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RESEARCH ARTICLE

Phytotherapeutic attenuation of patho-biochemical and oxidative alterations using *Artemisia annua* L. plant extract in experimentally induced *E. coli* (O101) infection in poultry birds

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ABSTRACT

The present experimental study was planned to investigate the effect of *Artemisia annua* (*A. annua*) against experimental *E. coli* (O101) organisms intraperitoneally in Delham Red (DR) chicks. In the experiment study, 300 day-old Delham Red (DR) birds were randomly divided into 6 groups. Group I acted as a control group, group II was given *E. coli* infection only, and groups III, IV, and V were administered with both *E. coli* and 70% aqua-ethanolic extract of *A. annua* at the dose rates of 500 mg, 1000 mg and 2000 mg/L water, respectively. The group VI was provided with 70% aqua-ethanolic extract of *A. annua* only at the dose rate of 2000 mg/L of water. *E. coli* (O101) infection was given to the birds intraperitoneally on the 7th day of age. The 70% aqua-ethanolic extract of *A. annua* was given in drinking water to birds from 0 days to day 14. The birds from each group were sacrificed at 1, 3, 5, 7, 10 and 14 days post-infection (DPI). The values of biochemical parameters such as ALT, AST and creatinine were increased, whereas concentrations of total protein and albumin were decreased in group II (*E. coli* infection only) as compared to group I (control group). However, the clinical signs and serum biochemical values in the groups III, IV and V were significantly lower in a dose-dependent manner as compared to group II (*E. coli* infection only). The gross pathology comprised fibrinous perihepatitis, fibrinous pericarditis, air sacculitis, splenomegaly and peritonitis with higher severity in group II, and there was a significant reduction in the gross lesions in groups III, IV and V in a dose-dependent manner. Microscopically, the liver and heart of group II showed severe perihepatitis, pericarditis, vacuolar changes, leukocytic infiltration, degenerative changes and enhanced cytoplasmic granularity. Similarly, in group II, the microscopic lesions in the spleen were characterized by reticuloendothelial cell hyperplasia and an increase in eosinophilic coagulum material. The microscopic lesions in the liver, heart, spleen and air sacs were of less severity in groups III, IV and V, which were attributed to the antibacterial effect of the plant extract used in the present study.

Keywords: *Artemisia annua, E. coli* (O101), hepatoprotective, liver damage, oxidative stress, serum biochemistry

INTRODUCTION

Avian colibacillosis is an infectious disease of poultry and is caused by *E. coli*, regarded as one of the main reasons for heavy morbidity and mortality in birds. Colibacillosis occurs in almost all species of domestic and wild birds as a highly acute fatal septicaemic disease[1]. Colibacillosis is characterised by colisepticemia, coligranuloma (Hjarre's disease), omphalitis/yolk sac infection, coliform cellulitis, peritonitis, salpingitis, haemorrhagic septicaemia, orchitis, osteomyelitis/synovitis, panophthalmitis, enteritis, and swollen head syndrome. In its acute form, it causes septicaemia that results in death and its subacute form, it causes pericarditis, perihepatitis, air saculitis, and other abnormalities. Various organ surfaces of birds get covered with a fibrin layer as an inflammatory response including the heart, liver, intestines, ovary, oviduct and lungs[2] .

Antibiotic administration is the most common and fastest approach for treating *E.coli* infection in broiler chickens, however, the main problem is the emergence of drug-resistant strains to the medications employed [3]. Antimicrobial drugs added to feed at low doses (sub-therapeutic dose) over a prolonged period may cause resistance development[4,5]. Antimicrobial resistance (AMR) for *E. coli* strains is a severe threat to public health because they could be transferred to humans through the food chain[6,7]. The WHO has suggested creating and utilising environment-friendly alternative techniques to prevent infections in poultry and other food-producing animals[10]. Therefore, it is important to develop or identify more potent, more natural and environment-friendly products to prevent or treat avian colibacillosis. The plant-based dietary supplement may turn out to be an effective and useful tool for treating and preventing *E. coli* infections as well as growth promoter.

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Artemisia annua L. (Annual mugwort, Sweet Wormwood, Sweet Annie, Sweet Sage wort,

Annual Wormwood) is an annual herb used for a very long time in Chinese and Hindu traditional medicine in Asia[11]. The naturally occurring bioactive components in *Artemisia annua* include monoterpenes, diterpenes, sterols and triterpenes, sesquiterpenes, phenylpropanoids, flavonoids, aliphatic (hydrocarbons, aldehydes and acids), aromatic (alcohols, ketones and acids). *A. annua* possesses various biological activities such as antibacterial, antifungal, anti-inflammatory, anticancer, antiviral, antiparasitic, anti-ulcerogenic, anti-adipogenic, anti-asthmatic, anti-osteoporotic, anti-nocicepetive and immunoregulatory etc[13].

Therefore, the current study was conducted to assess the antimicrobial properties of *Artemisia annua* L. plant extract against experimental *E. coli* infection in broiler chicks.

MATERIALS AND METHODS

Collection and Identification of plant material

In this study, *Artemisia annua* plant free from any dirt and dust was collected from Lahaul & Spiti (32.6192N, 77.3784E) district of Himachal Pradesh, India. Identification of the plant was done at CSIR-IHBT (Institute of Himalayan Bioresource Technology), Palampur, Himachal Pradesh, India.

Assessment of inhibition of bacterial growth

The 70% aqua-ethanolic extract of *Artemisia annua* plant was prepared as per the standard procedure[2]. *E.coli* organism was procured from the Department of Public Health, Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar, Haryana, India. The serotyping of the isolate was done at the Central Research Institute (CRI), Kasauli and maintained in the Microbiology laboratory of the Department of Veterinary Pathology, DGCN COVAS, Palampur, Himachal Pradesh, India. The disc diffusion method was used to assess the antibacterial action of the plant extract.

DMSO, which was taken as a solvent for impregnating the discs, was used as a negative control. The isolated organism was tested against various antibiotics like Gentamicin (GEN50), Amikacin (AK30), Oxytetracycline (O30), Ciprofloxacin (CIP30), Levofloxacin (LE5), Enrofloxacin (EX10), Ceftriaxone (CTR10 and CTR30), Amoxicillin & Salbactum (AMS30/15), Amoxicillin & Clavulanic acid (AMC20/10). Among all the antibiotics used in the *in vitro* trial, Ceftriaxone (CTR10) determined to be the most effective and thus the disc impregnated with Ceftriaxone (CTR10) served as the positive control. The empty sterile disc was mounted on inoculated plates to check for any inhibition.

Viable bacterial count

The viable count of the bacteria was determined by counting the colonies on EMB plates by performing serial dilutions of the *Escherichia coli* O101 culture, which was incubated in the nutrient broth for 18 hours. The serial dilutions were made in normal saline solution (NSS) diluted from 10^{-1} to 10^{-7} and from each dilution 0.1ml was uniformly distributed across EMB plates. These plates were incubated for 24 to 36 hours at 37^0C and after incubation; these plates were counted for colony-forming units (cfu) per ml.

Determination of LD_{50} of *E. coli* O101

The study was conducted on day-old chicks (N=48) procured from the University Poultry farm, COVAS, CSKHPKV, Palampur, India. The birds were randomly divided into six groups, each group have 8 birds. On the 7th day, the *E.coli* organism was given to the birds through the intraperitoneal route. In the first five groups, the birds were infected with 1ml of normal saline solution with different concentrations of organisms (3.3×10^8, 3.3×10^7, 3.3×10^6, 3.3×10^5, and 3.3×10^4 cfu/ml, respectively). The sixth group acts as a control, having no infection. The birds were closely observed for up to 10 days for any mortality.

Animal experimentation

The final experiment was conducted on 300; day-old Delham Red (DR) birds procured from Animal Genetics and Breeding Department of DGCN COVAS, Palampur and were reared under strict hygienic conditions. Birds were purchased and maintained as per the recommendations of CCSEA. On the 7th day, infection was induced to the birds intraperitoneally and this day was

Table 1. Experimental design

Group	Treatment	Dosing level of infection + 70% aqua-ethanolic extract	No. of birds
I	Feed only (control)	0+0	50
II	*E. coli* infection only	3.6×10^7cfu/ ml +0	50
III	*E. coli* infection + 70% aqua-ethanolic extract of *Artemisia annua*	3.6×10^7cfu/ml+500 mg extract per litre of water	50
IV	*E. coli* infection + 70% aquaethanolic extract of *Artemisia annua*	3.6×10^7cfu/ml+1000 mg extract per litre of water	50
V	*E. coli* infection + 70% aqua-ethanolic extract of *Artemisia annua*	3.6×10^7cfu/ml+2000 mg per litre of water	50
VI	70% aqua-ethanolic extract of *Artemisia annua*	0 + 2000 mg per litre of water	50

considered as the 0 DPI. Table 1 provides an overview of the various treatments administered to each group. The birds were repeatedly monitored throughout the experimental trial up to 14 DPI (21 days of age).

Clinical symptoms, mortality and body weight

The clinical symptoms to be observed at least three times a day included loss of appetite, restlessness, dullness, weight loss and diarrhoea. The mortality pattern was recorded throughout the experimental trial. To determine the effect of 70% aqua-ethanolic extract of *Artemisia annua* on the body weight, three birds from each treatment group were weighed on 0, 1, 2, 3, 5, 7, and 14 DPI and weight was recorded.

Serum Biochemistry

The blood samples were collected at 1, 3, 5, 7, 10 and 14 DPI from 3 birds in each group by cardiac puncture before sacrifice for the estimation of serum biochemicals like aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine (CRT), albumin (ALB) and total protein (TP) by using a semi-automatic biochemistry analyzer (Model AGAPPE MISPA neo).

Gross pathological examination

A detailed necropsy examination was done on the birds that were sacrificed and died throughout the experiment. Gross lesions were properly recorded, photographed and scored per a modified protocol given by Thakur et al.,[2]. After detailed necropsy examination, approximately 0.5 cm representative tissue sections from the liver, heart, spleen, and air sacs were collected for histopathological examinations in 10% neutral buffered formalin (NBF) from three chicks of each group at 1, 3, 5, 7, 10 and 14 DPI.

Histopathological examination

The microscopic lesions were scored as per the modified protocol given by Thakur et al.[2] The fixed tissues were washed overnight in running tap water, processed in various grades of alcohol, cleared in benzene, and embedded in paraffin wax. The sections were cut into a thickness of 3 to 5 micron and stained with routine Haematoxylin and Eosin stain (H&E) as per the standard protocol[14].

Reisolation of *E. coli*

Reisolation attempts for *E. coli* organisms were made at different intervals from the birds, which were sacrificed at different intervals. The obtained heart and liver swabs were aseptically collected and streaked directly on EMB plates. The plates were further incubated at 37^0C for 24 to 48 hours and subsequently checked for any bacterial growth.

Statistical analysis of data

The statistical evaluation of the results were subjected to ANOVA using the GraphPad Prism (10.2.1) statistical software and the means were compared using Tukey's test ($P \leq 0.05$).

RESULTS

***In vitro* antimicrobial activity of *Artemisia annua extract* against *E. coli* (O101)**

Ceftriaxone (CTR 10) was found to be the most effective among all antibiotics used in the *in vitro* study followed by Ceftriaxone (CTR 30) and Gentamycin (GEN 50). *Artemisia annua* extract showed a zone of inhibition measuring 18 mm at 200 mg/ml concentration and 70% aqua-ethanolic extract of *Artemisia annua* was further used in poultry birds (DR) infected with *E. coli* (O101).

Clinical Signs and mortality

The birds in-group I i.e. control group and group VI i.e. highest dose of 70% aqua-ethanolic extract of *Artemisia annua* were completely healthy and active throughout the experiment. The clinical symptoms in *Artemisia annua* treated groups appeared after 18h post-infection and included reduced feed and water intake, ruffled feather, abdominal breathing and whitish diarrhoea. The intensity of clinical symptoms was however much less in the groups III, IV and V in a dose-dependent manner in comparison to the group given infection alone i.e. group II.

No mortality was observed in group I and group VI throughout the experiment. The mortality in groups III, IV and V was relatively lower as compared with group II. The decline in the mortality rate in the groups III, IV and V reflects the antimicrobial potential of *Artemisia annua* against *E. coli* in infected birds.

Effect on body weight

In all six groups i.e. I, II, III, IV, V and VI, there was sequential growth in the body weight as the experiment progressed. The group IV and V exhibited a significant increase ($P \leq 0.05$) in the values of body weight at 5 DPI while the birds in group III showed a significant increase ($P \leq 0.05$) in body weight at 14 DPI in comparison to group II kept on plain infection only. The decrease in body weight in group II was linked to a decrease in feed consumption.

Biochemical changes

The serum ALT and AST values were significantly ($P \leq 0.05$) found to decline in the groups treated with the 70% aqua-ethanolic extract of *Artemisia annua* in a dose-dependent manner as compared with the group given *E. coli* infection only (group II) on days 3, 5 and 14 DPI. The group IV and V exhibited a significant reduction ($P \leq 0.05$) in the values of serum ALT at 7 DPI as compared with group II. An increase in the values of serum albumin was observed in the groups treated with *Artemisia annua* extract at different time intervals in a dose-dependent

manner. However, a significant increase (P≤0.05) in serum albumin was observed in group V treated with the highest dose of *Artemisia annua* extract (2000 mg) at 3DPI as compared with the group II administered with plain *E. coli* infection only.

Gross pathological examination

The lesions were in general, comprised of fibrinous perihepatitis, fibrinous pericarditis, airsacculitis, splenomegaly and peritonitis. The total gross lesion score and mean lesion score of fibrinous perihepatitis were higher in-group II throughout the experiment as compared to other treatment groups at different DPI. Severe fibrinous pericarditis was indicated by a thickened pericardial layer and caused adhesions to the epicardial surface of the heart in the birds of group II. Group II had the highest total gross lesion and mean lesion scores for the air sacs as compared to the other treatment groups at different time intervals post-infection (Fig. 1).

Histopathological changes

The histopathological examination of the liver in group II (*E. coli* infection only) included fibrin deposition, vaculoar changes, sinusoidal congestion, heterophilic and mononuclear cell infiltration, degenerative changes and increased cytoplasmic granularity. The intensity of microscopic lesions in the liver was highest in group II at 3, 5 and 7 DPI. Similar trend was noticed in other treatment groups III, IV and V but the intensity of lesions in liver was reduced in dose-dependent manner. Group V showed the lowest intensity of lesions among different treatment groups (Fig. 2). The lesions in the heart comprised of fibrinous pericarditis, infiltration of heterophils and mononuclear cells, congestion, muscle fibre degeneration and increased cytoplasmic granularity. Maximum lesion score intensity was observed in group II at 3, 5 and 7 DPI. Similar changes were observed in treatment groups III, IV and V but the changes were of lesser severity as compared to group II (*E. coli* infection only). Minimum microscopic lesion score was seen in group V among various treatment groups (Fig. 3). The lesions present in the

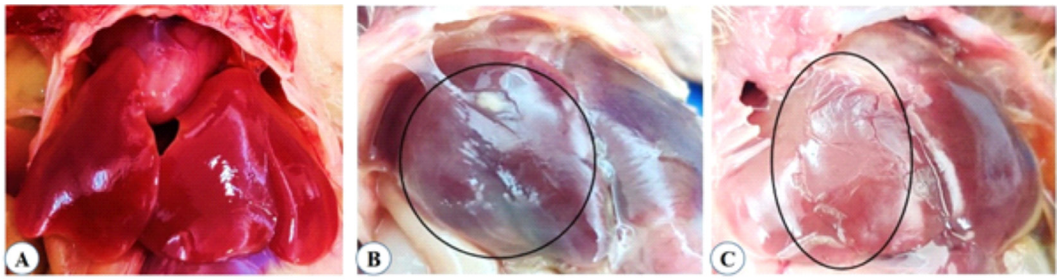

Fig.1. Gross pathology depicting the effect of different treatments on the liver of poultry birds. **A.** Control group showing normal liver. **B.** Group II treated with *E. coli* infection only depicting severe fibrinous perihepatitis. **C.** Group V treated with *E. coli* infection along with aqua-ethanolic extract of *Artemisia annua* exhibiting minimal fibrinous perihepatitis

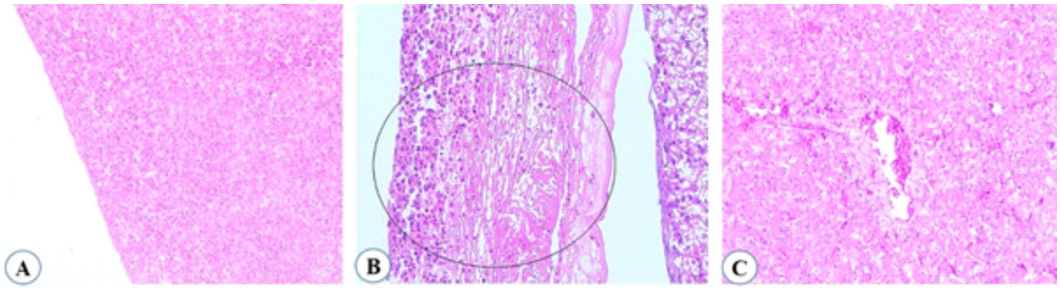

Fig.2. Histopathology depicting the effect of different treatments on the liver of poultry birds. **A.** Control group with normal architecture. **B.** Group II treated with *E. coli* infection only exhibiting severe fibrin deposition. **C.** Group V treated with *E. coli* infection along with aqua-ethanolic extract of *Artemisia annua* exhibiting mild fibrin deposition along with congestion

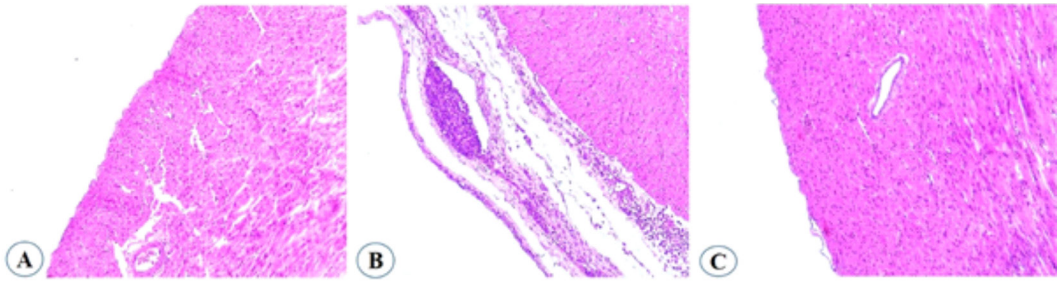

Fig.3. Histopathology depicting the effect of different treatments on the heart of poultry birds. **A.** Control group with normal architecture. **B.** Group II treated with *E. coli* infection only exhibiting severe fibrin deposition. **C.** Group V treated with *E. coli* infection along with aqua-ethanolic extract of *Artemisia annua* exhibiting mild fibrin deposition

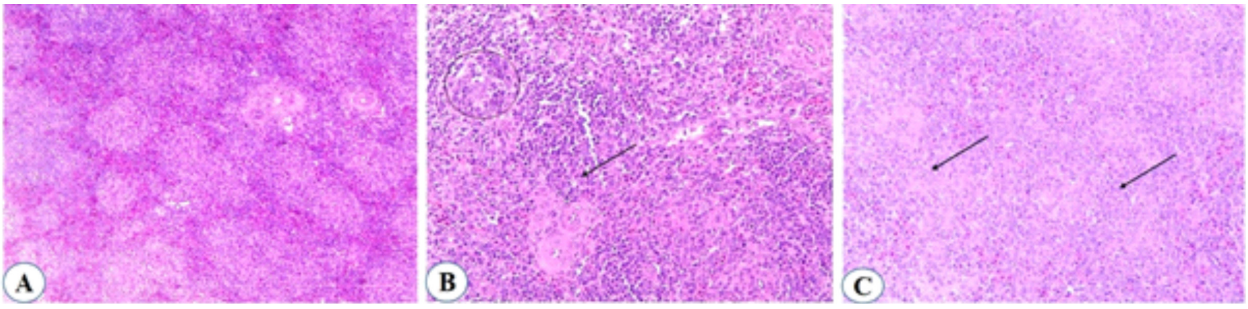

Fig.4. Histopathology depicting the effect of different treatments on the spleen of poultry birds. **A.** Control group with normal architecture. **B.** Group II treated with *E. coli* infection only exhibiting severe reticuloendothelial cell hyperplasia, and an increase in eosinophilic coagulum. **C.** Group V treated with *E. coli* infection along with aqua-ethanolic extract of *Artemisia annua* exhibiting mild reticuloendothelial cell hyperplasia

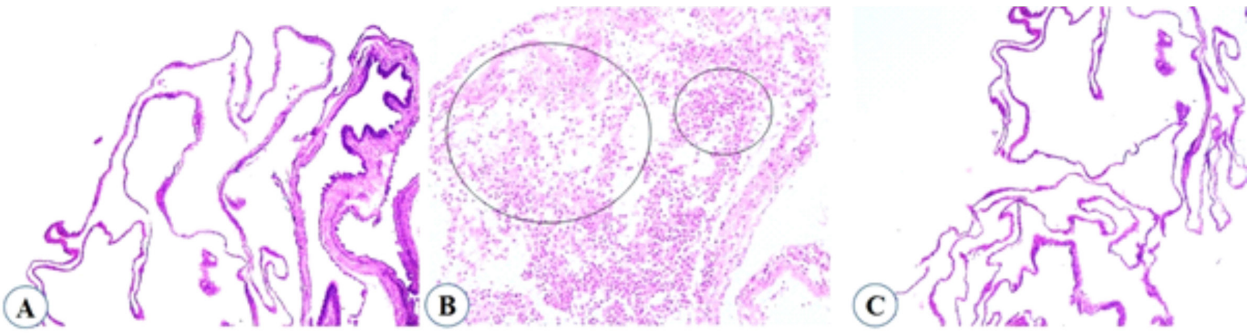

Fig.5. Histopathology depicting the effect of different treatments on the air sacs of poultry birds. **A.** Control group with normal architecture. **B.** Group II treated with *E. coli* infection only exhibiting severe fibrinous air sacculitis along with the infiltration of heterophils. **C.** Group V treated with *E. coli* infection along with aqua-ethanolic extract of *Artemisia annua* showing almost normal air sacs

spleen were reticuloendothelial cell hyperplasia, an increase in eosinophilic coagulum and congestion.

The maximum severity of lesions in the spleen were present in group II at 3, 5 and 7 DPI. The lesions intensity was reduced in dose-dependent manner in treatment groups III, IV and V. Group V showed lesions of reduced intensity in the spleen (Fig. 4). In the air sacs, fibrinous airsacculitis, infiltration of heterophils and mononuclear cells and congestion was observed largely at 3, 5 and 7 DPI in group II. In treatment groups III, IV and V similar trend was noticed but the severity of lesions was much lower than group II and the effect of plant extract was also dose-dependent (Fig. 5).

DISCUSSION

In the present study, *Artemisia annua* ought to show antibacterial activity by disc diffusion method with a zone of inhibition measuring 18 mm at 200 mg/ml concentration. The antimicrobial activity of aqua-ethanolic extract of *Artemisia annua* against *E. coli* were in accordance with Ikram et al.[15]Therefore, 70% aqua-ethanolic extract of *Artemisia annua* was further planned to be used in poultry birds infected with *E. coli* (O101).

In the *in vivo* study the clinical symptoms and mortality pattern were monitored regularly. The clinical symptoms, in general, in all the infected groups include a reduced feed and water intake, dullness, depression, huddling, ruffling of feathers, reluctance to move, and whitish watery diarrhoea. The mortality was reduced in the groups treated with *Artemisia annua* extract as compared with the group II provided with *E. coli* (O101).

The mean serum activity of ALT, AST and creatinine appeared increased whereas concentrations of total protein and albumin were lower in the infected groups as compared to the control group. But these values were highest the group treated with *E. coli* (O101) as compared with the groups provided with *Artemisia annua* extract in a dose dependent manner.

An elevation in the values of serum ALT in the *E. coli* infected group is associated with hepatocellular damage resulting into the alteration in cell membrane permeability and allowing the cytoplasmic ALT to leak into the circulation in accordance with previous study concluded by earlier workers[2]. The increase in the values of serum AST signify a damage to the hepatic system causing leakage of the enzyme into circulation. The increased level of serum creatinine in the infected group II as compared with the control and treatment groups may be associated with the renal damage and the finding of

our research has a correlation with previous observations documented by earlier[2]. The mean serum total protein values were lowest in group II as compared to the other treated group III, IV, V and VI. These findings were in accordance with the observations of Thakur *et al*.[2].

Fibrin deposition on the liver surface may be related to the endothelial damage of the blood vessels causing leakage of fibrin. The total gross lesion score and mean lesion score of fibrinous perihepatitis was higher in the group II throughout the experiment as compared to other treatment groups at different DPI. The similar type of fibrinous changes on hepatic parenchyma in *E coli* infection was observed in several studies conducted earlier[19]. In group III, IV, and V the total gross lesion score and mean lesion score were comparatively lower as compared to the group II given *E. coli* infection only at different time intervals post infection. The decline in the intensity of fibrin deposition on the liver may be related to antimicrobial potential and hepatoprotective effect of the *Artemisia annua* plant as reported in previous study[2]. Severe fibrinous pericarditis indicated by thickened pericardial layer and caused adhesions with epicardial surface of the heart were profoundly reported in the group II provided with *E coli* infection as compared with the other treatment groups. The severity of airsacculitis in the treatment groups III, IV and V treated with *Artemisia annua* extract showed a dose-dependent decline as compared to the group II given *E. coli* infection only.

The hepatocytes in the group II treated with *E. coli* infection only were markedly swollen with degenerative changes and increased cytoplasmic granularity. The fibrin layer was seen with moderate to severe intensity along with mononuclear cells and occasional heterophils. The total microscopic lesions and the mean microscopic lesion score of fibrinous perihepatitis, vacuolar changes and leukocytic infiltration in the liver were comparatively lower in all the treatment groups as compared to the group II given with *E. coli* infection only.

Severe fibrinous pericarditis in association with leukocytic infiltration predominantly MNCs were observed in the group provided with *E. coli* infection only. The inflammatory response majorly involved mononuclear cell infiltration admixed with few heterophils and fibroblast cells. Fibrinous pericarditis was however comparatively milder in the groups provided with *Artemisia annua* as compared to the group II administered with *E. coli* infection only. In group II, the total microscopic lesions and mean lesion score of reticuloendothelial cell hyperplasia and eosinophilic coagulum in the spleen were found to be higher as compared to the other groups at different intervals post-infection. In group V, severity of total microscopic lesion score and mean lesion score of reticuloendothelial cell hyperplasia and the presence of eosinophilic coagulum in the spleen was lower as compared to all the other treatment groups.

CONCLUSIONS

The 70% aqua-ethanolic extract of aerial parts of *Artemisia annua* exhibited antibacterial activity against *E. coli* (O101) as indicated by the results of *in vitro* as well *in vivo* studies. In the *in vivo* experiment, the extract significantly reduced serum liver enzyme levels, indicating notable hepatoprotective effects. Treated groups also exhibited markedly reduced gross and microscopic lesions on the liver, heart, air sacs and spleen, confirming the effective antibacterial and tissue-protective properties. In addition, the overall clinical response of the treated birds improved, as evidenced by reduced mortality, minimal clinical manifestations, and better feed intake. These findings collectively support the therapeutic potential of the 70% aqua-ethanolic extract of *Artemisia annua* as a natural antimicrobial, hepatoprotective, cardioprotective, and immunoprotective agent against *E. coli* (O101) infections in poultry birds.

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REFERENCES

1. Sara GR, Sur SK, Mitra M, Nag NC and Das AK. 1995. "Colibacillosis in capitive birds at Alipore Zoo". *J Anim Health* **34(1):** 43-45.
2. Thakur, S., Kumar, R., Asrani, R. K., Thakur, M., Patel, S. K., Patil, R. D., Obaidullah, A. J., & Emran, T. B. 2024. "Hepatoprotective and cardioprotective effect of *Artemisia nilagirica* leaf extract on *E. coli* challenged broiler chicken". *Heliyon*, **10(4)**, e25709. https://doi.org/10.1016/j.heliyon.2024.e25709
3. Saidi, B., Mafirakureva, P and Mbanga J. 2013. "Antimicrobial resistance of Escherichia coli isolated from chickens with colibacillosis in and around Harare, Zimbabwe". *Avian dis* **57(1):** 152-4.
4. Apata, DF. 2009. "Antibiotic resistance in poultry". *Inter J Poult Sci* **8(4):** 404-8.
5. Diarra, M.S and Malouin F. 2014. "Antibiotics in Canadian poultry productions and anticipated alternatives". *Front Microbiol* **17: 5**:282.
6. Akond, M.A., Alam, S., Hassan, S.M and Shirin M. 2009. "Antibiotic resistance of *Escherichia coli* isolated from poultry and poultry environment of Bangladesh". *Inter J Food safety*

11: 19-23.
7. Murray, M., Salvatierra, G., Dávila-Barclay, A., Ayzanoa, B., Castillo-Vilcahuaman, C., Huang, M., Pajuelo, M.J., Lescano, A.G., Cabrera, L., Calderón, M and Berg D.E. 2021. "Market chickens as a source of antibiotic-resistant Escherichia coli in a Peri-urban community in Lima, Peru". *Front Microbiol* **2(12):** 635871.
8. Nhung, N.T., Chansiripornchai, N and Carrique-Mas J.J. 2017. "Antimicrobial resistance in bacterial poultry pathogens: a review". *Front Vet Sci* **10(4):**126.
9. Aworh, M.K., Kwaga, J.K., Hendriksen, R.S., Okolocha, E.C and Thakur, S. 2021. "Genetic relatedness of multidrug resistant Escherichia coli isolated from humans, chickens and poultry environments". *Antimicrob Resist & Infec Control* **10(1):** 1-13.
10. WHO. 2001. "Antimicrobials in animal feed- a threat to human use". *WHO Drug Information* **15:** 160-162.
11. Ekiert. H., Świątkowska, J., Klin, P., Rzepiela, A and Szopa, A. 2021. "*Artemisia annua*– Importance in Traditional Medicine and Current State of Knowledge on the Chemistry, Biological Activity and Possible Applications". *Planta Medica* **87(08):** 584-99.
12. Gupta, P.C., Dutta, B., Pant, D., Joshi, Pand and Lohar, D.R. 2009. "*In vitro* antibacterial activity of *Artemisia annua Linn*. growing in India". *Inter J Green Pharm (IJGP)***3(3).**
13. Feng, X., Cao, S., Qiu, F and Zhang, B. 2020. "Traditional application and modern pharmacological research of Artemisia annua L". *Pharmacol & Therap* **216:** 107650.
14. Luna, L.G. 1968. "Manual of Histological Staining Methods. *Armed Forces Insti of Pathol*. New York, NY: McGraw Hill Book Company.
15. Ikram, M., Jan, G., Khan, S.D., Jan, F.G., Ullah, A., Shaheen, S., Ijaz, F., Rehman,S., Bahadar, S., Ziaulhaq, A.A and Iqbal, Z. 2015. "Antimicrobial activity and phytochemical screening of *Artemisia annua L*. and *Millotus philippensis (Lam.) Mull. Arg.* Leaves". *Am Eurasian J Agricul & Environ Sci* **15(12):** 2437-2441.