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Histomorphological Changes Associated with Different Doses of Chinese Propolis in the Bursa of Fabricius of Chickens

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ABSTRACT

This experiment was carried out to investigate the effect of dietary supplementation of Chinese propolis on the histological structure of bursa of Fabricius in Ross 308 broiler chickens. Eighty chicks were divided into 5 groups, 16 chicks each. Group 1 was fed only on basic diet and kept as control while groups 2, 3, 4 and 5 were fed on basic diet and received ether extract of propolis (EEP) in a dose of 100, 250, 500 or 750 mg/kg diet respectively. The treatment started from the first day after hatching and extended to day 42 where all birds were sacrificed and bursa of Fabricius were removed, processed and examined histologically. Chickens received EEP in a dose of 100 and 250 mg/kg diet showed an increase in size of the bursal folds and bursal lymphoid follicles with minimal regressive changes into the bursa such as a slight increase in the amount of inter-follicular connective tissue. Higher doses of EEP (500 and 750 mg/kg diet) produced substantial changes into the bursa such as degeneration in lymphatic follicles represented by cyst formation, liquifactive necrosis and significant increase in inter-follicular connective tissue. Our findings suggest that high doses of EEP led to faster bursal involution with subsequent negative impact on the humoral immune status of chicken.

Introduction

Propolis, also called blue glue, is a yellow-green to brown sticky resinous gum produced by honey bees (Ghisalberti, 1979). It has a very complex composition but many of its biological and pharmacological activities are thought to be due to flavonoids, stilbens, phenolic acids and its esters (Banskota *et al*., 2001; Isla *et al*., 2005). Propolis was suggested to be used as antiviral agent against many viruses (Harish *et al*., 1997), antimicrobial agent against broad spectrum of microbes (Burdock, 1998; Abdel-Mohsein *et al*., 2014; Mahmoud *et al*., 2014), anticancer agent (Mitamura *et al*., 1996), antiinflammatory agent (Bueno-Silva *et al*., 2013), antioxidant (Simões *et al*., 2004), as well as immunomodulatory agent (Dimov *et al*., 1992).

Bursa of Fabricius is one of the key organs of immunity in birds. It represents, as a primary lymphoid organ, the main source for B-lymphocytes production. In addition, it plays a central role in building up the immune system of birds especially in the first few weeks of life. The structure of the bursa reflects the humoral immune status of the bird because it is responsible for B-lymphocytes, and hence immunoglobulins production (Bickford *et al*., 1985). On the other hand, vaccination against viruses is the main firewall against viral diseases in chicken. Successful vaccination is totally dependent on the immune status of the birds at the time of vaccination. Adjuvants were used to en-

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hance the immune response of the bursa and consequently the whole immune status of the birds which in turn should potentiate the response to vaccines. Commonly used adjuvants like Freund's adjuvant or aluminum adjuvant have many disadvantages (Steven *et al*., 2009). Propolis was suggested by many authors as a strong immune adjuvant in many animal models including birds (Fischer *et al*., 2007; Sun *et al*., 2008; Fan *et al*., 2014). Biologically active components of propolis showed positive effects on the general immune status of the birds (Yuan *et al*., 2012) as well as on enhancing the immune response against viral challenges like Newcastle disease (Yuan *et al*., 2012; Chen *et al*., 2014). It was found to promote lymphocytic proliferation, up-regulate IL-2 and IL-6 as well as promoting development of immune organs in immunosuppressive chickens (Fan *et al*., 2013 and 2014).

In the course of poultry feeding programs, it was thought that propolis as a food supplement, promotes the immune response of poultry especially during the first few weeks after hatching (Yuan *et al*., 2012; Chen *et al*., 2014; Fan *et al*., 2013 and 2014). While propolis is generally considered safe when used in the recommended doses (Burdock, 1998), some authors suggested a possible toxic effect of some propolis components (Frenkel *et al*., 1993; Nieva Moreno *et al*., 2005). This motivated the authors to study morphological changes of the bursa of Fabricius in chicken subjected to various doses of propolis during the period between 1 and 42 days of age to certify the possible immunotoxic effect of propolis.

Materials and methods

All procedures of animal care and use in this study were approved by the Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt.

Ether extract of propolis (EEP)

Ether extracted propolis was purchased from Dalian Tianshan Industrial Co.™, Ltd. Changjiang Road, Dalian, Liaoning, China.

Birds and treatments

Eighty, one day old broiler chicks (Ross 308) were divided into five completely randomized groups of 16 birds each. Continuous lightning program (23 hours lightning and 1 hour darkness) was applied. Every possible effort was performed to minimize birds suffering. Diet and water were offered *ad libitum*. Group 1 was fed on basal diet with no additives and was kept as control. Groups 2, 3, 4, and 5 were fed on basal diet plus 100, 250, 500 or 750 mg propolis/kg diet respectively. The addition of propolis to the diet started at the first day after hatching and continued till day 42. Chickens were vaccinated against Newcastle disease at days 6, 14, 21, and 32 and against infectious bursal disease at days 10, 18 and 25 in drinking water. All birds were humanly sacrificed and dissected at age of 42 days and the bursa of Fabricius of both control and treated chickens were removed and fixed in neutral buffered 10 % formalin and embedded in paraffin. Serial paraffin sections were cut at 5 microns thickness, stained with hematoxylin and eosin stain (Bancroft and Stevens, 1990), examined and photographed. All presented micrographs are labeled according to the used microscopic magnification before enlarging.

Results

Group 1 (Control group)

The tunica mucosa of the bursal wall was thrown into large and small longitudinal folds (plicae) arranged into an interdigitating manner. The lamina epithelialis was of the pseudostratified columnar variety. Each fold presented a central lamina of connective tissue flanked on each side by lymphatic follicles. The lymph follicles, varying both in shape and size, were composed of a wide light medullary portion (germinal center) and a dark cortical portion (Fig. 1A). Delicate sub-epithelial connective tissue layer was demonstrated. Some lymph follicles were observed in close continuity with the surface epithelium. Each bursal fold (pilica) was further divided into several folia (leaflets) separated by clefts of variable depth.

Group 2 (100 mg/kg diet propolis)

The bursal folds demonstrated an increment both in length and width especially the small folds presented in between the large ones (Fig. 1B) along with slight increment into the amount of the interfollicular connective tissue. In addition, many lym-

phatic follicles were demonstrated pursuing a juxta position to the surface epithelium.

Group 3 (250 mg/kg diet propolis)

The bursal mucosal folds increased in size (Fig. 1C). The lymphatic follicles increased both in number and size per unite area. Few small cysts could be demonstrated within some lymph follicles containing basophilic cell debris. Numerous lymph follicles were observed opening into the bottom of the mucosal invagination (Fig. 1D).

Group 4 (500 mg/kg diet propolis)

The bursal folds presented abundant deep, simple or compound clefts which oriented the lateral borders into many leaflets (Fig. 2A). Many lymphatic follicles were demonstrated in contact with the surface epithelium. Lymph follicles were decreased in size and seen mostly to open into the bottom of the mucosal clefts (Fig. 2B). The sub-epithelial connective tissue appeared relatively wide and the inter-follicular connective tissue was plentiful (Fig. 2C). Some degenerating follicles present medullary variable sized cysts containing necrotic debris (Fig. 2D).

Group 5 (750 mg/kg diet propolis)

The bursal folds showed abundant deep simple or compound clefts more than the previous groups (Fig. 3A). The lymph follicles decrease in size and mostly appeared with several foci of liquifactive necrosis (Fig. 3B). The inter-follicular connective tissue was prominent especially at the tips of the mucosal folds. This was accompanied by depletion of lymphocytes within the lymph follicles. Several medullary cysts of various sizes demonstrated themselves along the mucosa. The cysts contained necrotic foci (Fig. 3C).

Discussion

Many authors noted that a variety of factors may influence bursal development, including hormones, infectious agent, environmental factors and exogenous chemicals (Marzo *et al*., 1990; Mase and Oishi, 1991; Milićević *et al*., 2002). The present investigation revealed that propolis addition to

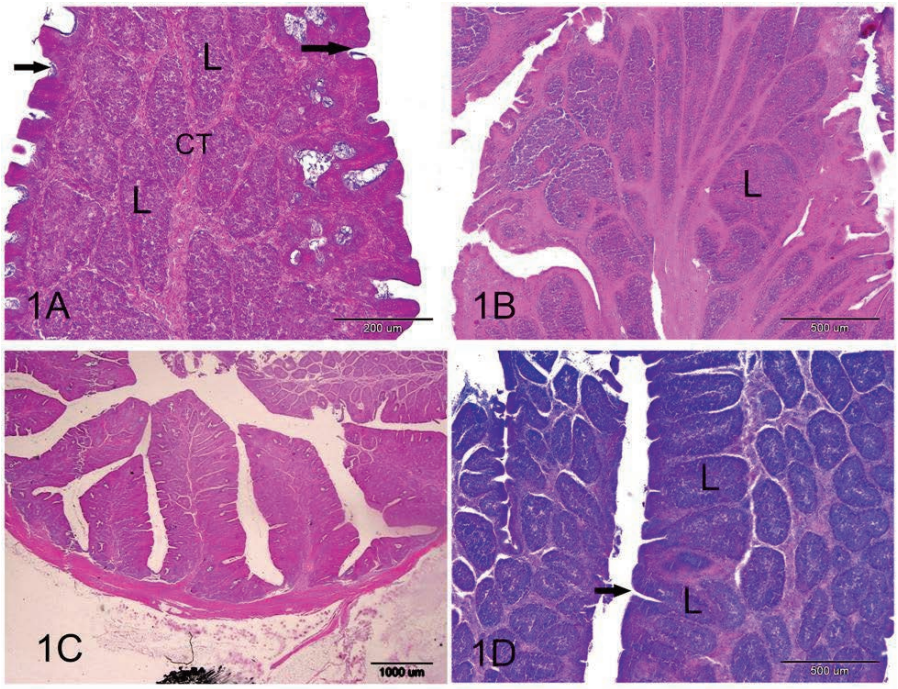

Fig. 1. A) H&E stained section of a portion of the bursal wall in a chicken of untreated group showing a mucosal fold further subdivided into several leaflets separated by clefts (arrows) of variable depths. Lymphatic follicles (L) are separated by thin layer of connective tissue (CT). B) H&E stained section of a bursal fold in a chicken of the second group (100 mg/kg body diet), showing lymphocyte follicles (L) structurally comparable to control group. C) H&E stained section of bursal mucosal folds in a chicken of the third group (250 mg/kg diet), showing numerous lymph follicles of various shapes. D) H&E stained section of bursal mucosa in a chicken of the third group (250 mg/kg diet) showing numerous lymph follicles (L) opening into the bottom of the mucosal clefts (arrow).

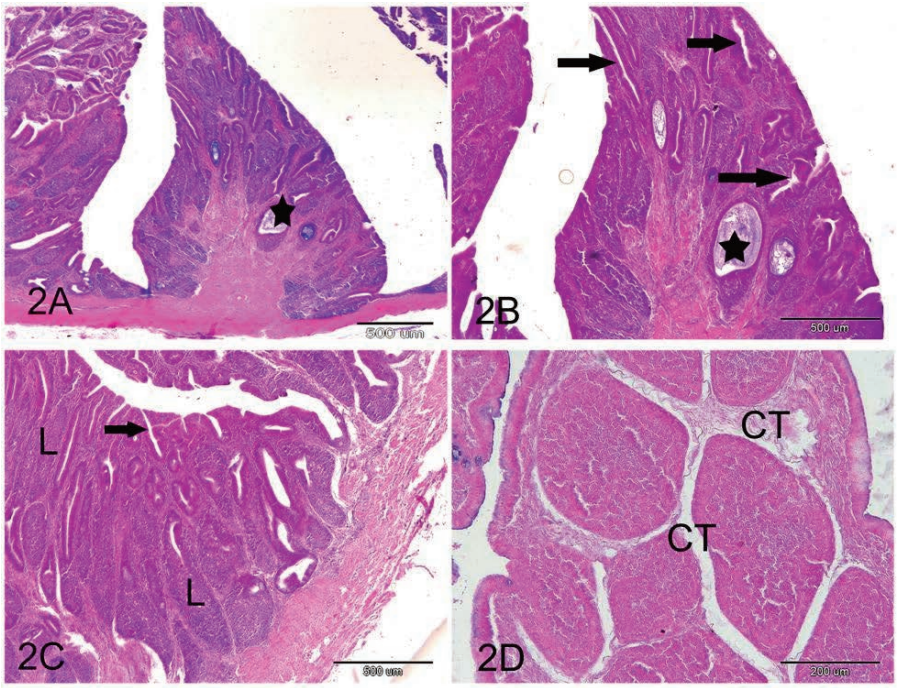

Fig. 2. A and B) H&E stained section of bursal mucosal folds in a chicken of the fourth group (500 mg/kg diet), showing numerous deep clefts (arrows) which oriented the lateral borders into many leaflets. Notice the follicular cysts (stars). C) H&E stained section of bursal mucosa in a chicken of the fourth group (500 mg/kg diet), showing numerous lymph follicles (L) opening into the bottom of the mucosal clefts (arrow). D) H&E stained section of bursal mucosal folds in a chicken of the fourth group (500 mg/kg diet), showing plentiful follicular connective tissue (CT).

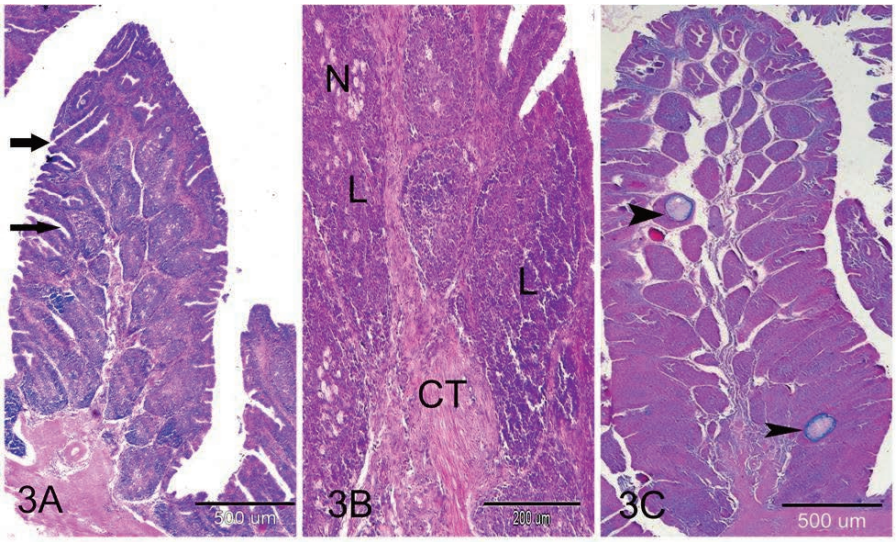

Fig. 3. A) H&E stained section of bursal mucosal folds in a chicken of the fifth group (750 mg/kg diet), showing numerous, deep, simple or compound clefts at the borders (arrows). B) H&E stained section of a portion of the mucosal folds in a chicken of the fifth group (750 mg/kg diet), showing liquifactive necrotic foci (N) into the lymph follicles (L) along with prominent fibrosis and connective tissue (CT) formation. C) H&E stained section of bursal mucosal folds in a chicken of the fifth group (750 mg/kg diet), showing medullary cysts of various sizes filled with mucoid secretion (arrowheads).

the ration of chickens at age between 1 and 42 days after hatching at various doses (100, 250, 500 and 750 mg/kg diet), influences bursal development in chickens leading to various forms of bursal involution.

The signs of involution were very few in the second group receiving propolis in a dose of 100 mg/kg diet and were in the form of slight increment into the amount of inter-follicular connective tissue. Besides, some lymphatic follicles appeared taking a juxta position to the surface epithelium. In the third group receiving a dose of 250 mg/kg diet mild regressive changes were observed in the form of few cysts within the follicular medulla. The amount of inter-follicular connective tissue was slightly increased. Meanwhile, the lymphatic folli-

cles increased in size and number indicating positive effects of propolis on the structure of the bursa. These findings are in agreement with previous reports suggesting immune enhancing effects of propolis (Yuan *et al*., 2012; Chen *et al*., 2014; Fan *et al*., 2013 and 2014). In a previous work by our group we proved that propolis can enhance the immune status of chickens subjected to stress conditions like heat stress (Abdel-Mohsein *et al*., 2014; Mahmoud *et al*., 2014).

However, signs of involution were obviously observed in those groups receiving propolis in doses of 500 or 750 mg/kg diet (group 4 and 5). These signs were represented by the appearance of abundant deep mucosal folds within the apex and both sides of the folds. Additionally the lymph follicles presented liquifactive necrotic foci within the medullary elements and lymphocytic exhaustion was also evident. Fragmentation and depletion of the cortical zones with cysts formation was also observed. The inter-follicular connective tissue was markedly increased and sub-epithelial connective tissue was relatively wide. These findings suggest a possible toxic effect of high doses of propolis on the bursa of Fabricius. Although propolis is generally considered safe, as concluded from several animal experimental models and different dosing patterns (Ikeno *et al*., 1991; De Castro and Higashi, 1995), some authors referred to a possible toxicity of some of its constituents such as caffeic acid phenyl ester (CAPE; Frenkel *et al*., 1993), benzyl cinnamate and benzyl benzoate (Popova *et al*., 2002). Moreover, Tinoco *et al*. (2013) reported 70% in vitro leukocytes death after propolis treatment. It is well known that the physiological involution of the bursa becomes histologically evident after 18 weeks of age (Bickford *et al*., 1985). The histological picture of the bursa of Fabricius in chickens received propolis in a dose of 750 mg/kg diet for only 7 weeks simulates more or less that picture of 24 week old normal (untreated) chickens described by Bickford *et al*. (1985). This proves that propolis in high doses can induce degenerative changes in the bursa and hasten the bursal involution process, a fact which was observed in the present investigation.

Conclusion

Propolis stimulates the activity of the bursa when used in low doses (100-250 mg/kg diet) and enhances the regressive changes in the bursa when used by high doses (500-750 mg/kg diet) and subsequently affecting humoral immunity. It is economically worthy to use this supplement at the concentration (250 mg/kg diet) as a tool to improve the immune status in poultry. Further work is needed to determine the exact component(s) and mechanism behind this bursal regression acceleration.

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