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Ameliorating effect of some herbal plants on the side effects of diclazuril drug in chickens

A thesis Presented by

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Summary

The present study was carried out to investigate the ameliorating effect of herbal plants as (*thymus vulgaris* powder and *artemisia annua* powder) to the adverse effect of diclazuril on performance of broilers, oocyst output, drug residue in tissues, some serum and biochemical constituents, hematological parameters, immunological status, additionally, its effect on cell gene as well as histopathological pictures in *E. tenella* experimentally infected broiler chickens.

The experiment was carried out on total of one hundred and five (105) one - day old broiler chicks with average weight 40-gm. Chickens fed on commercial ration, which prepared according to National Research Council (National Research Council, 2004). Chickens were reared under standard hygienic conditions. Feeds and water were *adlibtium* to chickens. Chickens were vaccinated as recommended program, and divided into7 groups (each group 15 chick) as following:

First group served as control group fed only on basal diet without any treatment (negative control group). The second group was served as an apositive control (infected and non-treated). The third group administrated water mixed with standard dose of diclazuril (1 ppm), while the fourth group was fed on basal ration mixed with *thymus vulgaris* powder at 5gm/ kg^{-1} ration and the fifth group was fed on basal ration mixed with *thymus vulgaris* powder at 5gm/ kg^{-1} in addition of water mixed with standard dose of diclazuril 1 ppm .The sixth group was fed on basal ration mixed with *artemesia powder* at 3gm/ kg^{-1} , The seventh group was fed on basal ration mixed with *artemesia powder* at 3gm / kg^{-1} in addition of water mixed with standard dose of diclazuril 1 ppm. The experiment continued for six weeks.

Oocyst inoculation:

Each groups except first group were directly inoculated orally with the help of a rubber oral gage, with 1 ml solution containing about 1×10^3 of sporulated oocytes of *E. tenella* on the 15 day of age, Fecal droppings were daily collected from the all birds of all groups for 6 successive days between 6-11 days post infection (PI), and the oocysts were counted in 1 gram (g) of faecal matter by the Mc-Master technique. The results revealed that chickens were experimentally infected with *E. tenella* and non-treated (G2) showed more oocyst output as compared to other groups (G3, G5, G6, and G7). However effect of diclazuril alone (G3) or with *thymus vulgaris* powder (G5) or *artemisia annua* powder alone (G6) on oocyst output has significant improved effect when compared to infected non treated group (G2), with reduction % is (71.26, 71.68, 71.6 respectively) and has failed to give extra parasites clear when compared with *artemisia annua* group (G7). Feeding chickens with t *thymus vulgaris* powder alone (G4) has no significant effect on oocyst output, when compared to (G2) (infected non treated group) and low significant effect when compared to negative control group (G1) and with other groups, with reduction % 0.25, while chickens feed *artemisia annua* powder with diclazuril (G7) showed highly significant improved effect on reduce oocyst output when compared to other groups and also delayed release oocyst, and increase reduction % of oocyst output (87.53). It concludes that supplementing broilers with *artemisia annua* powder (3gm/ kg) with diclazuril caused potent anticoccidial effect.

Growth performance parameters:

At the end of each week, 5 tagged chicks were weighted and body weight, body gain, feed intake as well as, FCR were recorded. The

weekly evaluation of growth performance revealed that positive control group (G2) (infected and non-treated) showed a low significant effect on body weight and body weight gain after infection, while feed consumption of chicken showed low significant effect after infection till 5th week of age when compared to control negative group (G1), while medication with diclazuril alone (G3) has no significant effect on body weight, body weight gain, feed consumption and FCR of chicken when compared to control negative group (G1), and showed improved effect when compared to infected and non-treated group (G2) after infection with *E.tenella*, however it has a low significant effect on body weight of chicken when compared to G5 (*thymus vulgaris* powder+ diclazuril) and with G7(*artemisia annua* powder+ diclazuril) at last two weeks. While chickens feeding ration mixed with *thymus vulgaris* powder (G4) has a significantly improved effect on body weight before infection with oocyst when compared to control negative group (G1) and diclazuril treated group (G3). However, it had a significantly decreased effect on body weight and weight gain of chicken after infection, while body weight gain returned at the 4th week of age also feeding ration containing *thymus vulgaris* powder to chickens (G4) has no significant effect on FCR and feed consumption except at 4th week showed decreased effect in feed intake of chicken when compared to control negative group (G1) and diclazuril treated group (G3). Where when giving *thymus vulgaris* powder with diclazuril (G5) it showed significantly improved effect on body weight and weight gain of chicken, While administration of *thymus vulgaris* powder with diclazuril (G5) resulted in a significant improvement effect at end of experiment on FCR and at 4th and 6th week feed consumption showed decreased effect in feed intake of chicken when compared to control negative group (G1) and diclazuril treated group (G3). Furthermore chickens feeding ration mixed *artemisia annua*

powder at (3gm/kg-1) (G6) has no significant effect on body weight, weight gain, feed consumption (except at 4th week showed decreased effect in feed intake) and FCR of chicken when compared to control negative group (G1) and diclazuril treated group (G3). However feeding chickens with *artemisia annua* powder in combination with diclazuril (G7) showed significantly improved effect on body weight of chicken at last two weeks, and FCR at end of experiment when compared to control negative group (G1) and diclazuril group (G3), and has no significant effect on feed consumption of chicken (except at 4th week showed decreased effect in feed intake) when compared to control negative group (G1) and diclazuril group (G3). The result concluded that *thymus vulgaris* powder and diclazuril can be successfully used in practice as a natural feed additive for broiler chicken.

Blood samples: at the end of the experiment, the first blood samples were taken from the wing vein of five birds in each group adding to anticoagulant EDTA (1mg/1ml) to be used in hematological studies. The second portion of the blood sample was taken at 7th day post infection by jugular puncture, and at the end of the experiment into simple centrifuge tubes without anticoagulant, left to clot then centrifuged at 3000 r.p.m for 15 minutes to get separate serum, serum samples were kept at -20$^{C^{o}}$ until assayed for biochemical analysis.

i- Hematological parameter:

The result revealed that chickens which were inoculated by *E. tenella* and non-treated (G2) showed lowered significant effect on all blood pictures (RBCs, Hb and PCV) and showed a marked increase of the total leucocytic count, and eosinophil with decrease in lymphocyte count in comparison with control non-infected group (G1). while chickens

medicated with diclazuril alone (G3) and/ or *thymus vulgaris* powder (G4, G5), *artemisia annua* powder (G6, G7) has no significant difference effect on (RBCs), Hb and PCV in comparison with control negative group (G1). However they show improved significant effect in comparison with infected non treated group (G2). Chicken treated with diclazuril (G3) has no significant effect on WBCs, heterophil, monocyte, and basophil in comparison with control non-infected group (G1), however, it had improved significant effect in comparison with infected non treated group (G2), but it has increase effect on eosinophil (eosinophilia) and decreased significant effect in lymphocyte when compared to control negative group (G1). Moreover feeding *thymus vulgaris* powder alone (G4) and when given with diclazuril (G5) showed no significant differential effect on WBCs, heterophil, monocyte and basophil in comparison with control non-infected group (G1) and diclazuril group (G3), however, it shows increase the significant effect of eosinophil (eosinophilia) in comparison with (G1), and improved significant effect of lymphocyte in comparison with diclazuril group (G3). Furthermore feeding ration mixed with *artemisia annua* powder (G6) or with diclazuril (G7) on WBCs, heterophil, monocyte, lymphocyte, eosinophil and basophil has no significant difference effect in comparison with (G1). The result concluded that diclazuril effect on immune system of birds by induction of slight immunosuppressive effect which is represented by decreased lymphocyte and *thymus vulgaris* powder ameliorate this defect.

Biochemical parameter:

i- Kidney functions:

The results showed that significant increase in level of uric acid and creatinine in (infected and non-treated group (G2) in comparison with control non infected group (G1). However administration of diclazuril alone or in combination with either *thymus vulgaris* powder or *artemisia annua* powder improved these effect and the levels of uric acid and creatinine become the same as control non infected group (G1).

ii- Liver function (ALT, alkaline phosphatase (ALP) and AST):

The result revealed that chickens experimentally infected with *E. tenella* and non- treated group (G2) showed increased effect on ALT, ALP, and AST at 7th day post infection and end of the experiment in comparison with control non-infected group (G1). While chickens medicated with diclazuril (G3) had no significant effect on ALT, ALP, and AST at 7th day post infection and end of the experiment in comparison with control non-infected group (G1) and showed improvement effect in comparison with infected non treated group (G2). Moreover at 7th day post infection, chicken feeding with *thymus vulgaris* powder (G4) has significantly improved effect on ALT while the end of the experiment did not affect ALT, ALP and AST, while when feeding with diclazuril (G5) showed improvement effect on ALT, ALP and AST at 7th day post infection and end of the experiment in comparison with the control group (G1) and diclazuril group (G3). On the other side addition of *Artemisia annua* powder to feed of chicken (G6) has no significant effect on ALT and AST; however it showed significant improved effect on ALP at 7th day post infection in comparison with the control group. While, group feeding *Artemisia annua* powder with diclazuril (G7)

showed significant improved effect on ALP at 7th day post infection while at the end of the experiment ALT, ALP, and AST showed significant improved effect in comparison with the control group and diclazuril treated group (G3).The result concluded that diclazuril did not show any significant difference of all examined biochemical parameters (liver and kidney function) when compared with non-infected non-treated chickens.

iii- Effect of diclazuril and/ or *thymus vulgaris* powder, *artemisia annua* powder on Protein profiles:

The result revealed that infected, non-treated group (G2) and diclazuril group (G3) had decrease effect on total protein and albumin at 7th day post infection and end of the experiment, in comparison with control non-infected group (G1). However at 7th day post infection feeding *thymus vulgaris* powder (G4) alone showed decrease in total protein and globulin with increased in A/G, while when given with diclazuril (G5) has improved significant effect on total protein, Albumin in comparison with control non-infected group (G1), where at end of experiment feeding *thymus vulgaris* powder (G4) has no significant difference effect on total protein, albumin, globulin and A/G in comparison with control non-infected group (G1), and showed improvement effect when compared with diclazuril group (G3), however when given *thymus vulgaris* powder with diclazuril (G5) showed improved significant different effect on total protein in comparison with control non-infected group (G1) and diclazuril group (G3). Furthermore, *Artemisia annua* (G6) and with diclazuril (G7) were showed no significant differential effect on total protein, Albumin, Globulin, and A/G at 7th day post infection and at the end of the experiment *Artemisia annua* (G6) showed significant decrease in total protein in comparison with control group (G1). Also it was showed improvement effect when

compared with diclazuril group (G3), moreover (G7) at end of experiment showed improved significant effect on total protein and albumin in comparison with diclazuril group (G3). The result concluded that diclazuril effect on immune system of birds by induction of slight immunosuppressive effect which is represented by decreased values of total protein associated with decreased serum albumin.

Immunological studies:

Effect of diclazuril and/ or *thymus vulgaris* powder, *Artemisia annua* powder on Haemagglution inhibition antibody titer (HIT) at 7th day post infection and at end of experiment:

The result revealed that infected, non-treated group (G2) and diclazuril treated group (G3) at 7th day post infection and at the end of experiment had decrease significant effect on HIT in comparison with control non infected group (G1). Furthermore it was seen that feeding broiler with *thymus vulgaris* powder (G4) or *artemisia annua* powder (G6) and when feeding *thymus vulgaris* powder or *artemisia annua* powder with diclazuril (G5, G7) at 7th day post infection and at the end of experiment showed improvement significant effect in comparison with diclazuril treated group (G3), and infected, non-treated group (G2). The result concluded that diclazuril effect on immune system of birds by induction of slight immunosuppressive effect which is represented by decreased HIT of chicken.

Estimation of drug residue and (Genotoxicity) (Comet assay):

At the end of experiment tissue samples were taken from liver, intestine, caecum and suspended in phosphate buffer saline for comet assay.

Also other samples were taken from (liver, breast and thigh muscles) and were kept at -20 ^{Co} till the time of estimation of drug residue.

Chickens treated with diclazuril (G3), showed presence of diclazuril residue in liver, thigh and breast muscle, and residue in liver was higher than in muscle. While chickens feed *thymus vulgaris* powder with diclazuril (G5) or feed *artemisia annua* powder with diclazuril (G7) both had improvement significant effect on decrease residue in tissue of broiler, when compare with diclazuril group (G3).

Also broiler infected non-treated group (G2) and diclazuril group (G3) cause positive genotoxicity of these tissues. While when given *thymus vulgaris* powder with or without diclazuril (G4, G5) cause negative genotoxicity of these tissue. However *thymus vulgaris* powder alone (G4) cause positive genotoxicity only in caecum gene. Furthermore administration of *artemisia annua* powder with or without diclazuril (G6, G7) cause negative genotoxicity of this tissue when compared with control negative group (G1) and diclazuril treated group (G3).

Histopathological examination:

Tissue samples of liver, kidney, intestine and caecum were fixed in buffer formalin solution 10 % for histopathological examination at 7th day post infection and at end of experiment. The result revealed that in experimentally infected untreated group showed severs degenerative changed in all tissue where diclazuril induced various pathological changes in tissues ranged from degeneration to necrosis of these tissues. However these changed will improve when supplementing broilers with *thymus vulgaris* powder (5gm/ kg) and/ or *artemisia annua* powder (3gm/ kg) with diclazuril.