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Original article

APIGENIN MODULATES CARDIORENAL DISORDERS IN COCKEREL CHICKENS EXPERIMENTALLY INFECTED WITH NEWCASTLE DISEASE VIRUS (KUDU 113 STRAIN)

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Summary

Esan, O. O., D. C. Ngoma, O. T. Jeremiah, F. M. Rajemison, O. A. Oladele, T.O. Ajibade, V. A. Aliyu, T. O. Omobowale, A. A. Oyagbemi, A. Adeogun & T. A. Jarikire, 2026. Apigenin modulates cardiorenal disorders in cockerel chickens experimentally infected with Newcastle disease virus (KUDU 113 strain). *Bulg. J. Vet. Med.,* **29**, No 1, 99–117.

Newcastle disease (ND) is a highly contagious viral disease of avian species. It spreads easily despite widespread vaccination and remains endemic in the majority of poultry-producing regions of the world. Apigenin, a flavonoid, has antioxidant, anti-inflammatory, antibacterial, antiviral, and anti-tumour properties. One-hundred-day-old cockerel chickens were assigned into six groups of 15 each – Group A (control); Group B: ND virus ($10^{6.5}$ per 0.1 mL) only; Groups C & D received 100 mg/kg and 200 mg/kg of apigenin orally in addition to ND ($10^{6.5}$ per 0.1 mL) while groups E and F were administered only apigenin 100 mg/kg and 200 mg/kg, respectively for seven days. Results showed significantly ($P<0.05$) increased heart rate, PR interval, and QRS complex of the ND-infected group when compared with the control one. There were significant ($P<0.05$) differences between infected and control groups in lymphocytes, serum K+, blood urea nitrogen and creatinine. The cardiac GSH, GST, GPx, and renal SOD activities increased in the ND-treated group, while MDA and H_2O_2 significantly increased in the infected, but ameliorated substantially in the treated groups. Immunohistochemistry revealed increased expression of cardiac troponin and TNF-α in the cardiac tissue, while the higher immune-positivity of ACE-2 and COX-2 in the kidney of the infected chicks were ameliorated in the treated groups. Therefore, apigenin feed supplementation could protect against ND due to its antioxidant, cardio-renal protective, and immune-boosting properties.

Key words: apigenin, cockerel chickens, immunohistochemistry, Newcastle disease, oxidative stress

INTRODUCTION

Poultry farming is a significant global industry involved in the production of an important aspect of human food chain (Gržinić *et al.,* 2023). Poultry meat and eggs are crucial for nutrition and food security due to their extensive consumption across various nations, ethnicity, customs, and faiths. Poultry is the livestock industry's most efficient sub-sector in terms of resource utilisation, providing safe source of animal protein to fill the gap of the increasing global demand for white meat with minimal environmental pollution and use of natural resources (Mottet & Tempio, 2017). The rapid growth of this sector is often hindered by a multitude of issues, such as disease and nutritional deficiencies, leading to substantial financial setbacks.

Newcastle disease virus (NDV), is highly infectious and an important member in the paramyxoviridae family that affects poultry, including domestic chickens and other avian species (Alexander *et al*., 1997). The primary modes of NDV transmission are the oral and respiratory routes. Viruses are commonly transmitted through body fluids of infected birds, such as faeces, nasal discharge, and respiratory droplets (Murcia *et al.,* 2009). Moreover, contaminated clothing, tools, or feed might serve as an indirect or mechanical means for individuals and other animals to transmit the virus to unaffected flocks (Alexander *et al.,* 2004). The clinical presentation of Newcastle disease (ND) includes a wide range of clinical signs that might vary depending on the severity of the virus strain, the age and breed of the infected chickens, and the presence of co-infections. Typical clinical signs include respiratory distress or discomfort, sneezing, nasal discharge, diarrhoea, weakness, poor appetite, increased thirst, tremors, paralysis, and significant mortality rates. In the nervous forms of the disease, neurological abnormalities manifest as neck twisting, torticollis, wing paralysis, or complete paralysis (Aldous *et al.,* 2003). Essential measures have been used to restrict Newcastle disease outbreaks through preventive and control strategies. Vaccination is regarded as the most crucial strategy for effective control.

Flavonoids encompass one of the largest categories of specialised metabolites including many compounds such as apigenin, hesperidin, quercetin, and catechin (Ghitti *et al.,* 2022). It was suggested that the inclusion of flavonoids in the diets of chickens could enhance the nutritional, microbiological, and sensory qualities of the resulting meats and eggs (Tan *et al.,* 2022). This was achieved by decrease in the triglyceride and cholesterol levels in meat and eggs, resulting in a beneficial modification of their fatty acid composition (Kamboh *et al*., 2018). Flavonoids are recognised for their ability to disrupt the cytoplasmic membrane, resulting in both bacteriostatic and bactericidal actions (Ullah *et al.,* 2020). Moreover, they also stop a lot of microbes from using energy and making nucleic acids. Furthermore, their antiviral properties are via the induction of structural alterations, which enable them to serve as substitutes for antiviral drugs (Ahmad *et al*., 2015).

Apigenin is a flavone and a prevalent flavonoid scientifically referred to as 4,5,7-trihydroxyflavone. It is a natural flavonoid present in various fruits, vegetables, functional foods, and medicinal plants such as onions, grapefruits, oranges, parsley, and chamomile (Salehi *et al*., 2019). Its high permeability and low solubility in water empower it to pass effortlessly through the host plasma mem-

brane. It is generally acknowledged that this bioactive flavonoid has anti-inflammatory, antioxidant, and antineoplastic properties (Chen *et al.,* 2023). Multiple studies, have demonstrated cytotoxic and cytostatic effects of apigenin on a range of cancer cells as well as for prevention of hypertension, atherogenesis, and liver injury caused by ischaemia/ reperfusion (Shukla *et al*., 2010). Presence of apigenin in biological system leads to upregulation of antioxidant enzymes such as catalase, superoxide dismutase (SOD), and GSH-synthase, which in turn serves to mitigate electrophilic and oxidative stress in cells (Salehi *et al*., 2019). In addition, it promotes the expression of genes that encode phase II enzymes by suppressing the NADPH oxidase complex and the inflammatory genes it affects downstream. It also enhances the expression of Nrf-2's nuclear translocation as reported by Huang *et al*. (2013).

Studies have demonstrated that apigenin exhibits antiviral properties against many viral families, such as Poxviridae and Flaviviridae (Hakobyan *et al.,* 2016). Thus, this study was conducted to investigate the effect of apigenin supplementation in the amelioration of experimentally induced Newcastle disease in cockerel chickens.

MATERIALS AND METHODS

Experimental animals

One-hundred day-old cockerel chickens purchased from CHI farms limited, Ibadan, Oyo State and the birds were accommodated in the Avian experimental animal unit of the Department of Veterinary Medicine, University of Ibadan. The cockerel chickens were brooded for two weeks before being raised up to four weeks, fed age-appropriate poultry feed (Ultima chicken feed®, Ibadan) and served clean drinking water *ad libitum*. The cockerels were housed in spacious open sided cages that provided all necessary conditions for their comfort as specified by the Animal Use and Care Research Ethics Committee (ACUREC) University of Ibadan. The study was granted approval by the University of Ibadan Animal Use and Care Research Ethics Committee (ACUREC) with approval number: UI-ACUREC/044-0523/31 and all the set-out protocols were strictly followed in the course of this experiment.

Experimental materials and chemicals

Powdered form of apigenin was purchased from AK Scientific (United States). The Newcastle disease challenge virus used for this study was the KUDU-113 strain (genotype XVII) of duck origin obtained from the National Veterinary Research Institute (NVRI) Vom, Jos, Nigeria. The LD_{50} titre was $10^{8.5}$ per mL of the viral stock solution and the infective dose administered was obtained by making 1:10 dilution using sterile phosphate buffered saline to obtain $10^{6.5}$ per 0.1 mL. Each infected bird received 0.1 mL via the intraocular route. One g and two g apigenin were dissolved in 10 mL each of distilled water to obtain 100 and 200 mg/mL liquid forms that were administered via oral gavage. All other chemicals used were of analytical grade and obtained from British Drug Houses (Poole, Dorset, UK).

Experimental design

Four weeks-old, uniform-weight cockerels were divided into six groups, with each group consisting of 15 cockerel chickens. Group A functioned as control group and received neither ND virus nor apigenin. Group B served as the infected group and received 0.1 mL of $10^{6.5}$/0.1 mL Newcas-

tle disease virus KUDU-113 strain intraocularly only. Similarly, groups C and D were administered 0.1 mL of Newcastle disease virus KUDU-113 $10^{6.5}$/0.1 mL intraocularly and in addition, received 100 mg/kg and 200 mg/kg of apigenin via oral gavage respectively for seven days post viral challenge. Groups E and F received only 100 mg/kg and 200 mg/kg of apigenin via oral gavage respectively for seven consecutive days.

Electrocardiographic recording

The ECG machine used was A 6/7 lead computer ECG equipment (EDAN VE-1010, Shanghai, China). The ECG was recorded, as previously reported by Esan *et al.* (2023). The Lead II characteristics were recorded for every bird, which included heart rate, P wave duration, PR interval, QRS duration, QT duration, and QT segment Bazett's correction.

Haematological and biochemical analyses

Five chickens were chosen at random for each group. Using a sterile syringe, 5 mL of blood was collected from the jugular vein. Two (2) milliliters of blood were then placed into separate lithium heparinised tubes to conduct a complete blood count assay. The blood samples were analysed using the method outlined by Samour (2006). Various parameters such as packed cell volume (PCV), haemoglobin (Hb), red blood cell counts (RBC), white blood cell count (WBC), thrombocyte counts, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), lymphocyte count, neutrophil count, monocyte count, and basophil count were determined. Similarly, electrolytes such as sodium, potassium, chloride, and bicarbonate were assayed and kidney function test was performed to measure urea and creatinine levels as described by Manterys *et al.*, (2016) and Adeyemo *et al*., (2018).

Tissue samples of kidney and heart were homogenised after the initial slicing into small fragments using a Teflon homogenizer in a homogenising solution consisting of 0.1M phosphate buffer at pH 7.4. Post mitochondrial fractions (PMFs) were obtained by subjecting the homogenate to centrifugation at a speed of 10,000 g for 10 min at a temperature of –4 °C in a refrigerated centrifuge. The liquid portion was gathered for biochemical analysis. The following assays were done on the tissue samples; lipid peroxidation (malondialdehyde, MDA), hydrogen peroxide generation (H_2O_2), superoxide dismutase (SOD), reduced glutathione (GSH), glutathione S-transferase (GST), glutathione peroxidase (GPx) as described by Oyagbemi *et al.* (2017).

Histology and immunohistochemistry

Thin slices of heart and kidney tissues fixed in 10% formalin were embedded in paraffin wax, sectioned into 5–6 μm thick sections, mounted on glass slides and stained with haematoxylin and eosin (H&E) following the protocol previously outlined by Drury *et al.* (1976).

Immunohistochemistry was performed as described by Esan *et al*. (2023a) with slight modifications, using cardiac troponin polyclonal antibody and tumour necrosis factor alpha (TNF-α)monoclonal antibody for heart tissue and renal angiotensin converting enzyme-2 (ACE-2) and renal cyclooxygenase-2 (COX-2) polyclonal antibodies for kidney tissue. The 2-step Poly-HRP Anti-Mouse/Rabbit IgG Detection System with DAB solution (Catalog No: E-IR-R217, Elabscience Biotechnology®, China) was used. The procedure involved evaluating the expres-

sion levels of the antibodies by first dewaxing the fixed histopathological slides and rehydrating the mounted tissues, which were rinsed in running water for 5 min. The slides were then immersed in phosphate-buffered saline (PBS) and subjected to antigen retrieval by intermittent boiling in a microwave oven. Endogenous peroxidase activity was blocked by adding hydrogen peroxide, followed by incubation in a humidified chamber as per manufacturer's instructions. Cardiac troponin, TNF-α antibodies were applied to heart slides and ACE-2 and COX-2 antibodies – to kidney slides, and incubated for 2 h as directed. Diaminobenzidine (DAB) was then added to the slides, and the reaction was stopped using deionised water (Esan *et al.* 2023b). The slides were immersed in haematoxylin in a glass jar for 3 s and then rinsed with PBS before the slides were sequentially immersed in 80%, 90%, and 100% ethanol, followed by xylene (100%) for 2 min each. After drying, DPX mountant was applied to each slide. The sections were observed using a Leica light microscope (Leica LAS-EZ®) equipped with a digital camera and Leica software application suite v.3.4.

Statistical analysis

Mean ± standard deviation (SD) of data were determined using GraphPad 7.0. Comparison between groups was carried out by one-way analysis of variance (ANOVA) and Tukey's multiple comparisons post-test for multiple comparisons at $P<0.05$.

RESULTS

Electrocardiographic results

Data shown in Table 1 indicates statistically significant ($P<0.05$) increase in heart rate, P wave, PR interval, QRS complex and QTc in NDV-exposed chicks compared to controls. Chicks exposed to NDV and treated with apigenin (100 mg/kg and 200 mg/kg) showed significant lower heart rate, P wave, QRS complex, QTc, and PR interval when compared to the NDV-exposed chickens.

Haematology and blood biochemistry

There was no significant difference in the packed cell volumes, red and white blood cell counts showed non-significant changes in all groups when compared to the

Table 1. Effect of Newcastle disease and apigenin on electrocardiography of cockerel chickens from Group A (control), Group B (ND infected only), Group C (ND infected+100 mg/kg apigenin), Group D (ND infected+200 mg/kg apigenin), Group E (100 mg/kg apigenin only) & Group F (200 mg/kg apigenin only). Data are presented as mean±SD (n=5)

Group	Heart rate (bpm)	P wave (ms)	PR interval (ms)	QRS complex (ms)	QTc(ms)
Group A	214.00± 15.06	27.50±3.70	51.00±4.55	30.75±3.50	167.75±8.96
Group B	251.50±17.68^{a}	43.00±2.83^{a}	69.50±9.19^{a}	40.00±9.90^{a}	227.00±16.97^{a}
Group C	216.50±8.39^{b}	32.50±5.07^{b}	58.25±7.18^{b}	33.50±1.29	183.50±22.13^{b}
Group D	204.20±6.38^{b}	25.25±4.50^{bc}	55.50±2.08^{b}	23.40±7.06^{bc}	158.80±18.21^{bc}
Group E	210.25±3.40^{b}	26.50±4.65^{bc}	58.67±8.59^{b}	28.75±3.77^{b}	174.00±14.73^{b}
Group F	228.25±8.66^{bde}	29.00±2.00^{b}	68.50±7.14^{acde}	29.60±4.16^{b}	165.50±6.76^{b}

Superscripts indicates significant difference at $P<0.05$ when compared with: (a) Control group (Group A); (b) Newcastle disease infected (ND) group only (Group B). Superscripts c, d, e indicate significant difference across groups.

Table 2. Effect of Newcastle disease and apigenin on haematology profile of cockerel chickens from Group A (control), Group B (ND infected only), Group C (ND infected+100 mg/kg apigenin), Group D (ND infected+200 mg/kg apigenin), Group E (100 mg/kg apigenin only) & Group F (200 mg/kg apigenin only). Data are presented as mean±SD (n=5)

Parameters	Group A	Group B	Group C	Group D	Group E	Group F
Packed cell volume (%)	31.4±1.67	33.2±3.60	30.6±5.10	29±3.0	30.8±2.50	32.4±1.95
Red blood cells (10^{12}/L)	3.08±0.20	3.41±0.31	3.21±0.29	3.24±0.25	2.97±0.23	3.16±0.19
White blood cells (10^{9}/L)	15.6±4.30	18.9±3.50	17.5±3.80	15.5±2.40	17.5±2.80	14.81±2.50
Haemoglobin (g/L)	102±5.3	112.4±10.5	102.2±16.7	97.4±9.6	100.5±8.3	103.4±6.5
Lymphocytes (%)	67.0±3.27	60.2±3.27[a]	63.4±6.07	63.0±3.39	64.25±3.80	66.0±3.16
Heterophils (%)	25.8±4.97	29.0±5.24	31.2±8.38	32.6±4.34	29.5±4.20	28.0±3.74
Eosinophils (%)	3±0.71	2.4±2.07	3.6±1.95	4.2±0.84	4.5±1.29	3.2±1.30
Basophils (%)	0.4±0.55	0.4±0.55	0.2±0.45	0.2±0.48	0.25±0.50	0±0
Monocytes (%)	3.85±0.84	3.0±0.71	1.6±0.89[a]	3.0±0.71	1.5±1.0[a]	2.8±0.48
Thrombocytes (10^{9}/L)	3.12±0.58	2.73±0.64	2.43±0.67[e]	1.90±2.72[ad]	3.93±0.37[b]	3.7±0.75[cd]

Superscripts indicates significant difference at $P<0.05$ when compared with: (a) Control group (Group A); (b) Newcastle disease infected (ND) group only (Group B). Superscripts c, d, e indicate significant difference across groups.

Table 3. Effect of Newcastle disease and apigenin on electrolyte profile of cockerel chickens from Group A (control), Group B (ND infected only), Group C (ND infected+100 mg/kg apigenin), Group D (ND infected+200 mg/kg apigenin), Group E (100 mg/kg apigenin only) & Group F (200 mg/kg apigenin only). Data are presented as mean±SD (n=5)

Groups	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)	HCO_3 (mmol/L)
Group A	127.6±3.05	2.74±0.27	91.0±4.20	25.8±1.10
Group B	133.6±3.36	3.48±0.28[acd]	95.0±5.00	25.0±1.00
Group C	129.4±3.05	2.78±0.33	92.0±2.74	25.4±0.90
Group D	129.2±3.42	2.72±0.42	90.0±7.91	26.2±1.10
Group E	133.0±1.58	3.22±0.19	100.0±3.54	23.4±1.14[ad]
Group F	134.4±3.65	3.32±0.51	98.0±6.71	23.8±1.79[ad]

Superscripts indicates significant difference at P<0.05 when compared with: (a) Control group (Group A). Superscripts c, d, indicate significant difference across groups.

control group (Group A). The lymphocyte percentage in the NDV infected group was significantly (P<0.05) lower when compared with the control group, while treatments with apigenin in groups C (100 mg/kg) and D (200 mg/kg) had non-significant effects in relation to the infected group. The monocyte percentage in controls was significantly (P<0.05) higher that the values obtained for apigenin treated groups as shown in Table 2. Similarly, there was a significant (P<0.05) increase in thrombocyte counts when the control group was compared with group E and a decrease in groups C and D respectively.

Table 3 showed significant (P<0.05) increase in serum potassium in the NDV-exposed chickens when compared to the controls. Administration of NDV and apigenin (100 mg/kg and 200 mg/kg) did restore the levels of serum potassium towards the control group value. Serum bicarbonates were significantly (P<0.05) increased in apigenin-treated chickens (100 mg/kg and 200 mg/kg) when compared to the control group.

The values of urea and creatinine levels were significantly (P<0.05) increased in cockerel chickens exposed to NDV when compared to the control. The administration of apigenin resulted in significant reduction of urea concentrations in NDV + apigenin (100 mg/kg) and NDV + apigenin (200 mg/kg) birds when compared to NDV-exposed chickens (Table 4). Significantly (P<0.05) increased creatinine occurred in chicks treated with 200 mg/kg apigenin alone vs controls.

Table 4. Effect of Newcastle disease and apigenin on kidney function of cockerel chickens from Group A (control), Group B (ND infected only), Group C (ND infected+100 mg/kg apigenin), Group D (ND infected+200 mg/kg apigenin), Group E (100 mg/kg apigenin only) & Group F (200 mg/kg apigenin only). Data are presented as mean±SD (n=5)

Groups	Urea (mmol/L)	Creatinine (μmol/L)
Group A	2.16±0.26	30.1±8.0
Group B	3.10±0.35[a]	47.8±4.4[a]
Group C	2.27±0.35[b]	37.1±7.1
Group D	2.33±0.26[b]	38.7±4.4
Group E	2.40±0.23[b]	35.4±8.8
Group F	2.73±0.35	47.8±8.0[a]

Superscripts indicates significant difference at P<0.05 when compared with: (a) Control group (Group A); (b) Newcastle disease infected (ND) group only (Group B).

Cardiac and renal markers of oxidative stress

Cardiac MDA content was significantly ($P<0.05$) increased in NDV-exposed cockerel chickens when compared to Group A and the chickens treated only with apigenin (Group E and F, respectively). These values were reduced to near normal control values in the NDV+apigenin treated groups (Table 5). There was significant ($P<0.05$) increase in cardiac and renal H_2O_2 in NDV-exposed cockerel chicks when compared to the control group and significantly lower values in NDV+apigenin (100 mg/kg) and NDV+ apigenin (200 mg/kg), groups, respectively.

Cardiac and renal markers of antioxidant defense system

The level of GSH and activities of GST in the heart tissues of NDV-exposed chickens were significantly ($P<0.05$) reduced in comparison to control Group A. The treatment with apigenin at 100 mg/kg (Group C) and 200 mg/kg (Group D) increased insignificantly the GSH and GST activity in cardiac tissues.

The level of H_2O_2 generation in the kidney was significantly ($P<0.05$) increased in NDV-exposed cockerel chickens when compared to the control Group A and the groups treated with both doses of apigenin as shown in Table 6. The level of GST activities in the renal tissue of NDV-exposed chickens was significantly ($P<0.05$) reduced in comparison to group F that received 200 mg/kg apigenin only. The GPx levels in NDV+ apigenin (100 mg/kg) and NDV+apigenin (200 mg/kg) groups were significantly ($P<0.05$) higher than in controls. Similarly, the groups that received apigenin had significantly ($P<0.05$) higher levels in respect to the control group. The SOD activities in the group exposed to NDV were significantly lower than both the control and the apigenin only groups (Table 6).

Table 5. Effect of Newcastle disease and apigenin on cardiac and renal oxidative stress markers of cockerel chickens from Group A (control), Group B (ND infected only), Group C (ND infected+100 mg/kg apigenin), Group D (ND infected+200 mg/kg apigenin), Group E (100 mg/kg apigenin only) & Group F (200 mg/kg apigenin only). Data are presented as mean±SD (n=5)

Groups	Heart		Kidney	
	H_2O_2 (μmol/min/mg protein)	MDA (μmol/mg protein)	H_2O_2 (μmol/min/mg protein)	MDA (μmol/mg protein)
Group A	32.94±3.39	2.01±1.25	26.48±6.84	1.27±0.18
Group B	36.75±7.20^{cf}	6.53±5.44^{aef}	55.08±3.03^{acdef}	1.29±0.19
Group C	30.90±1.04	3.48±0.29	24.57±3.99^{b}	1.10±0.67
Group D	31.06±0.47	3.00±0.40	34.44±6.06^{b}	0.97±1.26
Group E	31.71±0.58	2.44±0.64	25.86±3.24^{b}	1.26±0.73
Group F	29.43±7.95^{ab}	2.64±0.43	29.72±1.41^{b}	2.12±2.63

Superscripts indicates significant difference at $P<0.05$ when compared with: (a) Control group (Group A); (b) Newcastle disease infected (ND) group only (Group B). Superscripts c, d, e, f indicate significant difference across groups.

Table 6. Effect of Newcastle disease and apigenin on cardiac and renal antioxidant defense system of cockerel chickens from Group A (control), Group B (ND infected only), Group C (ND infected+100 mg/kg apigenin), Group D (ND infected+200 mg/kg apigenin), Group E (100 mg/kg apigenin only) & Group F (200 mg/kg apigenin only). Data are presented as mean±SD (n=5)

Groups	GSH (U/mg protein)	GST (U/mg protein)	GPx (U/mg protein)	SOD (U/mg protein)
Heart				
Group A	35.43±1.22	21.32±7.26	800.18±27.24	160.00±14.14
Group B	30.45±1.23^{ad}	8.08±2.07^{a}	761.56±43.12^{cd}	147.50±17.08
Group C	31.61±0.80^{a}	9.42±1.54^{a}	508.26±62.60^{a}	145±21.21
Group D	31.55±0.41^{a}	13.65±2.58	1065.04±83.36^{a}	105±7.07^{af}
Group E	32.47±1.00^{af}	11.58±3.68^{a}	585.45±94.55^{acd}	130±14.14
Group F	29.70±1.47^{b}	14.02±1.85	875.82±86.21^{ce}	213.33±5.77^{abce}
Kidney				
Group A	28.29±2.77	9.46±0.29	137.09±7.80	290.00±26.46
Group B	28.94±2.93	8.50±4.00	169.58±12.90	200.00±10.00^{af}
Group C	26.18±1.30	7.05±1.12^{f}	160.38±7.00	226.70±15.28
Group D	26.13±2.15	7.86±0.72	193.88±20.70^{a}	222.50±29.86
Group E	27.70±2.73	10.13±1.73	145.321±2.69^{df}	243.33±20.82
Group F	29.45±2.07	10.16±0.99	103.75±3.880^{bcd}	379.50±0.71^{bcde}

Superscripts indicates significant difference at $P<0.05$ when compared with: (a) Control group (Group A); (b) Newcastle disease infected (ND) group only (Group B). Superscripts c, d, e, f indicate significant difference across groups.

Histopathological and immuno-histochemical results

Fig. 1 illustrates the histology of the heart, while Fig. 2 presents the histological details of kidneys. There was multifocal myofibre degeneration and inflammation in the ND-infected group and mild myofibre degeneration and inflammation in the treated group. No observable lesions were found in the control groups. The renal tissue showed mild to moderate tubular epithelial necrosis and inflammation in the ND-infected group. However, the groups treated with apigenin revealed no visible lesions.

The immunohistochemistry studies on renal angiontensin converting enzyme-2 (ACE-2) and renal cyclooxygenase-2 (COX-2) immunolocalisation in the kidney revealed higher expressions in the NDV-infected cockerel chicks when compared to the control group and groups co-treated with apigenin (Fig. 3 and 4). The immunohistochemistry of cardiac tissues showed an increased expression of cardiac troponin in the heart as well as cardiac tumour necrosis factor alpha (TNF-α) in group B infected with NDV when compared to the control and those supplemented with apigenin (Fig. 5 and 6).

DISCUSSION

Naturally occurring or synthetic compounds capable of modulating immune responses in order to confer greater protection against infectious agents have been of immense interest in human and veterinary medicine (Kehrli & Roth, 1990).

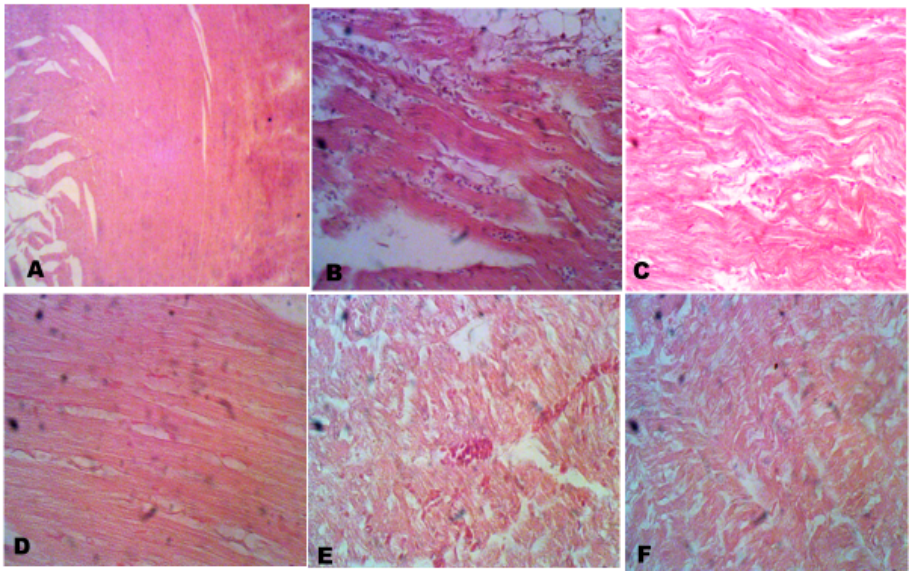

Fig. 1. Histology of cardiac tissues. Group A (control), Group B (ND infected only), Group C (ND infected+100 mg/kg apigenin), Group D (ND infected+200 mg/kg apigenin), Group E (100 mg/kg apigenin only) & Group F (200 mg/kg apigenin only). Haematoxylin and eosin staining; magnification ×400.

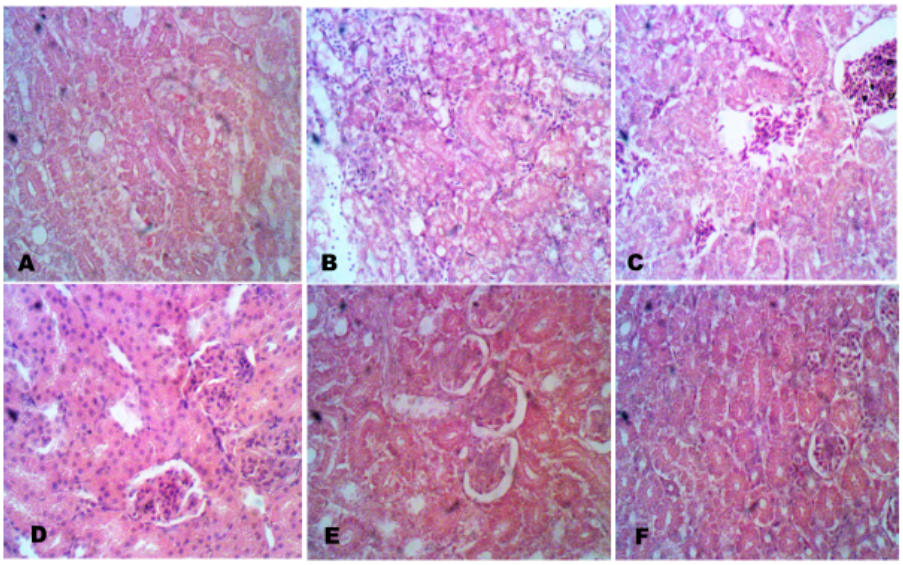

Fig. 2. Histology of renal tissues. Group A (control), Group B (ND infected only), Group C (ND infected+100 mg/kg apigenin), Group D (ND infected+200 mg/kg apigenin), Group E (100 mg/kg apigenin only) & Group F (200 mg/kg apigenin only). Haematoxylin and eosin staining; magnification ×400.

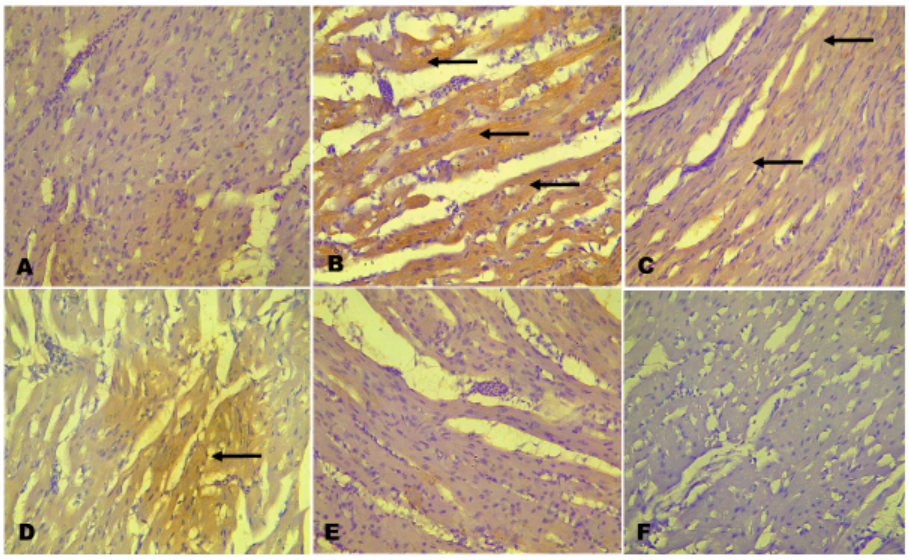

Fig. 3. Immunohistochemistry of cardiac troponin. Group A (control), Group B (ND infected only), Group C (ND infected+100 mg/kg apigenin), Group D (ND infected+200 mg/kg apigenin), Group E (100 mg/kg apigenin only) & Group F (200 mg/kg apigenin only). High-definition haematoxylin staining; magnification ×400. Arrows indicate areas of immunopositive reactions.

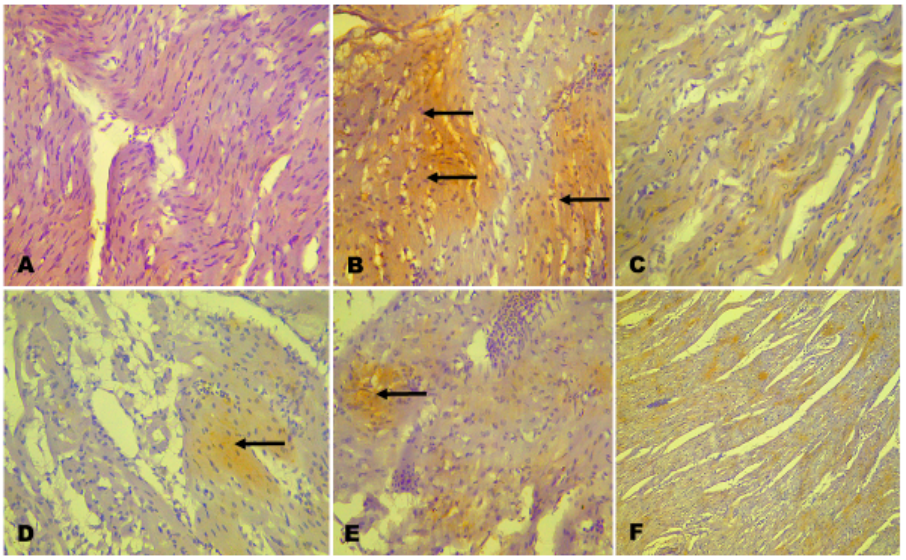

Fig. 4. Immunohistochemistry of cardiac tumour necrosis factor alpha (TNF-α). Group A (control), Group B (ND infected only), Group C (ND infected+100 mg/kg apigenin), Group D (ND infected+200 mg/kg apigenin), Group E (100 mg/kg apigenin only) & Group F (200 mg/kg apigenin only). High-definition haematoxylin staining; magnification ×400. Arrows indicate areas of immunopositive reactions.

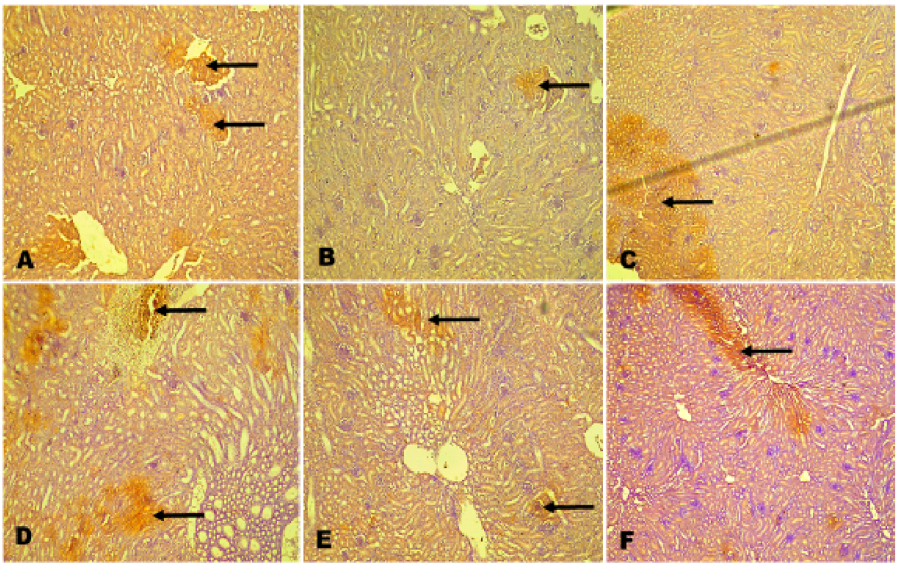

Fig. 5. Immunohistochemistry of renal angiotensin converting enzyme 2 (ACE2). Group A (control), Group B (ND infected only), Group C (ND infected+100 mg/kg apigenin), Group D (ND infected+200 mg/kg apigenin), Group E (100 mg/kg apigenin only) & Group F (200 mg/kg apigenin only). High-definition haematoxylin staining; magnification ×400. Arrows indicate areas of immunopositive reactions.

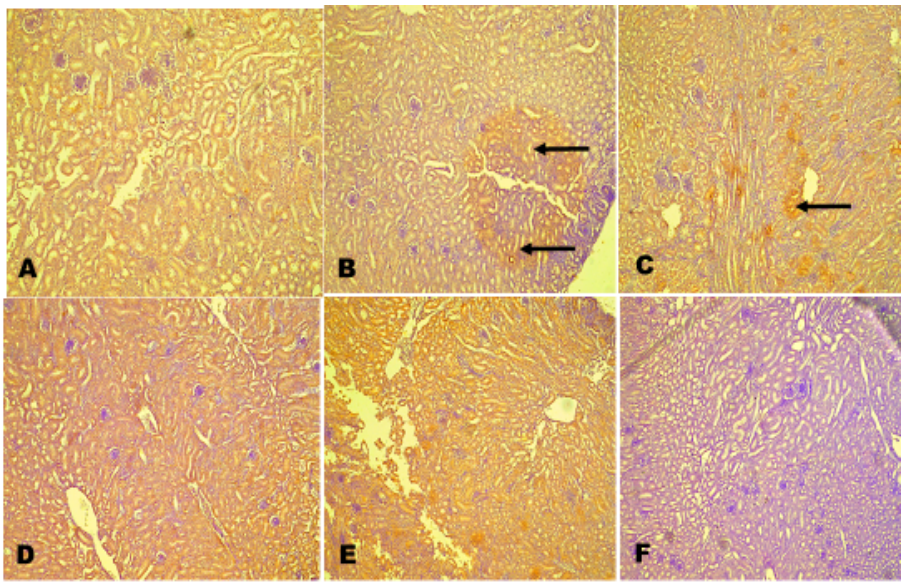

Fig. 6. Immunohistochemistry of renal cyclooxygenase 2 (COX-2). Group A (control), Group B (ND infected only), Group C (ND infected+100 mg/kg apigenin), Group D (ND infected+200 mg/kg apigenin), Group E (100 mg/kg apigenin only) & Group F (200 mg/kg apigenin only). High-definition haematoxylin staining; magnification ×400. Arrows indicate areas of immunopositive reactions.

Multiple mechanisms, including organ or tissue tropism, resistance to the host's immune system, and replication efficacy, are involved in the mechanism of action of velogenic NDV infection (Zhang *et al.,* 2023). This may be responsible for the significantly altered cardiac function, as evident from increased heart rate, prolonged P wave, PR interval, QRS complex, and QTc in this study. The infected group had a mean heart rate that was markedly higher than the control group. Similarly, the atria depolarisation (P wave) duration increased significantly in the infected group, suggesting atrial depolarisation abnormalities. Systemic hypertension, mitral valve dysfunction, aortic stenosis, and left ventricular hypertrophy have all been associated with anomalies in the left atrium (Platonov, 2012). Furthermore, the increased PR interval and the QRS complex elongation observed in the infected group may be consistent with first-degree atrioventricular block (AV). A first-degree AV is characterised by an ECG display of sinus rhythm with a steady increase in the PR interval and a corresponding increase in the QRS complexes without any dropped QRS complex. This is indicative of a delay in the electrical signal as it passes through the AV node and into the ventricles. First-degree AV block is a quite common ailment that is generally regarded as non-life-threatening (Oldroyd *et al.,* 2023). Treatment with apigenin significantly reversed the adverse effects of NDV on ECG parameters, with higher doses providing better outcomes. Group D, which received 200 mg/kg of apigenin, demonstrated the most significant restoration of normal ECG patterns. This is in consonant with similar experiments where flavonoid bioactive compounds such as hawthorn was used to improve pulmonary hypertension complications in chicken underscoring the cardioprotective effect of apigenin (Ahmadipour *et al.,* 2020). These improvements may be attributed to the antioxidant and anti-inflammatory properties of apigenin.

The haematology results showed depletion of lymphocytes in the infected group when compared with the control one. Lymphocyte depletion has been reported in Newcastle disease virus infection in chickens (Kristeen-Teo *et al.,* 2017, Hamisu *et al.,* 2021; Zhang *et al.,* 2023). The decreased lymphocyte count was significantly restored towards normal in the treated groups. Ferdous & Scott, (2023) demonstrated that the Newcastle disease virus not only infects chicken thrombocytes but also hinders their capacity for migration and phagocytosis that was similar to the findings in this study.

Urea, creatinine and potassium levels increased significantly in the infected group but were ameliorated in the treatment groups in a similar manner to the results obtained by Haleem *et al.,* (2024) in chickens and Zahid *et al.* (2022) in ducks. The increase in urea and creatinine in the infected group could be attributed to impaired renal function while hyperkalemia seen in this experiment may be responsible for the irregular heart beat seen on the ECG.

NDV causes a highly devastating and contagious disease in poultry, and oxidative stress by generating reactive oxygen species (ROS) mainly attributed to the increased H_2O_2 (Rehman *et al*. 2018). In this study, there were significant increases in H_2O_2 generation in the kidney and heart of the experimental birds. Reshi *et al.* (2014) described that most viral infections increase the production of ROS leading to overproduction, which may cause DNA damage and ultimately, disruption of the

cell functions. This study showed increase in H_2O_2 in group B (ND-infected only) in the heart and the kidneys which agreed with previous study by Subbaiah *et al.* (2011) that demonstrated increased H_2O_2 generation in chicken infected with NDV. Sahindokuyucu-Kocasari *et al.* (2021) revealed the potential of apigenin to increase liver and kidney expression of anti-inflammatory transcription factor. Our study showed a significant reduction in the level of H_2O_2 in studied organs in the apigenin-treated groups and the groups that were administered apigenin only had their activities of H_2O_2 within the range for the control group, thus showing the non-toxic properties of apigenin.

An increase in the level of lipid peroxidation as measured by the quantity of malondialdehyde (MDA) showed that ND-induced oxidative stress was observed in the heart and kidney in agreement with the studies of Kobroob *et al.* (2018) and Eweda *et al.* (2020). Lipid peroxidation of biological membranes leads to loss of membrane fluidity, increase in membrane permeability, altered receptor function and changes in membrane potential. This study showed that the level of MDA significantly decreased in all organs in both the ND-treated groups with apigenin and the groups treated only with apigenin as compared to the control group. This finding is similar to the study of Xu *et al*., (2020) that reported decrease in the production of lipid peroxidation product MDA in one diffusion model.

GSH is one of the non-enzymatic antioxidants that respond during oxidative stress. This study showed a decreased GSH activity in the heart and kidney as sign of ongoing oxidative stress caused by ND. This is similar to the findings of the study by Subbaiah *et al*., (2011) on perturbations in the antioxidant metabolism during (NDV infection in chickens and the protective role of vitamin E. Subbaiah *et al*. (2011) also reported that the levels of GSH were significantly reduced in NDV-infected tissues similarly to the findings in this study. However, Sahindokuyucu-Kocasari *et al.* (2021) reported that apigenin treatment of liver and kidney injuries in mice significantly increased the level of GSH in tissues in the apigenin-treated groups. This agrees with this study as ND group co-treated with apigenin (Groups C and D) showed improvement in the activity of GSH. Apigenin-only treated groups (Groups E and F) had higher GSH level, close to that of the control group.

The significant increase in GPx of kidney, as well as kidney and heart GST and SOD o in this study can be caused by increased antioxidant enzyme activity by Nuclear factor erythroid-2 (Nrf2). The first step is the modification of the keap 1 segment of Nrf2-Keap1 pathway. Nrf2 is usually low when not activated by stress factors. In the presence of ROS such as superoxide, hydroxyl, peroxyl radicals, the sulfhydryl groups on the keap1 segment oxidise cysteine residues such as Cys 151. This changes the conformation of Keap1 segment and prevent it from binding to Nrf2. As Nrf2 is released within the cytosol, it enters the nucleus to heterodimerise with Maf proteins and further bind to regulatory gene known as antioxidant response elements (ARE). The substances that initiate response at the ARE include environmental contaminants and some oxidants such as nitric oxide and hydrogen peroxide. The Nrf2-Maf-ARE complex then initiate subsequent specific antioxidant and detoxification genes including those of GST and other enzymes involved in antioxidant defense; thereby increasing antioxidant activities and inactivating free radicals (Baird &

Dinkova-Kostova, 2011; Oyagbemi *et al*., 2017). In this study, the activity of GPx decreased in ND-infected organs such as kidney and heart in line with the study of Subbaiah *et al.* (2011) where the decreased GSH levels may lead to decreased activity levels of GPx in virus-infected tissues. GPx is the enzyme that catalyses the detoxification of H_2O_2 and other hydroperoxide to water and oxygen. However, the observed increased level of GPx in the groups treated with ND+ apigenin and apigenin only was in line with the findings of Sahindokuyucu-Kocasari *et al.* (2021). This may be as a result of adaptive response to the antioxidant Nrf2 activation. The ND-infected birds co-treated with apigenin had a significant increase in the level of GPx in concordance with the report of Sahindokuyucu-Kocasari *et al.* (2021). The apigenin co-treated chicks had GPx values close and higher than those of the control group.

The GST and SOD activities of all groups were significantly increased when compared to their respective control groups in response to the oxidative stress induced by ND via adaptive response. They protect tissues from the damage caused by oxidative stress by detoxifying various substrates generated from cellular oxidative processes. This finding contradicts the study done by Subbaiah *et al.* (2011) who reported significantly lowered SOD activity in the brain and liver of NDV-infected chickens when compared with the control group; this may lead to the accumulation of super-oxides. This finding may result from the difference in the constituent and properties of vitamin E and apigenin used in the two studies. GST plays a key role in phase II detoxification mechanisms. The decrease in the activity of this enzyme suggests improper scavenging activity of free radicals during viral infection (Sahindokuyucu-Kocasari *et al.,* 2021). In the present study, the activity of GST was also significantly increased in the organs of ND-infected and co-treated chickens as compared with control birds. Apigenin restored the levels of both GST and SOD in studied organs because there was a significant increase in GST and SOD in the groups treated only with apigenin (E and F) vs controls. This was in line with the study of Sahindokuyucu-Kocasari *et al.* (2021) who reported a significant increase in the level of GST and SOD in the organs of apigenin-treated mice groups in relation to the control group.

The immunohistochemistry studies showed that the renal angiontensin converting enzyme-2 (ACE-2) and renal cyclooxygenase-2 (COX-2) immunolocalisation in the kidney revealed higher expressions in the NDV-infected group B broiler chickens when compared to the control group and groups co-treated with apigenin. Angiotensin I is converted to angiotensin II by the enzyme ACE, which narrows blood vessels and may result in hypertension (Ebirim *et al.* 2022). The elevated kidney ACE-2 expression indicated damage linked to NDV while the reduction in the immunolocalisation of renal ACE-2 in the apigenin-treated groups indicated the beneficial effects of apigenin on the renal tissue. COX-2 is known to participate in the synthesis of prostaglandin which promotes tumour growth and stimulates signalling pathways that control angiogenesis, apoptosis and cell proliferation, as well as cancer formation (Maksimowski *et al.,* 2020). In this study, COX-2 was downregulated by the treatment of Newcastle disease infected groups with apigenin.

There was an increased expression of cardiac troponin in the heart as well as

cardiac tumour necrosis factor alpha (TNF-α) in chicks infected with NDV in relation to both controls and infected birds co-administered with apigenin. The cardiac troponin is a protein found in the heart muscle that controls the calcium-mediated interaction between actin and myosin (Sharma *et al.,* 2004). A previous study reported that apigenin can inhibit oxidative stress-induced myocardial injury, but the mechanism have not been fully elucidated and need further exploration (Chen *et al.,* 2016). This study also reported that apigenin reduced isoproterenol-induced myocardial remodelling and inhibited the excessive accumulation of ROS and oxidative stress-induced cardiomyocyte apoptosis, thereby exerting satisfactory myocardial protect function (Wang *et al.,* 2022).

The present study demonstrated that exogenous administration of NDV to chickens affected vital organs, such as the kidney and the heart and induced substantial oxidative stress as evidenced by an increase in the hydrogen peroxide and malondialdehyde concentrations and alterations in the antioxidant metabolism status in the vital organs like the kidneys and the heart. Apigenin is widely found in various vegetables and fruits. This natural flavonoid with anti-inflammatory, antioxidant, anticancer, antiviral and other biological functions exhibited cardioprotective, nephroprotective, anti-inflammatory and antioxidant effects. When administered to the NDV-infected cockerel chickens, it restored and initiated revitalisation of heart and kidney cells that were destroyed and altered by NDV.

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