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Efficacy of *Azadirachta indica* Extract against Chicken Infectious Anemia Virus in Broiler Chickens: Study on Oxidative Stress, Hematological and Immunological Parameters

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ABSTRACT

Chicken infectious anemia virus (CIAV) is a significant immunosuppressive pathogen that compromises both cellular and humoral immune functions in poultry. The present study aimed to evaluate the immunosuppressive effects of neem liquid extract, acyclovir, and haemocare on CIAV infection by assessing hematological parameters, oxidative stress markers, phagocytic activities, DNA damage, and bone marrow analysis. One hundred and five one-day-old broiler chickens of both sexes were divided into seven equal groups. Group 1 was the control; uninfected and untreated, Group 2 was infected intramuscularly (IM) with 1 mL ($10^{4.5}$ TCID50 /0.1 mL) of CIAV-infected cell culture supernatant per chicken at 12 days of age, Group 3 was pre-treated with acyclovir (10 mg/kg IM daily for three days) starting at 9 days of age, infected with 1 mL of CIAV-infected cells at 12 days of age, Group 4 was pre-treated with neem (50 mL/Liter) in drinking water from day one until the end of the experiment, infected with 1 mL of CIAV-infected cells at 12 days of age, and subsequently treated with acyclovir (10 mg/kg IM daily) for two weeks starting at 15 days of age after the appearance of clinical signs, Group 5 was pre-treated with neem (50 mL/Liter), infected with 1 mL of CIAV-infected cells, and then post-treated with neem and hemocare (5 mL per 100 chickens in drinking water) for two weeks, commencing from day 15, Group 6 was pre-treated with neem (50 mL/Liter), infected with CIAV, and post-treated with a combination of neem, acyclovir, and hemocare (5 mL per 100 chickens), and Group 7 was infected with CIAV and post-treated with acyclovir at 10 mg/kg IM daily for two weeks. The results indicated normocytic normochromic anemia, with decreased antioxidant and phagocytic activities, and increased DNA damage in thymus tissue in CIAV-infected chickens. In conclusion, pre- and post-treatment with neem liquid extract, either alone or combined with acyclovir and haemocare, improved hematological parameters, oxidative stress markers, and phagocytic activities, while reducing DNA damage caused by CIAV infection in broiler chickens.

Keywords: Acyclovir, Chicken infectious anemia, Immunological study, Neem

INTRODUCTION

Schat (2003) noted that chicken infectious anemia virus (CIAV) significantly impacts chickens worldwide. There is no specific treatment for CIAV; instead, broad-spectrum antibiotics (Acyclovir), immunostimulant products (Neem), and hematinic medications (Haemocare) are administered to prevent secondary bacterial infections, boost the immune system, and support blood formation, respectively. Chicken anemia virus (CAV) causes a severe immunosuppressive disease in young chickens, characterized by anemia, extensive lymphoid tissue atrophy, loss of appetite, lethargy, depression, and, consequently, stunted growth and fatalities (Fatoba and Adeleke, 2019). The disease's economic impact results from high mortality rates as well as secondary and subclinical infections such as infectious bronchitis (IB; Zhang et al., 2015). Neem (*Azadirachta indica*), a member of the Meliaceae family, has a wide range of medicinal properties, as all parts of the tree have been shown to exhibit antioxidant, anti-inflammatory, and antibacterial effects. Moreover, it plays a role in preventing different diseases and treating conditions such as avian coccidiosis (Alzohairy, 2016). Additionally, neem leaf extracts are potent antivirals and have immunostimulant properties during the production cycle of broiler chickens (Hegazy et al., 2022).

Acyclovir, a guanosine analog antiviral medication, holds significant promise in veterinary medicine for both the prophylaxis and treatment of different diseases in a wide range of animal species, including poultry (Ahrens et al., 2013). On the World Health Organization list of essential medicines, acyclovir is mainly effective against herpes

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simplex virus infections, chicken pox, and shingles (Tavakkoli et al., 2014). The present study aimed to evaluate the effects of neem liquid extract alone and combined with acyclovir and hemocare to mitigate the detrimental effects associated with CIAV infection in broiler chickens.

MATERIALS AND METHODS

Ethical approval

The animal care and experimental protocols were approved by a research ethics committee of the Faculty of Veterinary Medicine, Zagazig University, Egypt, with approval number ZU-IACUC/2/F/365/2022.

Experimental animals

The present study utilized 105 one-day-old broiler chickens of mixed sexes, each weighing 45-47 grams. These chickens were sourced from non-immunized hens at the Dakahlia Poultry Company in Egypt. Throughout the study, the chickens were raised under established environmental and hygienic conditions to ensure their well-being and minimize confounding variables. The initial brooding temperature was maintained at 34°C, which was then gradually reduced to a constant 24 ± 2°C by the end of the third week. A continuous 24-hour photoperiod was applied. All chickens had *ad libitum* access to a well-balanced commercial ration and fresh water, in accordance with the rearing guidelines outlined by Vantress (2012). The basal diets provided to the chickens throughout the experimental period were formulated according to Vantress (2012) specifications, which were obtained from the Cairo Poultry Company (CPC), Egypt, and were adjusted based on the chickens' growth stages. The primary components of the basal diets included yellow maize and the proportion varied by growth stage; 58% for the starter stage (Days 1-10), 62% for the grower stage (Days 11-22), and 63.5% for the finisher stage (Days 23-30), soybean meal (48%), corn gluten (60%), soy vegetable oil, powdered limestone, calcium phosphate type 2, table salt, choline chloride and mixed vitamins (2955). These ingredients were consistently used to meet the nutritional requirements of the broiler chickens at each developmental stage.

Chicken infectious anemia virus

The CIAV was kindly obtained from the Animal Health Research Institute, Dokki, Egypt. Each chicken was infected with 1 mL of CIAV-infected cell culture supernatant ($10^{4.5}$ TCID50 /0.1 mL) intramuscularly at 12-day age (Gopal et al., 2015).

Chemical agents used for treatment

Neem extract

The neem liquid extract utilized in the present study was procured from Makin Company, a supplier specializing in natural raw materials for the food, pharmaceutical, and cosmetic industries. This extract was externally imported from Spain and certified as 100% pure and natural neem herbal liquid extract. It was supplied in one-liter containers and administered to the chickens at a concentration of 50 mL per liter of fresh drinking water (Durrani et al., 2008).

Acyclovir

Acyclovir 100% pure powder was obtained from Sigma Chemical Company, Egypt. At the dose of 10 mg/kg every 24 hours for 10 days, injected intramuscularly into the thigh muscle after being dissolved in distilled water (Mayahi and Talazade, 2010).

Hematinic agent

Hemocare syrup 10 mg iron /mL was purchased from Sina Pharmacy, Egypt. At a dose of 5 mL per 100 chickens in drinking water for two weeks (Bhatt et al., 2013). All the biochemical tests were performed using evaluation kits of Diamond Diagnostics, Egypt.

Experimental design

One hundred and five one-day-old broiler chickens of both sexes weighing from 45-47 grams were divided into seven equal groups with three replications in each group. Group 1 was used as a control, consisting of 15 broiler chickens per group. Group 2 was infected with 1mL ($10^{4.5}$ TCID50 /0.1 mL) CIAV cell culture supernatant (IM/chickens) from CIAV-infected cells at 12 days of age. Group 3 was given acyclovir (10 mg/kg/IM/day) for three days before infection, then infected with CIAV cell culture supernatant (1 mL/IM/chickens) from CIAV-infected cells at 12 days of age. Group 4 was administered neem liquid extract (50 mL/liter drinking water) from the first day to the end of the experiment (Day 30), then infected with CIAV (1 mL/IM/chickens) and treated at 15 days old with acyclovir (10 mg/kg IM daily) for two weeks. Group 5 was administered neem liquid extract (50 mL/liter drinking water), infected with CIAV (1 mL/IM/chickens), then post-treated with hemocare (5 mL/100 chickens in drinking water) from day 15 to day

28. Group 6 was pre-treated with neem liquid extract (50 mL/liter drinking water), then infected with CIAV (1 mL/IM/chickens), and post-treated with acyclovir (10 mg/kg IM daily) and hemocare (5 mL/100 chickens in drinking water) for two weeks. Group 7 was infected with CIAV (1 mL/IM/chickens) at 12 days of age and treated at 15 days old with acyclovir (10 mg/kg/IM/daily) for two weeks.

Blood samples

Blood samples were taken from each group at 15 and 30 days via the wing vein, with each sample split into three portions. The first portion (0.5 mL) was collected with dipotassium EDTA for hematological analysis. The second portion (2 mL) was gathered in sterile heparin solution for a phagocytic assay. The third portion (5 mL) was placed in a sterile centrifuge tube to clot and then centrifuged at 3000 rpm for 20 minutes. The clear sera were carefully separated and stored in a deep freezer at -20 ºC to analyze oxidative stress markers according to Coles (1986).

Tissue samples

All selected broiler chickens were humanely euthanized using an intraperitoneal injection of xylazine at 0.6 mL/kg body weight and ketamine at 0.7 mL/kg body weight. Following euthanasia, each chicken underwent a thorough necropsy, conducted as described by Kromann et al. (2022). This procedure involved a meticulous inspection of the external surfaces of the chickens, followed by the identification and documentation of any macroscopic lesions observed on internal organs. For further analysis, the thymus from each chicken was carefully collected and preserved in a solution of phosphate-buffered saline (PBS) with 1 µL of dimethyl sulfoxide (DMSO) per 10 µL of PBS. Additionally, the femoral bone was aseptically exposed to facilitate subsequent examination of the bone marrow.

Hematological examination

The total erythrocytic count ($x10^6$/µl), hemoglobin (Hb) concentration (g/dl), packed cell volume (PCV, %), mean corpuscular volume (MCV, fl), mean corpuscular hemoglobin (MCH, pg), and mean corpuscular hemoglobin concentration (MCHC, %), total and differential leukocytic counts ($x10^3$/µl), and platelet count ($x10^3$/µl) were measured for all groups of broiler chickens according to the methods outlined by Feldman et al. (2000).

Oxidative stress and antioxidant markers

Serum catalase (CAT, nmol/L), superoxide dismutase (SOD, U/mL), and lipid peroxidation (MDA, nmol/L) were assessed using enzymatic colorimetric methods with ready-to-use kits (Biodiagnostic, Egypt). The determination of serum CAT and SOD was performed according to Zhang et al. (2017) and MDA levels were evaluated based on Satoh (1978) in all groups.

Measurement of phagocytic activity

The phagocytic activity (%) of peripheral blood monocytes from all groups was measured using *Candida albicans* sourced from the Animal Health Research Institute, Egypt, following the methods outlined by Platt and Fineran (2015).

Evaluation of DNA damage

The DNA damage was evaluated in the thymus tissue of chickens from all experimental groups using the comet assay, following the methodology described by Langie et al. (2015). First, isolated thymus tissues were embedded in a mixture of 0.6% normal melting point and low melting point agarose gel on microscope slides. These slides were then immersed in a lysing solution (Comprising 2.5 M NaCl, 100 mM Na2EDTA, freshly added 1% Triton-X100, and 10% DMSO) at 4°C for one hour. This step facilitates the denaturation and unwinding of DNA. Next, the slides were placed in an electrophoresis buffer (300 mM NaOH, 1 mM Na2EDTA, pH 13.0) at 4°C for 30 minutes. Following electrophoresis, the slides underwent neutralization with a Tris-HCl buffer (400 mM Tris-HCl, pH 7.4). Finally, the DNA was stained with 20 µg/mL ethidium bromide, a fluorescent dye. The DNA damage was quantified by measuring tail length and tail moment using a Nikon Microscope-Eclipse E600 fluorescent microscope (Japan), equipped with a Y-FL EPI-Fluorescence attachment and a 40x objective. The microscope utilized an excitation filter of 515-560 nm and a barrier filter of 590 nm. An automatic digital imaging system running Comet assay™ software (Perceptive Instruments, UK) was used for image capture and analysis.

Examination of bone marrow

The femoral bones of chickens from all groups were exposed under aseptic conditions for histopathological examination of the bone marrow. Cells were washed two to three times with 199-medium (Sigma, USA) through repeated centrifugation at 3000 rpm for 10 minutes between each washing step and suspended using syringes with needles of different diameters. Smears of the cells were drawn on clean slides, fixed with methanol for 10 minutes, and

stained with Giemsa. At least 1000 cells were scored from each chicken to determine the total counts of myeloid and erythroid cells (Abouelella et al., 2007).

Statistical analysis

The current data was statistically analyzed by the variance method (ANOVA) using SPSS 18.0 software according to Tamhans and Dunlop (2000). The significant differences were tested using Duncan's multiple range tests to compare the means, which were significant at $p \leq 0.05$.

RESULTS

Clinical signs and mortality rate

During the experimental period, chickens in Group 1 remained healthy, exhibiting no adverse clinical signs. In contrast, Group 2 displayed significant clinical manifestations of disease, including depression, severe anemia, paleness, weakness, anorexia, and ruffled feathers, alongside notably poor weight gain. Additionally, Group 2 experienced a mortality rate of 13.3%. Conversely, chickens in Groups 3 to 7 demonstrated only mild clinical signs, and importantly, a statistically significant reduction in mortality rate ($p < 0.05$; Table 1), which suggested the therapeutic interventions had positive impacts on disease severity and survival.

Table 1. The mortality rate of 30-day-old broiler chickens of both sexes at 3 and 15 days post-infection

Groups	Total number of dead broiler chickens	Number of mortalities	Mortality rate (%)
Group 1 (C)	15	0	0
Group 2 (V)	15	2	13.3
Group 3 (AV)	15	0	0
Group 4 (NVA)	15	0	0
Group 5 (NVH)	15	1	6.6
Group 6 (NVAH)	15	0	0
Group 7 (VA)	15	0	0

Group 1 (C): Negative control, Group 2 (V): Chicken infectious anemia virus infected group, Group 3 (AV): Chicken pretreated with acyclovir then infected with chicken infectious anemia virus, Group 4 (NVA): Pretreated with Neem then infected with chicken infectious anemia virus then treated with acyclovir, Group 5 (NVH): Pretreated with neem then infected with chicken infectious anemia virus then treated with hemocare, Group 6 (NVAH): Pretreated with neem then infected with chicken infectious anemia virus then treated with acyclovir and hemocare, and Group7 (VA): Infected with chicken infectious anemia virus then treated with acyclovir.

Hematological results

Erythrogram

Chickens infected with CIAV exhibited a notable reduction ($p < 0.05$) in RBC count, HB concentration, and PCV, leading to the onset of normocytic normochromic anemia compared to the control group (Table 2). Additionally, groups 3 and 4 demonstrated normocytic normochromic anemia, although less severe than those observed in Group 2, three days post-infection (PI). After 15 days PI, chickens in groups 5, 6, and 7 illustrated a significant improvement in hematological parameters (RBC, Hb, PCV, MCV, MCH, and MCHC) compared to the infected chickens in Group 2 (Table 3).

Leukogram

Chickens infected with CIAV indicated a significant decrease ($p < 0.05$) in total leukocytic count (TLC), lymphocyte, heterophile, monocyte, eosinophile, basophile, and platelet count compared to the control group, three days PI (Table 4). While groups 3 and 4 had the same results, but less in severity compared to Group 2 ($p < 0.05$). In 15 days PI, there was an improvement ($p < 0.05$) in the leukocytic parameters (Lymphocyte, heterophile, monocyte, eosinophile, and basophile) and platelet count in groups 3 to 7, compared to Group 2 (Table 5).

Oxidative stress markers, phagocytic percent, and phagocytic index

Chickens infected with CIAV indicated a significant decrease ($p < 0.05$) in serum activities of CAT and SOD enzymes, along with a significant increase in MDA levels compared to the normal control group three days PI (Table 6). Moreover, groups 3 and 4 exhibited similar results, but with less severity compared to Group 2. There was an improvement in serum activities ($p < 0.05$) of CAT, and SOD enzymes with a reduction in MDA levels in chickens in groups 4,5,6 and 7 compared to Group 2 (Table 7) in 15 days PI. In addition to chicken infected with CIAV, there was a significant decrease ($p < 0.05$) in phagocytic percentage and phagocytic index compared with the normal control. Groups 3 and 4 indicated similar results, but with less severity ($p < 0.05$) compared to Group 2, three days PI (Table 6). Moreover, there was an improvement in phagocytic percentage and phagocytic index in groups 4,5, 6, and 7 compared to Group 2, 15 days PI (Table 7).

Evaluation of DNA damage by comet assay

Chickens infected with CIAV demonstrated significant DNA damage, characterized by a greatly reduced nuclear core and a large cloud of DNA fragments migrating away from the core, forming a characteristic comet tail ($p < 0.05$) when compared to Group 1. Groups 3 and 4 exhibited similar DNA damage, but it was less severe ($p < 0.05$) compared to Group 2, three days PI (Table 8). Furthermore, at 15 days PI, chickens in groups 4,5, 6, and 7displayed only a mild to moderate degree of DNA damage ($p < 0.05$) compared to the severely affected Group 2 (Table 9). This indicated that the applied treatments significantly mitigated CIAV-induced genotoxicity.

Examination of bone marrow

Control chickens (Group 1) demonstrated no lesions and normal bone marrow at 3 and 15 days PI (Figure 1). Meanwhile, CIAV-infected chickens (Group 2) showed severe depletion of bone marrow cells and replacement of erythrocytes by adipose tissue 3 and 15 days PI (Figure 2). Moreover, Group 3 indicated a depletion of bone marrow in 3 and 15 days PI (Figure 3). Group 4 illustrated moderate depletion of bone marrow three days PI (Figure 4), Group 5 demonstrated severe depletion of bone marrow and replacement of erythrocytes by adipose tissue 15 days PI (Figure 5). Group 6 illustrated mild depletion of bone marrow 15 days PI (Figure 6). Additionally, Group 7 indicated a slight depletion of bone marrow 15 days after PI (Figure 7).

Table 2. Erythrogram of 15-day-old broiler chickens of both sexes in groups 1-4 at three days post-infection

Parameters / Groups	RBC Count ($x10^6/\mu l$)	Hb (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (%)
Group 1 (C)	$3.21^{a} \pm 0.02$	$10.29^{a} \pm 0.03$	$33.43^{a} \pm 0.41$	$104.12^{a} \pm 0.75$	$32.05^{ab} \pm 0.23$	$30.78^{a} \pm 0.28$
Group 2 (V)	$2.56^{c} \pm 0.09$	$8.26^{d} \pm 0.17$	$27.0^{d} \pm 0.57$	$105.30^{a} \pm 1.83$	$32.24^{a} \pm 0.48$	$30.59^{a} \pm 0.11$
Group 3 (AV)	$3.03^{b} \pm 0.01$	$9.60^{b} \pm 0.05$	$31.03^{b} \pm 0.45$	$102.38^{a} \pm 0.75$	$31.65^{ab} \pm 0.26$	$30.88^{a} \pm 0.26$
Group 4 (NVA)	$2.86^{b} \pm 0.04$	$8.84^{c} \pm 0.02$	$29.22^{c} \pm 0.45$	$101.92^{a} \pm 0.88$	$30.85^{b} \pm 0.39$	$30.27^{a} \pm 0.41$

Group 1 (C): Negative control, Group 2 (V): Chicken infectious anemia virus infected group, Group 3 (AV): Chicken pretreated with acyclovir then infected with Chicken infectious anemia virus, Group 4 (NVA): Pretreated with neem then infected with chicken infectious anemia virus. RBC: Red blood cells, Hb: Hemoglobin, PCV: Packed cell volume, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration. ^{a,b,c,d} Means within the same column carrying different superscript letters are significantly different ($p \leq 0.05$).

Table 3. Erythrogram of 30-day-old broiler chickens of both sexes in all groups at 15 days post-infection

Parameters / Groups	RBC Count ($x10^6/\mu l$)	Hb (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (%)
Group 1 (C)	$3.32^{a} \pm 0.03$	$10.63^{a} \pm 0.07$	$34.50^{a} \pm 0.09$	$103.73^{a} \pm 1.34$	$31.96^{ab} \pm 0.16$	$30.82^{a} \pm 0.26$
Group 2 (V)	$2.60^{e} \pm 0.01$	$8.26^{e} \pm 0.23$	$27.03^{e} \pm 0.27$	$103.17^{a} \pm 0.62$	$31.55^{b} \pm 1.01$	$30.59^{a} \pm 1.09$
Group 3 (AV)	$3.10^{b} \pm 0.02$	$10.16^{b} \pm 0.08$	$32.16^{b} \pm 0.12$	$103.77^{a} \pm 1.11$	$32.79^{ab} \pm 0.06$	$31.60^{a} \pm 0.30$
Group 4 (NVA)	$3.00^{bc} \pm 0.02$	$9.95^{b} \pm 0.02$	$31.23^{c} \pm 0.14$	$104.00^{a} \pm 0.86$	$33.12^{a} \pm 0.22$	$31.85^{a} \pm 0.14$
Group 5 (NVH)	$2.79^{d} \pm 0.02$	$8.94^{d} \pm 0.02$	$29.50^{d} \pm 0.33$	$105.47^{a} \pm 0.71$	$31.91^{ab} \pm 0.19$	$30.30^{a} \pm 0.32$
Group 6 (NVAH)	$3.26^{a} \pm 0.04$	$10.56^{a} \pm 0.07$	$34.00^{a} \pm 0.10$	$104.10^{a} \pm 1.08$	$32.35^{ab} \pm 0.21$	$31.07^{a} \pm 0.11$
Group 7 (VA)	$2.91^{c} \pm 0.04$	$9.41^{c} \pm 0.08$	$30.56^{c} \pm 0.38$	$105.17^{a} \pm 0.61$	$32.28^{ab} \pm 0.32$	$30.81^{a} \pm 0.26$

Group1 (C): Negative control, Group 2 (V): Chicken infectious anemia virus infected group, Group 3 (AV): Chicken pretreated with acyclovir then infected with Chicken infectious anemia virus, Group 4 (NVA): Pretreated with neem then infected with chicken infectious anemia virus then treated with acyclovir, Group 5 (NVH): Pretreated with neem then infected with chicken infectious anemia virus then treated with hemocare, Group 6 (NVAH): Pretreated with neem then infected with chicken infectious anemia virus then treated with acyclovir and hemocare, and Group7 (VA): Infected with chicken infectious anemia virus then treated with acyclovir. RBC: Red blood cells, Hb: Hemoglobin, PCV: Packed cell volume, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration ^{a,b,c,d} Means within the same column carrying different superscript letters are significantly different ($p \leq 0.05$).

Table 4. Leukogram of 15-day-old broiler chickens of both sexes in groups 1-4 at three days post-infection

Parameters / Groups	TLC ($x10^3$/µl)	Differential leukocytic count					
		Lymphocytes ($x10^3$/µl)	Heterophile ($x10^3$/µl)	Monocytes ($x10^3$/µl)	Eosinophile ($x10^3$/µl)	Basophile ($x10^3$/µl)	Platelets ($x10^3$/µl)
Group 1 (C)	$25.3^{a} \pm 0.17$	$14.5^{a} \pm 0.05$	$7.30^{a} \pm 0.05$	$2.40^{a} \pm 0.05$	$0.85^{a} \pm 0.07$	0.25 ± 0.00	$46^{a} \pm 0.57$
Group 2 (V)	$14.62^{d} \pm 0.08$	$8.53^{d} \pm 0.20$	$4.16^{d} \pm 0.08$	$1.70^{c} \pm 0.05$	$0.23^{b} \pm 0.03$	-	$25^{d} \pm 1.73$
Group 3 (AV)	$20.92^{b} \pm 0.11$	$11.66^{b} \pm 0.17$	$6.80^{b} \pm 0.11$	$1.9^{b} \pm 0.05$	$0.35^{b} \pm 0.07$	0.20 ± 0.00	$40^{b} \pm 0.88$
Group 4 (NVA)	$17.77^{c} \pm 0.13$	$10.41^{c} \pm 0.21$	$5.32^{c} \pm 0.07$	$1.77^{bc} \pm 0.07$	$0.19^{b} \pm 0.05$	0.08 ± 0.00	$37^{c} \pm 0.45$

Group 1 (C): Negative control, Group 2 (V): Chicken infectious anemia virus infected group, Group 3 (AV): Chicken pretreated with acyclovir then infected with chicken infectious anemia virus, Group 4 (NVA): Pretreated with neem then infected with chicken infectious anemia virus. TLC: Total leukocyte count. ^{a,b,c,d} Means within the same column carrying different superscript letters are significantly different ($p \leq 0.05$).

Table 5. Leukogram of 30-day-old broiler chickens of both sexes in all groups at 15 days post-infection

Parameters / Groups	TLC ($x10^3$/µl)	Differential leukocytic count					
		Lymphocytes ($x10^3$/µl)	Heterophile ($x10^3$/µl)	Monocytes ($x10^3$/µl)	Eosinophile ($x10^3$/µl)	Basophile ($x10^3$/µl)	Platelets ($x10^3$/µl)
Group 1 (C)	$27.78^{a} \pm 0.14$	$15.81^{ab} \pm 0.10$	$8.31^{a} \pm 0.07$	$2.20^{a} \pm 0.11$	$1.06^{a} \pm 0.11$	$0.40^{a} \pm 0.16$	$46.66^{a} \pm 0.61$
Group 2 (V)	$13.61^{f} \pm 0.08$	$7.60^{f} \pm 0.10$	$4.57^{c} \pm 0.04$	$1.08^{d} \pm 0.04$	$0.36^{c} \pm 0.20$	-	$24.70^{e} \pm 1.04$
Group 3 (AV)	$27.0^{b} \pm 0.11$	$15.56^{bc} \pm 0.23$	$8.20^{a} \pm 0.11$	$2.10^{ab} \pm 0.05$	$0.96^{b} \pm 0.15$	$0.18^{a} \pm 0.00$	$43.6^{bc} \pm 0.34$
Group 4 (NVA)	$26.09^{c} \pm 0.11$	$15.30^{cd} \pm 0.05$	$8.23^{a} \pm 0.13$	$2.0^{ab} \pm 0.05$	$0.56^{bc} \pm 0.41$	-	$45^{ab} \pm 0.11$
Group 5 (NVH)	$24.74^{e} \pm 0.08$	$14.30^{e} \pm 0.08$	$7.48^{b} \pm 0.07$	$1.86^{b} \pm 0.04$	$0.80^{ab} \pm 0.06$	$0.30^{a} \pm 0.05$	$41.23^{ab} \pm 0.52$
Group 6 (NVAH)	$27.72^{a} \pm 0.15$	$16.20^{a} \pm 0.13$	$8.11^{a} \pm 0.06$	$2.20^{a} \pm 0.05$	$1.0^{a} \pm 0.1$	$0.21^{a} \pm 0.06$	$46.40^{a} \pm 0.30$
Group 7 (VA)	$25.80^{d} \pm 0.34$	$15.10^{d} \pm 0.20$	$8.20^{a} \pm 0.05$	$1.70^{c} \pm 0.15$	$0.80^{ab} \pm 0.10$	-	$25.80^{d} \pm 0.34$

Group1 (C): Negative control, Group2 (V): Chicken infectious anemia virus infected group, Group 3 (AV): Chicken pretreated with acyclovir then infected with chicken infectious anemia virus, Group 4 (NVA): Pretreated with neem then infected with chicken infectious anemia virus then treated with acyclovir, Group 5 (NVH): Pretreated with Neem then infected with Chicken infectious anemia virus then treated with hemocare, Group 6 (NVAH): Pretreated with neem then infected with chicken infectious anemia virus then treated with acyclovir and hemocare, and Group7 (VA): Infected with chicken infectious anemia virus then treated with acyclovir, TLC: Total leukocyte count. ^{a,b,c,d,e,f} Means within the same column carrying different superscript letters are significantly different ($p \leq 0.05$).

Table 6. Evaluation of serum catalase, superoxide dismutase, malondialdehyde, and phagocytic assay of 15-day-old broiler chickens of both sexes in groups 1-4 at three days post-infection

Parameters / Groups	CAT (nmol/l)	MDA (nmol/l)	SOD (U/ML)	Phagocytic (%)	Phagocytic Index
Group 1 (C)	$11.55^{a} \pm 0.71$	$0.62^{d} \pm 0.02$	$226.66^{a} \pm 12.03$	$63.66^{a} \pm 0.88$	$3.66^{a} \pm 0.04$
Group 2 (V)	$3.45^{c} \pm 0.12$	$7.64^{a} \pm 0.44$	$84.25^{c} \pm 4.04$	$41.66^{d} \pm 0.88$	$1.95^{d} \pm 0.02$
Group 3 (AV)	$8.95^{b} \pm 0.07$	$1.4^{c} \pm 0.11$	$188^{b} \pm 4.04$	$56.33^{b} \pm 0.88$	$2.81^{b} \pm 0.04$
Group 4 (NVA)	$7.83^{b} \pm 0.09$	$5.62^{b} \pm 0.01$	$99^{c} \pm 0.57$	$52.00^{c} \pm 1.15$	$2.56^{c} \pm 0.04$

Group 1 (C): Negative control, Group 2 (V): Chicken infectious anemia virus infected group, Group 3 (AV): Chicken pretreated with acyclovir then infected with chicken infectious anemia virus, Group 4 (NVA): Pretreated with neem then infected with chicken infectious anemia virus. CAT: Catalase enzyme, SOD: Superoxide dismutase and MDA: Malondialdehyde. ^{a,b,c,d} Means within the same column carrying different superscript letters are significantly different ($p \leq 0.05$).

Table 7. Evaluation of serum catalase, superoxide dismutase, and malondialdehyde, and phagocytic assay of 30-day-old broiler chickens of both sexes in all groups at 15 days post-infection

Parameters / Groups	CAT (nmol/l)	MDA (nmol/l)	SOD (U/ML)	Phagocytic (%)	Phagocytic Index
Group 1 (C)	$12.11^{a} \pm 0.36$	$0.65^{d} \pm 0.02$	$234.96^{a} \pm 3.96$	$67.33^{a} \pm 2.64$	$3.87^{a} \pm 0.04$
Group 2 (V)	$4.80^{c} \pm 0.07$	$7.38^{a} \pm 0.27$	$55.07^{d} \pm 3.95$	$45.66^{d} \pm 2.88$	$2.50^{e} \pm 0.11$
Group 3 (AV)	$12^{a} \pm 0.23$	$0.73^{d} \pm 0.02$	$232^{a} \pm 1.15$	$65.00^{ab} \pm 2.88$	$3.80^{a} \pm 0.11$
Group 4 (NVA)	$11.50^{a} \pm 0.28$	$0.78^{d} \pm 0.02$	$230^{a} \pm 1.73$	$63.16^{abc} \pm 0.16$	$3.79^{a} \pm 0.05$
Group 5 (NVH)	$9.88^{b} \pm 0.06$	$3.68^{b} \pm 0.05$	$169.33^{c} \pm 2.96$	$58.00^{c} \pm 0.57$	$2.87^{c} \pm 0.05$
Group 6 (NVAH)	$12.1^{a} \pm 0.45$	$0.70^{d} \pm 0.01$	$234.46^{a} \pm 0.55$	$66.60^{a} \pm 1.73$	$3.84^{a} \pm 0.11$
Group 7 (VA)	$10.48^{b} \pm 0.06$	$1.40^{c} \pm 0.03$	$210^{b} \pm 2.88$	$59.66^{bc} \pm 0.88$	$3.19^{b} \pm 0.05$

Group1 (C): Negative control, Group2 (V): Chicken infectious anemia virus infected group, Group 3 (AV): Chicken pretreated with acyclovir then infected with chicken infectious anemia virus, Group 4 (NVA): Pretreated with neem then infected with chicken infectious anemia virus then treated with acyclovir, Group 5 (NVH): Pretreated with neem then infected with chicken infectious anemia virus then treated with hemocare, Group 6 (NVAH): Pretreated with neem then infected with chicken infectious anemia virus then treated with acyclovir and hemocare, and Group7 (VA): Infected with Chicken infectious anemia virus then treated with acyclovir, CAT: Catalase enzyme, SOD: Superoxide dismutase and MDA: Malondialdehyde. ^{a,b,c,d,e} Means within the same column carrying different superscript letters are significantly different ($p \leq 0.05$).

Table 8. The DNA damage indices of 15-day-old broiler chickens of both sexes in groups 1-4 at three days post-infection

Parameters / Groups	Percentage of tailed	Tail length (PX)	Percentage of DNA in the tail	Tail moment	Olive tail Moment
Group 1 (C)	$9.30^{c} \pm 0.11$	$5.70^{b} \pm 0.65$	$8.07^{d} \pm 0.31$	$0.71^{b} \pm 0.08$	$1.32^{b} \pm 0.03$
Group 2 (V)	$14.76^{a} \pm 0.14$	$9.07^{a} \pm 1.81$	$22.40^{a} \pm 0.18$	$2.35^{a} \pm 0.39$	$2.91^{a} \pm 0.21$
Group 3 (AV)	$7.73^{d} \pm 0.14$	$5.49^{b} \pm 0.26$	$9.81^{c} \pm 0.15$	$0.77^{b} \pm 0.05$	$1.38^{b} \pm 0.05$
Group 4 (NVA)	$11.76^{b} \pm 0.14$	$11.35^{a} \pm 0.34$	$14.47^{b} \pm 0.66$	$1.81^{a} \pm 0.08$	$2.30^{a} \pm 0.08$

Group 1 (C): Negative control, Group2 (V): Chicken infectious anemia virus infected group, Group 3 (AV): Chicken pretreated with acyclovir then infected with chicken infectious anemia virus, Group 4 (NV): Pretreated with neem then infected with chicken infectious anemia virus. ^{a,b,c,d} Means within the same column carrying different superscript letters are significantly different ($p \leq 0.05$).

Table 9. The DNA damage indices (comet assay) of 30-day-old broiler chickens of both sexes in all groups at 15 days post-infection

Parameters / Groups	Percentage of tailed	Tail length (PX)	Percentage of DNA in the tail	Tail moment	Olive tail Moment
Group 1 (C)	$13.10^{c} \pm 0.45$	$7.01^{a} \pm 1.37$	$8.38^{bc} \pm 0.16$	$0.658^{ab} \pm 0.15$	$1.18^{b} \pm 0.06$
Group 2 (V)	$20.63^{a} \pm 0.31$	$5.88^{a} \pm 0.29$	$13.68^{a} \pm 0.38$	$0.794^{a} \pm 0.03$	$1.74^{ab} \pm 0.02$
Group 3 (AV)	$9.20^{d} \pm 0.15$	$6.45^{a} \pm 1.17$	$7.94^{c} \pm 0.65$	$0.433^{bc} \pm 0.04$	$0.98^{b} \pm 0.05$
Group 4 (NVA)	$15.13^{b} \pm 0.20$	$5.06^{a} \pm 0.20$	$9.28^{bc} \pm 1.23$	$0.390^{c} \pm 0.05$	$1.23^{b} \pm 0.14$
Group 5 (NVH)	$15.73^{b} \pm 0.15$	$7.48^{a} \pm 0.74$	$8.88^{bc} \pm 0.84$	$0.682^{ab} \pm 0.05$	$1.35^{b} \pm 0.13$
Group 6 (NVAH)	$12.70^{c} \pm 0.15$	$6.07^{a} \pm 0.20$	$10.76^{b} \pm 1.51$	$0.595^{abc} \pm 0.07$	$1.27^{b} \pm 0.17$
Group 7 (VA)	$15.03^{b} \pm 0.12$	$5.95^{a} \pm 0.73$	$10.35^{bc} \pm 0.14$	$0.602^{abc} \pm 0.07$	$1.19^{b} \pm 0.11$

Group1 (C): Negative control, Group2 (V): Chicken infectious anemia virus infected group, Group 3 (AV): Chicken pretreated with acyclovir then infected with chicken infectious anemia virus, Group 4 (NVA): Pretreated with neem then infected with chicken infectious anemia virus then treated with acyclovir, Group 5 (NVH): Pretreated with neem then infected with chicken infectious anemia virus then treated with hemocare, Group 6 (NVAH): Pretreated with neem then infected with chicken infectious anemia virus then treated with acyclovir and hemocare, and Group7 (VA): Infected with chicken infectious anemia virus then treated with acyclovir. ^{a,b,c,d} Means within the same column carrying different superscript letters are significantly different ($p \leq 0.05$).

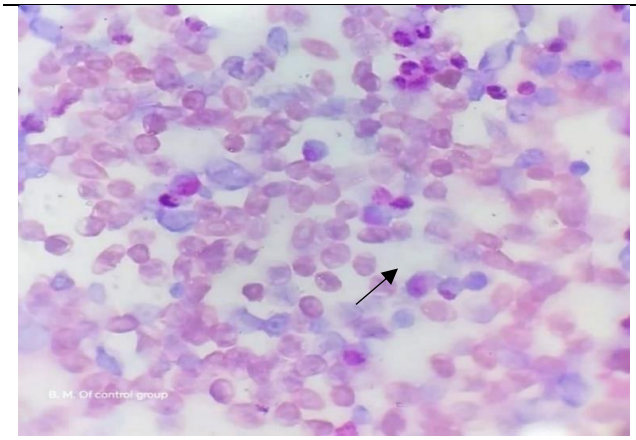

Figure 1. Bone marrow of control broiler chickens (Group 1). No lesions and normal bone marrow were observed in 3 and 15 days post-infection. Source: Authors of the present study.

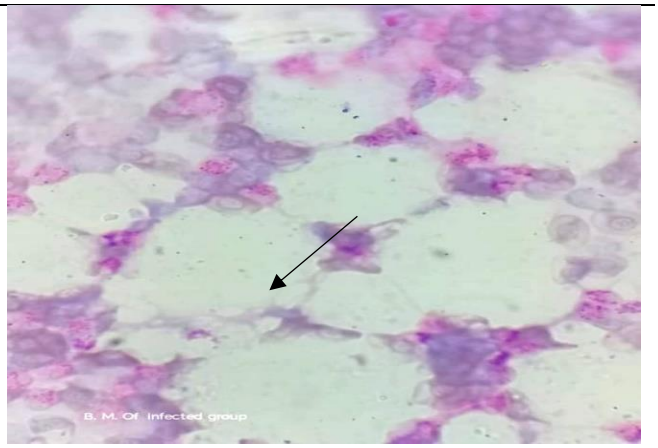

Figure 2. Bone marrow of broiler chickens infected with chicken infectious anemia virus (Group 2). Severe depletion of bone marrow cells and replacement of erythrocytes by adipose tissue occurred in 3 and 15 days post-infection. Source: Authors of the present study.

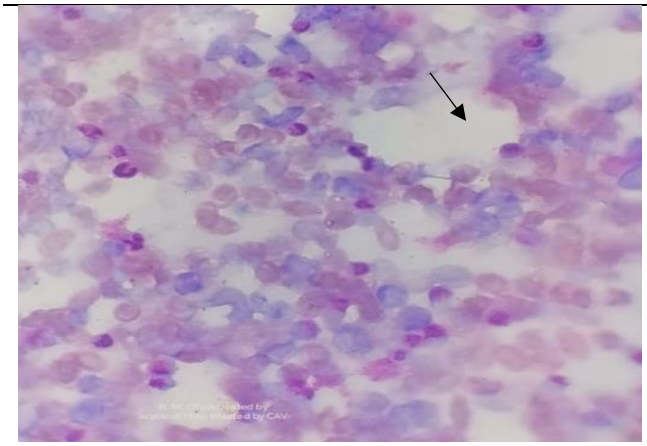

Figure 3. Bone marrow of broiler chickens infected with chicken infectious anemia virus and pretreated with acyclovir (Group 3). Few depletions of bone marrow were observed at 3 and 15 days post-infection. Source: Authors of the present study.

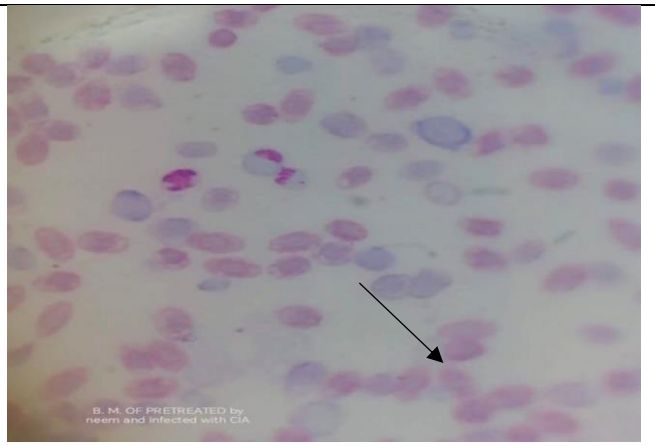

Figure 4. Bone marrow of 15-day-old broiler chicken infected with chicken infectious anemia virus and pretreated with neem (Group 4). Moderate depletion of bone marrow was observed three days post-infection. Source: Authors of the present study.

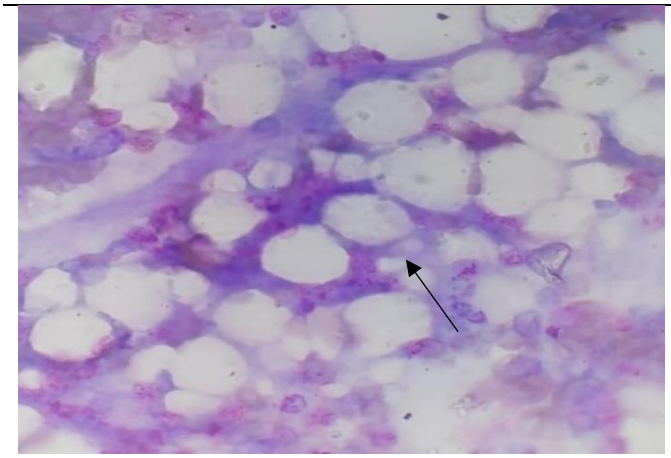

Figure 5. Bone marrow of 30-day-old broiler chickens infected with chicken infectious anemia virus and pretreated with neem and treated with hemocare (Group 5). Severe depletion of bone marrow and replacement of erythrocytes by adipose tissue were observed 15 days post-infection. Source: Authors of the present study.

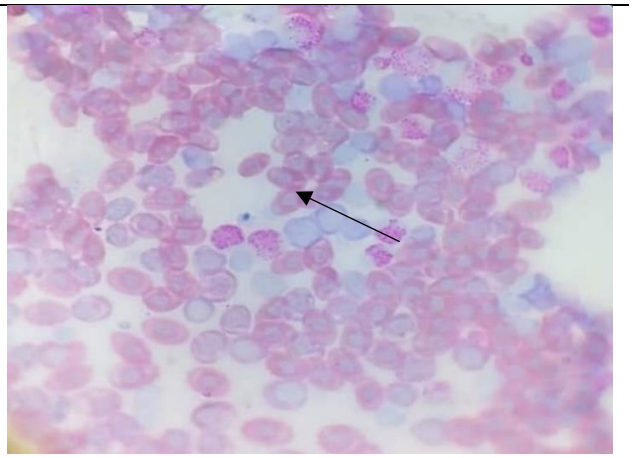

Figure 6. Bone marrow of 30-day-old broiler chickens infected with chicken infectious anemia virus, and pretreated with neem and treated with acyclovir and hemocare (Group 6). Mild depletion of bone marrow was observed 15 days post-infection. Source: Authors of the present study.

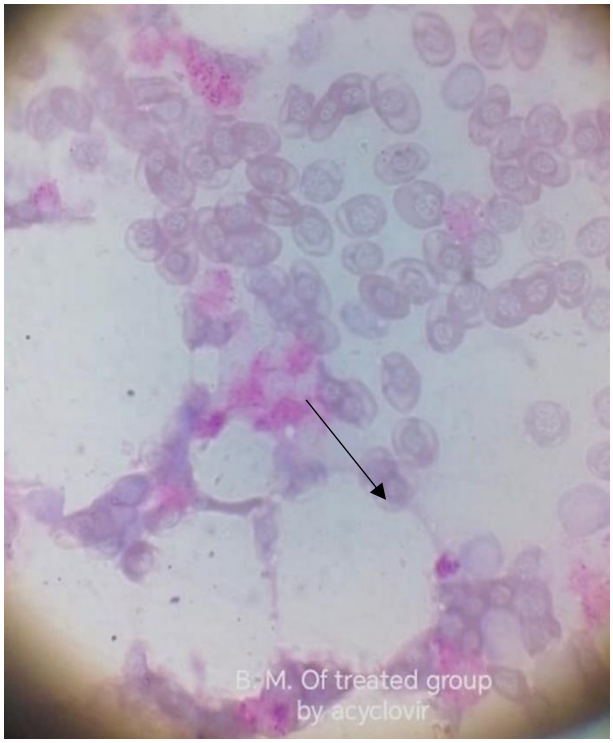

Figure 7. Bone marrow of 30-day-old broiler chickens infected with chicken infectious anemia virus and post-treated with acyclovir (Group 7). Slight depletion of bone marrow was observed 15 days post-infection. Source: Authors of the present study.

DISCUSSION

Chicken infectious anemia virus is a potent immunosuppressive agent that, along with other infectious agents, causes significant economic losses to the poultry industry worldwide (Andrabi et al., 2022).

The CIAV-infected chickens (Group 2) demonstrated a drooping aspect, lethargic appearance, pale combs and wattles, weakness, anemia, depression, ruffled feathers, stunted and growth retardation with high mortalities (13.3%). These clinical signs and mortality might be due to severe anemia and the immunosuppression effects of the CIAV that may lead to secondary bacterial infections (Von Bulow and Schat, 1997; Wang et al., 2025). The observed disappearance of severe clinical signs in Group 3 and their reduction or improvement in Group 7 can likely be attributed to the prophylactic and therapeutic administration of acyclovir. Acyclovir's mechanism of action involves its conversion to a monophosphate form by viral thymidine kinase. This active metabolite then acts as a potent inhibitor of viral DNA polymerase (Elion, 1982), effectively halting viral DNA replication and consequently reducing the pathological effects of the infection (Brigden and Whiteman, 1983). Clinical signs improved in groups 4, 5, and 6 pre- and post-treatment with neem liquid extract and/or acyclovir and hemocare, suggesting a multifaceted therapeutic effect. This improvement may be due to neem's antiviral effects reducing CIAV shedding or its virucidal activity, while acyclovir interferes with the virus replication and inactivation (Badam et al., 1999) or may block CIAV entry into the cell (Yerima et al., 2012). Neem is rich in triterpenoids and glycosides, which are responsible for antiviral potency (Hegazy et al., 2023), and the combined use of neem as a dietary additive has a better role in modulating the adverse effects of CIAV, which agrees with the findings of Abdulkareem et al. (2023), Hegazy et al. (2023), and Hemdan et al. (2023).

Regarding the erythrogram, Group 2 indicated a decrease in RBC count, Hb concentration, and PCV, indicating normocytic normochromic anemia at both 3 and 15 days PI. This change could be attributed to the infection with CIAV, which inhibited the hematopoietic functions of the bone marrow by targeting erythroid and lymphoid progenitor cells in the bone marrow and thymus, respectively (Adair, 2000). The direct effects of CIAV on the bone marrow led to aplasia and destruction in the erythrocytic series of the bone marrow cells as CIAV caused suppression of differentiation and proliferation of hemopoietic precursor cells, which drastically affected erythropoiesis, leading to anemia (Dhama et al., 2008), which agreed with the findings of Hegazy et al. (2022). The erythrogram results were corroborated by examination of the marrow tissue, which indicated a notable depletion of all hematopoietic progenitor cells. These findings aligned with Haridy et al. (2012), who reported that subclinical infections with CAV typically lacked both anemia and bone marrow lesions. The observed hematopoietic depletion in the current infected groups was consistent with the well-established direct cytotoxicity of CAV to bone marrow hematopoietic precursor cells in young chickens. Broiler chickens of 30 days of age, which received a prophylactic dose of acyclovir, showed improvement in

hematological parameters in 3 and 15 days PI. This improvement might be due to the antiviral effects of acyclovir (Wong et al., 2010), which decreased the destructive impacts of CIAV on bone marrow. The enhancement of blood parameters, including RBCs, Hb, and PCV, in groups 4, 5, and 6 may be attributed to components of the neem extract, such as flavonoids and quercetin, known for their hematopoietic properties (Raja et al., 2011), which agreed with the findings of Iyare and Obaji (2014) and El-Bolkiny et al. (2022). Group 4 showed improvement in hematological parameters, specifically RBCs, Hb, and PCV, at both 3 and 15 days PI. This positive effect might stem from the influence of neem liquid extract on the synthesis of hemoglobin within the bone marrow. It is hypothesized that neem's action involves promoting the release of erythropoietic factors, such as erythropoietin, from hepatic cells, thereby stimulating erythropoiesis (Ansari et al., 2012) with the effects of acyclovir. The present findings of improvement in hematological parameters agreed with those of Latheef et al. (2013). These hematological results were supported by examining bone marrow, which illustrated mild depletion of the erythroid and lymphoid progenitor cells. Group 5 indicated improvements in its erythrogram, likely due to the combined effects of hemocare and neem liquid extract. Hemocare is known to contribute to HB synthesis (Kumari et al., 2017), and this action, in conjunction with neem's potential to boost HB production, likely facilitated the observed recovery. These findings aligned with the previous studies by Ma et al. (2012), Bhatt et al. (2013), and Vijayakumar et al. (2019). Furthermore, Group 6, which received a prophylactic dose of neem liquid extract along with acyclovir and hemocare, and Group 7, treated solely with acyclovir post-infection, both exhibited improved erythrograms 15 days post-infection. This collective evidence strongly suggested the effectiveness of the different treatments in combating the hematological impacts of CIAV in chickens.

The current findings of leukogram analysis, which illustrated leukopenia, lymphopenia, heteropenia, monocytopenia, eosinopenia, basopenia, and thrombocytopenia at 3 and 15 days PI, were aligned with those of Nadeem et al. (2020). This condition is attributed to apoptosis in thymic and hematopoietic precursor cells (Basaraddi et al., 2013), stemming from the cytolytic actions of CIAV, which adversely affects the granulocytic lineage of bone marrow cells (Taniguchi et al., 1982). Hematopoietic precursor cells (Haemocytoblasts) and thymic precursor cells (Lymphoblasts) in the bone marrow and thymus cortex are primary targets of CIAV (Adair, 2000), leading to disrupted myelopoiesis and resulting in a significant decrease in myeloid cells and thrombocytopenia (Eltahir et al., 2011).

The enhancement of leukogram and platelet counts in groups 4-6 may be linked to the extract's components, such as flavonoids, quercetin, triterpenoids, and glycosides, which exhibit immunomodulatory properties and enhance the macrophage response, thereby stimulating the lymphatic system (Ray et al., 1996; Hegazy et al., 2023). The current study found improved leukogram and platelet counts at both 3 and 15 days PI. This positive outcome suggested that acyclovir, either alone or in combination with neem liquid extract, effectively reduced viral DNA replication. By inhibiting viral proliferation, these treatments likely diminished the overall infection load and the associated damaging effects of CIAV, which were consistent with the findings reported by Latheef et al. (2013). Hemocare, with its crucial iron content, plays a vital role in various enzyme systems that regulate cellular activities, including those essential for immune function (Camaschella, 2015). Both the hematinic properties of hemocare and the immunomodulatory effects of neem appeared to have ameliorated the CIAV-induced immunosuppression by fostering an improvement in leukocyte cell lineages. The current findings were further supported by the results from the bone marrow examination and aligned with the recent study by Mank et al. (2024).

Oxidative stress was induced by excessive hydrogen peroxide, superoxide, and hydroxyl radicals or/by insufficient antioxidative resistance (Macdonald et al., 2003). Malondialdehyde is one of the final products of polyunsaturated fatty acids peroxidation in body cells, and an increase in free radicals causes overproduction of MDA, which is commonly known as an oxidative stress marker (Gawel et al., 2004). The CAT and SOD enzymes play critical roles in the protection of body cells from oxidative damage by ROS (Murthy et al., 1981).

The current findings of reduced CAT and SOD activities, alongside elevated MDA levels, were likely attributable to the detrimental effects of CIAV. The CIAV infection is known to induce immunosuppression and apoptosis, processes that contribute to an increase in free radical production and oxidative stress within tissues. This heightened oxidative burden subsequently leads to the exhaustion of cellular antioxidant defenses, as evidenced by the decreased CAT and SOD levels (Schwar, 1996; Neven et al., 2023). This downregulation of the CAT is due to the apoptin–induced immunosuppression and apoptosis of the hematopoietic and lymphopoietic tissue (Chen et al., 2011). Group 3 and Group 4 demonstrated an improvement in serum levels of CAT, SOD, and MDA. This beneficial effect can be attributed to the antioxidant properties of acyclovir and/or the polyphenol content of neem liquid extract. neem, rich in flavonoids and phenolic acids, along with other active plant ingredients, likely enhanced the synthesis of endogenous antioxidant molecules and exerted direct free radical scavenging effects. These actions collectively alleviated the oxidative stress induced by CIAV, as supported by previous studies of Dkhil et al. (2012), Goyal and Brahma (2014). The current findings were consistent with those reported by Olugbenga et al. (2018) and Isler et al. (2002). Moreover, the CIAV-infected group indicated a significant reduction in phagocytic percent and phagocytic index. The observed impairment in

phagocytic activity aligned with the findings of Mohamed et al. (2016). This reduction is likely a direct consequence of the immunosuppressive effects of CIAV. Specifically, CIAV is known to impair macrophage functions, including their crucial role in phagocytosis (Tan and Tannock, 2005). The present results corroborate these earlier findings. Group 3 and Group 4 showed improvement in phagocytic percentage and index in 3 and 15 days PI. This improvement might be due to acyclovir enhancing phagocytosis (Stenseth et al.,1993), and neem might stimulate macrophage, cell-mediated immunity, and humoral immunity as reported by Nariman et al. (2015). Group 5 indicated less improvement in phagocytic percentage and index in 3 and 15 days PI, which might be due to the positive effects of hematinic on cell-mediated immunity masked by the interference of CIAV infection (Berger et al., 2000). This minor enhancement resulted solely from the effects of neem, consistent with findings by Walter et al. (1986), who stated that iron supplementation does not influence phagocytosis nor directly affect circulating neutrophils. However, it is necessary for neutrophil development in the bone marrow. Groups 6 and 7 indicated an improvement in phagocytic percentage and index due to the effective combination of neem, acyclovir, and haemocare in improving phagocytosis and immunity of broiler chickens.

Comet assay is widely known as a sensitive technique for studying DNA damage and repair. It is also called single-cell gel electrophoresis, which is based on agarose-embedded DNA electrophoresis, and this method is adapted to determine DNA damage in isolated cells (Gane et al., 2014). The current findings indicated that infected broiler chickens with high DNA damage in Group 2 showed migration of chromosomal DNA from the nucleus toward the anode, which was similar to the shape of a comet. The CIAV induces oxidative stress that causes direct or indirect DNA damage, chromosomal abnormalities, increased mutation rates, and apoptosis (Cadet et al., 2017). The current result aligned with the findings of Basaraddi et al. (2013), which indicated that the thymus serves as a primary organ for viral replication. These findings are evidenced by nucleosome fragmentation, an apoptosis indicator that rose at 7 and 10 days PI with CIAV. These results may stem from either the thymocytes' refractoriness to CIAV infection or their depletion in the thymus. This type of apoptosis plays a significant role in the pathogenesis of CIAV and is linked to the VP3 protein apoptin.

The current findings in groups 3-7 illustrated a reduction in DNA damage caused by CIAV in 3 and 15 days PI. The possible explanation is attributed to gene-protective effects of acyclovir against DNA damage by stopping the CIAV virus replication (Taylor and Gerriets, 2023). Neem has gene-protective effects by acting as a free radical scavenger and antioxidant agent, as neem contains several potent antioxidants, including carotenes, terpenoids, limonoids, quercetin, and sitosterol, which decrease oxidative stress and DNA damage (Alabi et al., 2013). Moreover, hematinic medicines stimulate the body's immune state and reduce the damaging effects of any invasive agents (Cherayil, 2010). Additionally, Pawson and Mehta (1998) reported that a lack of hematinics leads to a deficiency of vitamin B12 and folate coenzymes, which are necessary for DNA synthesis, so hematinic medications help in DNA synthesis.

CONCLUSION

Using neem liquid extract alone with a dose of 50 ml/liter fresh drinking water or in combination with acyclovir with a dose of (10 mg/kg /IM) every 24 hours for 10 days and hemocare with a dose of 5 mL per 100 chickens in drinking water for two weeks helped to improve and decrease the damaging effects of CIAV infection in chickens. Additionally, prophylaxis using acyclovir alone was effective in the inhibition of CIAV infection. It is recommended that future research be conducted *in vitro*, *in vivo*, and in clinical studies to evaluate the effects of neem liquid extract, acyclovir, and hemocare on the treatment of CIAV.

DECLARATIONS

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Authors' contributions

Nasr A M Nasr El-Deen, Rasha T M Alam, Ibtisam Mohammed Gamal El-Din, and Noha Gamal Amine Sakr were responsible for conceptualization, methodology, investigation, data analysis, drafting, and revising the manuscript. Nasr A M Nasr El-Deen, Rasha Alam, Ibtisam MG El-Din, and Noha Gamal Amine Sakr contributed to conceptualization, supervision, resource provision, and critical manuscript review and editing. All authors have read and approved the final edition of the manuscript before publication in the present journal.

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Competing interests

The authors have not declared any conflict of interest.

Ethical considerations

The present study was originally written by the authors and has not been published elsewhere. The authors checked the text of the article for plagiarism index and confirmed that the text is written based on their original scientific results.

Availability of data and materials

The data to support this study finding is available upon reasonable request to the corresponding author.

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