Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

J E Z S Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(3): 1504-1510

Received: 26-03-2017
Accepted: 27-04-2017

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Comparative studies between probiotic and vitamin E and selenium to reduce the effect of aflatoxin in broiler chicken

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Abstract
This study was conducted to investigate the protective role of both commercial products of probiotics + Vitamin E and selenium against the effects of aflatoxin in the broiler. A total of 200 chicks were divided into four groups (50 chicks each) as following: The T1 treated with probiotic with aflatoxin: T2 treated with vitamin E and selenium with aflatoxin: T3 non-treated group (fed on diet with aflatoxin): T4 control negative (diet without aflatoxin). The results revealed that the differences in the body weight of the T1 at 25 days (994 gm) and 35 day (1860 gm) were not significant as compared with corresponding estimations of 1011 and 1876 gm in the T4. Concerning the leukocyte count and H/L ratio results showed no significant difference between T2 (0.42) and T4 (0.41) compared with T1 (0.84) and T3 (1.29). The histopathological examination revealed that probiotics have the best impact compared with vitamin E+selenium. In conclusion: this study confirmed the protective role of probiotic on body weight and histopathological changes, while vitamin E and selenium had a protective role on leukocyte count.

Keywords: Aflatoxin, probiotics, vitamin E and selenium

Introduction
Aflatoxins are metabolites produced by some species of *Aspergillus* (e.g. *Aspergillus flavus and Aspergillus parasiticus* [1]. Also, aflatoxin will metabolize after ingestion by chicken resulting bad effects on the body such as poor growth, damage internal organs like liver and kidney, immunosuppression due to damage of lymphoid organs, increased susceptibility to environmental and microbial stresses, and high level of mortality [1,2]. Aflatoxins caused main problems in the poultry industry and extensive economic losses [3]. Therefore, different procedures adopted to reduce their effect on poultry [4]. The present study aimed to investigate the role of additives like probiotic and vitamin E and selenium in reducing the harmful effects of aflatoxin on body weight, vascular leukocytes, and tissue damage.
Probiotic means groups of beneficial microorganisms like bacteria, fungi, and yeast living in digestive system [5, 6]. One of the important functions of probiotics is to detoxification of mycotoxins by binding them to its own cell wall component [7]. Probiotic considered as one of the most important immunomodulatory agents by activation specific and nonspecific host immune response in poultry and this lead to reduce the immunosuppressive effects of aflatoxin [8]. The probiotic supplementation is beneficial for better body performance through improved body weight, weight gains, feed efficiency and feed conversion ratio as well as improve the economic efficiency in broiler chicks [9].
Vitamin E and selenium are nutrients that are involved in both metabolic and physiological processes, which are critical for poultry health and one of the main elements in the diet [10]. Vitamin E and Selenium plays a major role in the antioxidant defense system [11]. It has been suggested that there is a synergistic relationship between selenium and vitamin E, because of the vitamin E and selenium could remove the detoxification in the body by detoxifying hydroperoxides [12]. Vitamin E and selenium, has an effect in the cells of the immune system, like lymphocytes and macrophages and other inflammatory function through their influences on cytokines [13]. The supplementation of vitamin E and selenium to the diet of broiler could be increase the T cell population in the Thymus and spleen [14, 15].

2. Material and Methods

2.1 Chickens

A total of 200 chicks (Ross 308) mixed sexes were bred in the poultry field/Department of Pathology-College of Veterinary Medicine -University of Baghdad/Iraq during October-November 2016. The chicks were bred at complete hygiene conditions and provided by food, water (*ad libitum*). All birds subjected to complete vaccination programs during the period of the experiment (35 days).

2.2 Diet

All bird diets are contaminated with aflatoxin at the dose of 0.8 ppm except negative control - T4 and determined by ELISA and HPLC methods [16]. The chickens fed on the diet with aflatoxin during all the period of the experiment.

2.3 Experimental design

Two hundred chicks were used in this experiment. The birds divided into four equal groups (50 chicks each) with two replicates as the following*:*

T1: commercial products (Poultry star® of Biomin® company) of probiotics with concentration 5×10^{12} CFU /kg, supplied with drinking water *.*

T2: commercial products (UVEDCO ES®) of vitamin E 80 mg / Selenium 1.6 mg as 0.5ml/L was provided with drinking water*.*

T3: non-treated (control positive) group (feed on aflatoxin contaminated in dose (0.8ppm) (control positive).

T4: control negative (uncontaminated diet).

2.4 Body weight

The mean of ten samples from each group was weighed randomly for live body weight at ages 15, 25 and 35 days old, to show the difference in body weight.

2.5 Blood analysis

Blood samples were taken from chickens at age of 15, 25 and 35 days to measure the leukocyte differential count (Heterophil, Lymphocyte, Monocyte, Eosinophil, Basophil) as well as to measure the Heterophil / Lymphocyte ratio. A ten blood samples from each group stained with Wright stains and examined for 100 different cells*.*

2.6 Histopathology

Tissue samples (Thymus and Harderian gland) were taken from birds at 35 days to examine the histopathological changes. The samples were processed and stained with hematoxylin and eosin stain [17].

2.7 Statistics

All data were subjected to analysis of variance (ANOVA). Least significant diference was used to assess the significance difference among group [18].

3. Result and Discussion

3.1 Body weight

Table 1. Shows the results of body weight. The weight of control positive group was significantly ($P<0.05$) lowered than other groups followed by vitamin E group, while the probiotic treated group showed a significantly low body weight only at 15 days old as compared with control negative group.

Results showed that the probiotics have a pronounced effect against aflatoxin on body weight, and this suggested that the probiotic additives improved the intestinal microflora balance which removed the harmful effect (toxins and foreign substances) and makes a better environment [19]. Although the results agreed with Strompfova *et al*., [20] through increased the food substance analysis and increasing in the launch active ingredients of foodstuffs. As well as, probiotic improved the body performance through increasing the absorption of substances in the intestine by increasing the length villi and activated metabolic process and enzymes to get the most nutritional benefit [21, 22].

Though vitamin E and selenium has less effect than probiotic, but it enhanced the body weight comparing with control positive group and this may relate to the significant effect of vitamin E and selenium on feed conversion ratio and body weight [23, 24], also may be related to the ability of this additive to reduce the harmful effect of aflatoxin because of the vitamin E and selenium representative a great antioxidant agent [25].

3.2 Total differential count

The result of heterophil count was shown in Table (2). Results showed that, at 15, 25 and 35 days old, the control positive group was significantly ($P<0.05$) lower in the total differential count than other groups, followed by probiotics group. Vitamin E group showed no significant differences at ($P<0.05$) when compared with control negative group.

The result of lymphocyte count (Table 3) demonstrated that there is a significant decreasing ($P<0.05$) in control positive group when compared with negative control group at 15, 25 and 35 day, while the probiotic group showed significant differences ($P<0.05$) only in 25 and 35 days. Vitamin E/selenium group showed no significant differences compared with negative control group in 25 and 35 days. The result revealed that monocyte count (Table 4) increased significantly ($P<0.05$) at 15 days old. The control positive group has significant increasing ($P<0.05$) among other groups, but in 25 and 35 days old this group showed significant decrease ($P<0.05$) among other groups, while Vitamin E/selenium group showed significant differences ($P<0.05$) at 15 and 35 days old only and the probiotic group showed significant differences at 15 days old only comparing with negative control group*.*

The Eosinophil count (Table 5). Showed that at 15 days old the probiotic group showed a significant increasing ($P<0.05$) compared with other groups, while at 25 days old both probiotic and control positive groups showed a significant decreasing ($P<0.05$) compared with other groups. However, at 35 days old both probiotic and control positive group showed significant differences ($P<0.05$) compared with control negative group. Vitamin E/selenium group differed significantly ($P<0.05$) only at 35 days old compared with negative control group*.* Results in different days old in the same group indicated that both controls positive and negative showed no significant changes ($P<0.05$), while other groups show significant differences ($P<0.05$) at different ages.

The result of basophil count (Table 6) indicated no significant differences ($P<0.05$) among all groups and no differences in the same group at ages 15, 25 and 35 days old except the control positive group which showed significant differences ($P<0.05$) comparing with other groups.

In Table (7), the results of the heterophils / lymphocyte ratio showed that at 15, 25 and 35 days old, the control positive showed a significant increasing ($P<0.05$) compared with

other groups, while the probiotics group showed a significant increasing (P<0.05) at 25 and 35 days old only. Vitamin E/selenium group showed no significant differences compared with the negative group.

The result in the same group at different day showed a significant differences (P<0.05) in probiotic group at 15, 25 and 35 day old and control positive group at 35 day old only, while there is no significant differences (P<0.05) in these days at both vitamin E/selenium and control negative group.

In general, the leukocytes differential count (LDC) explained that the probiotics additive group has no significant effects in LDC and this result agreed with Shareef and Al-Dabbagh [26]. In a study included one hundred male broiler chicks treated with the different levels of probiotic (Saccharomyces cerevisiae), results revealed no significant differences in haematological parameters (differential count) [27]. However, Patterson and Burkholder [28] reported that the probiotics improved the blood picture when compared with control positive group due to enhanced the immunity. Moreover, the probiotic and prebiotics bind with aflatoxin and could prevent the the obsorption in the intestine [29].

Vitamin E/selenium groups recorded the best impact on LDC by decreasing heterophil and increasing lymphocyte that will lead to improving the heterophil lymphocyte ratio. This result is consistent with results obtained by Shlig [30] and Tayeb [31].

Finally, the results of the harmful effect of aflatoxin on total blood cells count agreed with other studies that showed the aflatoxin caused lymphocytopenia, monocytopenia and increasing the percentage of neutrophil counts [32, 33].

3.3 Histopathology

Thymus at 35 days old was normal in control negative group compared with the probiotic group (Figure. 1). The lesion at 35 days old in group of probiotic was severe and there are multiple focal areas of necrosis in the Thymus gland (Figure 2), while vitamin E and selenium group showed more severe lesion multiple areas of focal necrosis in cortex and medulla of Thymus lobules (Figure 3).The necrotic area appears sometimes as empty space or calcification may notice in the necrotic areas. The thymus in control positive group showed necrosis in addition to focal subcapsular granuloma consist of macrophages and epithelioid cells (Figure 4), control negative group showed normal thymus gland (Figure 5).

The results of Harderian gland at 35day old showed that the lesion of probiotic group was necrosis in the glandular lobes (Figure 6) and infiltration of large numbers of macrophage and lymphocyte in the interstitial tissue, also hyperplasia of epithelial forming a papillary projection, while in the vitamin E and selenium group, the lobules showed infiltration of large numbers of mononuclear cells between the alveoli and along the trabeculae (Figure 7) besides the congestion of blood vessels and hemorrhage. Also, dilation of some tubules with cellular debris in their Lumens and hyperplasia of glandular epithelia. The lesion in the control positive showed multiple focal areas of necrosis (Figure 8). Finally, control negative group showed normal Harderian gland (Figure 9).

The result of histopathological confirmed the changes in the lymphoid organs (Thymus, Harderian gland) with the presence of aflatoxin toxic at different ages and different additives. These histopathological results agreed with the results obtained by Menconi *et al*., [34] who reported that the probiotic additives have immunomodulatory and anti-inflammatory effects as they regulated the pro-inflammatory and anti-inflammatory cytokines Menconi *et al*., [34]. The role of probiotic in the local and cellular immunity (specific immunity) could be attributed to its ability to activate the production of IgA and T-cell and this will protect these organs [22]. Furthermore, Jaafer [35] showed that the probiotic treatment in chicken enhanced chicken humoral immune response through inducing the secondary lymphoid organs, such as Harderian and regulate the function of the T-cell which could lead to the maintenance of Thymus.

Concerning the histopathological changes in Thymus and Harderian gland for the group of the vitamin E and selenium, results revealed limited impacts. This is identical to some studies which suggested that the vitamin E and selenium additives reducing the effect of aflatoxin on immune response and lymphoid organs by modulating the metabolic end product or by activation the glutathione peroxidase (GSH-PH) as the aflatoxin is inactivated by binding with glutathione-s-transferase (GST) and excreted through urine and bile [36].

Table 1: The effect of the group and period on the body weight (gm) of broiler

Group / Day (gm)	Probiotic	Vit. E& selenium	C positive	C negative
15	370.4±6.99 C b	329.5 ± 8.41 C c	306 ± 1.54 C d	394.8 ± 6.68 C a
25	994 ± 20.12 B a	834.4 ± 9.48 B b	732.4 ± 15.48 B c	1011.6±10.55 B a
35	1860.6±76.17 A a	1600.1±50.88 A b	1158.6±33.69 A c	1876.6±20.43 A a

The differences in small letters horizontally refer to the significant differences (P<0.05).
The differences in capital letters vertical refer to the significant differences (P<0.05).

Table 2: The effect of the group and period on the no. of Heterophil

Group / Day (no.)	Probiotic	Vit. E& selenium	C positive	C negative
15	29.4 ± 2.52 B b	30.2 ± 1.94 A b	45.4 ± 2.21 B a	31.6 ± 1.44 A b
25	37.2 ± 1.43 A b	27.4 ± 1.60 B c	44.2 ± 1.94 B a	25.2 ± 3.68 A c
35	38.4 ± 1.44 A a	25.2 ± 2.58 C b	49 ± 1.73 A a	24.8 ± 3.49 A b

The differences in small letters horizontally refer to the presence of significant value at (P<0.05).
The differences in capital letters vertical refer to the presence of significant value at (P<0.05).

Table 3: The effect of the group and period on the no. of Lymphocyte

Group / Day(no.)	Probiotic	Vit. E& selenium	C positive	C negative
15	60.6 ± 2.58 A a	65 ± 2.42 A a	42.4 ± 2.19 A b	60.2 ± 1.77 A a
25	51.8 ± 2.09 B b	59.4 ± 3.25 B a	43.8 ± 1.83 A c	62 ± 2.76 A a
35	45.6 ± 2.25 C b	59.8 ± 2.09 B a	37.4 ± 1.60 B c	60.2 ± 1.85 A a

The differences in small letters horizontally refer to the significant differences (P<0.05).
The differences in capital letters vertical refer to the significant differences (P<0.05).

Table 4: The effect of the group and period on the no. of Monocyte

Group / Day(no.)	Probiotic	Vit. E& selenium	C positive	C negative
15	5.5 ± 0.02 C c	6.6 ± 0.51 C b	8.4 ± 0.75 A a	4.2 ± 0.20 B d
25	8 ± 1.09 B a	9.4 ± 0.75 A a	7.2 ± 0.49 B b	9.4 ± 1.97 A a
35	10.6 ± 0.45 A a	8.8 ± 0.92 B b	7.0 ± 0.45 B c	10.2 ± 0.56 A a

The differences in small letters horizontally refer to the significant differences (P<0.05).
The differences in capital letters vertical refer to the significant differences (P<0.05).

Table 5: The effect of the group and period on the no. of Eosinophil

Group / Day(no.)	Probiotic	Vit. E& selenium	C positive	C negative
15	3.9 ± 0.51 A a	3 ± 0.32 B b	2 ± 0.37 A c	3.2 ± 0.37 A b
25	2.6 ± 0.24 B b	3 ± 0.32 B a	2.2 ± 0.58 A b	3 ± 0.32 A a
35	3.8 ± 0.37 A b	5 ± 0.84 A a	2.4 ± 0.24 A c	3.6 ± 0.51 A b

The differences in small letters horizontally refer to the significant differences (P<0.05).
The differences in capital letters vertical refer to the significant differences (P<0.05).

Table 6: The effect of the group and period on the no. of Basophile

Group / Day(no.)	Probiotic	Vit. E& selenium	C positive	C negative
15	0.6 ± 0.40 B b	0.8 ± 0.20 B b	1.8 ± 0.45 C a	0.8 ± 0.20 A b
25	0.4 ± 0.24 B b	0.8 ± 0.15 B b	2.6 ± 0.40 B a	0.4 ± 0.24 A b
35	1.6 ± 0.24 A b	1.2 ± 0.37 A b	3.2 ± 0.37 A a	1.2 ± 0.37 A b

The differences in small letters horizontally refer to the significant differences (P<0.05).
The differences in capital letters vertical refer to the significant differences (P<0.05).

Table 7: The effect of the group and period on the H/L ratio

Group / Day (no.)	Probiotic	Vit.E& selenium	C positive	C negative
15	0.48 ± 0.07 A b	0.46 ± 0.04 A b	1.07 ± 0.08 B a	0.52 ± 0.03 A b
25	0.71 ± 0.04 B b	0.46 ± 0.06 A c	1.00 ± 0.08 B a	0.40 ± 0.07 A c
35	0.84 ± 0.02 C b	0.42 ± 0.06 A c	1.29 ± 0.09 A a	0.41 ± 0.06 A c

The differences in small letters horizontally refer to the significant differences (P<0.05).
The differences in capital letters vertical refer to the significant differences (P<0.05).

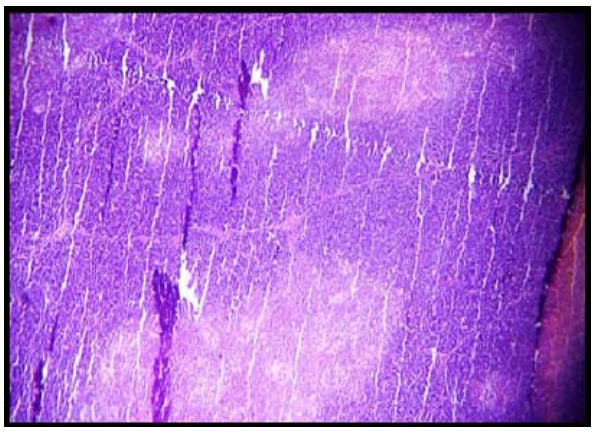

Fig 1: Histological section in Thymus1st group at (35day old): showed normal section (H&E Stain-X20).

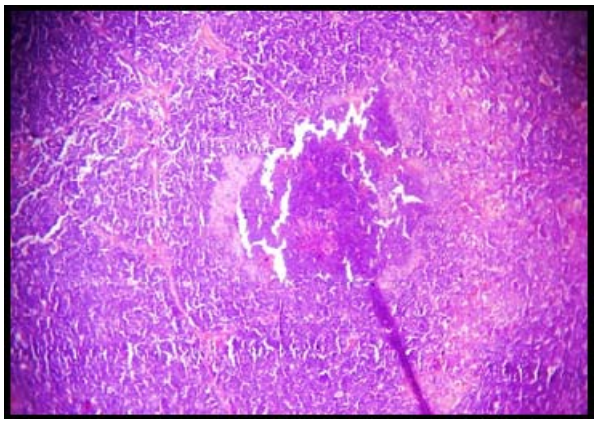

Fig 2: Histological section in Thymus1st group at (35day old): Necrosis (arrpw) in the thymic lobules (H&E Stain-X20)

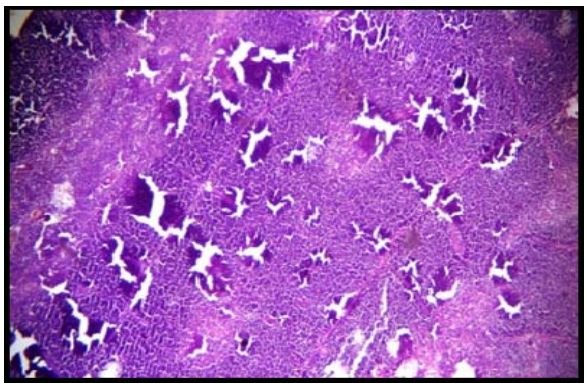

Fig 3: Histological section in Thymus2nd group at(35day old) multiple area of focal necrosis (arrow) (H&E Stain-X10)

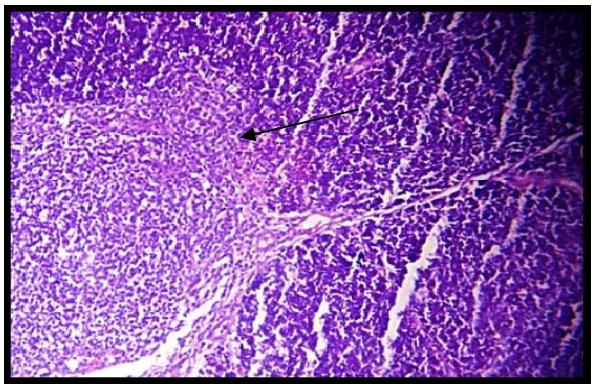

Fig 4: Histological section in Thymus3rd group at(35day old): focal sub capsular granuloma (arrow) (H&E Stain-X20)

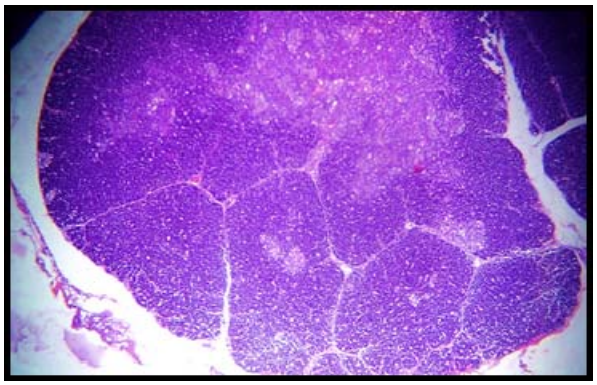

Fig 5: Histological section in Thymus4th group(3) in all ages:Normal architecture(H&E Stain-X4)

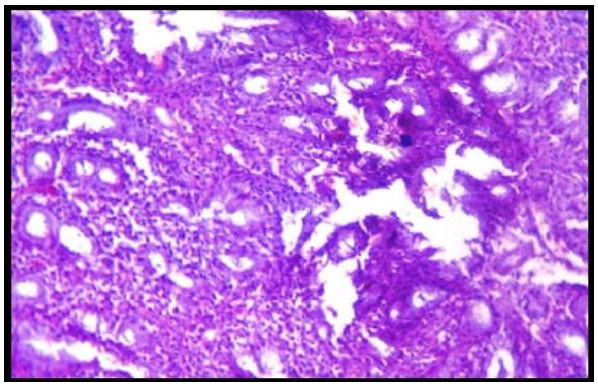

Fig 6: Histological sectionHarderian gland- 1st groupat (35day old): necrosis in the glandular lobes (arrow) (H&E Stain-X20)

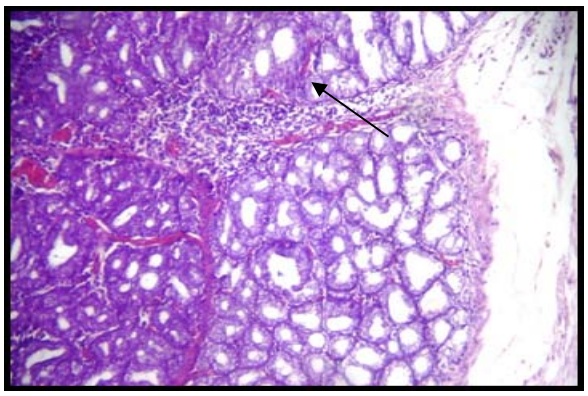

Fig 7: Histological sectionHarderian gland - 2nd groupat(35day old): infiltration large numberof mononuclear cells (arrow) (H&E Stain-X20)

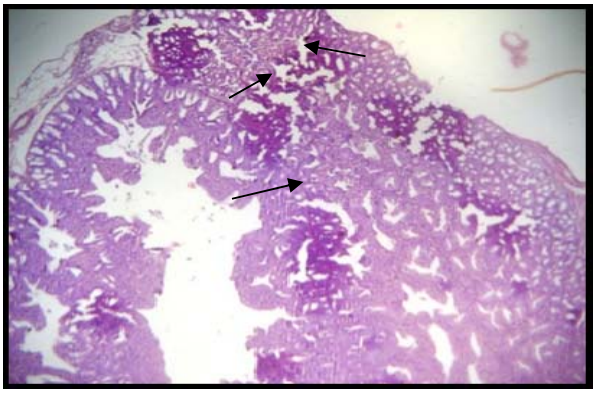

Fig 8: Histological sectionHarderian gland- 3rd group at (35day old): multiple focal necrosis (arrow) (H&E Stain-X4)

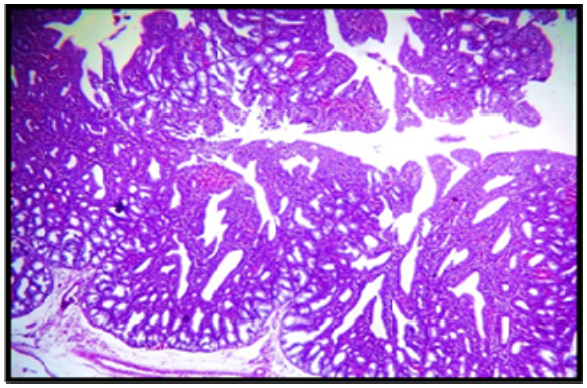

Fig 9: Histological sectionHarderian gland - 4th group at (35day old): normal architecture (H&E Stain–X4)

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