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# Some Biochemical and Haematological Studies on the Effect of Black seed and Curcumin in Vaccinated Broiler with Gumboro

**Azza A.Hassan[1], Ghada A. Abd El-Diem[2]**

[1]Biochemistry, Nutritional Deficiency and Toxicology Department, Animal Health Research Institute- Mansoura Lab, Egypt

[2]Poultry Diseases Department, Animal Health Research Institute- Mansoura Lab, Egypt

**Abstract:** ***This study was carried out to investigate the adverse effects of Gumborovaccine on performances, immunity and some biochemical parmeter of Broiler chicks. A total 120 broiler chicks were used in this study and divided into 6 groups each group contain 20 chicken and all chicks were weighted weekly till the end of the experiment. The results showed the broiler treated with Nigella sativa and curcumin produce increase in B.W, TLC andlymophcyte while decrease in neutrophil percent. Nigella sativa and curcumin induced increase in TP, Albumin , although NS and curcumin groups showed a significance decrease in ALT and AST, significantly increase in SOD, GSH, CAT and significantly decrease in MDA. Gumboro vaccine induced hepatic disorders and immunosuppressive revealed by decrease B.W, total Leucocytic countand lymophcyte percent, while increase in neutrophil percent. Gumboro vaccine induced decrease in total protein, Albumin , globulin increase in alanine aminotransferase (ALT) and Aspartate aminotransferase (AST), decrease in SOD, GSH, CAT, and significantly increase in MDA. This study indicated that treatment with Nigella sativa and Curcumin improved the adverse effects of Gumboro vaccine on performances, immunity, some biochemical parameters in serum and tissues, also decrease the risk of exposure to Gumboro vaccine, improve both of general health condition and immunostatus of chicks.***

**Keywords:** Black seed,Curcumin, Broiler, Gumboro vaccine

## 1. Introduction

Gumboro Disease (GD) called Infectious Bursal Disease (IBD) is a highly contagious viral disease , it was first recogenized in Gumboro district of Delware , USA (Cosgrove 1962) . In Egypt ,IBD was reported for first time by El-Sergany et al., (1974) Later on many outbreak were reported and described in various governorates with severe pathological lesions and high mortality up to 70% **(Madbouly et al.,1992 ).** One of the most critical disease is Gumboro which is the most important second viral disease after Newcastle . ***(Abdel-Rahman et al.,2007)*.**

Medicinal plants are widely used as home remedies and raw materials for the pharmaceutical industries. Herbal remedies are used in the prevention, treatment and cure of disorders and diseases since ancient times ***(Ashiq,et al., 2014)***. Nigella sativa is an annual herbaceous plant; seeds are black in color and taste slightly bitter. They are frequently used as a spice and added as a flavoring agent to bread, pickles and other dishes ***(Aljabre et al., 2005).*** The seeds of the plant are extensively used in traditional medicine in some countries, for the treatment of several diseases ***(Nostro et al., 2000).*** Recent scientific investigations on the seeds and their oil indicated a number of pharmacological activities including anti-carcinogenic ***(Rooney and Ryan, 2005)***, anti-ulcer ***(Kanter et al., 2005)***, anti-bacterial ***(Morsi, 2000)***, anti-fungal ***(Khan et al., 2003)***, anti-inflammatory, anti-pyretic and analgesic ***(Al-Ghamdi, 2001***).Curcumin (1,7-Bis 4-hydroxy-3-methoxy phenyl)-1-6- heptadiene-3,5-dione) , which gives the yellow colour to turmeric, was first isolated in 1815, and its structure as diferuloylmethane was determined in 1910 by Kazimierz Kostanecki, J. Miłobędzka and Wiktor Lampe ***(Shishodia et al., 2005).***Turmeric, *Curcuma longa* L. (family *Zingiberaceae*) is native to Southeast Asia and has a long history of therapeutic uses and a variety of important antimicrobial, antifungal, insecticidal, anti-inflammatory and antioxidant properties ***( Khattak et al., 2005).*** Turmeric has been found effective for controlling mycelial growth of many fungi ***(Singh et al., 2002),***and treatment of several diseases ***(Nostro et al., 2000).***

## 2. Material and Methods

### 2.1 Materials

**(1) Broiler chicks**

The present study was carried on a total number of 120 apparent healthy unsexed Broiler chicks obtained from El-Dakhelia Poultry Company.Broiler chicks were divided as the following: the first group: fed Broiler ration without any treatment (control group), the second group: Vaccinated with Gumboro. The third group : Fed with ration contain nigella sativa crushed seeds 1.5%. The fourth group vaccinated with Gumboro and fed with ration contain nigella sativa crushed seeds 1.5%. The fifth group**:** fed with ration contain curcumin powder 400 mg/kg diet. The sixth group vaccinated with Gumboro and fed with ration contain curcumin powder 400 mg/kg diet.

**Diet & Management**

Chicks were kept at the Animal House ofEl_Mansoura Veterinary Medicine for 30 days of experiment. The chicks were randomly divided into six groups. Each treatment group contained 20 chicks.According to **Hassan et al., (2003)** chicks were housed in wire battery cages of 86 L×50 W×25 H cm which were equally partitioned into 3 pens (29x50x25cm). The batteries were provided with feeders and drinker equipment, the chicks were allowed libitum access

to feed and water.Ventilation and temperature (22°C-31°C) were controlled to maintain bird comfort during the grow-out. Room were electrically heated and conditions of constant light were employed, provided 24 hours of lighting and checked three times daily for food, water and mortality.

### 2.2 Basal Experimental Diet

Diet was formulated to meet the nutritional requirements as suggested by the **(NRC, 1994),** to contain 24% CP and 2900 kcal ME/kg.Fresh feed were mixed weekly and not stored for more than one week. Neither antibiotic growth promoters nor anti-coccidials were added to any of the experimental diets.

| *Ingredient* | *Concentration (kg/100kg diet)* |
|---|---|
| Ground yellow corn | 55.780 |
| Soya bean meal | 31.960 |
| Fish meal | 1.000 |
| Corn gluten | 7.450 |
| Bran | 1.000 |
| DicalciumPhosphate(22%Ca&19%P) | 0.710 |
| Limeston(38% Ca) | 1.300 |
| Lysine (purity 98%) | 0.170 |
| DL – Methionine (purity 98%) | 0.070 |
| Iodized sodium chloride | 0.300 |
| Mineral& Vitamin premix | 0.300 |

The components of Basal experimental diet

Nigella sativa powder was purchased from Isis Company Registration No: COAE-Proc. COAE: Accreditation Certificate No.: DAKKs.ZE3307.00.According to Weiss (2002). The seeds contain about 35% carbohydrates, 21% protein and 35-38% fats. These fats are both saturated and unsaturated fatty acids and are present in oil in the following percentages: Saturated acids 18.1%,monounsaturated acids 23.8%, polyunsaturated acids 58.1%, other minerals present in the seed include thiamin, niacin, calcium, folacin, zinc and phosphorus.The main component in nigella sativa is thymoquinone. It contains up to 50% of this phytochemical compound, which has antioxidant properties. It also contains about 40% p-cymene and 15% a-pinene; naturally occurring organic compounds, as well as small amounts of other constituents. Nigella sativa was added freshly crushed every week to the feed in a dose 1.5%till the end after 30 days of the experement according to the plane of the work according to **(Boka, et al., 2013 and Tahan&Bayram 2011).** Curcumin powder (C21H20O6) . Molecular weight 368.39, Molecular Formula: 1, 6-heptadiene-3,5-dione-1,7-bis(4-hydroxy-3-methoxyphenyl)-(1E,6E) or diferuloylmethane.

The components of Curcumin

| | |
|---|---|
| Volatile (essential) oils | 3-7% |
| Fiber | 2-7% |
| Mineral matter | 3-7% |
| Protein | 6-8% |
| Fat | 5-10% |
| Moisture | 6-13% |
| Carbohydrates | 60-70% |

It was purchased from Research Lab Company imported from India Batch No.557A110713, curcuminpowder was added to the diet in adose rate 400mg/kg diet according to **(Tarasub et al., 2012).**All chicks were vaccinated by hot strian (IBD) vaccines produced by Ceva Company according to **(Giambrone and Ronald 1986).**

## 3. Growth Performance Study

All chicks were weighted weekly till the end of experiment.

**Sampling**

**a) Blood Sampling**

Two blood samples were collected after slaughter. The first one was collected in EDTA tubes to prevent blood coagulation and used for determination of total leucocytic count and differential leucocytic count. The second blood sample was collected into aclean and dry screw capped centrifuge tubes without anticoagulant and left to clot at room temperature, then centrifuged at 3000 r.p.m for collection of clear serum sample used for the biochemical analysis of serum total protein,serum albumin, serum globulin,serum liver enzymes (AST and ALT), kidney function( creatinine and uric acid).

**b) Tissues Sampling**

After the chicks were slaughtered liver tissue were separated then washed with normal saline for determination of Superoxide dismutase (SOD)**activity** according to***(Nishikimi et al., 1972).***, reduced glutathione (GSH)according to***Anderson*** **(1985) and Bartels (1971),** Catalase (CAT)according to**Aebi (1984),** and Malondialdhehyde MDA content according to***(Satoh, 1978)***).

**Blood parameters**

Total leucocytic counts were performed using the improved Neubauer hemocytometer according to the method described **by (Natt and Herrick., 1952).**Determination of differential leucocytic count was performed using the method described by **(Hoyer, 1993).**

**Serum parameters**

Determination of serum total proteins according to **(Doumas and and Biggs, 1971).** Determination of serum albumin according to **(Rodkey, 1965).** Determination of serum liver function **[**Alanine Aminotransferase (ALT) andAspartate aminotransferase (AST)] according to(Murray, 1984).Determination of serum kidney function(uricacidand Creatinine) according toBartels, (1971).

## 4. Results

**Table 1:** Effect of Nigllastaiva and Curcumin on the mean body weight in (g) in different groups.

| Days / Age Groups | 7days | 14days | 21day | 30day |
|---|---|---|---|---|
| G1(Control group). | 103 ± 9.6$^{a}$ | 395±13.5$^{b}$ | 780 ± 16.4$^{b}$ | 1080± 19.7$^{b}$ |
| Group2 ( Vaccinated group) | 101± 8.5$^{a}$ | 309 ± 14.2$^{d}$ | 635 ±14.3$^{d}$ | 935 ± 18.4$^{c}$ |
| Group3 ( Nigella sativa group) | 101.8±9.2$^{a}$ | 430± 16.7$^{a}$ | 870± 18.2$^{a}$ | 1160 ± 22.4$^{a}$ |
| Group4 ( Vaccinated + nigella sativa group ) | 100± 8.3 | 379±14.7$^{b}$ | 755± 15.6$^{b}$ | 998± 20.6$^{b}$ |
| Group5 (curcumin group) | 102 ± 9.4 | 401± 15.2$^{b}$ | 830 ± 16.4$^{a}$ | 1110 ± 20.3$^{b}$ |
| Group6 (Vaccinated + curcumin group) | 103 ± 8.7 | 358 ± 15.4$^{c}$ | 695 ± 14.2$^{c}$ | 1030±19.6$^{b}$ |

**Means with different letters in the same column are significantly different.**

**Table 2:** Effect of Nigella Stiva and Curcumin on the differnetial cell count of different groups

| Groups / Parameters | Leucocyti c count | Lymphocyte | Heterophils | monocyet | basophils | oesenophils |
|---|---|---|---|---|---|---|
| Group1 (control group). | 34.60±1.7$^{a}$ | 59±3.6$^{a}$ | 33.4±2.9$^{c}$ | 3.8±0. 2$^{a}$ | 0.9±0.04$^{b}$ | 3.5±0.2$^{a}$ |
| Group2 (Vaccinated group) | 25± 1.3$^{b}$ | 47.55± 2.9$^{c}$ | 45± 3.2$^{a}$ | 2.9± 0.1$^{c}$ | 1.4± 0.1$^{a}$ | 2 .85 ± 0. 2$^{b}$ |
| Group3 (Nigellasativa group) | 36.4±2.2$^{a}$ | 61.80± 4.8$^{a}$ | 30± 2.1$^{c}$ | 4.2± 0. 3$^{a}$ | 1.2± 0.1$^{a}$ | 3.8±0.3$^{a}$ |
| Group4 (Vaccinated + nigella sativa group ) | 32±1.9$^{a}$ | 54.15± 4.3$^{a}$ | 38.2±3.3$^{b}$ | 3.17± 0. 2$^{b}$ | 1.6 ± 0.1$^{a}$ | 2.88± 0. 2$^{b}$ |
| Group5 (curcumin group) | 32.40 ± 1.6$^{a}$ | 59.80±3.9$^{a}$ | 32.6±2.7$^{c}$ | 3.9± 0.3$^{a}$ | 1.2±0.1$^{a}$ | 2.95± 0. 2$^{b}$ |
| Group6 (Vaccinated + curcumin group) | 27.5± 1.8$^{b}$ | 52.60±3.2$^{b}$ | 39.5±3.3$^{b}$ | 3.73 ± 0. 2$^{a}$ | 1.3± 0.1 $^{a}$ | 2.87±0.2$^{b}$ |

Means with different letters in the same column are significantly different.

**Table 3:** Effect of Nigella Stiva and Curcumin on the serum biochemical parameters of different groups

| Parameters / Groups | Total protein(g/dl) | Albumin(g/dl) | Globulin(g/dl) | ALT(U/ l ) | AST(U/ l) | Criatinine(g/dl) | Uric acid(g/dl) |
|---|---|---|---|---|---|---|---|
| Group1(Control group). | 4.72±0.32$^{b}$ | 2.20±0.12$^{b}$ | 2.52±0.20$^{b}$ | 18.74±0.68$^{b}$ | 175±9.54$^{b}$ | 0.33±0.02$^{b}$ | 2.6±0.02$^{b}$ |
| Group2(Vaccinated group) | 3.6±0.22$^{c}$ | 1.83±0.07$^{c}$ | 1.77±0.15$^{d}$ | 32.11±1.12$^{a}$ | 203±10.2$^{a}$ | 1.08±0.05$^{a}$ | 3.9±0.04$^{a}$ |
| Group3( Nigella sativa group) | 5.22±0.42$^{a}$ | 2.51±0.15$^{a}$ | 2.71±0.27$^{a}$ | 15.43±0.62$^{b}$ | 154±9.73$^{c}$ | 0.22±0.02$^{b}$ | 2.0±0.01$^{b}$ |
| Group4( Vaccinated + nigella sativa group) | 4.49±0.28$^{b}$ | 2.05±0.09$^{bc}$ | 2.44±0.19$^{bc}$ | 23.24±1.04$^{ab}$ | 186±11.6$^{b}$ | 0.65±0.04$^{b}$ | 2.8±0.02$^{b}$ |
| Group5(curcumin group) | 4.96±0.26$^{b}$ | 2.17±0.0.6$^{b}$ | 2.79±0.02$^{b}$ | 16.33±0.9$^{b}$ | 169±13.5$^{b}$ | 0.28±0.3$^{b}$ | 2.2±0.02$^{b}$ |
| Group6(Vaccinated + curcumin group | 4.12±0.34$^{bc}$ | 1.98±0.05$^{bc}$ | 2.14±0.29$^{c}$ | 26.54±1.04$^{a}$ | 191±15.4$^{ab}$ | 0.78±0.04$^{b}$ | 3.1±0.03$^{b}$ |

**Means with different letters in the same column are significantly different.**

**Table 4:** Effect of Nigella Stiva and Curcumin on the liver tissue enzymatic antioxidantand non-enzymatic antioxidant system parameters of different groups

| Parameters / Groups | Total glutathione ng/g tissue | Reduced glutathione ng/g tissue | Oxidized glutathione ng/g tissue | Sodium oxide dismutase U/g tissue | Malondialdehyde MDA (M mol/g tissue | Ctalase U/g tissue |
|---|---|---|---|---|---|---|
| Group1 (Control roup). | 6.10±0.50$^{a}$ | 5.77±0.44$^{b}$ | 0.33±0.06$^{c}$ | 14.56±0.67$^{a}$ | 39.56±2.32$^{c}$ | 15.02±0.48$^{b}$ |
| Group2 ( Vaccinated group) | 3.18±0.09$^{c}$ | 2.36±0.07$^{d}$ | 0.82±0.02$^{a}$ | 8.78±0.45$^{d}$ | 61.43±4.65$^{a}$ | 10.13±0.55$^{d}$ |
| Group3 ( Nigella sativa group) | 6.91±0.40$^{a}$ | 6.63±0.37$^{a}$ | 0.28±0.03$^{c}$ | 15.82±0.75$^{a}$ | 36.89±3.2$^{c}$ | 17.21±0.96$^{a}$ |
| Group4 ( Vaccinated + nigella sativa group) | 4.82±0.30$^{b}$ | 4.34±0.26$^{c}$ | 0.48±0.04$^{b}$ | 14.89±0.54$^{a}$ | 45.3±0.43$^{b}$ | 13.54±0.65$^{c}$ |
| Group5 (curcumin group) | 6.27±0.40$^{a}$ | 5.96±0.37$^{b}$ | 0.31±0.03$^{c}$ | 12.87±0.64$^{b}$ | 38.24±2.30$^{c}$ | 15.82±0.73$^{b}$ |
| Group6 ( Vaccinated + curcumingroup ) | 4.52±0.30$^{b}$ | 3.94±0.26$^{c}$ | 0.58±0.04$^{b}$ | 11.12±0.43$^{c}$ | 49.77±3.48$^{b}$ | 12.31±0.57$^{c}$ |

Means with different letters in the same column are significantly different.

## 5. Discussion

Herbal medicine or herbalism (Nigella sativa and curcumin) is a time-honored practice of natural medicine that is older than mankind itself. The practice of using traditional herbal medicine based therapy is nowadays gaining more attention worldwide in both human and animal health care systems. Among the livestock sectors, poultry production systems are the most intensively reared with developments especially in the areas of nutrition, disease control, immunomodulators, genetic improvement, management and organization of dietary requirements along with the pressure of increasing demand for poultry products as well as threats of emerging pathogens**( Kuldeep et al., 2015).** This study showed that vaccinated group G2 which received Gumboro vaccine was decrease in body weight when compared to control group

and other groups as shown in **table (1)**, these results agreed with the findings of ***(Shehata et al., 2009*** **and Kuldeep et al., 2015.)**. Decreasing of growth rate by Gumboro vaccine may be due to disturbance of one or more basic metabolic processes (carbohydrate, lipid and or protein metabolism) in the liver and loss of appetite ***(Cheeke and Shull, 1985)***. In the present study the curcumin treated group G4 show increase in body weight when compared to vaccinated group G2 as shown in **table (1)** these result are agree with (***Sharma et al., 2011),*** which explained that the co-administration with curcumin improve bodyweight**..**Chicks of G2 showed lower leucocyte levels; evidently this may be due to a considerable decrease in lymphocyte count. This may be indicative of the deterioration of immune status in the chicks of this group in response to the effect of vaccine***Sharma et al., (2011)***.The elevation of the heterophil : lymphocyte ratio is a hematological indicator that animals are coping with chronic stress ***(Gross and Siegel, 1983)***. Induction of the hypothalamic-pituitary-adrenal axis has been shown not only to reduce the lymphocyte number but also to increase the number of neutrophils ***(Dhabhar et al., 1995)***. ), these results are confirmed with (***Sharma et al., 2011).***In the present study, vaccinated group G2 which received hot strian (IBD) vaccine showed decrease in total protein, albumin and globulin when compared to control non medicated group G1 as shown in **table (3)**, in this work, nigella stiva and curcumin treated group G3, G4 respectively, shows increase in total protein and albumin when compared to vaccinated group G2 as shown in **table (3) ,**these finding are in agreement with ***( El-Emam et al.,2011***). these finding is supported by ***(Mohapatra et al., 2011 andAshour et al., 2011),*** For the present study vaccinated group G2 which received hot strian (IBD) vaccines,showed increase in ALT and AST when compared to control non medicated group G1 **table (3)**. These result are in agreement with ***(Amiridumari et al., 2013; Selim et al., 2014).***According to ***Ozer et al., (2008).*** The elevation of AST and ALT may be due to significantly changed the stability of the lysosomal membrane, leading to a disorder of hepatocyte permeability in the liver ***(Varior and Philip, 2012).***Treatment with nigella stiva and curcumin showed marked recovery in vaccine treated chicks as shown previously, these finding is supported by***El-Agamy, (2010).***Curcumin showed a significant hepatoprotective activity by lowering the levels of serum marker enzymes ALT and AST. Treatment with nigella stiva and curcumin almost completely abolished the increase of serum ALT activity and decreased AST activity suggesting that curcumin and nigella stiva could effectively inhibit induced liver cell injury. The serum level of Nigella Sativa treated group G3 showed decrease in creatinine and uric acid when compared to group G2 table (3)these finding are supported by ***(Al-Ghasham et al., 2008).***It is obvious that administration of Nigella sativa may protect liver from free radical reactions (***Mona et al., 2002).***Creatine is synthesized in the liver, passes into circulation and is taken up almost entirely by skeletal muscle for conversion to creatine phosphate, and then both creatine and creatine phosphate are converted spontaneously into creatinine. Both are filtered at glomerulus. Although there may be some addtional secretion of creatinine by renal tubules, creatine is reabsorbed by the tubules at low plasma concentration. ***(MC Lauchlan, 1988)***. In the present study, curcumin treated group G4 show decrease in creatinine and uric acid when compared to vaccin treated group G2 as shown in table **(3)**, these results are supported with ***(Manikandan et al., 2011)*** . Curcumin administration showed a significant decrease in the levels of serum creatinine and uric acid. The curative effect of curcumin on the kidney markers can be attributed to its antioxidant property as it has been found that reactive oxygen species may be involved in the impairment of glomerular filtration rate(GRF) ***(Hughes et al., 1996)***.Concerning the present study, Nigella Sativa treated group G3 and curcumin treated G5 show an increase in SOD, GSH and CAT activity when compared to vaccinated group G2 as shown in **table (4)** and was decrease in MDA as shown in **table (4)**, these finding were in agreement with ***(Kanter et al., 2006)*** which found NS treatment decreased tissue and prevent inhibition of superoxide dismutase (SOD), reduced glutathione (GSH), and catalase (CAT) enzyme (***Nili-Ahmadabadi et al., 2011***), that found treatment by NS at doses of significantly prevented MDA production index of lipid peroxidation.Moreover, ***Azevedo-Martins et al., (2003)*** stated that SOD plays a role in the suppression of oxygen free-radical formation , SOD indicate that lipid peroxidation is one of the most prominent factors in carcinogenicity ***(Rastogi et al., 2001).Burits and Bucar, (2000)*** reported that NS acts as a donating agent in the DPPH assay and possesses hydroxyl radical scavenging properties in both the assay for non-enzymatic lipid peroxidation and the deoxyribose test. Nigella sativa oil also protect lipids against free-radical damage and may actually accelerate damage to other molecules ***(Burits and Bucar, 2000).***Thymoquinone, the main compound in NS oil, inhibits non-enzymatic lipid peroxidation in liposomes. In addition to thymoquinone, there are many other compounds in the oil possessing a strong antioxidative effect, such as p-cymene, m-cymene, α-thujene and carvacrol, which have been reported to possess antioxidant effects and radical scavenging properties ***(Burits and Bucar, 2000).***Nigella sativa has been encountered among the hepatoprotective herbs. Its active constituent TQ, considered as a potent antioxidant ***(Soliman et al., 2012),*** NS preserve the natural antioxidants in the cells by scavenging the superoxide anions ***(Badary et al., 2003).*** Current study show that curcumin treated group G4 show increase in SOD ,GSH and CAT activity when compared to vaccine treated group G2 as shown in **table (4)**, this results are in agreement with (***(Sharma et al., 2005 ; Mahmoud, 2013***) which reported that curcumin increased the activities of both serum and liver tissue SOD and GSH**,** and decrease in MDA when compared to vaccine treated group G2 as shown in table (4), these results are in agreement with ***(Mahmoud, 2013)***.The antioxidant enzymes superoxide dismutase and catalase play an important role in keeping homeostasis and protection against oxidative damage by removing the free radicals ***(Jia et al., 2009)***

## 6. Conclusion

We conclude that,IBD vaccine treatment resulted in haematological and biochemical changes typical of those reported previously. Treatment with Nigella sativa or Curcumin to chicks resulted in the restoration of the different parameters tested. The protective effects of Nigella sativa seeds may be due to the radical scavenging activity of

its components, whereas the protective effects of Curcumin may be due to the phenolic compounds present in it,. Consequently, NS and Curcumin are quite useful and reasonable during using Gumboro vaccine and during Gumboroinfection.particularly the immunosuppressive ones, safeguard poultry health and boost the productive performance of birds. It has been vivid also that the potential of medicinal herbs as the valuable source of therapeutics aids has attained a global significant place in the health system. So the sustainable present and future of poultry sector necessitates the optimum and accurate implementationof herbal remedies which overcome the demerits of extensive allopathic drugs.

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