perihepatitis), peritoneum (peritonitis) and oviduct (salpingitis), colisepticemia, coligranuloma, synovitis etc (Vandemaele *et al*., 2002). These diseases are responsible for significant economic losses to poultry industry (Gross,

Table 1: Mean values of different biochemical parameters of experimentally *E. coli* infected broiler chicken supplemented with 10 g/kg dry fruit extract of amla (mean ± S.E.)

Parameters	Groups	Post-infection days					
		0	3	7	14	21	28
AST (IU/L)	*E. coli* + Amla	144.53^{a}±7.33	281.15^{b}±5.45	331.47^{c}±2.84	316.03^{c}±2.17	292.97^{c}±3.02	265.67^{c}±3.48
	E. coli	173.18^{b}±7.40	298.57^{c}±3.41	382.18^{d}±2.77	339.27^{d}±2.10	310.72^{d}±2.53	289.88^{d}±3.75
	Amla	180.95^{b}±2.46	184.93^{a}±8.38	203.52^{b}±2.60	192.63^{b}±2.73	197.7^{b}±3.00	212.88^{b}±2.73
	Control	167.51^{b}±5.24	184.33^{a}±2.78	185.85^{a}±2.70	156.63^{a}±4.06	181.23^{a}±1.77	191.27^{a}±2.24
ALT(IU/L)	*E. coli* + Amla	4.03^{b}±0.72	6.20^{ab}±1.32	8.35 ^{b}±1.82	8.91 ^{a}±1.09	3.69 ^{a}±1.04	2.64 ^{a}±0.73
	E. coli	5.97^{ab}±1.34	8.36 ^{b}±1.53	9.60 ^{b}±1.14	11.78^{b}±0.71	5.60 ^{a}±0.37	5.02 ^{b}±0.46
	Amla	3.23^{a}±0.50	5.39 ^{a}±1.32	4.99 ^{a}±0.56	4.32 ^{a} ±0.51	4.24 ^{a}±0.43	3.21^{a}±0.40
	Control	3.40^{ab}±0.44	6.79 ^{a}±0.52	5.18 ^{a}±0.49	5.41 ^{a} ±0.60	4.27 ^{a}±0.44	3.67 ^{a} ±0.46
Total protein (g/dl)	*E. coli* + Amla	2.72^{a}±0.26	4.96 ^{a}±0.20	4.37^{b}±0.11	4.04^{b}±0.51	3.28^{ab}±0.42	3.53^{a}±0.39
	E. coli	2.52^{a}±0.11	4.41 ^{a}±0.15	4.00^{a}±0.07	3.05^{ab}±0.44	2.45^{a}±0.09	2.77^{a}±0.14
	Amla	2.51^{a}±0.10	3.18 ^{a}±0.23	3.26^{ab}±0.32	4.33^{c}±0.21	4.61^{a}±0.25	4.65^{b}±0.15
	Control	2.47^{a}±0.12	2.74 ^{a}±0.26	2.93^{a}±0.26	3.37^{b}±0.31	4.31^{ab}±0.11	4.45^{a}±0.19
Albumin(g/dl)	*E. coli* + Amla	1.51^{b}±0.09	1.26^{ab}±0.08	1.22^{a} ±0.07	1.26^{b}±0.20	1.59^{a}±0.20	3.42^{b}±0.54
	E. coli	1.34^{ab}±0.05	1.15^{a}±0.08	1.06^{a} ±0.15	1.19^{b}±0.08	1.16^{a}±0.26	3.22^{b}±0.42
	Amla	1.51^{b}±0.09	1.30^{a}±0.02	1.62^{b}±0.07	1.14^{b}±0.05	1.51^{a}±0.20	2.35^{ab}±0.13
	Control	1.18^{a}±0.04	1.22^{a}±0.05	1.34^{ab}±0.19	0.78^{a}±0.04	1.20^{a}±0.15	1.90^{a}±0.13
LDH(IU/L)	*E. coli* + Amla	282.70 ^{b} ±1.94	469.44^{c}±2.83	469.11^{b}±3.81	408.55^{b}±5.91	382.70^{c}±2.33	380.32^{c}±2.91
	E. coli	280.35 ^{a} ±2.29	539.24^{d}±3.82	486.57^{c}±2.88	480.75^{c}±4.19	465.82^{d}±2.92	363.21^{d}±4.53
	Amla	283.97^{ab} ±2.25	265.74 ^{a} ±3.03	276.84^{a}±2.72	320.15^{a}±3.87	336.07^{b}±4.30	318.16^{b}±4.94
	Control	290.73 ^{b} ±2.87	276.11^{b} ±2.49	276.92 ^{a} ±4.24	329.46 ^{a} ±4.99	259.95^{a}±3.54	282.22 ^{a} ±2.74
CPK(IU/L)	*E. coli* + Amla	1.53^{a}±0.22	0.38 ^{a}±0.04	0.28 ^{a}±0.03	1.26 ^{a}±0.08	1.14^{a}±0.07	1.13^{a}±0.20
	E. coli	1.75^{a}±0.12	0.43 ^{a}±0.01	0.43^{b}±0.07	1.39 ^{a}±0.07	1.31^{a}±0.09	1.25^{a}±0.07
	Amla	1.57^{a}±0.09	0.52^{ab}±0.03	0.48^{b}±0.03	1.38 ^{a}±0.11	1.19^{a}±0.05	1.08^{a}±0.06
	Control	1.59 ^{a}±0.10	0.61^{b}±0.07	0.52^{b}±0.03	1.38 ^{a}±0.13	1.27^{a}±0.06	1.09^{a}±0.06

AST: Aspartate transaminase, ALT: Alanine transaminase, LDH: Lactate dehydrogenase, CPK: Creatine phosphokinase

a, b, c, d : Means with unlike superscript letters in a column are significantly different, $P < 0.05$

E. coli + Amla*: Escherichia coli* infected and dry fruit extract of amla supplemented group

E. coli: Escherichia coli infected and without amla extract supplemented group,

Amla: only amla supplemented group

1961). *E. coli* turns pathogenic under adverse conditions of poor ventilation, overcrowding, immunosuppression etc (Goswami *et al*., 2004).

Rural population of India quite frequently uses medicinal plants for the treatment of some common infections of livestock and poultry. The medicinal properties of *Emblica officinalis* (*E. officinalis*) that is also commonly called as "Indian gooseberry" or "Amla" can be traced back in the ancient medical treatise like Ayurveda. Literature revealed that dietary addition of *E. officinalis* (Amla) fruit powder had a positive effect on growth performance in commercial broiler chickens (Patel *et al*., 2016). Heat stress leads to generation of free radicals and production of oxidative stress in almost every species (Lakhani *et al*., 2016). So, amla is the most potent source of ascorbic acid, tannins and flavonoids which helps to ameliorate the heat stress in goats (Kumar *et al*., 2010), cows (Ul-Haq *et al*., 2013) and buffaloes (Sunilkumar *et al*., 2010). Keeping in view the above facts, the present study will be undertaken with present study was undertaken in broiler chickens to study the effect of dry fruit extract of amla supplementation on biochemical changes.

MATERIALS AND METHODS

Ethical approval

The approval for conducting the experiment was taken from the Institutional Animal Ethics Committee (IAEC), Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar (1669/GO/abc/12/CPCSEA).

Experimental design

One hundred and sixty-eight day old healthy broiler chicks were procured from a local hatchery. All the birds were provided with fresh, clean drinking water and fed *ad libitum* throughout the experiment. The chicks were reared in the departmental animal house under strict hygienic conditions and were given feed and water ad libitum. These chicks were divided into two groups (A and B) containing eighty-four birds each. Diet of all the chicks of group A was supplemented with grinded dry fruit of Amla at the rate of 10g/kg of feed whereas, chicks of group B were given feed and water devoid of dry fruit extract of Amla supplementation throughout the experiment. After rearing for one week chicks of both the groups (A and B) were again divided into two subgroups (group A into A1 & A2 and group B into B1 & B2) containing 48 and 36 birds each, respectively. At the age of 7 days chicks of group A1 and B1 were injected intraperitoneally with standard infective dose of pathogenic *E. coli* (i.e. at the rate of 10^7 CFU of *E. coli*/0.5 ml). During the experiment blood was collected from six chicks from each group at day 0, 3, 7, 14, 21, 28 days post-infection (DPI) for biochemical studies. After collection of blood, Serum samples separated at different intervals were analysed for biochemical studies.

Dry fruit extract of Amla (*E. officinalis*)

Dry fruits of Amla were collected from local market and it was grinded and made into powdered form. It was mixed equally with broiler feed at the rate of 10g/kg of feed (Nakhajothi *et al*., 2009). The water provided to all the chicks was boiled and subsequently cooled. The feed and water were given *ad libitum*.

Preparation of *E. coli* inoculum

E. coli (serotype O120) isolated from naturally infected cases, was inoculated into Brain Heart Infusion (BHI) broth and incubated at 37°C for 24 h. Viable count of *E. coli* organism per ml of BHI was determined by surface spread method as described certain group of scientists (Cruickshank *et al.,* 1975). Serial 10 fold dilutions of the above culture were prepared in the sterile phosphate buffer saline (PBS) and 0.1ml of each dilution was pipetted onto three MacConkey's Lactose Agar (MLA) plates. The inoculum on the plates was spread with the help of a sterile spreader and then these plates were incubated at 37°C for 24 h. The average count of three plates of particular dilution having colonies in the range of 30-300 was calculated. This bacterial count for particular dilution was made in 0.1 ml, the inoculum that was used for each dilution. Then the viable count per ml was determined which was considered as Colony Forming Units (CFU) of the *E. coli*. The infective dose at the rate of 10^7 CFU of *E. coli*/0.5 ml was prepared that was used for the experiment as *E. coli* inoculum (Jindal *et al.,* 2003).

Biochemical studies

Serum samples collected at different intervals were analyzed for the following parameters:

Total protein and albumin concentrations

Total protein and albumin concentrations were analyzed using single step reagent kit employing chemistry analyzer employing Semiautomatic Biochemistry Analyzer (Erba Mannheim Chem-5 Plus, Transasia) and different kits procured from ERBA diagnostics Mannheim GmbH (Transasia Bio-Medicals Ltd.) (Doumas *et al*., 1972; Tietz, 1986a; Tietz, 1986b).

Aspartate transaminase (AST) activity: AST activity was measured by the standard methods of international Federation of Clinical Chemistry using single step reagent kits employing chemistry analyzer employing Semiautomatic Biochemistry Analyzer (Erba Mannheim Chem-5 Plus, Transasia) and different kits procured from ERBA diagnostics Mannheim GmbH (Transasia Bio-Medicals Ltd.) (Tietz, 1986a; Tietz, 1986b).

Alanine transaminase (ALT) activity: ALT activity was measured by the standard methods of international Federation of Clinical Chemistry using single step reagent kits employing chemistry analyzer employing Semiautomatic Biochemistry Analyzer (Erba Mannheim Chem-5 Plus, Transasia) and different kits procured from ERBA diagnostics Mannheim GmbH (Transasia Bio-Medicals Ltd.) (Tietz, 1986a; Tietz, 1986b).

Lactate dehydrogenase (LDH) activity: LDH activity was measured using single step reagent kits employing chemistry analyzer employing Semiautomatic Biochemistry Analyzer (Erba Mannheim Chem-5 Plus, Transasia) and different kits procured from ERBA diagnostics Mannheim GmbH (Transasia Bio-Medicals Ltd.) (Doumas *et al*., 1972; Tietz, 1986a; Tietz, 1986b).

Creatine phosphokinase (CPK) activity: CPK activity was measured using single step reagent kits employing chemistry analyzer employing Semiautomatic Biochemistry Analyzer (Erba Mannheim Chem-5 Plus, Transasia) and different kits procured from ERBA diagnostics Mannheim GmbH (Transasia Bio-Medicals Ltd.) (Tietz, 1986a).

RESULTS AND DISCUSSION

Mean values of biochemical parameters in different experimental groups are given in Table 1. The mean serum aspartate transaminase (AST) activities and mean serum alanine transaminase (ALT) activities of different groups are illustrated in Fig. 1 and 2. A significant increase ($P \leq 0.05$) in serum AST and ALT was observed in both the infected groups A1 and B1 as compared to non-infected groups A2 and B2. However, the values of mean serum ALT and AST were lower in group A1 (infected group with amla supplementation) as compared to group B1 (infected group without amla supplementation). Similar results have been reported by other workers in *E. coli* infection in birds (Eleiwa *et al*., 2011; Kamruzamman *et al*., 2012; Zaki *et al*., 2012; Abd El-Ghany and Ismail, 2014; Haq *et al*., 2015). The increase in serum AST is indicative of cellular injury to cardiac muscles and hepatocytes whereas elevated serum ALT is mostly due to hepatic injuries which have been noticed in the present study too as evidenced by pathological findings. Reports suggested that probiotics supplementation also increases the values of ALT and AST (Haq *et al*., 2015).

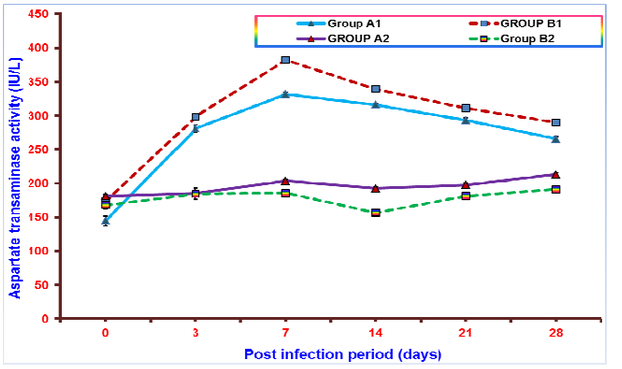

Fig. 1: Mean serum aspartate transaminase activities (IU/L) of broiler chicks in different experimental groups at different intervals

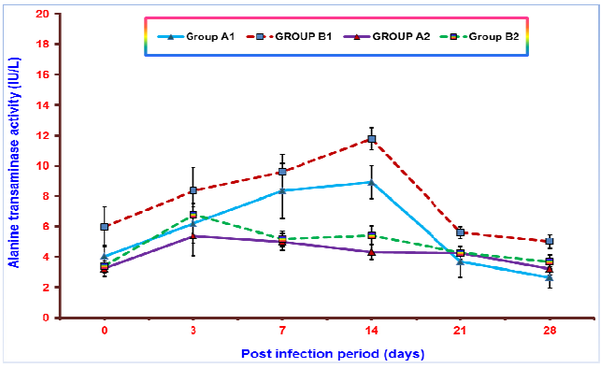

Fig. 2: Mean serum alanine transaminase activities (IU/L) of broiler chicks in different experimental groups at different intervals

Reports suggested that there was decrease in the levels of ALT and AST in the serum in the rats treated with *Phyllanthus emblica* along with lead acetate which might have activated the regeneration of hepatic cells indicating the hepatoprotective effect of the fruit (Jaiswal and Qureshi, 2010). There have been reports which concluded that cinnamon also increased the values of ALT and AST infected with *E. coli* infection (Tabatabaei *et al.,* 2015).

Mean total serum proteins concentration and albumin of different groups at various intervals are illustrated in Fig. 3 and 4. Studies on the total serum protein (TSP) and albumin revealed that there was a non-significant decrease in TSP and albumin concentrations were observed in both the infected groups as compared to control. This decrease in amla supplemented infected group (A1) was non-significantly less in comparison to the infected group without amla supplementation (B1). Decrease in these parameters in *E. coli* infection has been reported by other workers also (Christie and Halliday, 1979; Saini, 2004; Arshad *et al.,* 2007; Zaki *et al*., 2012; Kumari *et al.,* 2014; Haq *et al*., 2015). This is because of hypoproteinemia which may be due to renal disease leading to protein loss, liver damage which causes failure in plasma protein synthesis and congestive heart failure (Blood *et al.,* 1994). Liver is a site for albumin synthesis.

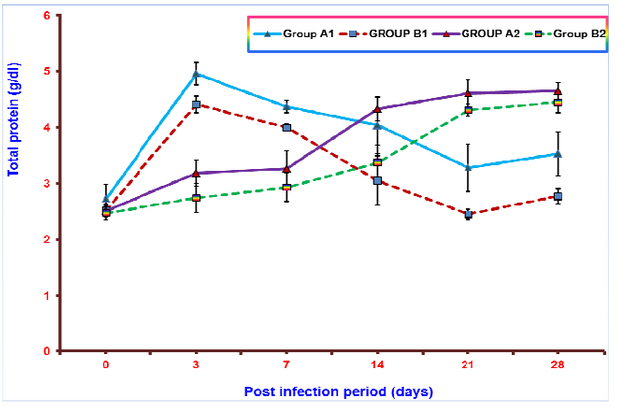

Fig. 3: Mean total serum protein concentration (g/dl) of broiler chicks in different experimental groups at different intervals

However, this decrease was significantly lower in amla supplemented group indicating the hepatoprotective effect of amla extract supplementation. A group of scientists observed a non-significant decrease in protein concentration and non-significant increase in albumin concentration in vitamin C supplemented Japanese quails (Gursu *et al*., 2004). Similar finding were also reported in the amla supplemented Vanaraja chicks (Kumar *et al.,* 2010). However, in one of the earlier research, some workers reported a significant increase in the concentration of albumin in amla fed rats (Rao *et al*., 2005).

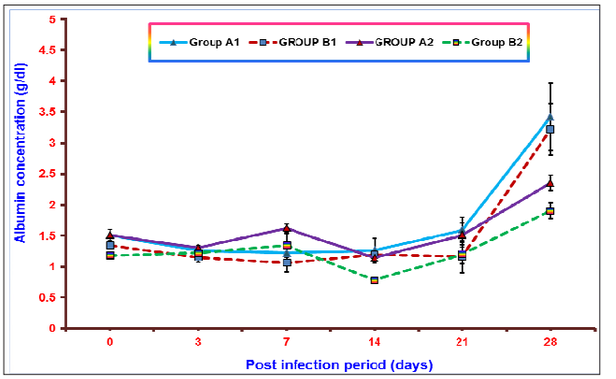

Fig. 4: Mean serum albumin concentration (g/dl) of broiler chicks in different experimental groups at different intervals

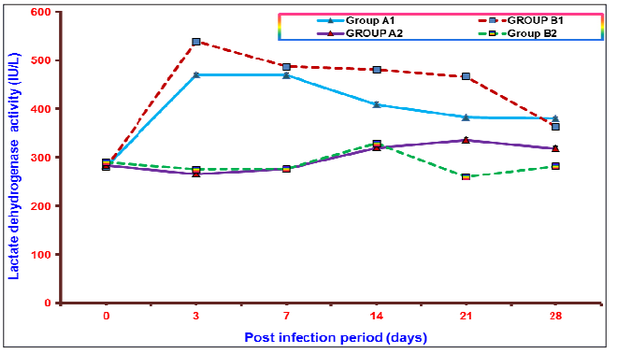

Fig. 5: Mean serum lactate dehydrogenase (IU/L) in broiler chicks of different groups at different intervals

Mean lactate dehydrogenase (LDH) levels of different groups at various intervals are illustrated in Fig. 5. Studies on lactate dehydrogenase (LDH) levels revealed that there was a significant increase in the mean serum lactate dehydrogenase activity in both the infected groups but this increase was more in the group with infection alone. Level of LDH increases in many processes such as hepatocellular necrosis, myocardial damage, renal necrosis, pancreatic necrosis and hemolysis etc. (Benjamin, 2013). In the

present study hepatic and myocardial necrosis was observed. This increase was lower in amla supplemented group indicating hepatoprotective and cardioprotective effect of amla. Reports suggested that infection with *E. coli* significantly increased the levels of LDH as compared to control groups but supplementation of 200 mg/kg cinnamon extract in infected broilers significantly reduced the levels of LDH (Tabatabaei *et al*., 2015).

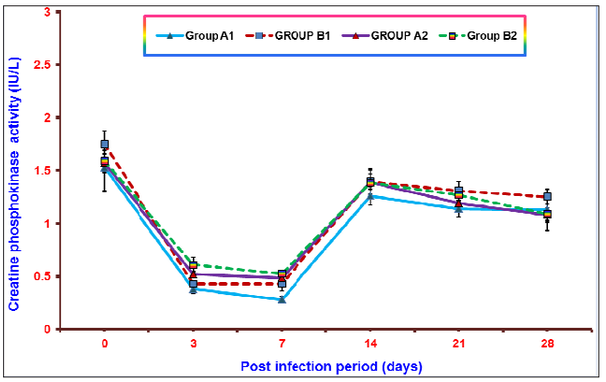

Fig. 6: Mean serum creatine phosphokinase (CPK) (IU/L) in broiler chicks of different groups at different intervals (Mean ± S.E.)

Mean serum creatine phosphokinase (CPK) concentrations of different groups at various intervals are illustrated in Fig. 6. A non-significant decrease in mean serum CPK concentrations was observed in both the infected groups as compared to control. This decrease was less in group A1 (Amla supplemented infected group) in comparison to the infected group B1 (without Amla supplementation). Creatine phosphokinase (CPK) functions in skeletal muscle, heart muscle and brain. Elevations in activities were mostly seen in muscle cell damage but literature reveals that muscle and liver damage occurs simultaneously. Elevated levels of liver enzymes in serum indicate the destruction of hepatocytes and dysfunction of the liver. These results are in accordance with some studies which concluded that the antioxidant properties of amla extracts and their effects on the oxidative stress in streptozotocin-induced diabetes in rats and accidentally found that serum level of creatine phosphokinase (CPK) was also reduced (Rao *et al*., 2005). Reports based on the study of CPK levels in *E. coli* inoculated group was significantly increased as compared to controls. Adding the powdered cinnamon extract at doses of 100 and 200 mg/kg of diet did not significantly change the levels of this enzyme as compared to those of control group but somehow it reduces the level. (Tabatabaei *et al*., 2015).

CONCLUSION

The present study was undertaken in broiler chickens to study the effect of dry fruit extract of amla supplementation on biochemical changes in relation to the severity of colibacillosis. Serum biochemical studies revealed significant increase in serum alanine transaminase, aspartate transaminase, lactate dehydrogenase activities, and decrease in creatine phosphokinase activity and non-significant decrease in total protein, albumin concentrations in both the infected groups. These changes were of significantly lower magnitude in amla supplemented group.

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Conflict of interest: The authors declare that they have no conflict of interest.

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