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Immunomodulating Effect of *Echinacea* and *Star Anise* in Protection and Treatment of Infectious Bronchitis Virus in Poultry

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Abstract

This study was carried out to evaluate the effect of two medicinal plants: *Echinacea purpurea* and *Star Anise* for treating and prevention of infectious bronchitis virus (IBV) in chickens via assessment of their immune stimulating effect in IBV challenged chicks. 160 one day old Cobb® unsexed broiler chicks with average body weight 46.3 g. Chicks were classified into 8 equal groups (20 of each). The 1st group served as a control negative, the 2nd group served as a control positive (infected with IBV virus at day 21, non- treated), the 3rd group received *Echinacea* from the 7th till 21st day and infected with IBV virus at day 21, the 4th group received *Star Anise* from the 7th till 21st day and infected with IBV virus at day 21, the 5th group received both *Echinacea* and *Star Anise* from the 7th till 21st day and infected with IBV virus at day 21, the 6th group infected with IBV virus at day 21, then received *Echinacea* from the 21st till 42nd, the 7th group infected with IBV virus at day 21, then received *Star Anise* from the 21st till 42nd, and the 8thgroup infected with IBV virus at day 21, then received both *Echinacea* and *Star Anise* from the 21st till 42nd. Estimation of the collected samples (blood and sera) were made at different periods (7th, 14th, 28th and 35th days) to determine the effects of the used drugs on some hematological, and biochemical parameters. In addition, tissue specimens from liver and trachea were taken for histopathological examination. The obtained results evoked a significant increase in WBCs, heterophiles, lymphocytes, monocytes and esinophils counts in the groups treated with *Echinacea* and *Star Anise* compared with that of the control group. Serum ALT, AST, serum urea and creatinine results revealed a significant increase in groups treated with *Echinacea* and *Star Anise* compared with the control group, while GSH, SOD and NO revealed decrease in groups treated with *Echinacea* and *Star Anise* compared with the control group. It could be concluded that the use of *Echinacea* and *Star Anise* as antivirals is positively beneficial in prevention and treatment of infectious bronchitis virus in poultry. Moreover, the use of the combination of both plants when used together have more powerful effects in the prevention and treatment of IBV in poultry.

KEYWORDS
Echinacea, *Star Anise*, Infectious bronchitis virus, Medicinal plants.

INTRODUCTION

Across the world, poultry is regarded as a good source of animal protein with a high biological value for human consumption. Therefore, it is important to control poultry health by controlling or preventing any diseases to achieve good production (Salehi, 2013). Poultry is exposed during its life to many infectious and deadly diseases, and the danger of poultry diseases lies in their sudden occurrence, rapid spread and difficulty in controlling them, especially viral diseases. Infectious bronchitis virus is one of the most prevalent viral diseases affecting poultry industry (Abd-El Moneim, 2012).

Infectious bronchitis virus (IBV), a Coronavirus, is found globally but only affects poultry. It not only affects the respiratory system but also the urogenital tract. Infection causes respiratory problems in infected birds, as well as a decrease in egg production in layers and breeders. Kidney injury is also possible. Infection can occur at any age, with a significant death rate in children (Shahnas *et al*., 2020; Bamford and Zuckerman, 2021).

In recent years, medicinal plants and their bioactive metabolites have become one of the primary foci of study in the hunt for effective and inexpensive medications to meet contemporary needs (Perera and Efferth, 2012). A range of plant metabolites can inhibit viral replication while having little or no influence on host physiology (Martin and Ernst, 2003; Hussain *et al*., 2017). Along with direct interferences with viral replication, these natural products have the ability to modify host immune responses to viral infections (Kurokawa *et al*., 2010). *Echinacea purpurea* (*E. purpurea*), a member of the Asteraceae family, is a medicinal plant having significant pharmacological and aesthetic characteristics (Gajalakshmi *et al*., 2012). *E. purpurea* was discovered to have powerful antioxidant, anti-inflammatory, and immunoregulatory properties (Lee *et al*., 2009). *Star Anise* is a spice that is used across the world. The plant was used in traditional medicine to treat skin inflammation, colic, vomiting, rheumatic problems, insomnia, antiulcer, and antibacterial properties (Wang *et al*., 2011; Sung *et al*., 2012; Hussaini *et al*., 2013). In sight of the previous facts, this study was undertaken to investigate the immunomodulatory and the antioxidant activities of *E. purpurea* and the *Star Anise* in the prevention of infectious bronchitis virus in chickens.

MATERIALS AND METHODS

Medicinal plants

Echinacea: One kilogram of *Echinacea purpurea* extract was brought from ABChem Company in Mansoura in powder form. *Echinacea* powder was added to drinking water (50 g/L) (Gra-

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shorn and Nasir, 2009).

Star Anise: One liter of *Star Anise* oil was brought from ABChem Company in Mansoura in liquid form. *Star Anise* essential oil was added to feed (20 ml/kg feed) (Ding *et al*., 2020).

Infectious bronchitis virus (IBV)

Standard IBV virus strain (IBV-EG-CH-CV10-2019-SP) was brought from Animal Health Research Institute, Dokki, Giza, Egypt).This strain (IBV Variant II live virus) has a Gene Bank accession number of MN651561.

The dose of challenge virus was adjusted to ensure a final estimated dose of $10^{5.6}$ EID_{50}/ml per bird and was administered intraocularly (Chousalkar *et al*., 2007).

Experimental chicks

One day old Cobb® broiler chicks (n=160) with average body weight 46.3 g, unsexed chicks were purchased from a commercial farm and classified randomly into 8 groups (each of 20 chicks) and maintained separately using wooden partitions. The chicks were reared in an open house under natural conditions, supported with wood shaving litter, water troughs and plastic feeders. They were supplied with a commercial diet (Dakahlia Poultry Company, Egypt). Starter diet was fed from the 1st to 14th day of age then grower diet for the next 14 days (14th-28th) and finally on finisher diet was fed from the 28th to 42nd days of age with 24 hours of light daily. Feed and water were provided ad-libitum throughout the experiment. Chicks were not given any medication or vaccination. All groups were kept under the same conditions and received the same procedures of management.

Group 1: Left as control –ve, received ration and drinking water free from any medicinal plants and non-infected.

Group 2: Control +ve, received ration and drinking water free from any medicinal plants and infected by IBV ($10^{5.6}$ EID_{50}/ml) at day 21 of age.

Group 3: Received *Echinacea* powder in drinking water (50 g /L) from the 7th till 21st day of age and infected by IBV ($10^{5.6}$ EID_{50}/ml) at day 21 of age.

Group 4: Received *Star Anise* oil in feed (20 ml/kg feed) from the 7th till 21st day of age and infected by IBV ($10^{5.6}$ EID_{50}/ml) at day 21 of age.

Group 5: Received both medicinal plants *Echinacea* powder (50 g /L) and *Star Anise* oil (20ml/kg feed) in drinking water and feed respectively from the 7th till 21st day of age and infected by IBV($10^{5.6}$ EID_{50}/ml) at day 21 of age.

Group 6: Infected by IBV ($10^{5.6}$ EID_{50}/ml) at day 21 of age and received *Echinacea* powder (50 g /L) in drinking water from the 21st till 42nd day of age.

Group 7: Infected by IBV ($10^{5.6}$ EID50/ml) at day 21 of age and received *Star Anise* essential oil in feed (20 ml/kg feed) from the 21st till 42nd day of age.

Group 8: Infected by IBV ($10^{5.6}$ EID_{50}/ml) at day 21 of age and received both medicinal plants *Echinacea* powder (50 g /L) and *Star Anise* oil (20 ml/kg feed) in drinking water and feed respectively from the 21st till 42nd day of age.

All methods used in the study were performed in accordance with the ethical guidelines and recommendations of the Research Ethics Committee, Faculty of Veterinary Medicine, Mansoura University.

Collection of Samples

The blood samples were collected at days 7, 14, 28 and 35. Blood was collected from the wing vein on EDTA for hematological studies. Other samples of blood were collected and allowed to clot for serum separation by centrifugation at 3000 rpm for 10 min for biochemical studies.

White blood cells count (WBCs) and differential leukocytic count, were determined according to Coles *et al*. (1986).

Liver enzymes included serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) based on the methods of Tietz (1976). Kidney function test included measuring serum urea (Vassault *et al*., 1986) and creatinine (Henry *et al*., 1964) levels.

Serum oxidative stress and antioxidant enzymes included reduced glutathione (Beutler, 1963) superoxide dismutase (Kei, 1987) and nitric oxide (Aebi, 1984).

Histopathological examination

Liver and tracheal tissues were collected after slaughtering of chicks and were fixed in 10% neutral buffered formalin for histopathological examination (Bancroft *et al*., 1990).

Statistical analysis

Data were analyzed using the statistical package for social science (SPSS), 15.0 software 2008) (Snedoecor and Cochoran, 1981).

RESULTS

Effect of medicinal plants on clinical signs and mortality rate of chicks infected with IBV

Clinical signs

All chicks in healthy control group (non-infected and non-treated) were healthy, viable and displaying no clinical symptoms throughout the experiment period. One day after IBV infection, all infected chicks displayed clinical symptoms such as: loss of appetite, depression, respiratory symptoms including: gasping, mouth breathing, sneezing, coughing and nasal discharge. The groups infected with IBV and treated with *Echinacea* and *Star Anise* showed milder degree of clinical symptoms compared with the control +ve group (Table 1).

Mortality rate

The mortality rate in each group was recorded throughout the experimental period as percentage (Table 1). The mortality rate was zero (0%) in the control –ve group all over experiment period and was zero (0%) in all groups in the first two weeks before IBV infection. Control +ve group revealed a high mortality rate after infection (70%) in the 3rd and 4th week of experiment. Treatment groups (G8, G7 and G6) showed low mortality rates (19%, 21% and 25% respectively) at the 3rd week and 9%, 11% and 10% respectively at the 4th week. Protective groups (G5, G4 and G3) showed low mortality rate compared with control + ve group as 30%, 32% and 35% respectively after infection at the 3rd week and 12%,15% and 20% at the 4th week after infection (Table 1).

Effects of the tested medicinal plants on some hematological parameters

On the first week before infection, the results evoked non-significant alterations on total and differential leucocytic count of all dosed groups along the sampling time compared with con

Table 1. The effect of oral administration of *Echinacea* (50 g /L drinking water) and *Star Anise* (20 ml/kg feed) at different times of the experiment on mortality rate and clinical signs in experimentally infected chickens with IBV (n=5).

Groups	7-21 days		21-28 days		28-42 days	
	Clinical signs	Mortality rate	Clinical signs	Mortality rate	Clinical signs	Mortality rate
G1	normal	0%	Normal	0%	normal	0%
G2	normal	0%	Sneezing, cough, gasping, tracheal rales, nasal discharge, and depression	70%	Depression and sneezing	70%
G3	normal	0%	Cough, sneezing and depression	35%	Depression	20%
G4	normal	0%	Depression, cough, and depression	32%	Depression	15%
G5	normal	0%	Depression and sneezing	30%	Depression	12%
G6	normal	0%	Depression and sneezing	25%	Depression	11%
G7	normal	0%	Depression and sneezing	21%	Depression	10%
G8	normal	0%	Depression and sneezing	19%	Depression	9%

Data are presented as Mean±S.E

The different letters within the same column are significant at P<0.05

G1: Control negative non-infected, non-treated, G2: Control positive infected with IBV virus at day 21, non- treated, G3: Received *Echinacea* from the 7th till 21st day infected with IBV virus at day 21, G4: Received *Star Anise* from the 7th till 21st day infected with IBV virus at day 21, G5: Received both *Echinacea* and *Star Anise* from the 7th till 21st day infected with IBV virus at day 21, G6: Infected with IBV virus at day 21, then received *Echinacea* from the 21st till 42nd, G7: Infected with IBV virus at day 21, then received *Star Anise* from the 21st till 42nd, G8: Infected with IBV virus at day 21, then received both *Echinacea* and *Star Anise* from the 21st till 42nd.

Table 2. The effect of oral administration of *Echinacea* (50 g /L drinking water) and *Star Anise* (20 ml/kg feed) at different times of the experiment on total and differential leukocytic count (heterophils, lymphocytes, eosinophils and monocytes) in experimentally infected chickens with IBV (n=5).

Groups	WBCs count (10^3/mm^3)				Heterophiles (%)				Lymphocytes (%)				Eosinophiles (%)				Monocytes (%)			
	Time pre-infection		Time post-infection		Time pre-infection		Time post-infection		Time pre-infection		Time post-infection		Time pre-infection		Time post-infection		Time pre-infection		Time post-infection	
	Day 7	Day 14	Day 28	Day 35	Day 7	Day 14	Day 28	Day 35	Day 7	Day 14	Day28	Day 35	Day 7	Day 14	Day 28	Day 35	Day 7	Day 14	Day 28	Day 35
G1	13.3±0.38^{d}	12.1±0.47^{d}	15.1±0.64^{a}	13.7±0.55^{ab}	23.00±4.04^{cd}	23.00±3.33^{bc}	23.00±1.21^{d}	22.00±0.88^{d}	64.00±1.98^{ab}	65.00±2.28^{c}	63.30±1.87^{d}	66.37±1.10^{d}	0.33±0.33^{a}	1.00±0.58^{a}	0.33±0.33^{b}	2.00±0.58^{a}	1.33±0.33^{b}	1.66±0.33^{b}	3.33±0.67^{bc}	3.00±0.58^{a}
G2	15.7±0.32^{b}	13.1±0.58^{cd}	12.1±0.64^{cd}	12.4±0.52^{bc}	15.00±2.52^{d}	19.00±0.58^{c}	32.00±1.21^{c}	32.00±1.53^{c}	64.00±4.19^{ab}	64.66±2.05^{c}	74.00±0.84^{c}	73.28±0.53^{c}	1.66±0.88^{a}	0.66±0.67^{a}	1.67±0.33^{a}	2.67±0.33^{a}	4.00±1.00^{a}	2.66±0.67^{b}	3.00±1.00^{bc}	2.33±1.00^{a}
G3	14±0.69^{bcd}	13.9±0.43^{bc}	12.6±0.21^{bc}	13.8±0.2^{ab}	35.00±2.84^{a}	36.00±2.01^{a}	32.00±1.21^{c}	33.00±0.88^{c}	63.00±0.58^{b}	71.72±2.08^{b}	75.73±1.32^{bc}	74.00±0.85^{bc}	1.00±0.58^{a}	0.67±0.33^{a}	1.66±0.33^{a}	1.33±0.88^{a}	2.33±0.33^{ab}	2.66±0.33^{b}	3.00±1.15^{bc}	3.33±0.33^{a}
G4	13.5±0.76^{bcd}	12.6±0.27^{cd}	11.8±0.17^{cd}	14.6±1.25^{a}	33.00±2.08^{a}	34.00±2.33^{a}	33.00±1.53^{c}	32.00±1.54^{c}	65.00±3.19^{ab}	71.66±0.60^{b}	74.67±0.65^{c}	73.28±0.66^{c}	1.33±0.88^{a}	1.00±0.58^{a}	1.66±0.34^{a}	2.67±0.33^{a}	3.33±0.67^{a}	2.33±0.33^{b}	2.33±0.33^{c}	2.33±0.33^{a}
G5	14.1±0.64^{cd}	12.3±0.46^{d}	12.1±0.46^{d}	15.3±0.63^{a}	31.00±2.84^{ab}	34.00±0.58^{a}	35.00±1.21^{bc}	35.00±1.53^{c}	66.00±3.56^{a}	75.33±0.91^{a}	76.33±0.48^{a}	76.66±0.18^{a}	0.66±0.33^{a}	0.67±0.66^{a}	0.33±0.34^{b}	2.00±0.00^{a}	2.66±0.33^{ab}	3.00±0.58^{ab}	3.67±1.45^{bc}	3.00±1.00^{a}
G6	13.5±0.32^{cd}	17.3±0.15^{a}	15.2±0.2^{a}	14.5±0.35^{a}	22.00±1.45^{cd}	22.00±1.45^{bc}	38.00±0.88^{ab}	37.00±1.53^{b}	65.00±0.88^{a}	65.00±0.79^{c}	74.63±0.93^{c}	75.41±0.89^{b}	0.66±0.66^{a}	0.67±0.67^{a}	1.33±0.33^{ab}	2.00±0.58^{a}	1.33±0.33^{b}	3.00±0.58^{ab}	5.66±1.20^{ab}	5.00±0.67^{a}
G7	17.8±0.64^{a}	16.9±0.35^{a}	16.1±0.42^{a}	14.8±0.4^{a}	23.00±1.45^{bc}	22.00±1.43^{bc}	40.00±0.58^{a}	40.00±0.88^{ab}	63.33±6.64^{b}	64.66±1.45^{c}	76.56±0.61^{ab}	74.00±1.17^{bc}	1.33±0.33^{a}	0.67±0.33^{a}	1.33±0.34^{ab}	2.33±0.33^{a}	2.33±0.88^{ab}	3.33±0.88^{ab}	5.00±0.58^{a}	5.11±1.00^{a}
G8	15.1±0.5^{bc}	15.1±0.52^{b}	17.5±0.35^{b}	15.6±0.29^{c}	25.00±1.50^{bc}	26.00±1.21^{b}	41.00±0.58^{a}	41.00±0.89^{a}	64.68±1.87^{ab}	66.54±2.23^{c}	77.50±0.17^{a}	75.34±0.62^{ab}	0.66±0.33^{a}	0.66±0.67^{a}	1.67±0.33^{a}	2.00±0.57^{a}	2.33±0.33^{ab}	4.67±0.33^{a}	6.66±0.33^{ab}	6.00±0.88^{a}

Data are presented as Mean±S.E

The different letters within the same column are significant at P<0.05

G1: Control negative non-infected, non-treated, G2: Control positive infected with IBV virus at day 21, non- treated, G3: Received *Echinacea* from the 7th till 21st day infected with IBV virus at day 21, G4: Received *Star Anise* from the 7th till 21st day infected with IBV virus at day 21, G5: Received both *Echinacea* and *Star Anise* from the 7th till 21st day infected with IBV virus at day 21, G6: Infected with IBV virus at day 21, then received *Echinacea* from the 21st till 42nd, G7: Infected with IBV virus at day 21, then received *Star Anise* from the 21st till 42nd, G8: Infected with IBV virus at day 21, then received both *Echinacea* and *Star Anise* from the 21st till 42nd.

trol group. After IBV infection on day 21, chicks experimentally infected with IBV and non treated elicited asignificant increase in WBCs count (P≤0.05) when compared with control –ve group. Treatment groups (G6, G7 and G8) showed decrease in total and differential leucocytic count during the 3rd and 4th weeks compared with the protective groups (G3, G4 and G5) as shown in Table 2.

Effects of the tested medicinal plants on serum liver enzymes (ALT and AST)

On the first week before infection, the results evoked non-significant alterations on ALT and AST activities. On the second week before infection, there were non- significant alterations on ALT and AST activities except the protective groups (G3, G4 and G5) that showed increase on serum ALT and AST activities compared with other groups. On the 3rd and 4th weeks after IBV infection, protective and treatment groups showed increase on ALT and AST activities compared with the control +ve groups. Treatment groups (G6, G7 and G8) showed increases on ALT and AST activities compared with the protective groups (G3, G4 and G5) as presented in Table 3.

Effects of the tested medicinal plants on serum urea and creatinine levels

On the first week before infection, the results exerted non-significant alterations on the levels of serum urea and creatinine. On second week before infection, non- significant alterations on serum ALT and AST activities were observed, except for the protective groups (G3, G4 and G5) that showed increases in the levels of serum urea and creatinine compared with other groups. On the 3rd and 4th week after IBV infection, the protective and treatment groups showed increase in serum urea and creatinine levels compared with the control positive group. Treatment groups (G6, G7 and G8) showed increases in serum urea and creatinine levels compared with the protective groups (G3, G4 and G5) as shown in Table 4.

Effects of the tested medicinal plants on serum oxidative stress and antioxidant enzymes

Serum reduced glutathione (GSH)

Our results showed that *Echinacea* and *Star Anise* treated groups showed a significant decrease in GSH level after treat-

Table 3. The effect of oral administration of *Echinacea* (50 g /L drinking water) and *Star Anise* (20 ml/kg feed) at different times of the experiment on serum AST and ALT levels in experimentally infected chickens with IBV (n=5)

Groups	ALT (U/L)				AST (U/L)			
	Time pre-infection		Time post-infection		Time pre-infection		Time post-infection	
	Day 7	Day 14	Day 28	Day 35	Day 7	Day 14	Day 28	Day 35
G1	20.67±0.88^{c}	20.33±0.88^{c}	24.67±0.88^{d}	25.00±0.58^{d}	42.00±0.58^{a}	38.3±0.88^{a}	36.6±0.88^{d}	38.00±1.15^{d}
G2	21.33±0.88^{bc}	24.00±0.58^{b}	43.67±2.02^{ab}	41.33±1.85^{a}	40.6±1.21^{ab}	38.00±0.58^{a}	108.3±0.88^{a}	105.00±0.58^{a}
G3	23.00±0.58^{bc}	31.00±0.58^{a}	42.67±1.45^{ab}	40.33±1.33^{ab}	38.6±1.21^{bc}	34.00±0.58^{b}	76.00±0.58^{c}	75.3±0.33^{c}
G4	22.00±1.53^{bc}	29.67±1.20^{a}	41.33±0.88^{b}	41.00±0.58^{a}	36.00±0.58^{cd}	33.00±0.58^{b}	75.00±0.58^{c}	76.00±0.58^{c}
G5	24.33±0.88^{ab}	29.00±0.58^{a}	41.00±0.58^{a}	40.33±1.85^{b}	35.3±0.88^{d}	31.00±0.58^{c}	77.3±0.88^{c}	77.00±0.58^{c}
G6	26.00±0.58^{a}	25.33±1.45^{b}	37.00±0.58^{c}	35.66±1.20^{c}	39.00±0.58^{b}	39.00±0.58^{a}	81.00±0.33^{b}	81.6±0.88^{b}
G7	24.00±0.58^{ab}	24.33±1.20^{b}	35.00±0.58^{c}	32.66±1.20^{c}	36.00±0.58^{cd}	39.00±0.58^{a}	81.3±0.33^{b}	81.3±0.88^{b}
G8	24.33±0.88^{ab}	25.33±1.20^{b}	34.00±0.58^{c}	33.00±1.53^{c}	35.3±0.88^{d}	39.6±0.88^{a}	85.30±0.58^{c}	85.6±0.33^{c}

Data are presented as Mean±S.E

The different letters within the same column are significant at P<0.05

G1: Control negative non-infected, non-treated, G2: Control positive infected with IBV virus at day 21, non- treated, G3: Received *Echinacea* from the 7th till 21st day infected with IBV virus at day 21, G4: Received *Star Anise* from the 7th till 21st day infected with IBV virus at day 21, G5: Received both *Echinacea* and *Star Anise* from the 7th till 21st day infected with IBV virus at day 21, G6: Infected with IBV virus at day 21, then received *Echinacea* from the 21st till 42nd, G7: Infected with IBV virus at day 21, then received *Star Anise* from the 21st till 42nd, G8: Infected with IBV virus at day 21, then received both *Echinacea* and *Star Anise* from the 21st till 42nd.

Table 4. The effect of oral administration of *Echinacea* (50 g/L drinking water) and *Star Anise* (20ml/kg feed) at different times of the experiment on serum creatinine and uric acid levels in experimentally infected chickens with IBV (n=5)

Groups	Creatinine (mg/dl)				Uric acid (mg/dl)			
	Time pre-infection		Time post-infection		Time pre-infection		Time post-infection	
	Day 7	Day 14	Day 28	Day 35	Day 7	Day 14	Day 28	Day 35
G1	0.7±0.06^{ab}	0.7±0.06^{ab}	0.7±0.03^{d}	0.5±0.15^{d}	21.3±0.67^{b}	23.33±0.88^{ab}	22.6±0.88^{d}	24.6±0.33^{d}
G2	0.8±0.05^{a}	0.6±0.05^{ab}	1.9±0.12^{a}	1.8±1.45^{a}	24.6±1.21^{a}	24.00±0.58^{ab}	80.3±0.33^{a}	80.00±0.58^{a}
G3	0.67±0.03^{ab}	1.7±1.15^{a}	1.2±0.03^{d}	1.2±0.12^{d}	24.00±0.58^{ab}	19.00±0.58^{c}	56.00±0.58^{c}	54.00±0.58^{c}
G4	0.7±0.06^{ab}	0.4±0.05^{ab}	1.3±0.02^{c}	1.3±1.05^{c}	24.00±1.53^{ab}	18.00±0.56^{c}	54.00±0.58^{c}	53.2±0.58^{c}
G5	0.5±0.1^{b}	0.2±0.05^{b}	1.1±0.08^{c}	1.00±0.05^{c}	23.3±0.88^{ab}	17.00±0.58^{c}	56.6±0.33^{c}	55.00±0.58^{c}
G6	0.6±0.05^{ab}	0.83±0.08^{ab}	1.2±0.11^{ab}	1.1±0.08^{ab}	26.00±0.58^{a}	25.00±0.58^{a}	77.00±0.31^{b}	74.00±0.58^{b}
G7	0.8±0.05^{a}	0.63±0.08^{ab}	1.3±0.12^{b}	1.2±0.25^{b}	25.00±0.58^{a}	24.00±0.58^{ab}	76.0±0.33^{b}	73.3±0.88^{b}
G8	0.7±0.11^{ab}	0.66±0.08^{ab}	1.00±0.05^{b}	1.00±0.08^{b}	24.00±0.58^{ab}	22.3±0.88^{b}	78.00±0.31^{b}	75.4±0.33^{b}

Data are presented as Mean±S.E

The different letters within the same column are significant at P<0.05

G1: Control negative non-infected, non-treated, G2: Control positive infected with IBV virus at day 21, non- treated, G3: Received *Echinacea* from the 7th till 21st day infected with IBV virus at day 21, G4: Received *Star Anise* from the 7th till 21st day infected with IBV virus at day 21, G5: Received both *Echinacea* and *Star Anise* from the 7th till 21st day infected with IBV virus at day 21, G6: Infected with IBV virus at day 21, then received *Echinacea* from the 21st till 42nd, G7: Infected with IBV virus at day 21, then received *Star Anise* from the 21st till 42nd, G8: Infected with IBV virus at day 21, then received both *Echinacea* and *Star Anise* from the 21st till 42nd.

ment compared to the control group (Table 5).

Serum superoxide dismutase (SOD)

The present work mirrored non-significant alterations on the first week before infection in serum superoxide dismutase (SOD) activity. On the second week before infection, non- significant alterations were detected in superoxide dismutase (SOD) activity except for the protective groups (G3, G4 and G5) that showed a decrease in the activity of serum SOD compared with other groups. On the 3rd and 4th weeks after IBV infection, protective and treatment groups showed decrease on serum SOD activity compared with the control positive group. Treatment groups (G6, G7, and G8) showed decrease in serum SOD activities compared with the protective groups (G3, G4 and G5) as shown in Table 5.

Serum nitric oxide (No)

Chicks experimentally infected with IBV and non-treated evoked a significant decrease in serum nitric oxide levels all over the experimental period when compared with healthy control group. Chicks experimentally infected with IBV and treated with *Echinacea* produced a significant decrease in serum nitric oxide levels (P≤0.05) when compared with control group. Infected chicks with IBV and treated with *Star Anise* showed decrease in serum nitric oxide levels (P≤0.05) when compared with control group (Table 5).

Evaluation of humeral immune response

ELISA antibody titer was statistically significantly higher in treatment groups G8> G7>G6 than protective groups G5>G4>G3 and both treatment and protective groups were higher when compared with control positive group (Table 6).

Histopathological examination

Tracheal lesions

The histological study showed different stages of inflammation, degeneration, and necrosis to the tracheal tissues. Our data revealed that G1 (control negative group) showed normal architecture of trachea. G2 (control positive group) showed thickened tracheal mucosa with focally sloughed, eroded epithelium, swollen and variably infiltrated with abundant lymphocytes, macrophages and heterophils beside expansion of submucosa with edema admixed with few inflammatory cells. G3 showed multifocal tracheal epithelial swollen with mild submucosal edema. G4 showed marked tracheal epithelial vacuolation expanded and compress the lamina propria with submucosal edema separated connective tissue from the underlying cartilage. G5 showed diffuse tracheal vacuolation with diffuse submucosal expansion with lymphocytic aggregations. G6 showed normal appearance

Table 5 The effect of oral administration of *Echinacea* (50 g/L drinking water) and *Star Anise* (20ml/kg feed) at different times of the experiment on serum nitric oxide (NO), superoxide dismutase (SOD) and redused glutathione (GSH) levels in experimentally infected chickens with IBV (n=5)

Groups	GSH (mmol/L)				No (µmol/L)				SOD (U/ml)			
	Time pre-infection		Time post-infection		Time pre-infection		Time post-infection		Time pre-infection		Time post-infection	
	Day7	Day14	Day28	Day35	Day7	Day14	Day28	Day35	Day7	Day14	Day28	Day35
G1	0.4±0.05^{a}	0.5±0.20^{a}	0.4±0.15^{a}	0.63±0.15^{a}	24.23±0.67^{a}	25.95±0.5^{a}	26.5±1.14^{a}	25.3±0.53	124.00±0.58^{a}	125.00±0.58^{a}	127.00±0.58^{a}	127.33±0.33
G2	0.5±0.21^{b}	0.68±0.06^{b}	1.82±0.15^{b}	1.92±0.23^{b}	25.24±0.5^{b}	26.37±0.78^{b}	23.24±0.53^{bc}	23.3±0.78	125.67±0.88^{b}	127.00±0.58^{b}	123.67±0.33^{b}	123.00±0.58
G3	0.63±0.15^{a}	0.55±0.10^{a}	0.77±0.06^{a}	0.77±0.21^{a}	23.4±0.95^{b}	29.95±0.66^{b}	22.34±1.8^{b}	22.25±0.9	122.00±0.58^{c}	121.33±0.33^{c}	118.00±0.58^{c}	118.66±0.33
G4	0.9±0.26^{a}	0.67±0.21^{a}	1.20±0.15^{a}	1.21±0.22^{a}	26.0±0.04^{c}	30.72±0.95^{c}	21.29±1.72^{c}	21.24±0.74	121.00±0.58^{c}	120.00±0.58^{c}	120.33±0.33^{c}	120.33±0.76
G5	0.7±0.23^{b}	0.70±0.06^{b}	0.82±0.32^{ab}	0.80±0.25^{b}	27.12±2.84^{b}	30.54±0.9^{b}	21.13±0.33^{bc}	21.17±0.5	125.00±0.58^{c}	124.00±0.58^{c}	120.66±1.20^{c}	120.23±0.58
G6	0.83±0.46^{a}	0.95±0.32^{a}	1.50±0.17^{a}	1.50±0.06^{a}	27.13±1.8^{c}	27.14±0.65^{c}	23.54±0.9^{c}	23.95±0.5	130.33±0.58^{b}	130.00±0.58^{b}	121.66±0.33^{c}	121.00±0.77
G7	0.53±0.06^{a}	0.65±0.26^{bc}	1.64±0.21^{ab}	1.63±0.32^{ab}	26.15±0.74^{b}	25.14±0.65^{b}	21.4±0.86^{b}	21.36±0.78	125.33±1.53^{b}	125.33±0.88^{bc}	122.00±0.58^{c}	122.34±0.89
G8	0.67±0.12^{a}	0.75±0.25^{a}	1.33±0.06^{a}	1.30±0.18^{a}	26.13±0.33^{a}	27.25±1.5^{a}	22.31±0.58^{a}	22.13±0.33	130.33±0.58^{a}	131.33±0.33^{a}	120.00±0.58^{a}	120.33±0.34

Data are presented as Mean±S.E

The different letters within the same column are significant at P<0.05

G1: Control negative non-infected, non-treated, G2: Control positive infected with IBV virus at day 21, non- treated, G3: Received *Echinacea* from the 7th till 21st day infected with IBV virus at day 21, G4: Received *Star Anise* from the 7th till 21st day infected with IBV virus at day 21, G5: Received both *Echinacea* and *Star Anise* from the 7th till 21st day infected with IBV virus at day 21, G6: Infected with IBV virus at day 21, then received *Echinacea* from the 21st till 42nd, G7: Infected with IBV virus at day 21, then received *Star Anise* from the 21st till 42nd, G8: Infected with IBV virus at day 21, then received both *Echinacea* and *Star Anise* from the 21st till 42nd.

Table 6. The effect of oral administration of *Echinacea* (50 g/L drinking water) and *Star Anise* (20ml/kg feed) at different times of the experiment on ELISA antibody titre in experimentally infected chickens with IBV (n=5)

Groups	Day 28	Day 35	Day 42
G1	360.00±2.65^{a}	365.00±5.57^{a}	357.33±6.42^{a}
G2	992.33±11.23^{b}	1044.00±22.47^{b}	14083.00±6.17^{b}
G3	1923.33±55.08^{c}	1946.70±64.29^{c}	1983.33±55.08^{c}
G4	1926.00±50.08^{c}	1948.00±58.29^{c}	1985.00±63.04^{c}
G5	1930.00±55.05^{c}	1952.00±51.07^{c}	1990.00±50.08^{c}
G6	2440.00±52.9^{d}	2366.70±40.4^{d}	2418.67±35.85^{d}
G7	2443.00±54.8^{d}	2370.00±44.4^{d}	2422.00±38.84^{d}
G8	2447.00±55.4^{d}	2373.00±45.3^{d}	2425.00±36.73^{d}

Data are presented as Mean±S.E

The different letters within the same column are significant at P<0.05

G1: Control negative non-infected, non-treated, G2: Control positive infected with IBV virus at day 21, non- treated, G3: Received *Echinacea* from the 7th till 21st day infected with IBV virus at day 21, G4: Received *Star Anise* from the 7th till 21st day infected with IBV virus at day 21, G5: Received both *Echinacea* and *Star Anise* from the 7th till 21st day infected with IBV virus at day 21, G6: Infected with IBV virus at day 21, then received *Echinacea* from the 21st till 42nd, G7: Infected with IBV virus at day 21, then received *Star Anise* from the 21st till 42nd, G8: Infected with IBV virus at day 21, then received both *Echinacea* and *Star Anise* from the 21st till 42nd.

of the tracheal mucosa with mild edema separated the submucosal connective tissue from the hyaline cartilage. G7 showed marked thickening of the tracheal mucosa with diffuse necrotic and swollen tracheal epithelial beside extensive inflammatory infiltrate that also diffusely expands and effaces the submucosa. The inflammatory infiltrate was composed of abundant lymphocytes and macrophages admixed with fewer heterophils, RBCs, esinophilic fibrillar material and edema. G8 showed moderate epithelial thickening with vacuolated epithelial cells, intercellular and submucosal edema mixed with few lymphocytes, macrophages and erythrocytes.

Liver lesions

Our data recorded that G1 (control negative group) showed normal histological appearance of hepatocytes. G2 (control positive group) showed focal area of moderate cellular inflammatory aggregates variably extend into the sinusoids, inset, inflammatory cells are composed of lymphocytes and plasma cells. G3 showed subendothelial collagen (fibrosis) occasionally bridges portal areas, proliferate and disrupts hepatic parenchymal architecture, inset, and expanded area of fibrous connective tissue proliferations. G4 showed congested and dilated blood vessels with massive perivascular lymphocytic aggregations admixed with fibroblast proliferations, inset, massive expansion of hepatic parenchyma by marked lymphocytes, fibroblast. G5 showed diffuse mildly swollen hepatocytes with mild subendothelial collagen proliferation, inset proliferated plump fibroblast with focally aggregated lymphocytes expanded into vacuolated hepatocytes. G6 showed normal architecture of up to 80% of hepatic parenchyma with few basophilic intranuclear inclusion body. G7 showed focal area of cellular infiltrates, inset, higher power showing focal lymphocytic aggregations, focal minimal to mild subendothelial portal bridging fibrosis, inset, and minimal fibroblast admixed with few lymphocytes around congested blood vessels. G8 showed minimal focal area of fibrosis with few hepatocytes contain inclusion body, inset, focal area of bridging and transmigrating fibroblast admixed with mild number of lymphocytes. Hepatocytes showing amphophilic to basophilic intranuclear inclusion bodies that either completely fill or expand the nucleus.

DISCUSSION

Experimental chicks infected with IBV showed various degree of clinical symptoms vary from mild to severe which manifested in the form of coughing, sneezing, depression, nasal discharge, mouth breathing, gasping and mild to moderate loss of appetite. These results agreed with Raimundas *et al*. (2019). The obtained results showed that *Echinacea* (50 g/L) in drinking water and *Star Anise* (20 ml/kg feed) significantly reduced clinical symptoms in

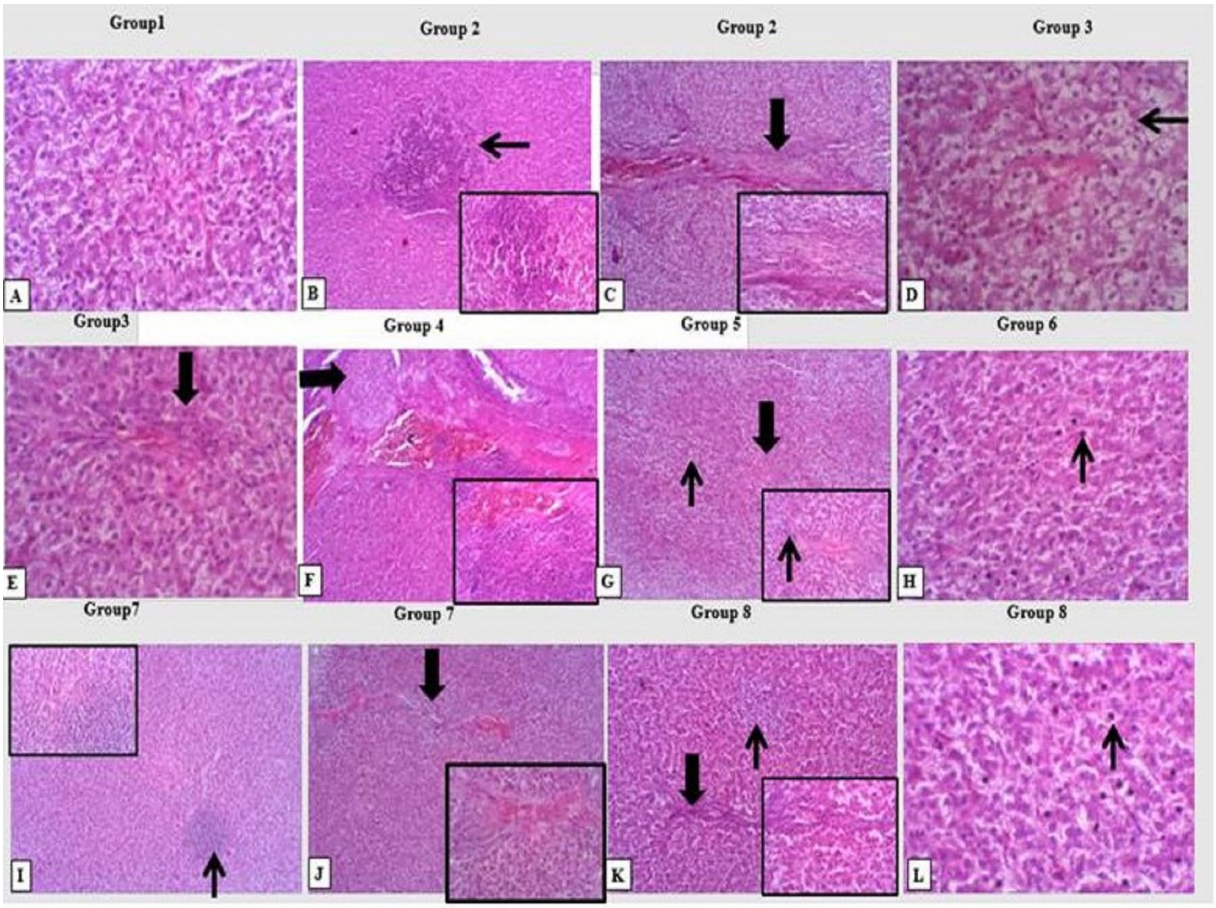

Fig. 1. Representative photomicrograph of trachea from different treatment groups. A) Group1 control group showing normal architecture of trachea. B) group 2 showing thickened tracheal mucosa with focally sloughed, eroded epithelium (thick arrow), swollen and variably infiltrated with abundant lymphocytes, macrophages and heterophils (arrowhead) beside expansion of submucosa with edema admixed with few inflammatory cells (thin arrow). C) group 3 showing multifocal tracheal epithelial swollen (thick arrow) with mild submucosal edema (thin arrow). D) Group 4 showing marked tracheal epithelial vacuolation (thick arrow) expanded and compress the lamina propria with submucosal edema separated connective tissue from the underlying cartilage (thin arrow). E) Diffuse tracheal vacuolation (thick arrow) with diffuse submucosal expansion with lymphocytic aggregations (thin arrow). F) Group 6 showing normally appearance tracheal mucosa with mild edema (thin arrows) separated submucosal connective tissue from hyaline cartilage. G) Group 7 showing marked thickening of tracheal mucosa with diffuse necrotic (thick arrow) and swollen tracheal epithelial (thin arrow) beside extensive inflammatory infiltrate (arrow heads) that also diffusely expands and effaces the submucosa. The inflammatory infiltrate is composed of abundant lymphocytes and macrophages admixed with fewer heterophils, RBCs, esinophilic fibrillar material and edema. H) Group 8 showing moderate epithelial thickening with vacuolated epithelial cells (thick arrows), intercellular and submucosal edema (thin arrow) admixed with few lymphocytes, macrophages and erythrocytes (arrowhead). A- H, H&E, 400X.

infected groups and improved flock productivity. Meanwhile, the combination between both medicinal plants at their doses in G5 and G8 resulted in more reduction of clinical symptoms.

Data from this study are in the same line with Saravanan and Deepthi (2019). They recorded that, IBV infection in chickens resulted in symptoms like watery eyes, mucus in the nares and trachea, sneezing, tracheal rales, and coughing. In layer birds, IB results in decreased egg production as well as quality of eggs, and some IBV variants cause interstitial nephritis. Infection with IBV causes ciliostasis in the trachea and predisposes to the secondary pathogens further complicating the disease.

In the present study, the mortality rate in the 8 tested groups revealed that after IBV infection the treatment groups (G8, G7 and G6) showed the lowest mortality rate followed by the protective groups (G5, G4 and G3). On the other hand, the highest mortality rate was recorded in the control positive group (G2). From these findings we concluded that the use of *Echinacea* and *Star Anise* (before and after virus infection) can't prevent the viral infection but can improve bird's resistance against viral infection and lowering the mortality rate in infected birds. This finding is in accordance with Yasmin *et al*. (2020).

In this study, the administration of *Echinacea* (50 g/L drinking water) and *Star Anise* (20 ml/kg feed) before and after IBV challenge showed a significant improvement in TLC and lymphocytic counts which significantly increased over time.

TLC was the highest in the treatment groups (G8, G7 and G6) followed by the protective groups (G5, G4 and G3) while the control positive group (G2) recorded a significant decrease in TLC and lymphocytic count. These results are in accordance with Hudson and Selvarani (2011) and Akram *et al*. (2011) who concluded that *Echinacea* and *Star Anise*, respectively, have potent antiviral effects which attributed to the stimulation of the immune system. In addition, Khattab *et al*. (2019) reported that there was an increase in lymphocytic cells of birds that supplemented with *Echinacea*. Also, Sherif *et al*. (2020) mentioned that there was an increase in lymphocytic cells of birds that supplemented with *Star Anise* oil. The improvement of TLC in the treatment groups (G8, G7 and G6) and protective groups (G5, G4 and G3) than the control positive group (G2) is in accordance with Al-Shammari (2011) and Saied *et al*. (2011) who reported that TLC increased in birds supplemented with *Echinacea* and *Star Anise*.

On first week before infection, the results evoked non- significant alterations in ALT and AST activities. On the second week before infection, non- significant alterations in ALT and AST activities were observed except for the protective groups (G3, G4 and G5) which showed increase in ALT and AST activities compared with the other groups which is in the line with Fathallah *et al*. (2020) and El-Ashram *et al*. (2020) that reported that medicinal plants *Echinacea* and *Star Anise* induced a significant increase in serum ALT and AST activities. On the 3rd and 4th weeks after IBV infection, the protective and treatment groups showed increases in ALT and AST activities compared with the control positive group. Treatment groups (G6, G7and G8) showed increases in ALT and AST activities compared with the protective groups (G3, G4 and G5).

In the present study, findings related to serum urea and creatinine levels are in accordance with Annahita *et al*. (2013) that stated that serum uric acid levels increased significantly after treatment with *Echinacea purpurea*. Treatment with *Star Anise* essential oil showed a significant ($p<0.05$) increases in serum creatinine, uric acid levels towards normalization (Ghozy *et al*., 2017).

It is well-known that the stress (especially the oxidative stress) affecting the cell components including lipids, proteins, carbohydrates and nucleic acids (Kalam *et al*., 2011). Oxidative stress also has been implicated in several diseases (Grune, 2000).

The overproduction of reactive oxygen species (ROS) could result in oxidative stress, that considered as an important mediator to induce damage to cell structures, including membranes, lipids, proteins and DNA (Valko *et al*., 2007).

In the current study, the protective and treatment groups that received medicinal plants *Echinacea* and *Star Anise* showed significant decrease in GSH level compared with control positive group and these results were in agreement with Mahmoud *et al*. (2022) who concluded that *Echinacea* has antioxidant, anti-in-

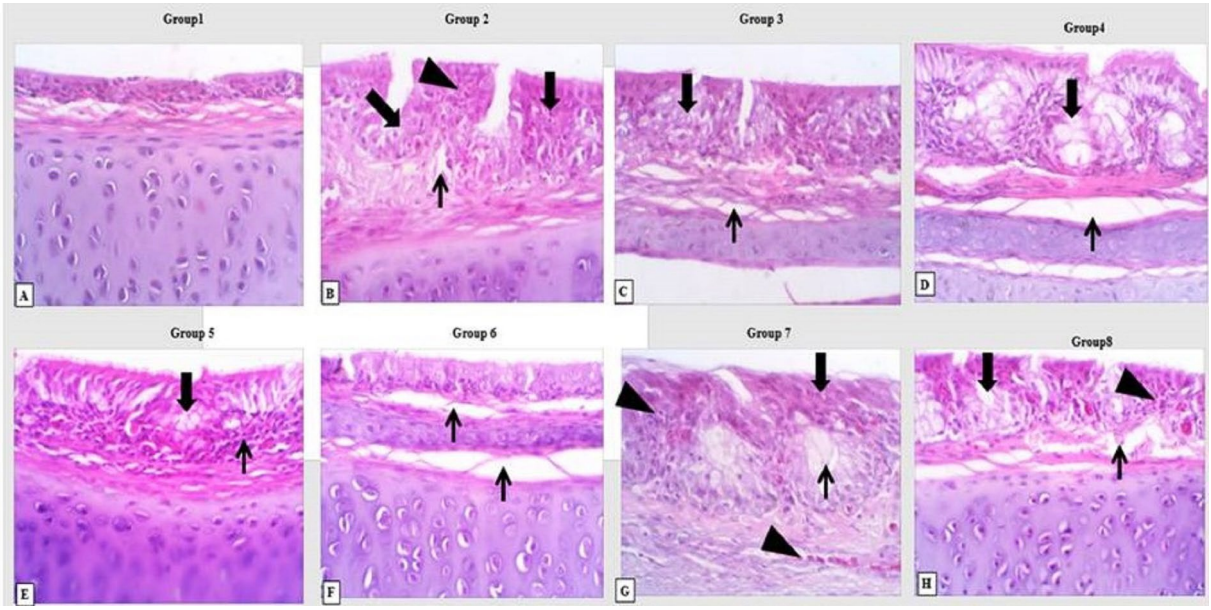

Fig. 2. Photomicrograph of poultry liver from different treatment groups. A) Control group showing normal histological appearance of hepatocytes. B) Control positive group showing focal area of moderate cellular inflammatory aggregates variably extend into the sinusoids (thin arrow), inset, inflammatory cells are composed of lymphocytes and plasma cells. C) Control positive group showing subendothelial collagen (fibrosis) (thick arrow) occasionally bridges portal areas, proliferate and disrupts hepatic parenchymal architecture, inset, and expanded area of fibrous connective tissue proliferations. D, E) Group 3 showing diffuse hepatic vacuolation (thin arrow) and minimal area of perivascular fibroblast proliferation with few lymphocytes' infiltrations (thick arrow), H&E, 400X. F) group 4 showing congested and dilated blood vessels (c) with massive perivascular lymphocytic aggregations admixed with fibroblast proliferations (thick arrow), inset, massive expansion of hepatic parenchyma by marked lymphocytes, fibroblast. G) Group 5 showing diffuse mildly swollen hepatocytes (thin arrow) with mild subendothelial collagen proliferation (thick arrow), inset proliferated plump fibroblast with focally aggregated lymphocytes expanded into a vacuolated hepatocytes (thin arrow). H) Group 6 showing normal architecture of up to 80% of hepatic parenchyma with few basophilic intranuclear inclusion body (thin arrow). I) group 7 showing focal area of cellular infiltrates (thin arrow), inset, higher power showing focal lymphocytic aggregations, H&E, 400X. J) Focal, minimal to mild subendothelial portal bridging fibrosis (thick arrow), inset, and minimal fibroblast admixed with few lymphocytes around congested blood vessels. K, L) Group 8 showing minimal focal area of fibrosis (thick arrow) with few hepatocytes contain inclusion body (thin arrow), inset, focal area of bridging and transmigrating fibroblast admixed with mild number of lymphocytes. L) Hepatocytes showing amphophilic to basophilic intranuclear inclusion bodies that either completely fill or expand the nucleus (thin arrow).

flammatory and immunostimulatory effects and could ameliorate the lead-induced immunotoxicity and oxidative stress, and also in agreement with Khaled *et al*. (2016) who found that the anise oil has a protective role in decreasing oxidative stress in favism-induced rats.

The obtained data is supported by Nematalla *et al*. (2011) who investigated the effect of *Echinacea* as an antioxidant on ageing markers by measuring the activity of glutathione-s-transferase (GST) and discovered a significant improvement in increased and decreased levels of GST markers and returned abnormal levels to normal.

The aforementioned findings were supported by the findings of Gholamreza *et al*. (2011), who discovered that the dried aerial part powder of *Echinacea purpurea* (EP) has a significant efficiency on serum total antioxidant activity in broiler chick. The use of 10 g EP/kg food improved the overall serum antioxidant activity in broiler chick. As a result, the plant with high potential should be tested for antioxidant activity, with antioxidant activity testing further broken down into elements such as preventing oxidation and scavenging free radicals.

Our findings are consistent with those of Iftikhar *et al*. (2022) who investigated the potential of *Star Anise* tea (SAT) on oxidative stress, obesity, and related biochemical parameters in a high-fat-sugar-diet induced obesity model in rats and discovered that SAT had strong protective effects against obesity and oxidative stress, particularly at higher doses.

Our study revealed that chicks experimentally infected with IBV and non-treated evoked a significant decrease in serum nitric oxide levels all over the experimental period when compared with healthy control group. The experimentally infected chicks with IBV and treated with *Echinacea* produced a significant decrease in serum nitric oxide levels ($P \leq 0.05$) when compared with control group. Infected chicks with IBV and treated with *Star Anise* showed decrease in serum nitric oxide levels ($P \leq 0.05$) when compared with control group. These findings agreed with those of Zilizhai *et al*. (2007), who revealed that alcohol extracts of *Echinacea* decrease macrophage production of nitric oxide and tumor necrosis factor-alpha and exhibited potential anti-inflammatory effect in vitro. Findings from this study were consistent with those of Olayinka and Anthony (2010), who reported that the level of nitric oxide was significantly reduced by the *Star Anise* crude extract.

The obtained findings for serum SOD activities were supported by those of Huang *et al*. (1996), who demonstrated that the protective enzymes against oxygen free radicals like CAT and SOD might be used as circumstantial evidence for increased ROS production. SOD activity can be used to detect oxidative stress-induced lipid peroxidation damage in the cell.

Findings from the present study are consistent with those of Iftikhar *et al*. (2022), who evaluated and validated the potential of polyphenol-rich *Star Anise* tea (SAT) on oxidative stress, obesity, and related biochemical parameters in a high-fat-sugar-diet (HFSD)-induced obesity paradigm in rats. The usual method of preparing SAT in warm water was used.

The present study showed that oral administration of *Echinacea* (50 g/L drinking water) and *Star Anise* (20 ml/kg feed) increased the antibody titer against IBV and the highest antibody titer was found in the protected treated group G8 and these findings were in agreement with Gurbuz *et al*. (2010) who confirmed that the antibody production against IBV was improved by administration of *Echinacea* to experimental chicks and pointed that this increase is attributed to *Echinacea* content.

The histological study showed different stages of inflammation, degeneration, and necrosis to the tracheal tissues.

According to Benyeda *et al*. (2010), IBV primarily affects the trachea and histopathologically results in epithelial cell loss and degeneration, lymphoid cell infiltration of the lamina propria, depletion of mucus secreting cells, heterophils exudate in the lumen, hyperplasia of mucus-secreting cells, and metaplasia and hyperplasia of epithelial cells.

The findings are consistent with those of Moustafa *et al*. (2016), who discovered that the tracheal lining epithelium was hyperplastic and displayed metaplasia. Sloughed, desquamated epithelium with few granulocytes was found in the tracheal lumen. The submucosa of the trachea was edematous, with mild to moderate lymphocyte infiltration. Endotheliosis was seen in the serosal blood vessels.

In accordance with the obtained results for the effect on the liver tissues, Moustafa *et al*. (2016) recorded that the hepatic tissue revealed multiple focal areas of necrosis which was replaced by lymphocyte and heterophils aggregation. The hepatocytes showed degenerative and fatty changes and the blood vessels were congested with perivascular edema and mononuclear cell infiltration. Some blood vessels contained micro thrombi and some of them were organized.

CONCLUSION

It could be concluded that the use of *Echinacea* and *Star Anise* as an antiviral is positively beneficial in the prevention and treatment of infectious bronchitis virus in poultry. Moreover, the use of the combination of both plants when used together have more powerful effects in prevention and treatment of IBV in poultry. So, it is recommended to use *Echinacea* and *Star Anise* in the prevention and treatment of IBV in poultry.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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