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Evaluation of the effectiveness of herbal immunopotentiator products on carcass traits in amelioration of heat stress in broiler chickens

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Abstract
The objective of this study was to find out whether certain herbal products, namely Herbal C at 10 g/kg feed, Osmo C at 10 g/kg feed, Heat Beat at 10 g/kg feed, and Immuplus AFS at 50 g/kg feed, were effective at minimizing heat stress in broiler chickens. Five dietary groups: Control, Herbal C, Osmo C, Heat Beat, and Immuplus AFS each comprised up of nine replicates of five chicks each were produced from a total of 225day old chicks. For about six weeks, poultry birds were reared in experimental shed with temperature and humidity index ranged from 93.78 to 97.16%. In each experimental groups, the carcass parameters including carcass weight, abdominal fat, giblet weight, tender and fillet weight and muscle pH, had non significantly greater values than the control. Additionally, histopathological studies on kidney, liver, spleen, thymus, and bursa of Fabricius revealed some degenerative changes in the control group, including shrunken glomeruli in the kidney, inflammatory cell infiltration in the perivascular and periductular region of the liver, congestion, and hemorrhages, as well as an indistinct vital change in the white and red pulp of the spleen. As a result of their immunopotential action, herbal immunopotentiator supplements only often underwent regenerative changes that suggested a protective effect. According to the findings of the current study, herbal treatments, in particular Immuplus AFS and Osmo C, may be recommended as natural alternatives for the alleviation of heat stress.

Keywords: Certain herbal products, amelioration, heat stress, broilers

Introduction
High ambient temperature compromises animal welfare and productivity due to heat stress (HS) experienced over summer months. Heat stress has become one of the major challenges facing global livestock production, especially in the warmer parts of the world (Rhoads *et al*., 2013) [1]. India has a tropical climate, which means that the winters are generally cold and the summers were hot. One of the major stressors in the development of poultry is a hot and humid environment. One of the livestock production sectors that significantly contributes to the production of human food is poultry production. Poultry products have recently increased in significance and popularity (Adbhai *et al*., 2019) [2]. For laying hens, the ideal ambient temperature is probably between 19 and 22 °C, and for broiler growth, between 18 and 22 °C (Charles and walker 2002) [3]. According to definition heat stress is a common environmental stressor that occurs when the amount of heat produced by an animal surpasses the animal's capacity to dissipate the heat to its surrounding environment (Lara and Rostagno, 2013) [4]. This imbalance between heat production and body heat loss occurs when the environmental temperature rises above the upper critical temperature of the thermoneutral zone (Bernabucci *et al*., 2010; Lara and Rostagno, 2013) [5, 4]. Heat stress symptoms are likely to manifest when birds are subjected to environmental temperatures above the thermoneutral zone, or above 30 °C.

Heat Stress is the result of an imbalance between heat production and heat loss mechanisms, therefore, in addition to physical modifications of the animal's environment to minimize the exposure to heat, various mitigation strategies need to be targeted to achieve homeothermy (Chauhan *et al*., 2015) [6]. The strategies to reduce Heat Stress have been broadly classified as environmental modifications, genetic selection for improving thermotolerance, and nutritional strategies to improve feed intake and decrease metabolic heat production. Heat Stress will potentially affect broiler meat quality by muscle pH decline rate (Gregory 2010) [7]. The magnitude of changes in carcass characteristics may vary between different species, while overall, Heat Stress-induced carcass yield loss has been estimated to cause extensive economic losses to livestock industries.

The cost and impracticality of cooling animal structures have made dietary changes more common. Ascorbic acid and tocopherol, in particular, have been proven in studies to be particularly effective in reducing the harmful effects of environmental stress (Sahin *et al* 2002) [8]. There are many techniques to mitigate the adverse effects of high ambient temperature on the performance of chickens. The adverse effects of high ambient temperature on the performance of chickens can be reduced in a variety of ways. Numerous research conducted recently have indicated that dietary supplements containing antioxidants can reduce heat stress. Additionally, using immunopotentiator products is shown to enhance performance under heat stress. However, the majority of these synthetic substances are known to have adverse side effects in addition to being expensive. By virtue of their composition, multiple herbal antioxidants can be used as a safer alternative. By improving antioxidative enzymatic systems, herbs like *Withania somnifera*, *Asparagus raecemosus*, *Phyllanthus emblica*, and *Ocimum sanctum* have been used to protect tissues from ROS while enhancing cell viability (Saravanan 2007) [9].

Materials and Methods Birds and management

The housing facility at the Poultry Experimental Station, Department of Livestock Farm Complex, College of Veterinary Science, Rajendranagar, Hyderabad located in the Deccan plateau of Southern India was where experimental birds were raised. The experimental shed's average temperature was between 38.5 °C and 41.69 °C, and its average relative humidity was between 60.92 and 70.27%. Temperature humidity index was calculated using the formula (Moraes *et al*. 2008) [10]:

During the trial period, the temperature humidity index varied between 93.78 and 97.16%. The chicks were raised in battery brooders (6'6" x 4') with an average floor space of 1 sq feet per bird under ideal brooding circumstances for 42 days. The entire study period was conducted using standard management procedures. At the hatchery, birds were vaccinated against Marek's disease, Newcastle disease (ND), the Infectious Bursal Disease of Georgia strain vaccine, and Lasota at 7 and 28 days of age.

Table 1: Average weekly data on temperature, humidity and temperature humidity index in experimental shed

Week	Temperature (°C)	Humidity (%)	Temperature-Humidity Index (%)
1st week	38.50b	68.94a	93.78b
2nd week	40.21a	70.27a	96.69a
3rd week	40.51a	63.21b	95.25ab
4th week	40.74a	64.23b	95.82a
5th week	41.69a	64.34b	97.16a
6th week	41.37a	60.92b	95.89a
S.Em	0.28	0.71	0.30
N	7.00	7.00	7.00
P value	0.01	0.00	0.01

Experimental design

Commercial diets based on maize and soybean meal were provided to the birds during the starter (0–21days) and finisher (2-42 days) phases. These diets included 21.5% and 19.5 percent crude protein and 3050 and 3150 kcal/kg of metabolizable energy (ME), respectively. The experimental animals were aged one day to 42 days. About 225 one day old Commercial male broiler chicks were randomly divided into five treatment groups of nine replicates with five chicks in each replication. The experimental diets included the basal diet supplemented with Herbal C, Osmo C, Heat Beat, and Immuplus AFS at 10 g/kg, 10 g/kg, 10 g/kg, and 50 g/kg of feed, respectively, while the control diet was made up of corn soya bean meal.

Materials and Methods

Slaughter Parameters

At 6th week, nine broiler chicks in total were chosen from each treated group. The broiler chickens were brought to out of sight of the other broiler chicks two hours before they were supposed to be sacrificed in order to reduce stress on the chickens. The carotid arteries were immediately severed after cervical dislocation, and the hens were then bled for 100 seconds before spending roughly 4 minutes in 60 °C of hot water. The feathers were subsequently eliminated by running through a mechanical picker with a rotating drum for 30 seconds. Feathers, feet, heads (cut at the first cervical vertebra), and shanks were removed after the blood had stopped, and evisceration was carried out. Weighing of the carcasses was done. Total carcass yield, weights of specific organs including the liver, kidney, spleen, thymus, and Bursa of Fabricius, tender and fillet weights, and abdominal fat were the variables examined. The data thus obtained from liver, heart and gizzard weight were calculated to arrive giblet yield. The giblet yield was determined using the weights of the liver, heart, and gizzard. The carcass yield was estimated as the live weight to carcass weight ratio. The statistical analysis was done by using Statistical Package for Social Sciences (SPSS) 15th version.

Histopathological studies

45 birds (5 groups x 9 replicates) were sacrificed at the end and their liver, kidney, spleen, thymus, and Bursa of Fabricius tissues were taken for histopathological studies. They were embedded in paraffin after being dried in a graduated ethanol series. Using a microtome, sections with a thickness of about 5 microns were cut out and stained with hematoxylin and eosin (Culling, 1963) [11].

Results Carcass Traits

The results of statistical analysis showed that consuming herbal immunopotentiator products had no appreciable impact on the carcass parameters (Expressed as % live weight), including relative carcass weight, abdominal fat, Giblet weight (Liver, heart, and gizzard), tender and fillet weight, and muscle pH. The relative yield of the carcass varied from 61.19 to 67.25% live weight, and the values for abdominal fat were 1.01 to 1.64% live weight and Giblet were 4.38 to 4.95% live weight, respectively. There was little distinction between the groups. There was no statistically significant (p>0.05) difference in the tender and fillet weights (breast muscle) across the experimental groups. The obtained values for these parameters ranged from 11.32 to 15.02% live weight. Muscle pH did not differ significantly (p>0.05) across the experimental group and ranges between 5.45 and 5.55. The obtained liver weights are between 2.17 and 2.52% of the live weight. No significant difference (p>0.05) existed between the groups. In contrast, group 4 supplemented with Heat Beat showed little rise with a mean value of 2.52% live weight when compared to all other experimental groups. The trend for kidney yield followed that of the liver yield, with Immuplus AFS supplementation producing a 0.57% increase

in live weight in kidney yield as compared to other treatment groups. The value ranges in the 0.39 to 0.57% live weight range. Heat Beat fed broiler chicks had spleen weight that was considerably greater (P 0.05) than Immuplus AFS fed animals (0.12% live weight). The spleen weights of the other supplementary groups, however, were comparable to those of the control group. Spleen relative weights varied from 0.09 to 0.15% of live weight. There was no statistically significant difference in the values for Thymus weight among the experimental groups (p >0.05). The values for thymus weight varied from 0.42 to 0.53% live weight. The bursa of Fabricus has a relative weight of 0.06 to 0.08% of live weight. The relative weights of the liver, kidney, thymus, and bursa of Fabricius were not substantially affected by dietary herbal immunopotentiator products (p > 0.05), although there was a significant difference in the relative weight of the spleen. The data on different carcass parameters of broilers at 6th week of age as influenced by different dietary treatments is presented in Table 2 & 3.

Table 2: Effect of dietary inclusion of herbal immunopotentiator products on carcass parameters based on organ weights (% live weight) at 6th week of age in commercial broiler chicken

Group	Carcass	Abdominal fat	Giblet wt	Tender and fillet wt	Muscle pH
Control	61.19	1.07	4.38	11.32	5.45
Herbal C	61.30	1.01	4.75	11.46	5.54
Osmo C	67.25	1.18	4.44	11.68	5.53
Heat Beat	63.86	1.64	4.70	11.52	5.46
Immuplus AFS	61.94	1.63	4.95	15.02	5.55
SEM	0.893	0.104	0.144	0.58	0.026
N	9.00	9.00	9.00	9.00	9.00
P Value	0.157	0.125	0.744	0.83	0.604

Means with different superscript in a column differ significantly: p<0.05, P-value: probability value. N: number of replicates; SEM: Standard Error Mean

Table 3: Effect of dietary inclusion of herbal immunopotentiator products on slaughter parameters based on organ weights (% live weight) at 6th week of age in commercial broiler chicken

Group	Liver	Kidney	Spleen	Thymus	Bursa of Fabricius
Control	2.17	0.51	0.09b	0.50	0.06
Herbal C	2.25	0.39	0.10b	0.44	0.08
Osmo C	2.27	0.40	0.11b	0.53	0.06
Heat Beat	2.52	0.49	0.15a	0.50	0.07
Immuplus AFS	2.18	0.57	0.12ab	0.42	0.08
SEM	0.072	0.030	0.007	0.031	0.006
N	9.00	9.00	9.00	9.00	9.00
P Value	0.563	0.236	0.02	0.779	0.558

Means with different superscript in a column differ significantly: P<0.05, P-value: probability value. N: number of replicates; SEM: Standard Error Mean

Histopathological Studies

The control group and other treated group, which were sacrificed on the 42nd day of the experiment, were examined for histopathological changes in the liver, kidney, spleen, thymus, and Bursa of Fabricius.

Group 1 (Control)

The glomeruli in the kidney sections of the control group (which was under heat stress) were smaller and more cellular. The liver of the control group exhibited considerable dilatation of the sinusoidal space as well as infiltration of an inflammatory perivascular and periductular region. The control group's region of the spleen had hemorrhages, congestion, and blurry white and red pulp. Thymus in the control group displayed depletion, follicular congestion, and sinusoidal dilation. Indistinct follicular differentiation and hypoplasia of bursal follicles were visible in the bursa of Fabricius in the Control group.

Group 2 (Herbal C)

Birds given Herbal C showed consistent Bowman's capsule, light congestion, and hemorrhages in their kidney sections. This group's liver sample had modest sinusoidal congestion and proliferation of fibrous tissue. While the thymus displayed mild medullary edema and a normal capsule, the spleen displayed normal capsule and mild splenic sinusoidal dilatation. The Bursa of Fabricius segment from this group displayed localized interfollicular edema and a slight growth of fibrous tissue.

Group 3 (Osmo C)

The kidney of the Osmo C supplemented birds had mildly dilated renal arteries and mildly cystic appearing tubules. While liver sections revealed a slight loss of hepatic cords, at the center hepatic cells had been restored. This group's spleen had somewhat more capillaries in the red pulp area and slightly different follicles (white and red pulp). The thymus showed moderate corpuscular connective tissue, a normal capsule, and mild interfollicular connective tissue alterations as well as mild medullary edema.

Group 4 (Heat Beat)

Bowman's capsule diminished and desquamation of epithelial cells were visible in the bird's kidney after using Heat Beat supplements. The liver tissue sample from this group showed normal portal triad with hepatic cord distortion. The spleen segment revealed prominent white and red pulp as well as a small patch of white pulp. The thymus, however, displayed well differentiated medulla, as well as typical cellularity at the medulla. The Bursa of Fabricius segment displayed homogeneous bursal follicles and typical follicular shape.

Group 5 (Immuplus AFS)

Birds given Immuplus AFS showed moderate renal capillary congestion and mild cystic dilatation of tubules in their kidney sections. The same group's liver displayed a homogenous nucleus, a normal portal triad, and mild dilated sinusoids. While the spleen showed congestion and minor hypoplasia, the thymus segment displayed mild thymic follicle enlargement and focal region follicle depression. In this group of birds, the bursa of Fabricius revealed normal epithelial cells with interfollicular connective tissue.

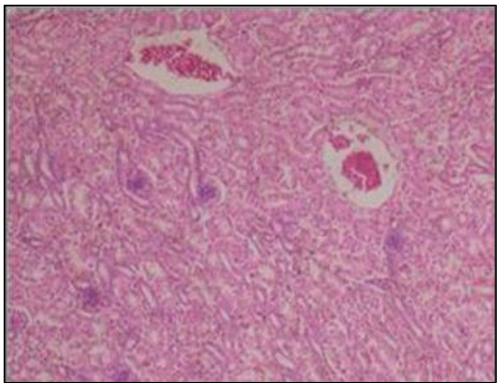

Fig 1: Photomicrograph of kidney showing shrunken glomerulus, hyper cellularity (Group1 control 42nd day), H&E X100

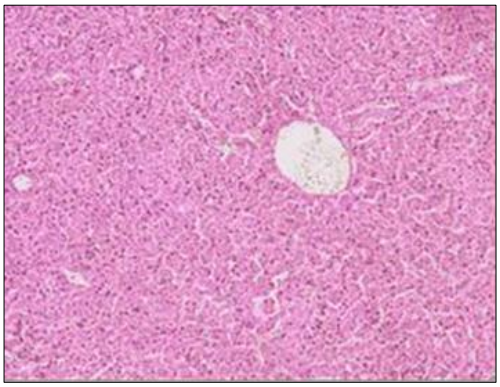

Fig 2: Photomicrograph of liver showing infiltration of inflammatory perivascular and periductular area, severe dilatation of sinusoidal space (Group1 control 42nd day), H&E X100

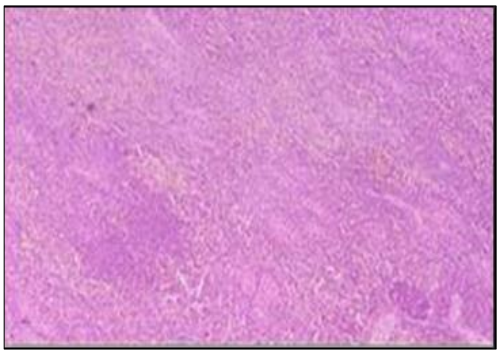

Fig 3: Photomicrograph of spleen showing congestion, haemorrhages and indistinct white and red pulp (Group 1control 42nd day), H&E X100

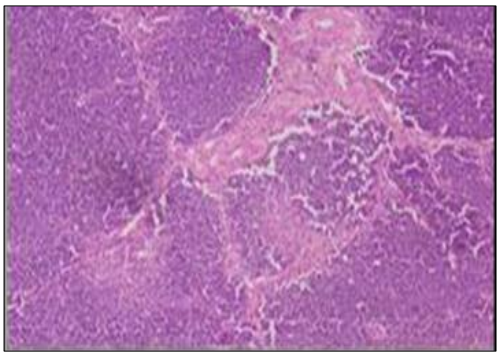

Fig 4: Photomicrograph of thymus showing depletion, congestion of follicles, dilation of sinusoids (Group1 control 42nd day), H&E X 100

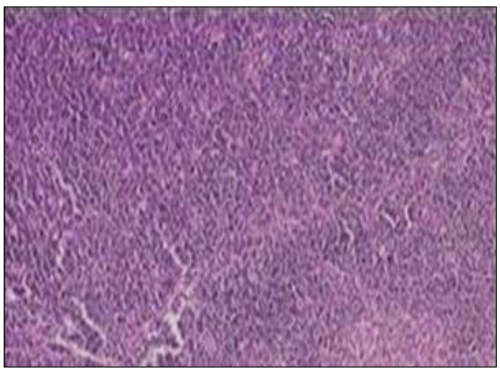

Fig 5: Photomicrograph of Bursa of Fabricius showing indistinct follicular differentiation and hypoplasia of bursal follicles (Group1 control 42nd day), H&E X 100

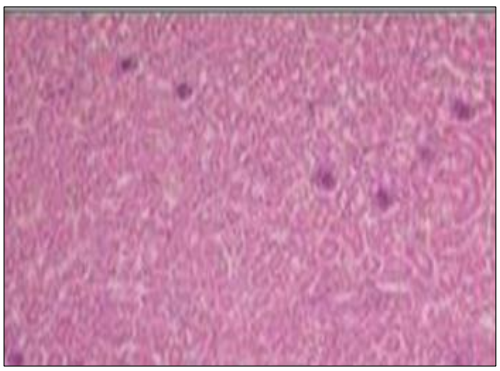

Fig 6: Photomicrograph of kidney showing uniform bowman's capsule, mild congestion and haemorrhages (Group 2 Herbal C 42nd day), H&E X 100

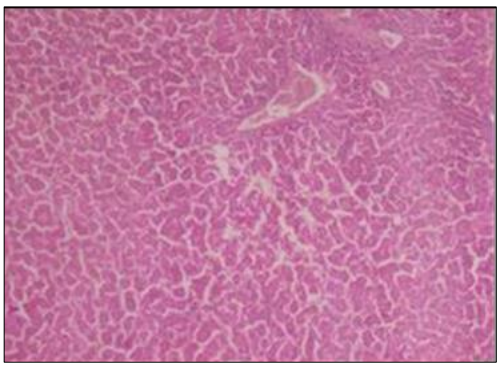

Fig 7: Photomicrograph of liver showing mild congestion of sinusoids, mild fibrous tissue proliferation (Group 2 Herbal C 42nd day), H&E X 100

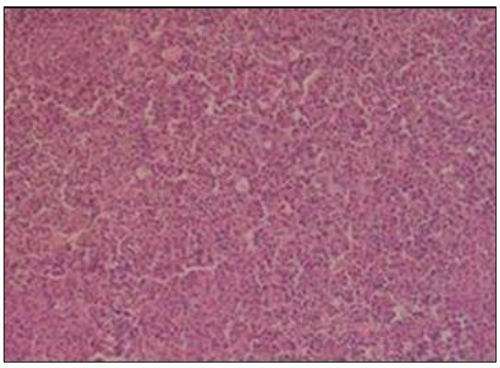

Fig 8: Photomicrograph of spleen showing normal capsule, mild splenic sinusoidal dilation (Group 2 Herbal C 42nd day), H&E X 100

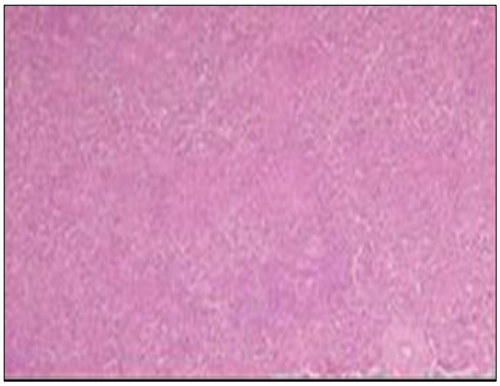

Fig 9: Photomicrograph of thymus showing mild medullary edema, normal capsule (Group 2 Herbal C 42nd day), H&E X 100

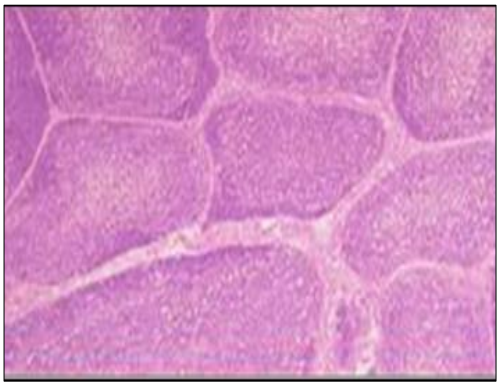

Fig 10: Photomicrograph of Bursa of Fabricius showing mild proliferation of fibrous tissue, focal interfollicular edema (Group 2 Herbal C 42nd day), H&E X 100

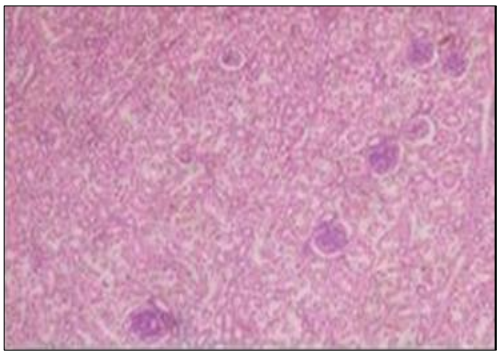

Fig 11: Photomicrograph of kidney showing mild dilatation of renal vessels, mild cystic appearance of tubules (Group3 Osmo C 42nd day), H&E X 100

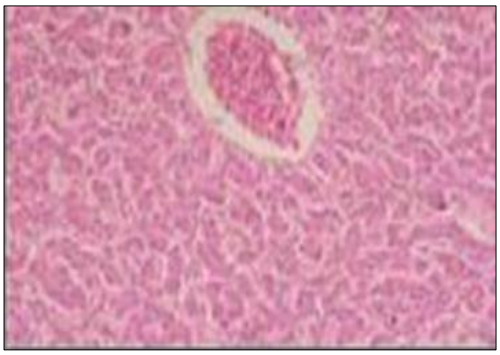

Fig 12: Photomicrograph of liver showing mild destruction of hepatic cords, restoration of hepatic cells at center (Group 3 Osmo C 42nd day), H&E X 100

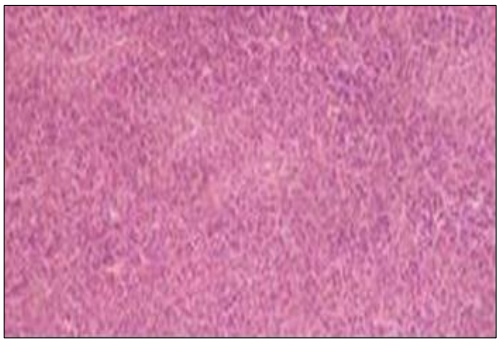

Fig 13: Photomicrograph of spleen showing mild increased number of capillaries at red pulp area, slight difference with follicles (white and red pulp) (Group 3 Osmo C 42nd day), H&E X 100

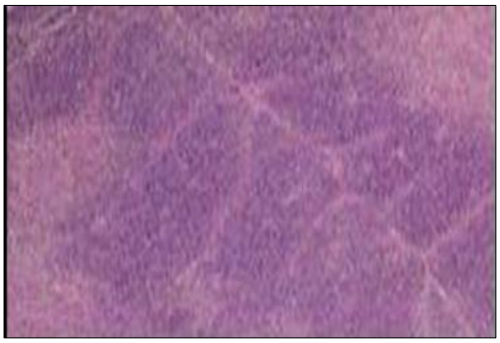

Fig 14: Photomicrograph of thymus showing mild corpuscular connective tissue, normal capsule (Group 3 Osmo C 42nd day), H&E X 100

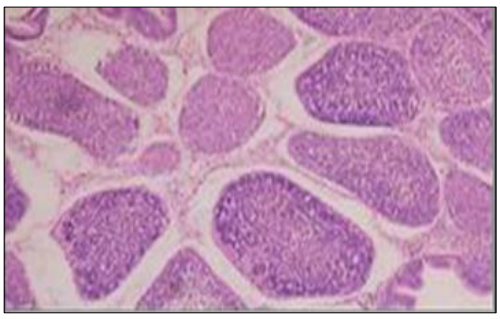

Fig 15: Photomicrograph of Bursa of Fabricius showing mild changes in inter follicular connective tissue, mild medullary edema (Group 3 Osmo C 42nd day), H&EX 100

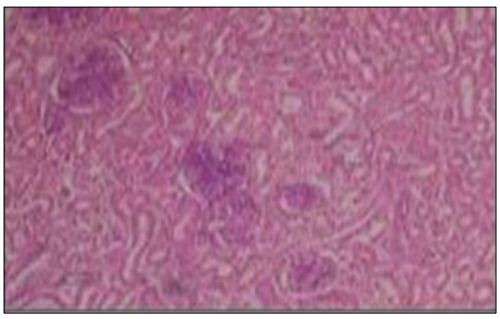

Fig 16: Photomicrograph of kidney showing desquamation of epithelial cells, decreased bowman's capsule (Group 4 Heat Beat 42nd day), H&E X 100

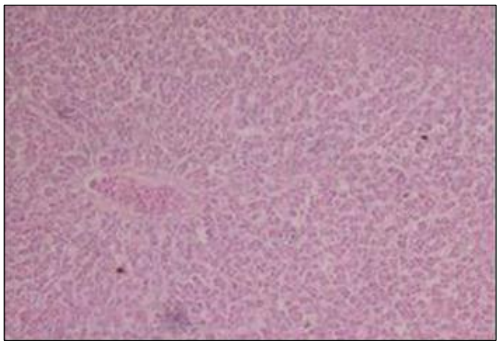

Fig 17: Photomicrograph of liver showing normal portal triad, distortion of hepatic cords (Group 4 Heat Beat 42nd day), H&E X 100

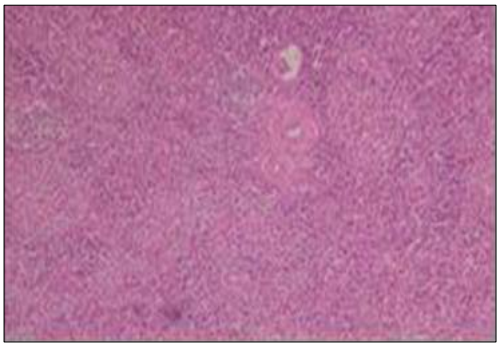

Fig 18: Photomicrograph of spleen showing distinct white and red pulp, narrow area of white pulp (Group 4 Heat Beat 42nd day), H&E X 100

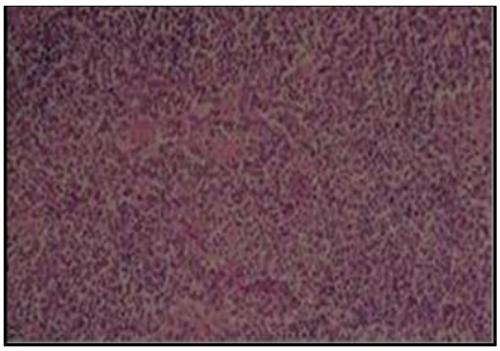

Fig 19: Photomicrograph of thymus showing normal cellularity at medulla, well differentiated cortex and medulla (Group 4 Heat Beat 42nd day), H&E X 100

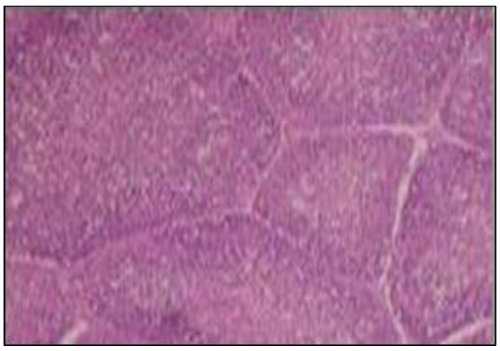

Fig 20: Photomicrograph of Bursa of fabricius showing normal follicular structure, uniform bursal follicles (Group 4 Heat Beat 42nd day), H&E X 100

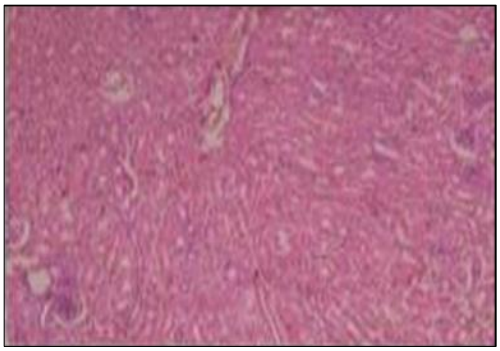

Fig 21: Photomicrograph of kidney showing mild congestion of renal capillaries, mild cystic dilation of tubules (Group 5 Immuplus AFS 42nd day), H&E X 100

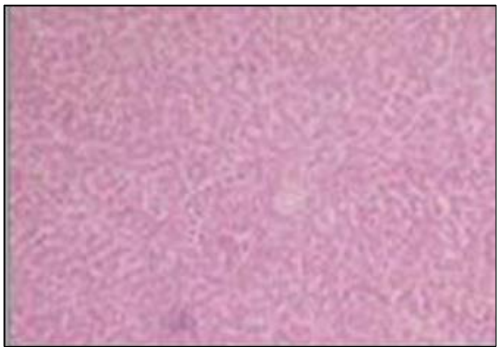

Fig 22: Photomicrograph of liver showing normal portal traid, uniform nucleus, mild dilatation and mild dilated sinusoids(Group 5 Immuplus AFS 42nd day), H&E X 100

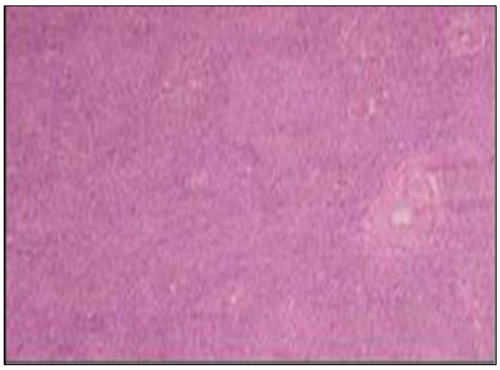

Fig 23: Photomicrograph of spleen showing congestion and mild hypoplasia (Group 5 Immuplus AFS 42nd day), H&E X 100

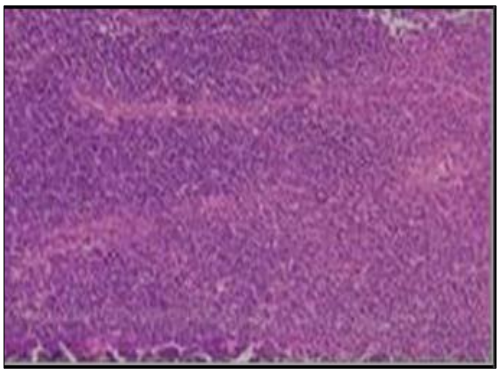

Fig 24: Photomicrograph of thymus showing mild dilation of thymic follicles, focal area depression of follicles (Group 5 Immuplus AFS 42nd day), H&E X 100

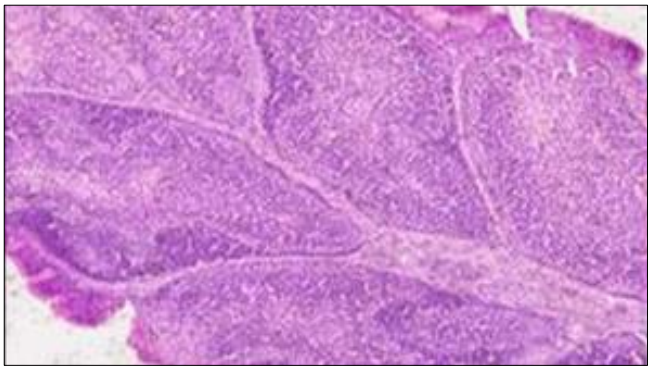

Fig 25: Photomicrograph of Bursa of Fabricius showing normal epithelial cells with interfollicular connective tissue (Group 5 Immuplus AFS 42nd day), H&E X 100

Discussion

An attempt was made in the present study to test the effectiveness of four herbal immunopotentiator products as dietary supplements in reducing heat stress. During the trial time, the typical THI that is comfortable for the birds should be around 75. Throughout the whole trial time, the THI was >90, indicating severe heat stress in the birds. In the present study, the carcass yield showed a marginal improvement in % carcass weight over control though the values were non-significant. Similar non-significant higher values were observed with other carcass parameters in treated group. Contrary to the present findings, Quarles and Adrian (1989) [12], Sahin and Kucuk, (2001) [13s] reported a significantly improved carcass characters in heat stressed Japanese quails when supplemented with vitamin C. Sahin *et al*. (2002) [8] also reported that dietary inclusion of vitamin C increased giblet weight in heat stressed broilers. It is reported that heat stress at 40 °C, for 1 hour had an effect of decline in pH (Wang *et al*., 2016) [14] and the findings of other reporters (Pedulwar *et al*., 2007) [15] showed improved dressing % yield of broiler birds when supplemented with Ashwagandha root powder. Heat stress is shown by the degenerative alterations in the control group's histopathological studies, which were absent in the group receiving herbal supplements. The herbal immunopotentiator products ability to fight off free radicals may be the cause of the absence of degenerative alterations in the group that received herbal supplements.

Conclusion

In conclusion, herbal supplements may be regarded as a safer substitute for synthetic antioxidants when it comes to combating heat stress induced histopathological alterations and enhancing its output through the summer.

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