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PROTECTIVE EFFECTS OF *EMBLICA OFFICINALIS* AND YEAST SELENIUM AGAINST HEAT STRESS IN BROILER CHICKENS: PERFORMANCE, HEMATOBIOCHEMICAL, ANTIOXIDANT IMMUNOLOGICAL, CORTICOSTERONE AND PATHOLOGICAL STUDY

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ABSTRACT:
This study was conducted to assess the protective effect of dietary supplementation of Emblica officinalis and yeast selenium on general performance, hematobiochemical, antioxidant studies, immunological, corticosterone and pathology of broiler chickens under heat stress. Total 45 broiler chickens were divided into 03 equal groups with 15 birds in each group. Group G-I was served as control fed with basal diet only, while G-II was treated with basal diet + E. officinalis fruit powder @ 0.3 % of feed and G-III was treated with basal diet + yeast selenium @ 0.3 mg/kg of feed. Dietary supplementation of E. officinalis fruit powder and yeast selenium alleviated heat stress adverse effects in terms of general performance, hematobiochemical, antioxidant, immunological, corticosterone and pathological parameters. However, the better results were found with yeast selenium supplementation compared to other groups.

KEYWORDS: *Emblica Officinalis*, Heat Stress, Selenium

INTRODUCTION

One of the most significant problems confronting the poultry business and one that has an impact on the performance of the birds is global warming, which can cause heat stress (Gregory, 2010). High ambient temperature influences several physiological as well as performance aspects. Various environmental factors like sunlight, thermal irradiation, air temperature, humidity, and housing conditions of fowl are responsible for heat stress (Lara and Rostagno, 2013). The most appropriate temperature for a performance/thermo-neutral zone is between 19-22ºC for laying hens and 18-22ºC for growing broilers (Charles and Walker, 2002). Heat stress increases the production of free radicals by causing lipid peroxidation in cell membranes and the release of stress hormones, which have a direct impact on protein catabolism, glucose, and lipid metabolisms (Hosseini-Mansoub et al., 2010; Imik et al., 2013). Therefore, heat stress causes large economic losses in chicken production every year due to factors including increased morbidity and mortality, slowed development, and immune system suppression (Lara and Rostagno, 2013).

Selenium (Se) is an essential trace element that performs a wide variety of functions. It is crucial to a variety of animal physiology, including birds. It has crucial functions in immune system health, growth, productivity, and stress reduction. Se research has garnered a lot of attention due to its primary roles as an antioxidant activity engaged in protection against damage brought on by free radicals and oxidative stress. Se is regarded as a significant antioxidant due to its role in the production of glutathione peroxidase (GPx). In the form of selenoproteins such GPx, superoxide dismutase (SOD), glutathione reductase (GR), selenoprotein P, and selenoprotein W, Se exerts its physiological function (Jayaprakash and Marshall, 2011). It is an integral constituent of a minimum of 25 selenoproteins within the body that regulate cellular redox and antioxidant defense enzyme systems and control free radicals of reactive oxygen species and thus reduces MDA levels (Eid et al., 2023). It could be supplemented in diets as inorganic minerals salts, typically as sodium selenite, organic form such as Se-enriched yeast, selenocysteine and selenometheionine. Usually, the organic forms of Se have better bioavailability and antioxidant properties than inorganic forms (Edens and Sefton, 2016). Additionally, organic forms of Se are

less toxic and more environmental friendly than inorganic forms (Kuricova et al., 2003). Organic selenium had improved result of feed conversion ratio when compared with inorganic selenium (Yang et al., 2012).
In addition, Amla (Emblica officinalis) is one of the herbs which is naturally used feed additives and have the potential to enhance broiler production. It is one of the richest sources of water-soluble vitamins, minerals, amino acids, tannins, and phenolic compounds (Yokozawa et al., 2007). The rapid growth rate in commercial broilers as well as heat stress accelerates oxidative stress due to increased free radical generation (Feng et al., 2008). The phenolic acids found in E. officinalis include tannic and acidic acids that contribute to the antioxidant activity, and additionally, to water-soluble vitamins (Kumar et al., 2006). Phyto-chemical analysis of Amla fruit powder provided evidence of the presence of medicinally important bioactive compounds which may be exploited beneficially to boost productivity in broilers (Patel et al., 2016). The present investigation evaluate the protective effect of E. officinalis fruit powder and yeast selenium supplementation in broiler chickens under heat stress in term of production performance, hematobiochemical, antioxidant, immunological, corticosterone and pathological parameters.

MATERIALS AND METHODS

2.1 Experimental Site and Location

The experiment was carried out in a poultry house of the Post Graduate Institute of Veterinary and Animal Science, Akola, Maharashtra Animal and Fishery Science University, Nagpur Maharashtra State (Latitude: 20°70' N. Longitude: 77°02' E, Altitude: 286 m), India, located in the central India from 11th April to 23rd May 2022. The birds were kept under natural conditions, without artificial control of the environment. As a result, they were subjected to the naturally prevailing conditions of high environmental temperature (exceeding 250C and 390C) and relative humidity (Approx. 47%), characteristic of the hot–dry season in the central zone of India.

2.2 Management of Birds

Forty five birds, belonging to the Cobb broiler strain, were procured procured from M/s. Amruta Hatcheries, Dastur Nagar, Near Gondbaba Temple, Amravati - 444606, Maharashtra, India (CPCSEA Reg No: Akola/IAEC/ 312/01/2000/06/22). The birds were vaccinated, via drinking water, against infectious bursal disease (on days 7 and 14) and Newcastle disease (on day 21) using Gumboro and Lasota vaccines, respectively. Each broiler chick was tagged, using a masking tape, on the leg for identification and proper recordings. Deworming was carried out with piperazine solution, dissolved in drinking water at a single oral dose of 160 mg/kg (Aliu, 2007). The broiler chickens were raised on deep litter system and maintained on pre-starter, starter and finisher feed with ample supply of drinking water, obtained from M/s. Shrikrupa Poultry feeds, Dastur Nagar, Near Gondbaba Temple, Amravati - 444606, Maharashtra, India. They were housed in a poultry pen, littered with wood shavings on concrete floor and measuring 8.7 × 7.5 × 1.91 m.

2.3 Experimental Design

The experiment was duly approved by Institutional Animal Ethics Committee of the Post Graduate Institute of Veterinary and Animal Sciences, Akola (PGIVAS, Akola/IAEC/312/01/2000/06/22, Date: 6th August, 2021). The experimental birds were acclimatized for 1-2 days before the start of experiment under standard hygienic and managemental conditions in Poultry Research Centre, PGIVAS, Akola. After acclimatization, chicks were randomly allotted into groups with 15 chicks in each group (Group I, II and III,). Group I was served as control, while group II was treated with basal diet + E. officinalis fruit powder @ 0.3 % of feed and group III was treated with basal diet + yeast selenium @ 0.3 mg/kg of feed The treatment was given for 42 days.

2.4 Collection of Blood and Serum Samples

On 35th and 42nd day of experiment blood sample from randomly selected six birds were collected from the jugular vein using the method given by Steven and Ridgway (1966). The different aliquots of blood were taken EDTA, heparinized and plain/clot activator vial.

2.5 Performance Parameters

The live body weights of all birds were recorded individually, accurately and group wise using electronic weighing balance at weekly interval (0, 7th, 14th, 21st, 28th, 35th and 42nd day) in morning hours before feeding. Records of feed offered and residue left over were recorded weekly. The average weekly body weight gain (BWG) (g) and feed conversion ratio (FCR) was calculated over this period (6 weeks). The average weekly body weight gain was calculated from the difference in body weight attained at the end and the start of the concerned period for each replicate of group. Feed conversion rate (FCR) was determined as feed intake (FI) divided by body weight gain (BWG).

2.6 Haematological Evaluation

Blood obtained in EDTA (1-2 mg/ml) was used for hematological analysis like total erythrocyte count (TEC), hemoglobin (Hb), packed cell volume (PCV), total leukocyte counts (TLC). The heterophil to lymphocyte (H:L) ratio was also determined. Thin blood smears were prepared from a fresh blood from all the birds for differential leukocyte counts (DLC). Immediately after collection of blood, haematological parameters were estimated using standard method.

2.7 Biochemical Evaluation

Blood collected in clot activator was used for separation of serum for further biochemical estimation. The serum was then separated by centrifugation at 3000 rpm for 15 minutes in an eppendorf tube. Immediately after collection of blood, serum enzyme tests like serum total protein (TP), albumin, globulin, aspartate transaminase (AST),

alanine transaminase (ALT), creatinine, blood glucose and cholesterol were estimated using standard method and readymade biochemical kits. Serum samples were kept at -20 0C for further analysis (HA, HI).

2.8 Antioxidant Enzymes Status

Blood collected in heparinized vial was used for MDA analysis. Membrane peroxidative damage in erythrocytes was measured in terms of malondialdehyde (MDA) production by the method of Rehman (1984).

2.9 Immunological Parameter

Humoral immune response and cell mediated immune response (CMl) in six birds of each group were evaluated. Humoral immune response was evaluated on the basis of HI titres against New castle disease (NDV) virus by beta procedure at the end of 5th week (Allan and Gough, 1974). Cell mediated immune response was judged by skin sensitivity test using Hapten-1-chloro 2-4 dinitrochlorobenzene (DNCB) as per procedure described by Valsala et al. (1981).

2.10 Serum Corticosterone Estimation

By using commercial kit Serum corticosterone level was evaluated from the serum collected at the end of experiment

2.11 Organ Weight Estimation (G)

After conducting detailed necropsy examination organs were removed carefully without damaging. Each organ was then rinsed with ice-cold saline, dried with blotting paper, and weighed on a digital weighing balance. Organs collected were heart, brain, liver, kidneys, spleen, thymus, bursa of Fabricius, pancreas and intestine.

2.12 Histopathological Observations

Birds were sacrificed at the end of the experiment i.e., on the 42nd day and tissues from visceral organs such as heart, brain, liver, kidneys, spleen, thymus, bursa of Fabricius, duodenum and ileum were collected in 10% formal saline solution for further histopathological investigation. Tissues from these organs were processed for routine histological techniques after fixation, utilizing water for hydration, alcohol for dehydration, xylene for clarifying, and impregnated with paraffin wax before embedding as per standard procedure described by Luna (1968). Sections of 4–6 µm were cut on a rotary microtome and stained with Haematoxylin and Eosin stain. The stained sections were then examined under microscope, and histopathological lesions were recorded for each organ.

2.13 Morphometrical Observation

All the light microscopic variables were viewed under 4X magnification by using microscope, image analysis software was used to analyze the images. Villus height (height from the tip of the villus to the crypt), villus width, crypt depth (crypt depth from the base of the villi to the sub mucosa) and the villus height to crypt depth ratio were calculated.

2.14 Statistical Analysis

The data was evaluated using equal Completely Randomized Design (CRD) for general performance, haematological and biochemical parameters as described by Snedecor and Cochran (1989). Means were compared as per Duncan's multiple range test and data were processed for statistical analysis using SPSS Software package (23.0). Values were expressed as Mean ± SE. Values were considered at probability of 5% and 1% level as significant and highly significant, respectively.

RESULTS

3.1 Growth Performance

The effects of heat stress and protective effect of dietary supplementation of E. officinalis and yeast selenium on growth performance and mortality of broiler. During the 6th week of the trial, the mortality seen in the current study was 20%, 13.33% and 6.66%, in GI, GII and GIII groups, respectively, within the anticipated range. According to the result of this (Table 1, 2, 3 & 4), heat stress group I showed a significantly ($P<0.05$) reduced average weekly body weight (BW), and average weekly body weight gain (BWG) and non significant decrease in average weekly feed intake (TFI) and average weekly feed conversion ratio (FCR) and increased the mortality rate compared to GII and GIII. While E. officinalis (GII) and yeast selenium (GIII) supplementation significantly ($P<0.05$) increased the average weekly body weight (BW), and average weekly body weight gain (BWG), non significant increase in average weekly feed intake (TFI), improvement in average weekly feed conversion ratio (FCR) and decreased the mortality rate, when compared with heat-stressed birds. The supplementation of yeast selenium revealed the greatest improvement than separate supplementation with E. officinalis (Amla powder).

3.2 Haematological Parameters

The effects of heat stress and preventive effects of E. officinalis and yeast selenium on the hematological parameters are shown in (Table 4, 6 & 7) in which, heat stressed group showed a significant ($P<0.05$) decline haemoglobin (Hb), packed cell valoume (PCV), total erythrocyte count (TLC) and significant ($P<0.05$) increase in mean corpuscular volume (MCV) but no significant change in mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) reflecting a picture of macrocytic normochromic anemia. Moreover, In case of differential leucocyte count there were a significant ($P<0.05$) increase in heterophil, heterophil: lymphocyte (H:L) ratio and basophil where as significant ($P<0.05$) reduction were noticed in lymphocyte and no as such change in monocyte and eosinophil count thus reflecting stress picture of leukogram. Amla powder and yeast selenium feed addition were able to restore the altered hematological parameters compared to the heat stressed group. Notably, administration of yeast selenium exhibited greater improvement than other treatment groups.

3.3 Biochemical Parameters

The effects of heat stress and alleviating effects of E. officinalis and yeast selenium on the selected biochemical parameters presented in (Table 9 & 10) showed that, heat stressed group caused a significant (P<0.05) decline in total proteins, albumin and globulin concentrations together with a significantly (P<0.05) elevated serum enzyme activities of aspartate transferase (AST/SGOT), alanine transaminase (ALT/SGPT), creatinine, total cholesterol, and glucose concentrations. Moreover, the dietary amla powder and yeast selenium additions were able to reverse the altered biochemical parameters. Notably, the best results observed in group received diet supplemented with yeast selenium.

3.4 Immune Responses

The deleterious effects of heat stress and the ameliorative effects of E. officinalis and yeast selenium on immune responses are shown in (Table 11 &12) implicated that heat stressed birds showed immunosuppressive effect through a significantly (P<0.05) decline HI titer against New castle disease virus (NDV) on both day 35th as well as on 42nd day of experiment. For cell mediated immune (CMI) response heat stressed group recorded significantly increased skin fold thickness after 24 and 48 hours when challenged by 2-4 dinitrochlorobenzene (DNCB). Furthermore, the best immunological enhancement obtained in birds given yeast selenium more than E. officinalis administered separately.

3.5 Antioxidant Enzymes Estimation

The effects of heat stress and ameliorative effects of E. officinalis and selenium on the oxidative stress and erythrocytic antioxidant biomarkers like lipid peroxidation (LPO) shown in (Table 13) illustrated, the heat stressed group revealed a significant (P<0.05) increase oxidative stress marker malonaldialdehyde (MDA) level. On the other hand these results were reversed by dietary supplementation with amla and yeast selenium as shown by a significant (P<0.05) reduction in oxidative stress marker MDA. Arguably, yeast selenium exhibit strongest antioxidant properties and seen to be proven than other treatments.

3.6 Corticosterone Estimation

The effects of heat stress and protective effects of E. officinalis and yeast selenium on serum corticosterone estimation shown in (Table 14) displayed, the heat stressed group revealed a significant (P<0.05) increase corticosterone level. On the other hand these results were reversed by dietary supplementation with amla and selenium as shown by a significant (P<0.05) reduction in corticosterone level. On other hand, yeast selenium displays strongest antioxidant and stress-relieving properties than other treatments.

3.7 Relative Organ Weight

The effects of heat stress and protective effects of E. officinalis and yeast selenium on the relative organ weight presented in (Table 15) showed that, heat stressed group caused a significant (P<0.05) decrease in relative organ weight of heart, liver, kidney, spleen, thymus, bursa of Fabricius, and intestine. However, supplementing E. officinalis and yeast selenium leads to increase in relative weight of above mentioned organ in broiler exposed to heat stress.

Table 1. Average weekly body weight (g) per bird in different groups during the experimental period.

Group	0 Day	1st Week	2nd Week	3rd Week	4th Week	5th Week	6th Week
G I	42.73 ±0.61	123.26 ±1.99^{c}	298.33 ±5.09^{b}	519.80 ±22.39^{b}	874.26 ±10.13^{c}	1201.00 ±17.46^{c}	1495.80 ±16.66^{c}
G II	42.46 ±0.68	131.06 ±1.14^{b}	310.13 ±8.63^{a}	561.80 ±11.20^{a}	925.80 ±7.40^{b}	1266.06 ±21.72^{b}	1601.40 ±19.90^{b}
G III	42.60 ±0.64	132.00 ±1.08^{b}	316.66 ±7.84^{a}	558.66 ±6.11^{a}	922.73 ±9.71^{b}	1259.20 ±10.55^{b}	1611.53 ±22.82^{b}
P value	-	**	**	**	**	**	**
CD (0.05)	NS	4.026	11.633	38.809	31.358	46.916	72.037

Values indicated mean ± S.E. Mean values with common alphabet as superscript do not differ significantly
*NS = Non significant -**Significant at 1% -* Significant at 5%*

Table 2. Average weekly body weight gain (g) per bird in different groups during the experimental period.

Group	1st Week	2nd Week	3rd Week	4th Week	5th Week	6th Week
G I	80.53 ±2.06^{c}	175.06 ±5.07	221.46 ±23.22^{b}	354.46 ±21.98^{d}	326.73 ±21.12	294.80 ±30.02^{b}
G II	88.60 ±1.45^{b}	179.06 ±9.13	251.66 ±11.78^{ab}	364.00 ±12.51^{c}	340.26 ±24.85	335.33 ±22.66^{ab}
G III	89.40 ±1.34^{b}	180.00 ±10.34	242.66 ±11.68^{b}	365.73 ±18.44^{b}	348.26 ±15.28	352.33 ±28.27^{a}
P value	**	NS	*	**	NS	**
CD (0.05)	4.545	-	30.304	0.000	-	42.523

Values indicated mean ± S.E. Mean values with common alphabet as superscript do

not differ significantly.
*NS = Non significant -**Significant at 1% -* Significant at 5%*

Table 3. Average weekly feed consumption (g) per bird in different groups during experimental period

Group	1st Week	2nd Week	3rd Week	4th Week	5th Week	6th Week	Total feed consumption	Pooled mean
G I	109.00	237.98	382.33	641.33	656.33	740.10	2767.07	461.17 ±104.62
G II	119.00	242.10	410.44	650.66	667.66	796.33	2886.19	481.03 ±108.95
G III	118.00	242.23	429.33	649.23	657.33	792.66	2888.78	481.46 ±107.69
P value	-	-	-	-	-	-	-	NS
CD (0.05)	-	-	-	-	-	-	-	-

Values indicated mean ± S.E. Mean values with common alphabet as superscript do not differ significantly.
*NS = Non significant -**Significant at 1% -* Significant at 5%*

Table 4. Average weekly feed conversion ratio (FCR) in different groups during the experimental period

Group	1st Week	2nd Week	3rd Week	4th Week	5th Week	6th Week	Pooled Mean
G I	1.35	1.35	1.72	1.80	2.00	2.51	1.78±0.17
G II	1.33	1.34	1.70	1.78	1.93	2.36	1.74±0.15
G III	1.33	1.34	1.69	1.77	1.91	2.26	1.71±0.14
P value	-	-	-	-	-	-	NS
CD (0.05)	-	-	-	-	-	-	-

Values indicated mean ± S.E. Mean values with common alphabet as superscript do not differ significantly.
*NS = Non significant -**Significant at 1% -* Significant at 5%*

Table 5. Mean haematological values of Hb, PCV and TEC in control and treatment groups

Treatment Group	Hb(g/dL)	PCV (%)	TEC (10^6/cumm)
G I	$6.76±0.24^b$	$26.00±1.12^b$	$2.55±0.12^b$
G II	$8.05±0.15^a$	$29.66±0.71^a$	$3.11±0.09^a$
G III	$7.90±0.29^a$	$29.83±1.01^a$	$3.13±0.10^a$
P value	**	**	**
CD(0.05)	0.649	2.792	0.317

Values indicated mean ± S.E. Mean values with common alphabet as superscript do not differ significantly.
*NS = Non significant -**Significant at 1% -* Significant at 5%*

Table 6. Mean erythrocyte indices like MCV, MCH and MCHC in control and treatment groups

Treatment Group	MCV (fL)	MCH (pg)	MCHC (gm/dL)
G I	$104.05±3.37^a$	26.72±1.22	26.41±1.94
G II	$103.80±8.77^b$	26.17±1.01	25.19±0.86
G III	$96.10±5.81^c$	25.48±1.73	26.57±1.06
P value	0.000	NS	NS
CD(0.05)	-	-	-

Values indicated mean ± S.E. Mean values with common alphabet as superscript do

not differ significantly.
*NS = Non significant -**Significant at 1% -* Significant at 5%*

Table 7. Mean haematological values related to leucocytes in control and different groups

Group	TLC (10^3/mm^3)	Heterophil (%)	Lymphocyte (%)	H:L Ratio	Monocyte (%)	Eosinophil (%)	Basophil (%)
G I	20.83 ±1.32^{c}	29.83 ±1.44^{a}	64.50 ±1.78^{b}	0.46 ±0.035^{a}	1.16 ±0.30	3.66 ±0.33	0.83 ±0.30^{a}
G II	26.00 ±0.70^{ab}	25.50 ±1.25^{b}	71.66 ±1.28^{a}	0.35 ±0.023^{b}	1.08 ±0.22	2.16 ±0.30	0.55 ±0.16^{b}
G III	24.83 ±0.70^{b}	26.16 ±0.90^{b}	69.33 ±0.88^{a}	0.37 ±0.017^{b}	1.33 ±0.42	2.5 ±0.56	0.66 ±0.21^{b}
P value	**	*	**	**	NS	NS	*
CD (0.05)	2.543	3.523	3.861	0.074	-	-	0.381

Values indicated mean ± S.E. Mean values with common alphabet as superscript do not differ significantly.
*NS = Non significant -**Significant at 1% -* Significant at 5%*

Table 8. Serum total protein, albumin and globulin level in the control and different treatment groups

Group	Total protein (g/dl)	Albumin(g/dl)	Globulin(g/dl)
G I	2.56±0.17^{b}	1.18±0.10	1.37±0.13^{b}
G II	3.14±0.17^{a}	1.45±0.08	1.69±0.12^{b}
G III	3.11±0.20^{a}	1.36±0.08	1.75±0.22^{ab}
P value	**	NS	*
CD(0.05)	0.510	-	0.450

Values indicated mean ± S.E. Mean values with common alphabet as superscript do not differ significantly.
*NS = Non significant -**Significant at 1% -* Significant at 5%*

Table 9. Serum AST, ALT, creatinine, cholesterol and glucose profile in different groups

GROUP	AST (IU/L)	ALT (IU/L)	CREATININE (mg/dL)	CHOLESTEROL (mg/dL)	GLUCOSE (mg/dL)
G I	220.65 ±5.09^{a}	48.83 ±1.43^{a}	0.43 ±0.01^{a}	169.6 ±0.84^{a}	198.16 ±2.07^{a}
G II	189.05 ±4.36^{b}	44.6 ±1.3 1^{b}	0.30 ±0.02^{b}	159.63 ±1.33^{b}	190.83 ±1.88^{b}
G III	184.71 ±5.28^{b}	44.83 ±1.26^{b}	0.22 ±0.05^{c}	161.36 ±1.21^{b}	182.5 ±2.26^{c}
P value	**	**	**	**	**
CD(0.05)	18.702	3.811	0.001	3.681	5.730

Values indicated mean ± S.E. Mean values with common alphabet as superscript do not differ significantly.
*NS = Non significant -**Significant at 1% -* Significant at 5%*

Table 10. Mean Haemagglutination Inhibition titer against NDV in the control and different treatment groups on 35th and 42nd day of experiment

GROUP	HI titer (35th day) $\log_2$	HI titer (42nd day) $\log_2$
G I	6.00±0.25^{b}	6.50±0.22^{b}
G II	6.66±0.21^{a}	7.33±0.21^{a}
G III	6.83±0.16^{a}	7.50±0.22^{a}
P value	**	*
CD(0.05)	0.602	0.632

Values indicated mean ± S.E. Mean values with common alphabet as superscript do not differ significantly.
*NS = Non significant -**Significant at 1% -* Significant at 5%*

Table 11. Cutaneous delayed hypersensitivity response to DNCB in the control and different treatment groups

GROUP	Thickness of skin (mm)		
	0 hrs	**24 hrs**	**48 hrs**
G I	0.57±0.01	2.31±0.16^{b}	1.75±0.16^{d}
G II	0.55±0.01	3.33±0.40^{a}	2.13±0.43^{c}
G III	0.55±0.01	3.13±0.21^{a}	2.2±0.12^{b}
P value	NS	**	**
CD(0.05)	-	0.810	0.002

Values indicated mean ± S.E. Mean values with common alphabet as superscript do not differ significantly.
*NS = Non significant -**Significant at 1% -* Significant at 5%*

Table 12. Mean values of LPO levels (erythrocytic antioxidant) in control and different treatment groups

GROUP	LPO level (nmol/mL)
G I	5.21±0.12^{a}
G II	3.53±0.10^{b}
G III	3.34±0.04^{bc}
P value	**
CD(0.05)	0.264

Values indicated mean ± S.E. Mean values with common alphabet as superscript do not differ significantly.
*NS = Non significant -**Significant at 1% -* Significant at 5%*

Table 13. Mean values of corticosterone in control and different treatment groups

GROUP	Corticosterone (ng/mL)
G I	100.47±2.43^{a}
G II	86.13±1.47^{b}
G III	85.70±0.97^{b}
P value	**
CD(0.05)	6.245

Values indicated mean ± S.E. Mean values with common alphabet as superscript do not differ significantly.
*NS = Non significant -**Significant at 1% -* Significant at 5%*

Table 14. Relative organ weight (g) in control and treatment groups

Group	Heart	Liver	Kidney	Spleen	Thymus	Bursa	Intestine
G I	0.35 ± 0.01^{b}	2.38 ±0.12^{c}	0.547 ± 0.021^{c}	0.061 ± 0.007^{b}	0.26 ± 0.02^{b}	0.074 ± 0.009^{d}	4.02 ± 0.33b
G II	0.41 ± 0.01^{b}	2.85 ± 0.12^{bc}	0.603 ± 0.074^{bc}	0.102 ± 0.012^{a}	0.36 ± 0.02^{a}	0.092 ± 0.007^{c}	4.48 ± 0.33c
G III	0.49 ± 0.01^{a}	3.32 ± 0.11^{ab}	0.722 ± 0.030^{ab}	0.093 ± 0.005^{a}	0.34 ± 0.03^{a}	0.095 ± 0.008^{b}	4.72 ± 0.23a
P value	**	**	**	**	*	**	**

CD (0.05)	0.066	0.506	0.131	0.026	0.077	0.000	0.000

Values indicated mean ± S.E. Mean values with common alphabet as superscript do not differ significantly
*NS = Non significant -**Significant at 1% -* Significant at 5%*

Table 15. Mean values of villi height, crypt depth, and villi height: crypt depth of duodenum and ileum of control and treatment groups

GROUP	Duodenum			Ileum		
	Villi height (µm)	Crypt depth (µm)	Villi height-Crypt depth ratio	Villi height (µm)	Crypt depth (µm)	Villi height-Crypt depth ratio
G I	1556.52 ±30.25^{b}	239.26 ±9.81^{a}	6.72 ±0.28^{b}	543.73 ±24.92^{b}	147.43 ±4.76^{a}	3.81 ±0.21^{d}
G II	1724.88 ±53.31^{a}	218.90 ±8.36^{b}	8.02 ±0.36^{a}	641.61 ±32.22^{a}	143.82 ±5.13^{b}	4.52 ±0.23^{c}
G III	1742.20 ±26.28^{a}	217.83 ±5.25^{b}	8.11 ±0.23^{a}	645.26 ±13.39^{a}	143.44 ±5.37^{c}	4.65 ±0.20^{b}
P value	*	**	*	**	**	**
CD(0.05)	152.55	6.255	1.131	54.180	0.466	0.220

Values indicated mean ± S.E. Mean values with common alphabet as superscript do not differ significantly
*NS = Non significant -**Significant at 1% -* Significant at 5%*

3.8 Gross Pathological Observations

The birds in the heat-stressed control group I had slightly pale and friable liver with granular texture (Fig 2). Focal areas of hepatocellular necrosis, and congestion were also recorded. Kidneys were slightly pale and enlarged with mild congestion in some of the birds. The bursa of Fabricius exhibited slight to moderate atrophy. There was moderate enlargement of heart with slight ventricular hypertrophy with excessive blood accumulation. Thymus in most of the group exhibited atrophic changes (Fig 3), but group I had more of these changes than the other groups. On opening, the intestine revealed minor haemorrhages at mucosa. Similar pathological changes were mild in other treatment groups. The necropsy investigation of the birds that died during the trial period revealed the pale, parboiled musculature mainly of pectoralis muscle (Fig 1), pale and friable liver, pale and enlarged kidneys along with mild intestinal haemorrhages.

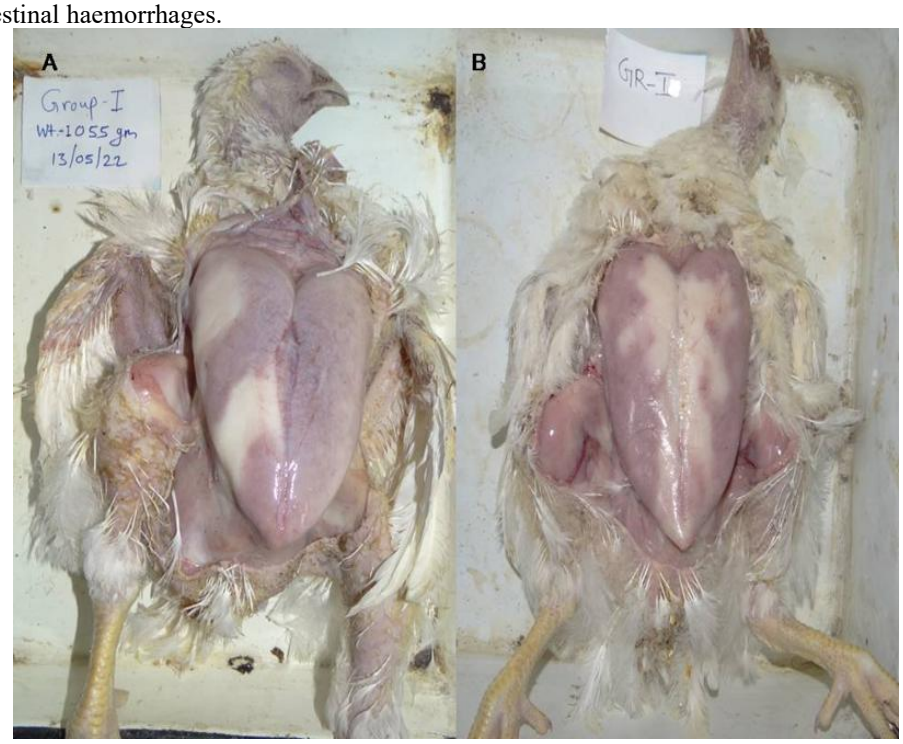


Figure 1. Gross image showing parboiled appearance of pectoralis muscle from group I (GI).

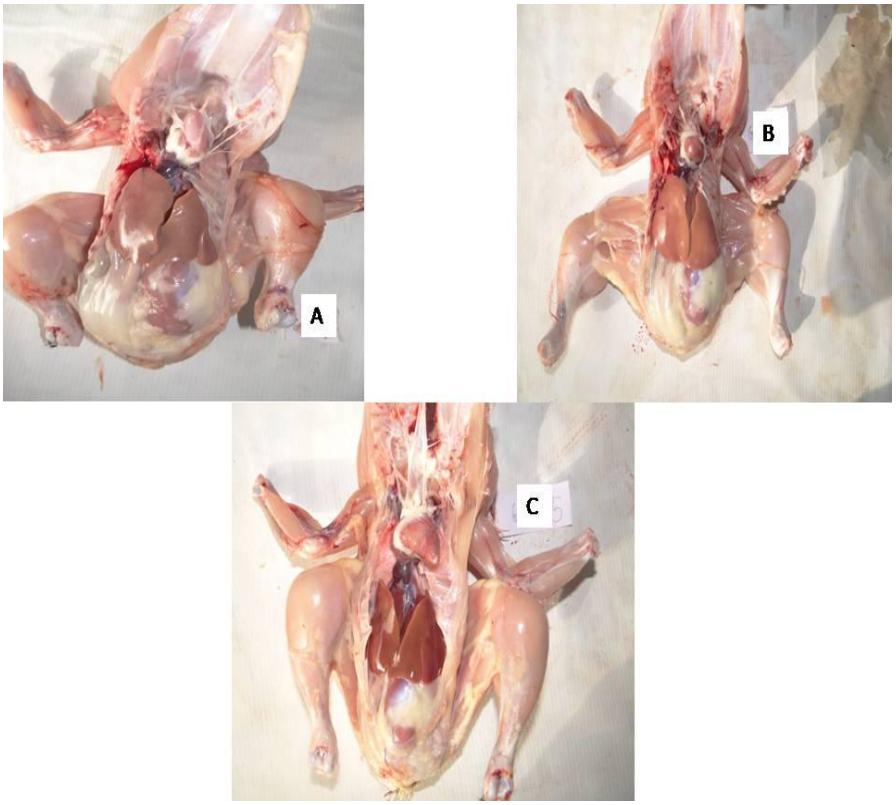


Figure 2. Liver : A) GI showing pale colour, B) GII showing mild pale and slight haemorrhages, C) GIII showing mild pale colour

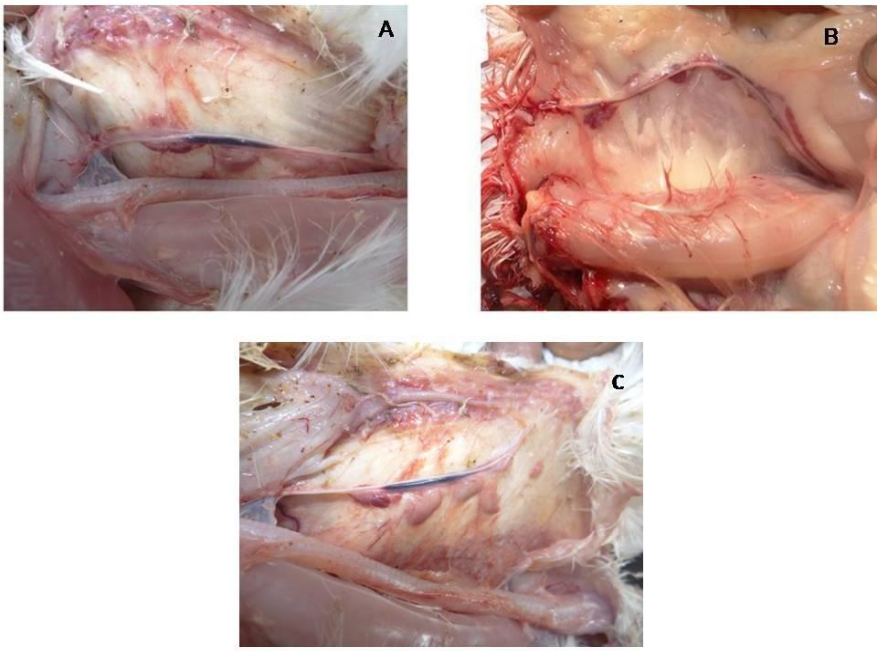


Figure 3. Thymus showing atrophy at various degrees in different groups.

3.9 Histopathological Observations

A marked widening of the sinusoidal spaces, moderate to severe granular and vacuolar changes, centrilobular necrosis, mononuclear cell infiltration, and prominent architectural losses were all visible in sections of the liver from GI along with the focal areas of the necrosis. Sections from GII and GIII contrasted with those from GI, exhibiting focal areas of necrosis, mild sinusoidal space separation, and mild to moderate granular and vacuolar degenerative changes in hepatocytes. In comparison to the other groups, GIII sections exhibited relatively fewer pathological alterations of lesser intensity.

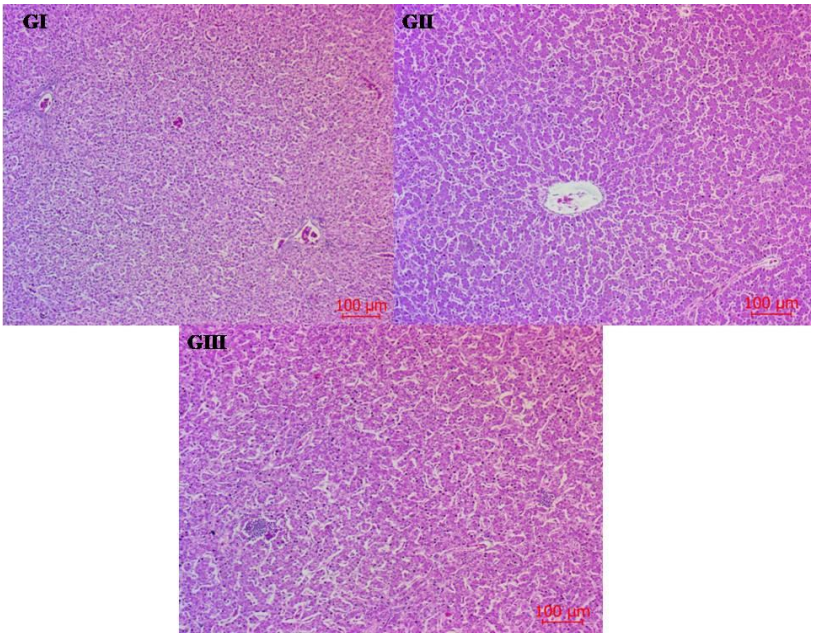


Figure 4. Microphotograph of Liver showing marked vacuolar and granular degeneration of hepatocytes, widening of the sinusoidal spaces, sinusoidal haemorrhages (H & E x100) (GI); moderate vacuolar and granular degenerative changes and sinusoidal dilatations (H & E x100) (GII); moderate vacuolar and granular degenerative changes, sinusoidal dilatations and congestion and focal mononuclear cell infiltration (H & E x100) (GIII).

Heart tissue sections from GI exhibited severe myofibrillar degeneration along with haemorrhages, and myofiber vacuolar changes. Additionally, loss in cross striations and focal areas of necrosis were observed. Microscopic investigation of tissue sections of the heart from treatment (GII and GIII) revealed mild cardiac degeneration with lesser intensity and separation of myofibers with limited infiltration of red blood cells and mononuclear cells.

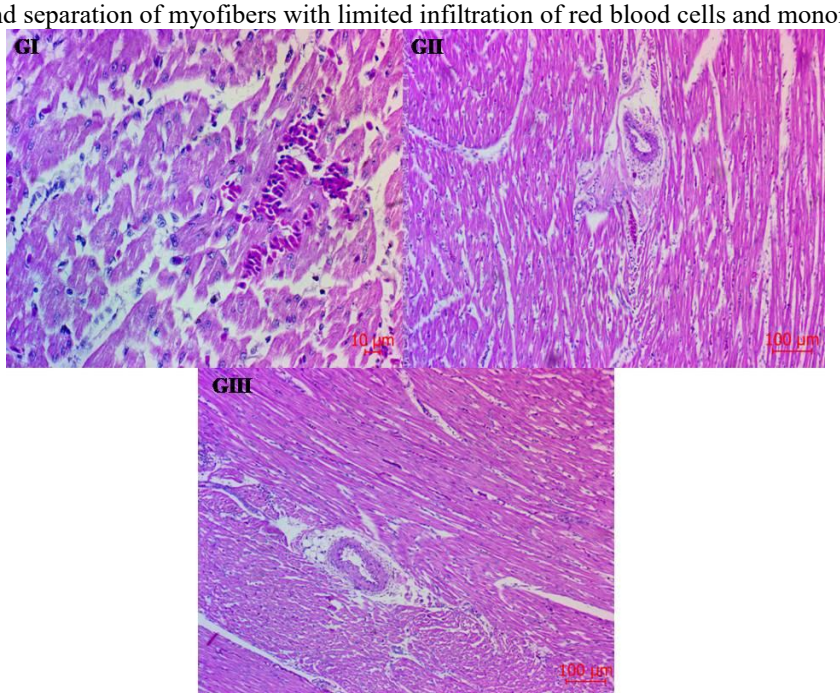


Figure 5. Microphotograph of Heart showing marked myofibrillar degeneration with loss of cross striations, granular changes in the myofibers and focal areas of haemorrhages with widening of interfibrillar space (H & E x400) (GI); mild degenerations, infiltration of inflammatory cell in between muscle bundles and separation of myofibres with minimal infiltration of mononuclear cells (H & E x100) (GII); milder separation of myofibres with minimal infiltration of mononuclear cells with minimal infiltration of inflammatory cell (H & E x100) (GIII).

Kidney tissue from GI displayed intertubular hemorrhages and blood vessel congestion. The tubular epithelium showed granular and vacuolar changes, focal tubular necrosis, and polymorphonuclear cell infiltration. Additionally, Detachment of the tubular epithelium from the basement membrane was also observed. Microscopic analysis of renal tissue sections from the treatment groups (GII and GIII) revealed mild to moderate granular and

vacuolar changes, mild intertubular haemorrhages, and slight tubular epithelial swelling. Compared to the other treatment groups, GIII exhibited fewer degenerative alterations with lesser intensity.

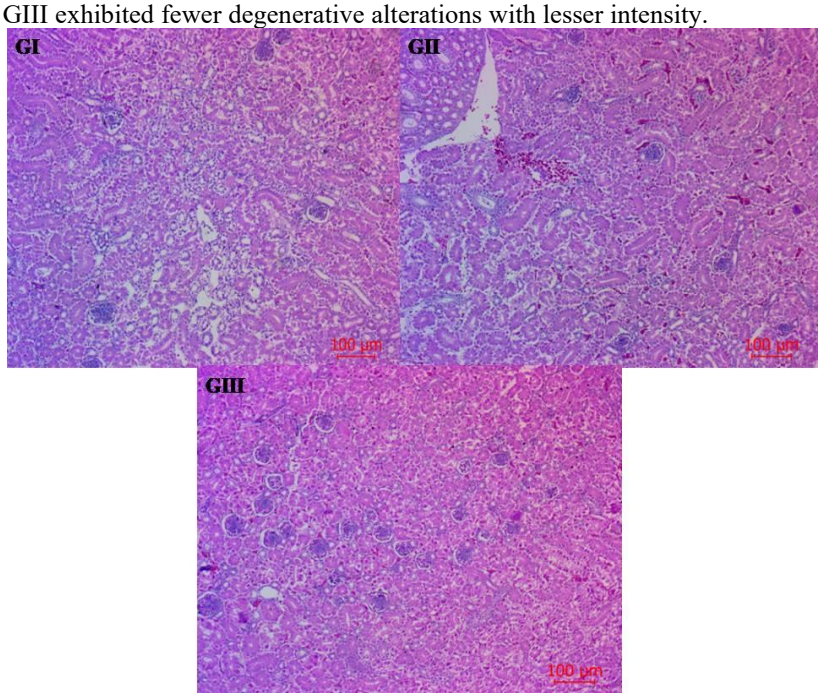


Figure 6. Microphotograph of Kidney showing extensive vacuolar and granular degenerative changes, multifocal areas of intertubular haemorrhages and focal areas of necrosis (H & E x100) (GI); mild degenerative changes in tubular epithelium with mild swelling of the tubular epithelium, with prominent intertubular haemorrhages (H & E x100) (GII); mild to moderate degenerative changes, intertubular haemorrhages and mild vacuolar changes (H & E x100) (GIII).

Microscopic analysis of sectional tissues of spleen, thymus and bursa of Fabricius from the heat-stressed control GI revealed moderate lymphoid depletion from follicles as well as notable hemorrhagic changes. The lymphoid follicles in the tissue sections from the GII and GIII groups revealed relatively less depleted lymphoid populations. Comparatively histological alterations of lesser intensity were present in GIII than in the other groups.

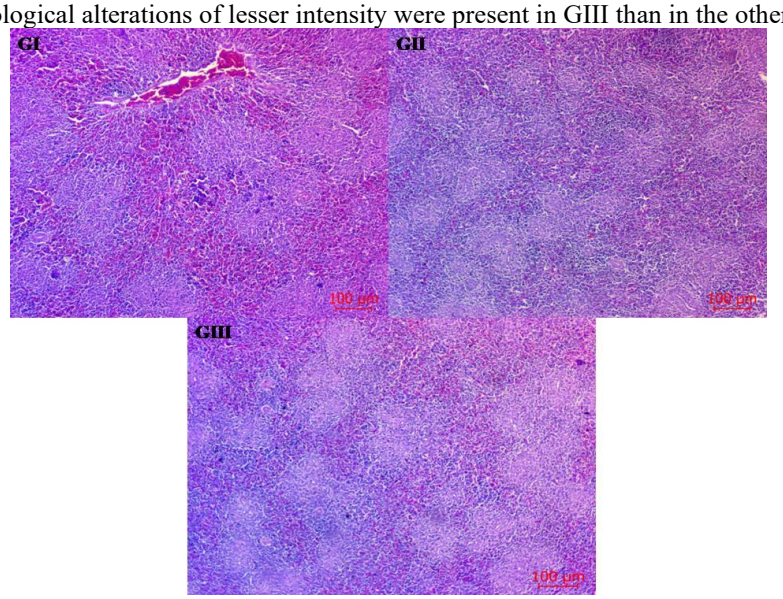


Figure 7. Microphotograph of Spleen showing moderate decrease cellularity of lymphoid cells in red and white pulp and haemorrhages with loss of distinction between white and red pulp (H& E x100) (GI); apparently normal splenic parenchyma with slight lymphoid cells population depletion (H& E x100) (GII); mild depletion of lymphoid cells with mild to moderate degenerative changes in the lymphoid follicle (H& E x100) (GIII).

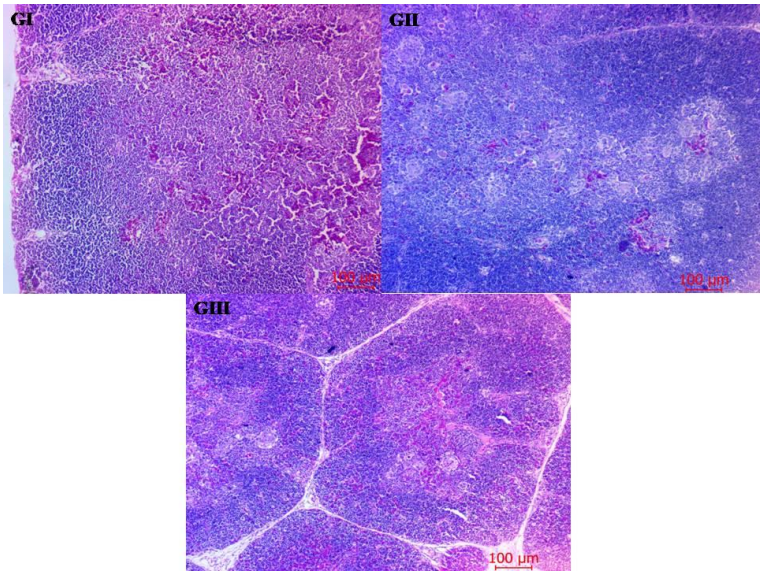


Figure 8. Microphotograph of Thymus showing mild to moderate loss of the lymphoid cells in cortex and medulla with multifocal areas of haemorrhages (H & E x100) (GI); mild loss lymphoid cell with apparently normal histoarchitecture of thymus (H & E x100) (GII); mild loss lymphoid cell in cortex with apparently normal histoarchitecture of thymus and mild edematous changes at centre of thymic follicle (H & E x100) (GIII).

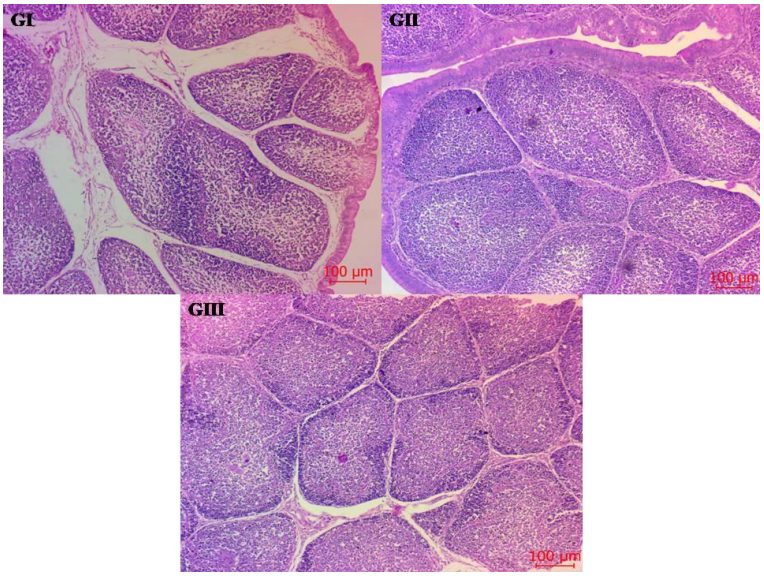


Figure 9. Microphotograph of Bursa of Fabricious showing marked depletion of lymphoid cell population in the bursal follicles along with marked increase in the interfollicular space (H & E x100) (GI); mild depletion of lymphoid cell population in the bursal follicles (H & E x100) (GII); mild depletion of lymphoid cell population in the bursal follicle with slight proliferations of intefollicular connective tissues (H & E x10) (GIII).

There was moderate neuronal degeneration and vacuolar alterations and increase in perivascular space of the cytoplasm of the neurons in the brain section from the heat exposed GI. An increase in Virchow-Robin spaces was noticeable, along with significant congestion, with hemorrhagic areas, and a notable rise in glial cells. Brain tissue from the GII, and GIII group revealed mild neuronal degeneration, vacuolar changes in the cytoplasm increase in perivascular space and congestion in blood vessels.

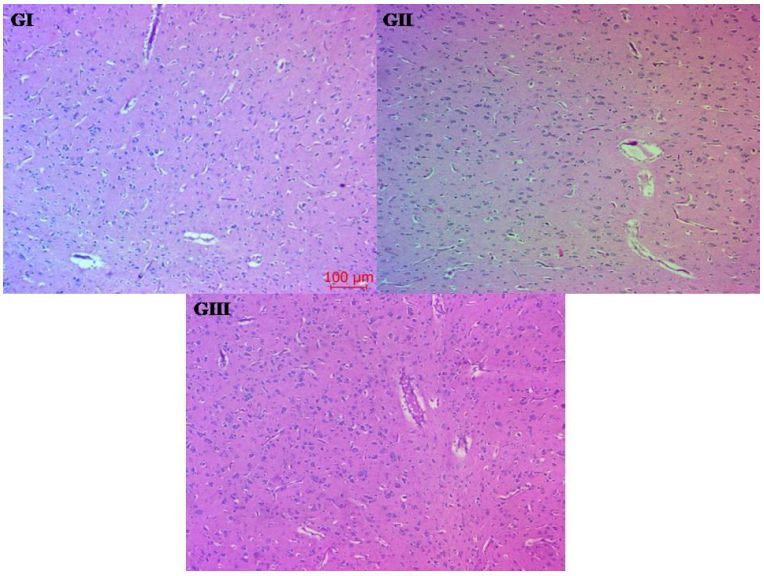


Figure 10. Microphotograph of Brain showing moderate neuronal degeneration and vacuolar changes and increase in perivascular space (H & E x100) (GI); mild neuronal degeneration and vacuolar changes increase in perivascular space (H & E x100) (GII); mild neuronal degeneration and vacuolar changes in the cytoplasm increase in perivascular space (H & E x100) (GIII).

The duodenum and ileum sections from GI underwent microscopic inspection, and the findings revealed mild to severe lamina propria degeneration, loss of histoarchitecture, inflammatory changes, necrosis, and desquamation of villi. Sections of duodenum and ileum from the GII and GIII groups revealed similar changes with mild intensity.

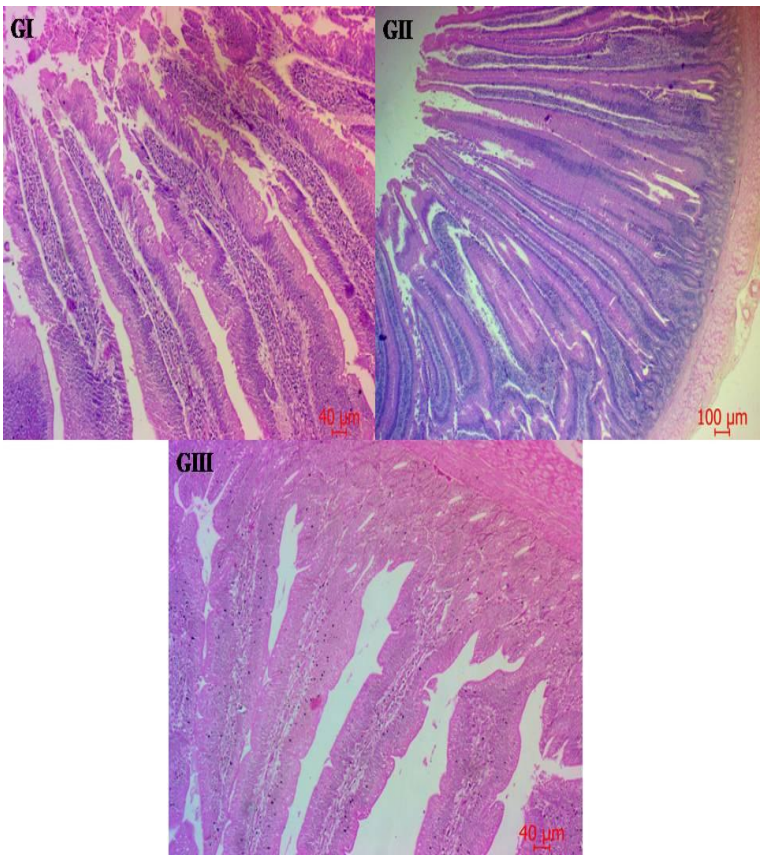


Figure 11. Microphotograph of Duodenum showing necrosis and desquamation at the tip of the villi with goblet cell hyperplasia, there is moderate mononuclear cells infiltration in lamina propria (H & E x100) (GI); desquamation at tip of villi with mild mononuclear cell infiltration in lamina propria (H & E x100) (GII); mild desquamation at tip of villi with mild mononuclear cell infiltration in lamina propria (H & E x100) (GIII).

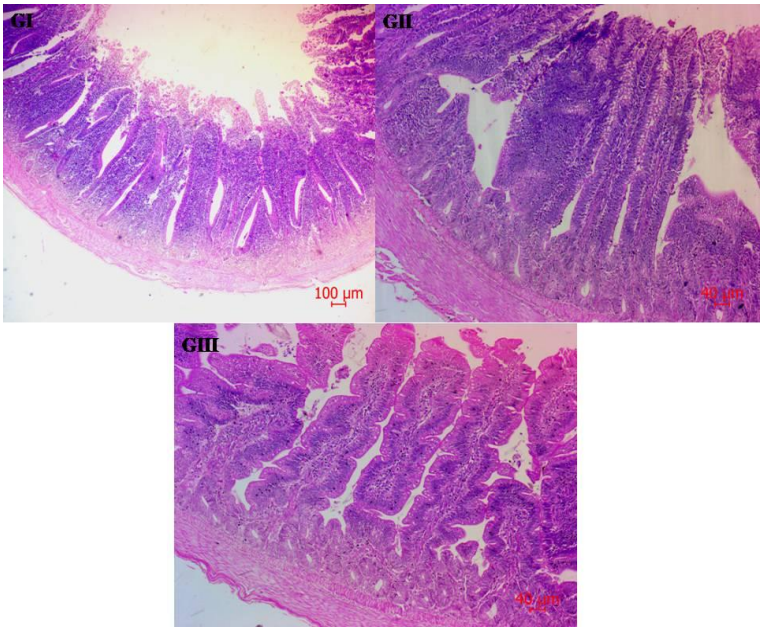


Figure 12. Microphotograph of Ileum showing moderate inflammatory changes in the villi with the loss of architecture and fusion of the villi with marked inflammatory cell infiltration in the lamina propria (H & E x100) (GI); mild inflammatory changes in the villi with goblet cell hyperplasia with moderate inflammatory cell infiltration in lamina propria (H & E x100) (GII); mild inflammatory changes in the villi with mild inflammatory cell infiltration in lamina propria (H & E x100) (GIII).

3.10 Histo-Morphometry

The duodenum and ileum of the heat-stressed control group I had significantly lower villi height and villi height:crypt depth ratios. The duodenum and ileum of the III group showed the most noticeably elevated villus height and villi height:crypt depth ratio, followed by the II groups. Group I displayed considerably rise in crypt depth. The III group showed the significant reduction in crypt depth, followed by the II and I groups.

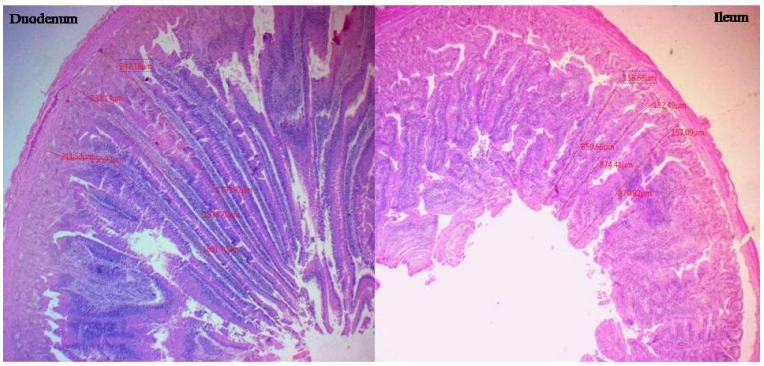


Figure 13. Duodenum showing increased villus height and decreased crypt depth (H & E x40) (GII); Ileum showing increased villus height and decreased crypt depth (H & E x40) (GIII).

CONCLUSION

Dietary supplementation with Emblica officinalis and selenium alleviated heat stress adverse effects in broiler birds. From aforementioned study it is very clear that E. officinalis and selenium exhibits hepatoprotective, cardioprotective, nephroprotective, immunomodulatory, antioxidant and anti-stress property. But Se yeast shows better result. Further study is also needed to understand the pathogenicity at molecular level.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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