**Egyptian Journal of Veterinary Sciences**

**https://ejvs.journals.ekb.eg/**

# Evaluation of the Effect of Neem Liquid Extract, Acyclovir and Haemocare against Chicken Infectious Anemia Virus Infection in Chickens

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**Abstract**

CHICKEN infectious anemia virus (CIAV) is a very important disease of chickens with worldwide distribution, it is accountable for the huge economic losses to farmers rearing chickens, as it affect all ages of chickens and the affected birds showed marked aplastic anemia, moderate to severe atrophy of the thymus, muscular hemorrhages and immunosuppression. The present work, investigated the effect of neem liquid extract, acyclovir and haemocare on the destructive effect of CIAV, through evaluation of the biochemical, inflammatory cytokine gene expression (IL-10) and histopathological examination. The current results showed elevation in the serum ALT, AST, ALP, creatinine, uric acid and in organic phosphorus in CIAV infected chickens. In addition to hypoproteinemia, hypoalbuminemia and hypoglobulinemia with reduction in serum calcium level and inflammatory cytokine (IL-10) gene expression. Histopathologically, CIAV infected chickens showed damaging effect in the liver, kidney and thymus tissues. While, the infected groups that pre and post treated with neem liquid extract alone and or in combination with acyclovir and haemocare showed an improvement in the altered biochemical parameters, inflammatory cytokine gene expression and histopathological findings of previous tissues. In conclusion, the present results proved the effective pre and post supplementation of neem liquid extract alone and or in combination with acyclovir and haemocare in modulating the adverse effect of CIAV infection in chickens, also pretreatment with acyclovir more effective than using it in treatment.

**Keywords:** CIAV, Neem liquid extract, Acyclovir, haemocare and biochemical studies.

## Introduction

Chicken infectious anemia virus CIAV is an infectious disease caused by a non-enveloped, icosahedral DNA virus which is one of the smallest known avian pathogen [1]. It belongs to genus Gyrovirus of family Circoviridae [2]. The transmission of CIAV occurs along both vertical and horizontal routes, and all ages are susceptible to it [3]. In field, the clinical disease generally occurs in chicks around two weeks of age without a maternal antibody against CIAV [4], or even in chickens with maternal antibody [5]. Chicken anemia virus causes an acute and immunosuppressive disease of young chickens, clinically characterized by anorexia, lethargy, depression, drooling of wings, ruffled feathers, pallor of comb and wattle, beak and mucous membranes, cutaneous, subcutaneous and intramuscular hemorrhages with high mortality [6]. Also, characterized by severe anemia, aplasia of bone marrow and generalized lymphoid atrophy [7]. Moreover, CIAV infection causes a severe defects in splenic T-lymphocyte functions and fall in interleukin production [8-10].

Neem (*Azadirachta indica*), is a member of the Meliaceae family, it has a therapeutic role in the treatment of various diseases based on the fact that neem has a complex of various constituents including nimbolinin, nimbin, nimbidin, nimbidol, salannin,

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(Received 19 March 2025, accepted 16 June 2025)
DOI: 10.21608/ejvs.2025.369766.2717

and quercetin, such types of ingredients play role in the diseases management through modulation of various genetic pathways and other activities [11]. It has a numerous biological and pharmacological activities, including antiviral, antibacterial, antifungal and anti-inflammatory [12, 13], also it has a hepatoprotective effect [14]. Leave extract of neem has shown virucidal activity via virus inactivation besides interfering at the early stage of its replication cycle [15].

Acyclovir is a synthetic analogue of a purine nucleoside that has antiviral action against some viruses, including varicella-zoster and herpes simplex. It is converted to its active form (acyclovir triphosphate) by virus thymidine kinase which specifically targets viral DNA that inhibit viral replication in the host [16]. Also, acyclovir even at concentrations up to 20 microgram/ml, did not depress cellular immune responses that are important for successful elimination of invading herpes group viruses [17]. Acyclovir is a better choice for treating viral diseases as it is available in drug stores and much less expensive than other antiviral drugs, moreover, it is available in both injectable and tablet forms, which offers more flexibility in treating animals that may not be trained to accept veterinary interventions [18].

## Material and Methods

*Experimental animals*

One day old chicks, from non-immunized hens were obtained from the Dakahlia Poultry Company. Chicks were raised in identical built-up litter poultry house with a source of heat to give a starting temperature of 34 ºC reduced gradually to a constant temperature of 24 (±2) ºC at the end of third week, continuous photoperiod was applied for 24 h., fed on a well-balanced commercial ration and water *ad-libitum*.

*Isolated agent, chemicals and reagents*

Chicken infectious anemia virus CIAV was kindly obtained from the Animal Health Research Institute, Dokki, Egypt. Challenged dose 1 ml of CIAV infected cell culture supernatant ($10^{4.5}$ TCID50 /0.1 ml) per bird intramuscularly [19] at12 day age. Neem liquid extract was obtained from Makin Co. Supplying Natural Materials for Industries of Food, Pharmacy & Cosmetics, externally imported from Spain.100% Natural & pure neem herbal liquid extract 1 liter. At dose of 50 ml/liter fresh drinking water [20]. Acyclovir was obtained from Sigma Chemical Company. At dose of 10 mg/kg every 24 hr for 10 days injected IM [21]. Haemocare was purchased from local pharmacy. At dose of 5 mL per 100 birds in drinking water for 2 weeks [22]. Biochemical commercial kits were purchased from Diamond Diagnostics, Egypt.

*Experimental design*

One hundred and five one day old, chicks were divided into 7 equal groups. Group 1, was kept as control. Group 2, was infected with CIAV (1 ml/IM/bird infected cell culture supernatant at 12 days age). Group 3, was given acyclovir (10 mg / kg/IM/day ) for 3 days before infection, then infected by CIAV by same dose as gp. (2). Group 4, was administrated neem liquid extract (50 ml/liter drinking water) from the 1st day to the end of the experiment, then infected with CIAV and treated at 15 day old with acyclovir for 2 weeks. Group 5, was administrated neem liquid extract then infected with CIAV and was post treated with haemocare (5 mL/100 birds in drinking water) from the 15 day age for 2 weeks. Group 6, was pre-treated with neem liquid extract, then infected with CIAV and post-treated with acyclovir and haemocare for 2 weeks as in gps. (3, 4 & 5). Group 7, was infected with CIAV at 12 day age and treated at 15 day old with acyclovir by the same dose and duration.

*Blood samples*

Blood samples were collected at 15 and 30 day from begin of the experiment, from the wing vein. 5 ml of blood was collected from each bird in dry clean centrifuge tube and left to clot, then centrifuged at 3000 r.p.m for 20 minutes, clear sera was separated carefully, and were kept at deep freezer for serum biochemical analysis and gene expression.

*Tissue samples*

After collection of blood samples chickens were sacrificed and tissue specimens from liver, kidney and thymus were collected and fixed in 10% neutral buffered formalin for histopathological examination.

*Estimation of serum hepatic function tests*

The serum activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined according to Reitman and Frankle [23]. The serum alkaline phosphatase activity (ALP) was determined according to Kind and King [24], while determination of serum total proteins (TP) and albumin (Alb) according to Grant et al. [25] and Drupt [26] respectively. The serum globulin (Glob) levels was calculated by subtracting the obtained albumin level from the obtained total proteins level according to Doumas [27].

*Estimation of renal function tests*

Determination of serum uric acid according to Fossati et al. [28] and determination of creatinine by photometric colorimetric test for kinetic measurement without deproteinization, according to Henry [29]. Also, determination of serum calcium

(Ca) and phosphorus (Ph) levels according to Tietz [30] and Daly et al. [31], respectively.

*Estimation of serum inflammatory cytokine gene expression (interleukin-10 (IL-10))*

Quantification of serum cytokines by real time PCR for quantifying of the expression levels of cytokines (IL-10), the DNA samples of the respective peripheral blood mononuclear cell (PBMCs) were subjected to real time PCR analysis using gene specific primers, and data analysis was performed by method of Livak and Schmittgen [32].

*Histopathological examination*

Liver, kidney and thymus were collected from all groups and fixed in 10 % neutral buffered formalin for 48 hours, then washed under running water. The washed specimens will be dehydrated by using increased graded concentrations of ethyl alcohol starting with 75% and ending with absolute alcohol. Samples will be cleared after immersing in xylol for 2hours. The cleared samples will be put in clear jar containing 50% paraffin and incubated for 2 hours at $37^0$C, thereafter the samples will placed in crucible containing soft paraffin and kept in an oven for 2 hours at $48^0$C. The samples will blocked in hard paraffin and cut in sections of about 5 micron thickness, stained with Hematoxylin and Eosin (H & E). The sections will mounted with Canada balsam and covered with cover slides to be ready for histopathological examination according to Bancroft [33].

*Statistical analysis*

The obtained data was statistically analyzed using "F" test one way ANOVA according to Tamhans and Dunlop [34] using "MSTAT-C" computer program. Means in the same column followed by different letters were statistically significant at $p \leq 0.05$ and the highest values were represented with the letter (a).

## Results

The liver tissue of the control showed normal tissue along different periods of the experiment (gp. 1, fig. 3. 1). In comparison, CIAV infected birds, the liver tissue showed vascular congestion, focal coagulative necrosis, fatty changes of hepatocytes, and endothelial hypertrophy 3 days PI, while showed necrotic foci replaced by mononuclear cells with cytoplasmic vacuolation of numerous hepatocytes 15 days PI, (gp. 2, fig. 3. 2& 3) respectively. Liver of infected groups pre and post treated with neem liquid extract alone or in combination with acyclovir and haemocare showed congestions of the central veins (gp. 3, fig. 3. 4) 3 &15 days PI. In addition, liver tissue showed vascular congestion, focal necrotic area replaced by mononuclear cells, and fatty change of numerous hepatocytes 3 days PI, while showed infiltration with mononuclear cells and numerous vacuolated hepatocytes 15 days PI, (gp. 4, fig. 3. 5& 6) respectively. Moreover, liver tissue of birds in gps. (5, 6 & 7) showed diffuse fatty change of hepatocytes (fig. 3. 7) also, showed numerous vacuolated hepatocytes, with presence of few hemosiderin pigment (fig. 3. 8) and vascular congestion (fig. 3. 9) 15 days PI respectively.

The kidney tissue of the control showed normal tissue along the different experimental periods (gp. 1, fig. 4. 10). In comparison, CIAV infected birds, the kidney tissue showed tubular necrosis and vascular congestion 3 days PI, and tubular necrosis, few regenerated tubular epithelia and minute interstitial haemorrhage 15 days PI, (gp. 2, fig. 4. 11& 12), respectively. While the kidney of infected groups pre and post treated with neem liquid extract alone or in combination with acyclovir and haemocare showed nearly normal histological structure (gp. 3, fig. 4. 13) 3 & 15 days PI. In addition, showed tubular necrosis with desquamation of the necrotic cells to the tubular lumens, and vascular congestion 3 days PI, while showed tubular necrosis and few regenerated tubular epithelia with minute interstitial haemorrhage 15 days PI, (gp. 4, fig. 4. 14& 15). Moreover, the kidney tissue of birds in gps. (5, 6& 7) showed vascular congestion and tubular necrosis (Figs. 4. 16 &17) respectively and tubular necrosis with desquamation of the necrotic cells to the tubular lumens besides interstitial minute haemorrhages (fig. 4. 18) 15 days PI respectively.

The thymus tissue of the control showed normal tissue along different experimental periods (gp. 1, fig. 5. 19). In comparison, in CIAV infected birds, the thymus tissue showed cortical lymphoid depletion together with proliferation of the reticuloendothelial cells 3 days PI, while showed cortical lymphoid necrosis together with cortical haemorrhage 15 days PI, (gp. 2, fig. 5. 20& 21) respectively. Thymus tissue of infected groups pre and post treated with neem liquid extract alone or in combination with acyclovir and haemocare showed vascular congestion (gp. 3, fig. 5. 22) 3 & 15 days PI. In addition, lymphoid depletion and necrosis with increased connective tissue elements 3 days PI, while showed vascular congestion 15 days PI, (gp. 4, fig. 5. 23 & 24) respectively. Moreover, the thymus tissue of birds in gps. (5, 6& 7) showed marked lymphoid depletion (fig. 4. 25), with vascular congestion (fig. 4. 26) and lymphoid depletion together with vascular congestion (fig. 4. 27) 15 days PI, respectively.

## Discussion

Viral infections that impair the immune system have a huge financial impact on the poultry industry, chicken infectious anemia virus (CIAV) is one of the

most common immunosuppressive diseases in poultry, also CIAV has emerged as a major poultry virus, causing serious financial harm to the global poultry industry, furthermore, earlier research has revealed that CIAV is widely distributed in Egypt's commercial poultry sectors [35].

The treatment and immunomodulatory strategies with haematinics and immunostimulants for checking clinical pathology and improving the immunity in birds have been suggested for its control [36].

Neem liquid extract is a potent antiviral and has immunostimulant properties during the production cycle of broilers when used in combination with CIAV vaccination [37].

In this study the hepatorenal protective effect of neem liquid extract pre-post supplementation alone and or in combination with acyclovir and haemocare on CIAV infected birds was evaluated.

The current results revealed significant elevation in serum activities of ALT, AST and ALP, with a significant reduction in the serum total protein, albumin and globulin 3 and 15 days PI in CIAV infected birds, the increase in liver enzyme may be due to CIAV infection induced degeneration and necrosis in hepatocyte, so increase permeability of cell and release the hepatic enzymes into circulation [38, 39], also hypoproteinemia may be due to the decrease in feed intake by infected chicks and /or decreased synthesis by damaged hepatocyte, also it may be due to increased protein loss by damaged kidney. The current result agree with Hegazy et al. [40] who found that bird infected with CIAV showed hypoproteinemia, hypoalbuminemia, hypoglobulinemia and a decrease in the A/G ratio. Also, [19] and [41] reported that chicks exposed to CIAV showed an increase in the activities of ALT, AST and ALP enzymes with hypoproteinemia and hypoalbuminemia. The present result supported by histopathological findings as liver tissue showed vascular congestion, focal coagulative necrosis, fatty change of hepatocytes and endothelial hypertrophy.

The present results demonstrated that neem liquid extract pre and post treatment alone and or in combination with acyclovir and haemocare in CIAV infected birds significantly ameliorated the altered hepatic parameters by decreasing the elevation in ALT, AST and ALP, also increasing the reduction in total protein, albumin and globulin. This improvement may be related to the ability of acyclovir in reducing viral DNA replication so decreases infection and damaging effect of the virus [42], also the immunomodulatory, hepatoprotective and hepatostimulatory effect of neem liquid extract, it has antioxidant properties and the ability to normalize the impaired membrane function, the active constituent of neem liquid extract plays a key role in the reduction of hepatic necrosis and preserving the structural integrity of the hepatocellular structures [14, 43, 44]. These results were confirmed by the improvements in the histopathological examination of liver tissue.

In addition, CIAV infection lead to elevation in the serum creatinine, uric acid and phosphorus levels with reduction in serum calcium level 3 and 15 days PI, these changes may be attributed to the renal damage and muscular injuries caused by CIAV and decrease renal blood flow so decrease glomerular filtration rate leading to the increase in serum creatinine levels and uric acid with alterations in phosphorus and calcium levels [45], and these results confirmed by histopathological findings of kidney tissue which showed tubular necrosis and vascular congestion. The present result agree with [7, 36, 41 and 46] who reported that chicks infected with CAV showed significant decrease in serum calcium level with a significant increase in serum creatinine, uric acid and inorganic phosphorus levels.

Neem liquid extract pre and post treatment alone and or in combination with acyclovir and haemocare significantly improved altered renal parameters, due to virostatic effect of acyclovir against DNA and RNA viruses [47]. This effect protects renal cell from damage, also neem liquid extract decreases damaging effect of virus with acyclovir and enhances renal function as well as enhances effective utilization of proteins in the diets [48-50], and moreover, neem has a nephroprotective effects [51]. These results partially agree with [52] who reported that neem leaves enhanced renal function in White New Zealand rabbits infected with *E. coli*. These results confirmed with histopathological study of kidney.

Inflammatory cytokine Interleukin (IL)-10 is one of the anti-inflammatory cytokines [53] which is important in controlling viral immunity. It acts as an immunoregulator, inhibiting proinflammatory responses from innate and adaptive immunity and preventing tissue damage due to exacerbated adaptive immune response. However, viruses have evolved mechanisms that exploit the immunoregulatory function of (IL)-10 for immune evasion, suppression and tolerance promoting their own survival, also, (IL)-10 can be produced by all immune cells, and it can also modulate the function of these cells [54].

Chickens infected with CIAV (gp. 2) revealed a significant reduction of mRNA gene expression of IL [10], 3 and 15 days PI, this due to impairment of cytotoxic T lymphocyte development through destruction of lymphoid precursors by the virus, as T lymphocytes are a major target of CIAV with effect on the downstream adaptive immunity [55, 56].

Current results were confirmed by histopathological findings of thymus tissues which showed cortical lymphoid depletion together with proliferation of the reticuloendothelial cells 3 days PI, and showed cortical lymphoid necrosis together with cortical hemorrhage 15 days PI. These results agree with [8] and [10] who found that natural exposure of 3-week-old chickens to CIAV resulted in impaired functional activity of interleukins. The improvement of mRNA gene expression of IL 10 in CIAV infected groups pre and post treated with neem liquid extract alone and or in combination could be related to rich of azadiractoids, a highly active liminoid terpenoids, which possesses anti-inflammatory properties [57]. Also, may be due to, the ability of neem liquid extract to increase the activity of natural killer cells, which are potent cytotoxic cells particularly against virus-infected cells [58)]. Our results confirmed by histopathological findings of thymus tissues. These changes agree with [59] who showed an increase of anti-inflammatory cytokines (IL-10) in neem leaf extract in dextran sodium sulfate induced colitis in rats.

Moreover, the improvement may be due to the effect of acyclovir alone or in combination with neem liquid extract in reducing viral load, so decreasing the infection and damaging effect of the virus on lymphoid precursos (60 & 48). Also, haemocare able to maintain immune system, as normal development and function of T- cells depend on an adequate supply of iron, zinc and copper [61].

## Conclusion

Infection with CIAV produced hepatorenal damage with alteration in inflammatory cytokines. Neem liquid extract demonstrated a protective effects by improving the altered examined parameters. Moreover, combination between neem liquid extract with acyclovir and haemocare more effective in improving the damaging effect of the viral disease than use each of them alone.

*Acknowledgments*

Great thanks and sincerest appreciation to Prof. Dr. Mohammed M. M. Metwally Department of pathology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt. for his help for histopathological studies.

*Funding statement*

This study didn't receive any funding support

*Declaration of Conflict of Interest*

The authors declare that there is no conflict of interest.

*Ethical of approval*

The management and treatment procedures were done according to the Guidelines for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH) and approved by a research ethics committee of the Faculty of Veterinary Medicine, Zagazig University with approval number: ZU-IACUC/2/F/365/2022.

**TABLE 1. Serum hepatic parameters (mean value ±SE) of chicken in gps. (1-4) 3 days PI.**

| Parameters<br>Groups | ALT U/L | AST U/L | ALP U/L | Total protein (TP) g/dl | Albumin (Alb) g/dl | Globulin (Glob) g/dl |
|---|---|---|---|---|---|---|
| GP1 (C) | $8.95^{d}$±0.40 | $88.97^{d}$±1.00 | $46.21^{d}$±1.14 | $4.60^{a}$±0.277 | $2.59^{a}$±0.148 | $2.01^{a}$±0.12 |
| GP2 (V) | $27.08^{a}$±0.77 | $136.42^{a}$±1.88 | $90.44^{a}$±0.57 | $2.76^{c}$±0.091 | $1.53^{c}$±0.03 | $1.23^{c}$±0.08 |
| GP3 (AV) | $11.71^{c}$±0.08 | $105.60^{c}$±0.70 | $57.59^{c}$±1.15 | $3.45^{b}$±0.12 | $1.81^{bc}$±0.08 | $1.64^{b}$±0.11 |
| GP4 (NV) | $20.24^{b}$±0.60 | $118.60^{b}$±1.43 | $86.81^{b}$±1.15 | $2.96^{bc}$±0.03 | $2.05^{b}$±0.07 | $0.91^{c}$±0.07 |

$^{a,b,c,d}$ Means within the same column carrying different superscript letters are significantly different at $p \leq 0.05$.
Group1 (C) control negative, Group2 (V) chicken infectious anemia virus (CIAV) infected group, Group 3 (AV) chicken pretreated with Acyclovir then infected with CIAV, Group 4 (NVA) pretreated with Neem then infected with CIAV.

Serum Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Total proteins (TP) Albumin (Alb) and Globulin(Glob).

**TABLE 2. Serum hepatic parameters (mean value ±SE) of all groups (1-7) 15 days PI.**

| Parameters/ Groups | ALT U/l | AST U/l | ALP U/l | Total protein (TP) g/dl | Albumin (Alb) g/dl | Globulin (Glob) g/dl |
|---|---|---|---|---|---|---|
| GP1 (C) | $8.25^{d}$±0.34 | $82.73^{e}$±0.23 | $47.60^{d}$±1.15 | $4.64^{a}$±0.11 | $2.48^{a}$±0.11 | $2.16^{a}$±0.05 |
| GP2 (V) | $27.13^{a}$ ±0.40 | $132.76^{a}$ ±1.18 | $94.53^{a}$±2.30 | $2.63^{d}$±0.11 | $1.18^{d}$±0.05 | $1.45^{c}$±0.05 |
| GP3 (AV) | $8.80^{d}$ ±0.05 | $84.66^{de}$ ±0.13 | $47.20^{d}$±1.15 | $4.55^{a}$±0.28 | $2.41^{a}$±0.05 | $2.14^{ab}$±0.00 |
| GP4 (NVA) | $9.60^{c}$ ±0.05 | $85.50^{d}$ ±0.28 | $51.96^{c}$±0.57 | $4.38^{ab}$±0.05 | $2.29^{ab}$±0.05 | $2.08^{ab}$±0.01 |
| GP5 (NVH) | $11.00^{b}$ ±0.17 | $104.5^{b}$ ±0.38 | $63.43^{b}$±0.57 | $3.20^{c}$±0.11 | $1.93^{c}$±0.23 | $1.26^{d}$±0.01 |
| GP6 (NVAH) | $8.42^{d}$ ±0.13 | $82.80^{e}$ ±0.35 | $47.50^{d}$±0.57 | $4.60^{a}$±0.05 | $2.40^{a}$±0.10 | $2.20^{a}$±0.05 |
| GP7 (VA) | $10.46^{b}$ ±0.19 | $92.0^{c}$ ±0.1.15 | $52.96^{c}$±1.15 | $4.08^{b}$±0.05 | $2.03^{bc}$±0.02 | $2.05^{b}$±0.02 |

$^{a,b,c,d}$ Means within the same column carrying different superscript letters are significantly different at $p \leq 0.05$.

Group1 ( C ) control negative , Group2 (V) chicken infectious anemia virus (CIAV) infected group, Group 3 (AV) chicken pretreated with Acyclovir then infected with CIAV, Group 4 (NVA) pretreated with Neem then infected with CIAV then treated with acyclovir, Group 5 ( NVH) pretreated with Neem then infected with CIAV then treated with hemocare, Group 6 pretreated with Neem then infected with CIAV then treated with acyclovir and hemocare,and Group7 (VA) infected with CIAV then treated with Acyclovir.

**TABLE 3. Serum renal function tests (mean value ±SE) of chickens in gps. (1-4) 3 days PI.**

| Parameters/ Groups | Uric acid mg/dl | Creatinine mg/dl | (Calcium) Ca | (Phosphorus) Ph |
|---|---|---|---|---|
| GP1 (C ) | $3.81^{d}$ ±0.08 | $0.39^{d}$±0.02 | $8.85^{a}$±0.08 | $4.55^{c}$±0.06 |
| GP2 (V) | $7.98^{a}$ ±0.10 | $1.21^{a}$±0.02 | $6.25^{d}$±0.18 | $8.05^{a}$ ±0.16 |
| GP3 (AV) | $4.5^{c}$±0.28 | $0.77^{c}$±0.02 | $7.71^{b}$±0.06 | $6.11^{b}$±0.11 |
| GP4 (NV) | $6.16^{b}$ ±0.08 | $1.00^{b}$ ±0.02 | $7.18^{c}$±0.10 | $6.42^{b}$ ±0.14 |

$^{a,b,c,d}$ Means within the same column carrying different superscript letters are significantly different at $p \leq 0.05$.
Group1 (C) control negative, Group2 (V) chicken infectious anemia virus (CIAV) infected group, Group 3 (AV) chicken pretreated with Acyclovir then infected with CIAV, Group 4 (NVA) pretreated with Neem then infected with CIAV.

**TABLE 4. Serum renal function tests (mean value ±SE) of all groups (1-7) 15 days PI.**

| Parameters/ Groups | Uric acid mg/dl | Creatinine mg/dl | (Calcium) Ca | (Phosphorus) Ph |
|---|---|---|---|---|
| GP1 (C) | $4.56^{d}$±0.03 | $0.506^{d}$ ±0.01 | $8.79^{a}$±0.17 | $4.80^{d}$±0.08 |
| GP2 (V) | $8.84^{a}$±0.02 | $1.26^{a}$ ±0.06 | $6.42^{d}$±0.23 | $7.70^{a}$ ±0.13 |
| GP3 (AV) | $4.62^{d}$±0.03 | $0.51^{d}$ ±0.00 | $8.68^{a}$ ±0.05 | $4.75^{d}$±0.01 |
| GP4 (NVA) | $4.69^{d}$±0.08 | $0.56^{cd}$ ±0.00 | $8.57^{a}$±0.10 | $5.02^{cd}$±0.06 |
| GP5 (NVH) | $6.62^{b}$±0.06 | $0.736^{b}$±0.00 | $7.02^{c}$±0.05 | $6.41^{b}$ ±0.11 |
| GP6 (NVAH) | $4.64^{d}$ ±0.03 | $0.490^{d}$ ±0.01 | $8.70^{a}$ ±0.05 | $4.80^{d}$±0.04 |
| GP7 (VA) | $5.01^{c}$±0.00 | $0.62^{c}$ ±0.01 | $8.07^{b}$±0.05 | $5.14^{c}$ ±0.106 |

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

**TABLE 5. Serum inflammatory cytokine gene expression interleukin – 10 (mean value ±SE) of chickens in gps. (1-7) 3 and 15 days PI.**

| Parameters/ Groups | Interleukin (IL)-10 | |
|---|---|---|
| | 3 days PI | 15 days PI |
| GP1 (C) | $1.00^{a} \pm 000$ | $1.00^{a} \pm 000$ |
| GP2 (V) | $0.113^{d} \pm 0.08$ | $0.051^{g} \pm 0.02$ |
| GP3 (AV) | $0.540^{b} \pm 0.01$ | $0.574^{e} \pm 0.05$ |
| GP4 (NVA) | $0.441^{c} \pm 0.07$ | $0.796^{d} \pm 0.04$ |
| GP5 (NVH) | - | $0.895^{b} \pm 0.07$ |
| GP6 (NVAH) | - | $0.876^{c} \pm 0.01$ |
| GP7 (VA) | - | $0.436^{f} \pm 0.05$ |

$^{a,b,c,d,e,f,g}$ Means within the same column carrying different superscript letters are significantly different at $p \leq 0.05$.

Group1 ( C ) control negative , Group2 (V) chicken infectious anemia virus (CIAV) infected group, Group 3 (AV) chicken pretreated with Acyclovir then infected with CIAV, Group 4 (NVA) pretreated with Neem then infected with CIAV then treated with acyclovir, Group 5 ( NVH) pretreated with Neem then infected with CIAV then treated with hemocare, Group 6 pretreated with Neem then infected with CIAV then treated with acyclovir and hemocare,and Group7 (VA) infected with CIAV then treated with Acyclovir.

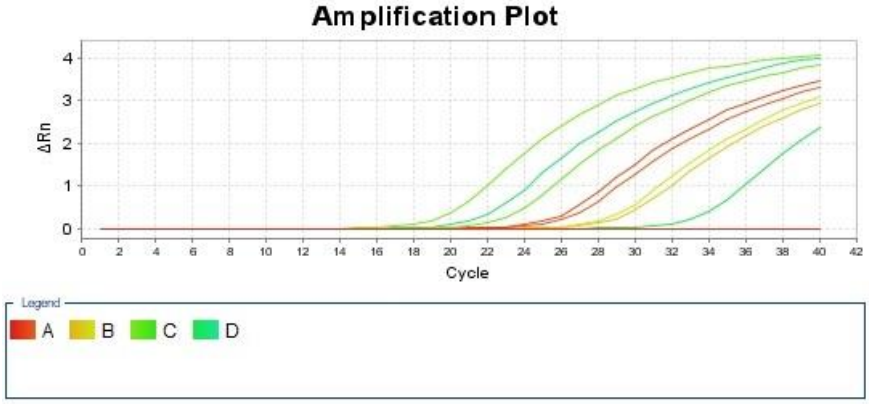


**Fig . 1. Assessment of serum inflammatory cytokine gene expression interleukin -10 (IL10) of chickens in all groups (1-4) 3 days PI.**

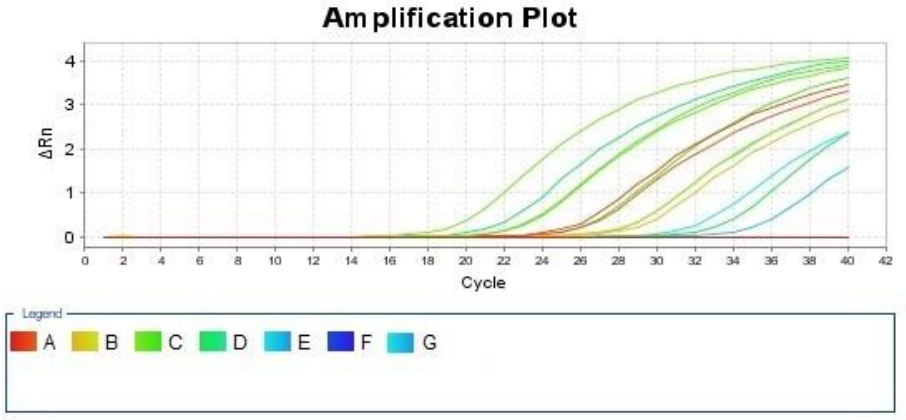


**Fig. 2. Assessment of serum inflammatory cytokine gene expression interleukin -10 (IL10) of chickens in all groups (1-7) 15 days PI.**

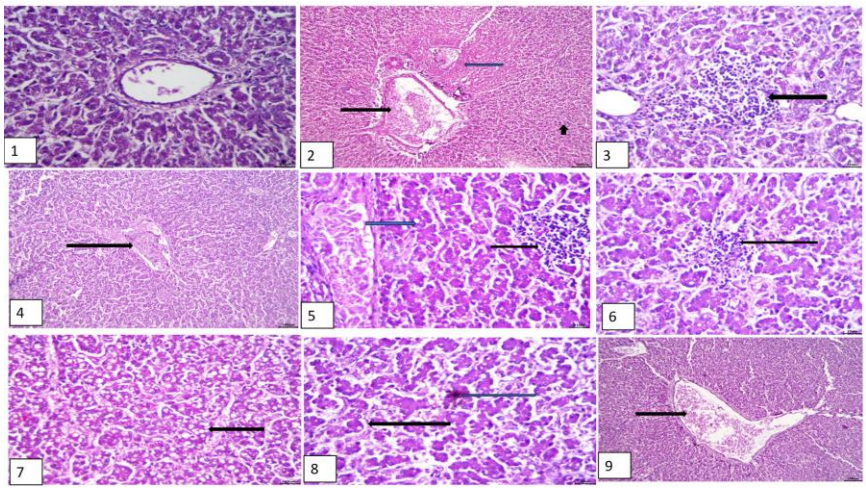

**Fig. 3. Photomicrographs of liver tissue of control (gp.1), CIAV infected bird (gp.2) and infected birds pre and post treated with neem liquid extract alone and or in combination with acyclovir and haemocare (gps.3-7) 3 and 15 days PI. Photo.** (1) liver tissue of gp.1 showed normal histological structure 3 and 15 day PI, (H&E 100X). Photo. (2) liver tissue of gp.2 showed vascular congestion (black arrow), focal coagulative necrosis, (blue arrow) fatty change of hepatocytes (arrow head) and endothelial hypertrophy 3 days PI, (H&E 25X)**,** moreover, photo. (3) showed necrotic foci replaced by mononuclear cells, with cytoplasmic vacuolation of numerous hepatocytes 15 days PI, (H&E 100X). Photo. (4) liver tissue of gp. 3 showed congestions of the central veins 3 and 15 days PI, (H&E 25X). Photo. (5) liver tissue of gp. 4 showed vascular congestion, focal necrotic area replaced by mononuclear cells (black arrow), and fatty change of numerous hepatocytes (blue arrow) 3 days PI, (H&E 100X), while photo. (6) showed infiltration with mononuclear cells and numerous vacuolated hepatocytes 15 days PI, (H&E 100X). Photo. (7) liver tissue of gp. 5 showed diffuse fatty change of hepatocytes 15 days PI, (H&E 100X). Photo. (8) liver tissue of gp. 6 showed numerous vacuolated hepatocytes(black arrow), with presence of few hemosiderin pigment (blue arrow) 15 days PI, (H&E 100X). Photo. (9) liver tissue of gp.7 showed vascular congestion 15 days PI, (H&E 25X).

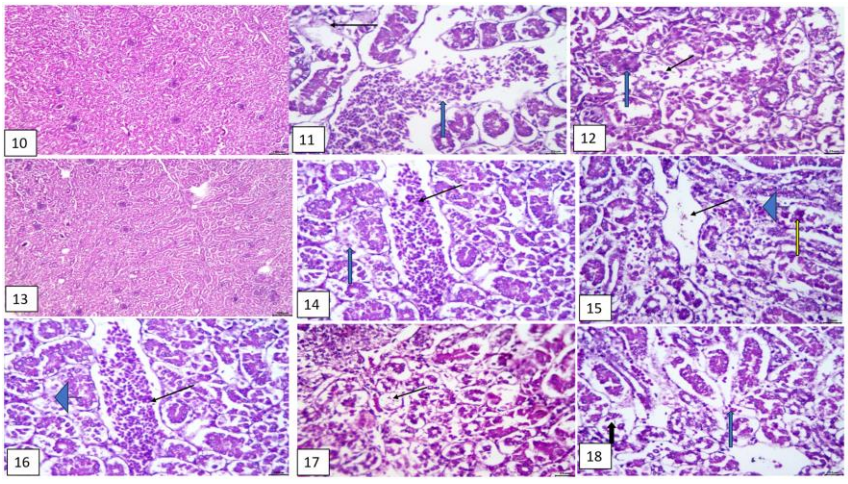

**Fig.4. Photomicrograph of kidney tissue (cortical convoluted tubules) of control (gp.1), CIAV infected bird (gp.2) and infected birds pre and post treated with neem liquid extract alone and or in combination with acyclovir and haemocare (gps.3-7) 3 and 15 days PI.** Photo. (10) kidney tissue (cortical convoluted tubules) of gp. (1) showed normal histological structure 3 and 15 day PI, (H&E 25 X). Photo. (11) kidney tissue (cortical convoluted tubules) of gp. (2) showed tubular necrosis ( black arrow) and vascular congestion (blue arrow) 3 days PI, (H &E 100 X), while photo. (12) showed tubular necrosis (black arrow), few regenerated tubular epithelia (blue arrow) with minute interstitial hemorrhage 15 days PI, (H&E 100 X). Photo. (13) kidney tissue (cortical convoluted tubules) of gp. (3) showed normal histology 3 and 15 days PI, (H&E 25 X). Photo. (14) kidney tissue (cortical convoluted tubules) of gp. (4) showed tubular necrosis with desquamation of the necrotic cells to the tubular lumens (blue arrow) and vascular congestion (black arrow) 3 days PI, (H&E 100 X), while photo. (15) showed vascular congestion (black arrow), tubular necrosis with cellular casts ( yellow arrow) besides interstitial minute hemorrhages (blue arrow) 15 days PI, (H&E 100X). Photo. (16) kidney tissue (cortical convoluted tubules) of gp. (5) showed vascular congestion (black arrow) and tubular necrosis (blue arrow) 15 days PI, (H&E 100 X). Photo. (17) kidney tissue (cortical convoluted tubules) of gp. (6) showed tubular necrosis 15 days PI, (H&E 100 X). Photo. (18) kidney tissue (cortical convoluted tubules) of gp. (7) showed tubular necrosis with desquamation of the necrotic cells to the tubular lumens (black arrow) besides interstitial minute hemorrhages (blue arrow) 15 days PI, (H&E 100 X).

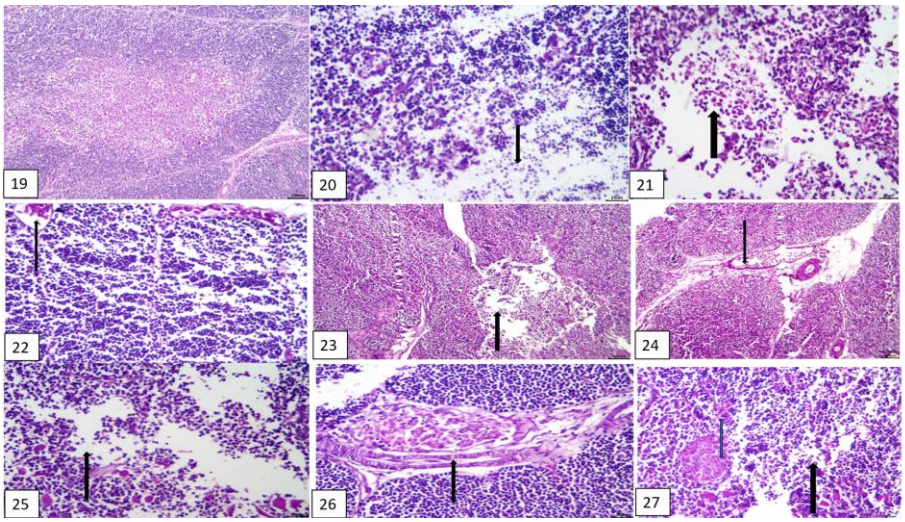


**Fig. 5. Photomicrograph of thymus tissue of control (gp.1), CIAV infected bird (gp.2) and infected birds pre and post treated with neem liquid extract alone and or in combination with acyclovir and haemocare (gps.3-7) 3 and15 days PI. Photo. (19) thymus tissue of gp**. (1) showed normal histological structure 3 and15 day PI, (H&E 25X). Photo. (20) thymus tissue of gp. (2) showed cortical lymphoid depletion together with proliferation of the reticuloendothelial cells 3 days PI, (H&E 100X), while photo. (21) showed cortical lymphoid necrosis together with cortical hemorrhage 15 days PI, (H&E 100X). Photo. (22) thymus tissue of gp. (3) showed vascular congestion 3 and15 days PI, (H&E 100X). Photo. (23) thymus tissue of gp. (4) showed lymphoid depletion and necrosis, with increased the connective tissue elements 3 days PI, (H&E 25X), while photo. (24) showed vascular congestion 15 days PI, (H&E 100X). Photo. (25) thymus tissue of gp. (5) showed marked lymphoid depletion 15 days PI, (H&E 100X). Photo. (26) thymus tissue of gp. (6) showed vascular congestion 15 days PI, (H&E 100X). Photo. (27) thymus tissue of gp. (7) showed lymphoid depletion (black arrow) together with vascular congestion (blue arrow) 15 days PI, (H&E 100X).

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# تقييم تأثير مستخلص النيم السائل، وأسيكلوفير، وهيموكير ضد عدوى فيروس فقر الدم المعدي في الدجاج

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**الملخص**

فيروس فقر الدم المعدي للدجاج (CIAV) هو مرض خطير للغاية يصيب الدجاج مع انتشار عالمي، وهو مسؤول عن الخسائر الاقتصادية الفادحة لمربي الدجاج، حيث يصيب جميع أعمار الدجاج وأظهرت الطيور المصابة فقر دم لا تنسجي ملحوظ، وضمور متوسط إلى شديد في الغدة الزعترية ونخاع العظم، ونزيف عضلي، وتثبيط المناعة. في دراستنا، قمنا بالتحقيق في تأثير مستخلص النيم السائل، والأسيكلوفير، وهيموكير على التأثير المدمر لـ CIAV، من خلال تقييم التعبير البيوكيميائي لجين السيتوكين الالتهابي (IL-10) والفحص النسيجي. أظهرت نتائجنا ارتفاعًا في مصل ALT وAST وALP والكرياتينين وحمض اليوريك والفوسفور العضوي في الدجاج المصاب بـ CIAV. بالإضافة إلى نقص بروتين الدم، ونقص ألبومين الدم ونقص غلوبولين الدم مع انخفاض في مستوى الكالسيوم في المصل والتعبير الجيني للسيتوكين الالتهابي (IL-10). من الناحية النسيجية، أظهرت الدجاجات المصابة بفيروس CIAV تأثيرًا ضارًا في أنسجة الكبد والكلى والغدة الزعترية. بينما أظهرت المجموعات المصابة التي عولجت قبل وبعد العلاج بمستخلص النيم السائل وحده أو بالاشتراك مع الأسيكلوفير وهيموكير تحسنًا في المعايير الكيميائية الحيوية المتغيرة، والتعبير الجيني للسايتوكين الالتهابي، والنتائج النسيجية المرضية للأنسجة السابقة. في الختام، أثبتت نتائجنا فعالية المكملات قبل وبعد العلاج بمستخلص النيم السائل وحده أو بالاشتراك مع الأسيكلوفير وهيموكير في تعديل الآثار الجانبية لعدوى CIAV في الدجاج، كما أن العلاج المسبق بأسيكلوفير أكثر فعالية من استخدامه في العلاج.

**الكلمات المفتاحية** : CIAV، مستخلص النيم السائل، الأسيكلوفير، هيموكير، والدراسات الكيميائية الحيوية.