Histopathological patterns in experimentally avian coccidiosis after treatment with Urtica dioica L. (Urticaceae)

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ABSTRACT

This study was designed to investigate the effect of the herb type Urtica dioica L on the pathogenesis in chicken meat (Rose strain) were experimentally infected by 1500 oocyst of Eimeria tenella at 22 day old age. It divided randomly into three equal groups at 7 days. The first group represented the positive control, while the second and third groups were treated with herbs at a concentration of 1% and 5% in the feed and aqueous leave extract of Urtica dioica L respectively, every day of the experiment. Histopathological examination of liver, spleen, intestines and brain of control group revealed hepatic cell necrosis; complete lymphoid depletion in splenic and bursal tissue associated with neuronal degeneration and accompanied by presence numerous degenerated merozoites in ecem. While the histopathological examination in treated groups showed well developed proliferation lesions mainly when treatment with water. Examination revealed major histopathological changes in fodder treatment group, vascular congestion and sinusoidal dilation with periodical MNCs infiltration, bursa epithelial hyperplasia with reactive lymphoid hyperplasia in splenic tissue together with pyers patches lymphoid depletion with evidence of cystic for tomatum in infected chickens mainly at 48 old day.

INTRODUCTION

In the last decade plant extracts were widely investigated, medicinal plants stimulate and increase the secretion of digestive enzyme from liver and pancreas(1). Also, they were used for controlling avian coccidiosis and improving poultry performance worldwide, easy usage, non-side effects(2). And because of the development of coccidial resistance to the medical products and the potential harmful effects on human health, there is need to find out the safe alternatives for the control of avian coccidiosis and the use of herbal remedies in poultry diets has been proposed because of their natural stimulation of the immune system, enhanced growth performance and/or anticoccidial effects, antioxidant, anti-fungi and etc(1).

Since the Urtica dioica leaves containing high crude protein , amino acid , calcium and amino phosphorous and they have certain advantages for basic materials of formulate feed for livestock and poultry (3); also the nettle effects were create appetite stimulation and stimulate the growth performance and/or anticoccidial effects, anti-oxidant, anti-fungi and etc(1).

The leave extract of Urtica dioica can cause a little increasing in the main morphometric indices of liver
such as area of hepatocyte , in the periportal zone liver and the other possibility for the hepatoprotective effects of Urtica dioica L. (Urticaceae) may be related to antioxidant activity to decrease and prevent liver damage , and lower liver enzyme depends on the antiinflammatory effect of Urtica dioica (5).

The aim of the present study was to investigate the effects of dried and aqueous extract Urtica dioica a medical plant as feed additive on histopathological lesions in broiler chickens organs( liver, spleen, bursal gland, cecum and brain) experimentally infected with Eimeria tenella, a highly pathogenic Eimeria species that causes caecal coccidiosis.

Materials and Methods

1- Birds preparation The environmental conditions of temperature and humidity One hundred and fifty Ross chicks at one day old were fed with anticoccidial free feed. The environmental conditions were exactly same for all the groups. The temperature at about 3.7.3°C was maintained according to standard program . The birds were kept in 3 cages (5 x 5m). Each cage was equipped with feeders and drinkers. There was no mortalities recorded. To prevention of chickens against Newcastle and bursal infectious disease, all of the chicks were vaccinated at 11 and 14 days of age, respectively.

2 -Preparation of herbal extracts. The dried parts of medicinal plants (leaves and stems) were used. Nettle was supplied from local market and has been ranked by the National Iraqi Institute for herbs in Abu Ghrab, cleaned and milled ,then stored in clean nylon bags. They were extracted according to Harbone and Mabray (6).

3- Eimeria tenella challenge Collected samples from cecum from broiler naturally infected with coccidiosis (local isolates of E.tenella) from different regions in the province of Baghdad after adding solution Potassium Dichromate concentration of 2.5% , and was purified by single oocysts infections by flotation methods (7). The isolation and increase parasite diagnosed oocysts parasite in laboratory branch parasites/college of veterinary medicine -University of Baghdad according to the method (8).The oocysts were preserved in 2.5% potassium dichromate solution to induce sporulation and kept in a refrigerator (3–5°C) until use(9). Each bird was challenged with 1500 oocysts /chicken of E.tenella at the age of 22nd day. (excepted control group).

4- Experimental design. This experimental was conducted in poultry felid in College of Veterinary Medicine -University of Baghdad. experimental chicks were randomly divided into 3 groups (pen) of 50 birds each, from days 1 to 21, the birds were fed a starter diet and from days 22 to 48, a grower diet in mash were formulated using National Research Council (NRC) (10).Challenge of each bird was carried out by administering infected with Eimeria tenella at a dose rate of 1500 sporulated oocyte / ml in saline water per bird orally directly into the crop via an oral gavage on the 22nd day of age. The control group with no Urtica dioica supplement, no additives (control positive). The second group basal diet containing 1% of (Urtica dioica L.) at day 17 mixed with other ingredients, and 3rd group basal diet put water was containing aqueous extract of 0.5% of Urtica dioica at day 17 to end of experimental period.

Histopathological examination When the chicks reached 37, 48 days of age, were selected at random and killed for pathological examination, the liver, spleen, bursal glands, cecum and brain were isolated and the tissue samples were preserved in 10% neutral buffered formalin and were later processed using standard histopathological techniques.(11).

Results: 1-Control group at 37day old, the hall mark histopathological feature of liver showed sever vacuolar degeneration predominately fatty change with sinusoid dilation, (fig.1) together with per portal cellular infiltration consist mainly of MNCs. Also, the result showed destruction in splenic tissue characterized mainly by lymphoid depletion in white pulp (fig.2) as well as congestion of splenic red pulp with sinus congestion. However, sections at 48 day old, That showed sinusoid dilation and congestion associated with disrupted hepatic cord,(fig.4), and epithelial hepatic hyperplasia with cellular inflammatory infiltrate in sub capsular rejoin associated with slight fibrosis, individual cell necrosis of some hepatocytes(fig.5), as well as massive necrosis and congestion with hemorrhage in both splenic tissue associated with slight fibro muscular hyper atrophy of splenic tissue.(fig.6 &7), also there was Sever distraction in the bursa follicles associated with sever necrotic changes in the medullary region and blood vesicles congestion in sub scapular layer. (fig. 8),but demonstrate sever epithelial ling distraction of sub mucosal glands associated with cellular infiltration in cecum.(fig.9).
Fig.(1)The liver showed severe vacuolar degeneration of hepatic cords with severe sinusoid dilation( ) and congestion( ) at 37 day. (H&E stain X40).

Fig(2)Similar observation seen in splenic tissue of infected control group that reveal massive destruction in splenic tissue ( ), mainly characterized by lymphoid depletion white pulp, at 37 day (H&E stain, X40), and observed in bursal tissue associated with severe loss depletion bursal lymphoid follicles, both cortical and medullar lymphoid depletion.

Fig(3)There was individual cell necrosis of some hepatocytes( ) together with sinusoid dilation and congestion in liver associated ( ) with disrupted hepatic cord, at 37 day (H&E stain, X40).

Fig(4)The splenic tissue showed massive necrosis( ) and congestion with hemorrhage associated with slight fibro muscular hyper atrophy of splenic artery( ), at 37 day. (H&E stain, X40).

Fig(5)Sever distraction in the bursa follicles associated with sever necrotic changes in the medullary region ( ) at 37 day. (H&E stain, X20).

The histopathological examination of control group at 48 day, demonstrate sever epithelial ling distraction of sub mucosal glands associated with cellular infiltration in cecum and number of degenerated merozoite developed in some mucosal glands resulting in cystic distension of these glands with necrotic tissue debris.

Fig(6): Cecum section changes showed cystic distention of degenerated mucous gland, containing number of degenerated merozoites with cellular infiltration in the Lomina Propria ( ) at 48 day (H&E staine.X40).
Fig (7): with Cellular infiltration in lamina propria resulting in submucosal thickness of cecum tissue, at 48 days (H&E stain, X40).

Fig (8): The brain section showed moderate to severe neuronal degenerative changes with blood vessels congestion (H&E stain, X40).

2. The microscopical appearance in group 2 (*Urtica Dioica*, treated in food 1% at 37 day old) there was massive diffused necrosis in the liver parenchyma associated with severe congestion of blood vessels and sinusoids.

Fig (9): liver showed moderate heterophilic MNC cellular aggregate mainly around bile duct together with various degree of sloughing of its epithelium, also there was loss of parenchyma with atrophy of surviving them, at 37 days. (H&E stain, X40).

Fig (10): The spleen showed massive hemorrhage and congestion in red pulp with massive lymphoid depletion in white pulp, at 37 days (H&E stain, X40).

Fig (11): There is severe destruction in all structures of treated bursal tissue involving lymphoid tissue that showed severe lymphocytosis specially in the cortex with severe dilation of Lumina propria, fig (11&12) and slight hyperplasia with slight squamous metaplasia in the epithelial mucosa, at 37 days. (H&E stain, X40).

Fig (12): wide space between bursal follicles with extensive cortical media lymphoid depletion, at 37 days (H&E stain, X20).
Fig (13): While in the latest day (48 day) the histopathological lesion of the group 2 that treated with *Urtica dioica* in food there was severe congestion and dilation of central vein ( ) together with slight per central fibrosis of the liver tissue, at 48 day. (H&E stain, X40).

Fig (14): Another section there was MNc cells infiltration ( ) with slight fibrosis that seen mainly in portal area in liver, at 48 day (H&E stain, X40).

Fig (15): The spleen showed slight reactive lymphoid hyperplasia ( ) of white pulp together with hemorrhage in red pulp ( ), at 48 day. (H&E stain, X20).

Fig (16): And slight cellular infiltrate in red pulp with muscular hypertrophy of splenic blood vessels ( ) at 48 day (H&E stain, X20).

The bursal tissue show similarity to previous group except accompanied with slight cortical lymphoid by severe distension and dilation in Lamina propria with congestion and slight hyperplasia of cortical lymphoid follicle, at 48 day. (H&E stain, X20).

The cecum in this group at 48 day showed severe epithelial ling distraction of sub mucosal glands associated with cellular infiltration.

Fig (17): MNc infiltrate in some dilated sinusoid ( ) and per duct of cellular infiltration ( ) as well as necrotic lesion may also absorbed in the cecum at surface epithelium with subepithilum heterophilic infiltration with blood vessels congesting ( ) and goblet cell hyperplasia, also PMNCs, eosinphill and MNc infiltrate in the top of villi, at 48 day, fig (18).
Fig.(18) blood vessels congesting ( )and goblet cell hyperplasia, also PMNCs, eosinophil and MNCs infiltration in the top of villi, at 48 day.

Fig(19): there is intensive submucosal accombined ( ) depletion fibrosis, at 48 day. (H&E, stain).

The brain showed severe hemorrhage and blood vessels congestion and meninges cerebral together with neuronal degeneration as well as necrosis in other neurons, at 48 day. (H&E stain, X40).

Fig(20): also associated with cystic formation that containing cellular depress and the neurons showed various degree of necrosis (central chromatolysis picnosis), at 48 day. (H&E, stain, X40).

The Microscopical section in the liver of group 3 (Urtica dioica L.) treated in water (37 day old) showed severe congestion in portal area associated with focal cellular aggregation with sinusoids congestion and dilatation of blood vessels with inflammatory cellular in filter together with slight portal fibrosis.

Fig(21): Multifocal cellular aggregation consist mainly of heterophils ( ) and lymphocytes together with slight dilation of sinusoid, at 37 day. (H&E stain, X20).

Fig(22): diffused and focal necrosis of parenchyma together with atrophy of surviving hepatocyte of liver, at 37 day. (H&E stain, X40).

Fig(23): the section showed lymphoid depletion of splenic white pulp with slight cellular infiltration ( ), at 37 day. (H&E stain, X20).

Fig(24): The main feature characterized by moderate lymphoid depletion mainly observed in the medullar area associated with degenerative changes ( ), at 37 day. (H&E stain, X40).
Fig.(25): Sever hemorrhage and congestion of meningeas and cerebral parenchyma ( ) with slight vacuolar degeneration and clear view of cerebral edema ( ) with congestion of blood vesicles, at 37 day (H&E stain, X40).

The group 3 that treated with Urtica dioica extract watery (48 day old) showed multifocal aggregation of mononuclear cells in hepatic tissue mainly around blood vessels in portal region.

Fig(26): Focal mononuclear cellular aggregated in liver parenchyma mainly around blood vessels in portal area with proliferation of kupffer cells, at 48 day (H&E stain, X40).

There was variable degree of reaction lymphoid hyperplasia were demonstrated in splenic tissue of this group, as in fig(19), at 38 day (H&E stain, X20).

Fig(27): and also the result showed fibrous thickening of splenic capsule in addition to slight congestion of red pulp and medial thickening and proliferation of splenic arterioles, at 48 day (H&E stain, X40).

Fig(28): Great lymphoid hyperplasia of bursal tissue associated with fibrous thickening of intestinal connective tissue ( ), at 48 day (H&E stain, X20).

Fig(29): The predominant cecum feature was intense sub mucosal and lamina propria MNCs and PMNCs infiltration ( ) that result in great thickness of Lamina Propria, together with marked sub epithelial heterophilic and MNCs that consist of macrophage, plasma cells infiltration ( ) with on clear lesion in the epithelial vili (fig.32), at 48 day (H&E stain, X40).

Fig(30): Sever mononuclear peri-vascular cuffing of cerebral blood vessels with slight congestion ( ) and dilation of blood vessels with per vascular MNCs cuffing together with MNCs infiltration ( ) in cerebral parenchyma of brain at 48 day (H&E, stain X 40).
Discussion:

The microscopical observation of control revealed morphological alteration of the splenic arteriole wall, which were subject of clear in duration while the cytoplasm of monocyte showed increased acidophlic and contained vacuoles this data were in consistence with Patial et al.,(12).The general effects include changes in the cellular kinetics and morphology of the villi. The pathological changes are mainly due to the second generation schizonts (13). By the fifth or sixth day the caeca are dilated, the contents containing unclotted and partly clotted blood, schizonts. In primary infection, numbers of heterophils and mast cells were increased during the acute inflammation process which indicates mast cells play a role as primary inflammatory cells(14). Heterophils predominated when necrosis was extensive;otherwise,mononuclear cells were the main inflammatory cells(5).

The current data of control group showed sever morphological changes in the internal examined organs and the caeca are dilated, the numbers of heterophils and mast cells were increased during the acute inflammation process which indicates mast cells (14). Heterophils predominated when necrosis was extensive;otherwise mononuclear cells were the main inflammatory cells(5).

Urtica dioica can stimulate lymphocytic proliferation as well as of neutrophils hepatoprotective changes(16) and these observation in consistence with our observations mainly in the examined organs of aqueous Urtica dioica extract in 48 days old groups particularly in liver and spleen as well as splenic lesions in second group that fed on Urtica dioica in feed. At 37 days old showed similar pathological changes but lesser than the previous group, however, other researches Harput et al., (17) demonstrate that aqueous Urtica dioica extract stimulate the proliferation of T- lymphocyte and suppressed the production of lipopolysaccharide (LPS) stimulated macrophages without affecting cell viability, while Richemann et al.,(18) showed that part of the anti-inflammatory affected of Urtica dioica extract might be a scribed to its inhibitory effected on NF-cappa-B-activation.

According to above observation concerning the effects of even when it's applied in small dose of an Urtica dioica on the lymphoid organsbroilers infected with E.tenella parasite, while the liver and bursal gland morphology attention was also paid to the structure and appearance of blood vessels. In chicken from experimental groups in 48 days the severity of lesions in arteries was significantly lower than in birds from groups in 37 days. Riehemann et al., (19) were suggested the effective of feed additive; Alisma canaliculatum with probiotics (ACP) on the growth performance, meat composition, oxidative stability, and fatty acid composition of broiler meat. No similar studies on birds seem to be an available on bird for comparison of results. However, in the present study none of these organs (liver, spleen, bursa, cecum and brain) were effected significantly by level of Urtica dioica extract in drinking water compared to the studies on mice and rats absence of frank necrotic changes. This may be due to anti-oxidant and anti-inflammatory protective properties of Urtica dioica(20), in addition there was evidence of clear perivascular MNCs aggregation in brain tissue mainly (that treated Urtica dioica extract 0.5% at age 37 day) and (that treated Urtica dioica extract 0.5% at age 48day), this observation may indicate has possibly anti apoptotic supplement promoting cell survival in brain which can be preventive against later injuries(21).

Liver in the (treated with Urtica dioica in food 1% at aged 37day) may explain morphological alteration characterized by sever vacuolar degeneration mainly in peripheral zone of portal area may indicate the possibility for hepatoprotective effects of Urtica dioica extract may be related to antioxidant activity to prevent liver damage depends on the anti inflammatory effect of it(5).

The Flavonide play an important role as antioxidant which acts as transition metal ion chelaters because the free radical generation is mainly catalyzed , however excessive intake of flavonoids which cause decrease in essential trace elements [Cu and Zn] and their related enzyme activities(22) which lead to long-terms of supraphysiological doses of flavonoids increased gastrointestinal absorption of essential elements (Zn-cu-Fe) and their tissue, availability in brain and liver. This effected seems to be different with vibrations of structural(13).

Phenolic compounds of nettle are very important plant constituent because of their scavenging ability which is due to their hydroxyl group and stabilizing lipid peroxidation in the cell membrane (22) together with elevated but partial inhibitory effects on cyclooxygenase and lipoxygenase derived reaction, additionally , isolated phenolic acid from Urtica dioica to inhibit leukotriene B4-synthesis in a concentration dependent manner in vitro(23).

In conclusion, our study has shown that experimental diet treatment at 0.5% level might have potential efficacy on immunity in Urtica dioica extract mixed diet is able to stimulate the immune parameters in Urtica dioica herb. These results suggested that Urtica dioica extract may provide a new therapeutic value in specific and nongeneric immunity in the broiler chicks infected experimentally with E.tenella and low doses certainly did not cause any pathological lesion in liver. However, the effect of Urtica dioica mixed diet used as an immunostimulant by oral delivery has to be further studies.

Reference: