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Effects of prophylactic treatment with methanol leaf extract of *Anacardium occidentale* on some serum biochemistry parameters of chickens infected with velogenic Newcastle disease virus

Jacinta N. Omeke[1*], Iniobong C. Ugochukwu[1], John I. Ihedioha[1], Aruh O. Anaga[2], Wilfred S. Ezema[1], John O. A. Okoye[1]

[1]Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria.

[2]Department of Veterinary Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria.

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Abstract

This study evaluated the effects of prophylactic treatment with methanol leaf extract of *Anacardium occidentale* (MLEAO) on the serum biochemistry parameters of chickens infected with velogenic Newcastle disease virus (vNDV). One hundred and twenty five chickens, randomly assigned into five groups (A – E) of 25 each, were used for the study. Chickens in groups A, B and C were each drenched with 300, 150 and 75 mg/kg body weight of MLEAO, respectively for 7 days. Thereafter, chickens in groups A, B, C and D were infected with vNDV. Group D was the untreated infected control, while Group E was the untreated uninfected control. Blood samples for serum biochemistry assay were collected on day 0 (baseline), day 7 of the prophylactic treatment (PT), and on days 3 and 6 post-infection (PI) with vNDV, and biochemical assays were done following standard procedures. Results showed that on day 7 of the PT, there were significant ($p < 0.05$) alterations in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activity, and levels of serum proteins, albumins, uric acid and calcium in some of the MLEAO-treated groups especially group A, and on days 3 and 6 PI, the significant ($p < 0.05$) alterations in AST, ALP, proteins, albumins, globulins, uric acid, calcium and phosphorus persisted in mainly groups A and B. Chickens in Group C had significantly ($p < 0.05$) higher serum protein and globulin levels when compared to those of Groups A, B and D. It was concluded that treatment of chickens with MLEAO at 300 mg/kg dose mainly and to a lesser extent at 150 mg/kg led to adverse serum biochemical outcomes such as elevation of serum AST activity and levels of serum uric acid, and depression of serum ALP activity and levels of serum proteins, albumins, globulin, calcium and phosphorus, while its administration at 75 mg/kg dose led to stabilized cell membrane permeability and enhanced total protein and globulin synthesis.

Keywords: *Anacardium occidentale*; Methanol extract; Newcastle disease virus infection; Prophylaxis, Chicken; Serum biochemistry.

***Correspondence:** Jacinta N. Omeke**;** E-mail: jacinta.omeke@unn.edu.ng Phone: +2348037932717

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Introduction

Newcastle disease (ND) constitutes a major disease problem of poultry worldwide (Awan *et al.,* 1994). The disease is caused by Newcastle disease virus (NDV), an avian *Orthoavula* virus 1 that belongs to the genus *Orthoavula* virus, in the subfamily *Avulavirinae*, family *Paramyxoviridae* and order *Mononegavirales* (Walker *et al.,* 2019). Newcastle disease alone has been known to account for more than 50% of total losses in Africa's poultry flocks (Musa *et al.,* 2009). It was first reported in Nigeria in 1952 in Ibadan (Hill *et al.,* 1953), and since then, the disease has become the most important disease of chickens throughout the country, with annual epidemics being recorded in highly susceptible poultry flocks (Sa'idu *et al.,* 2006).

The NDV infects nearly all avian species, causing diseases that range in severity from inapparent, mild, acute to peracute (Okorie-Kanu *et al.,* 2016; Omeke *et al.,* 2018*a*). Based on the severity of clinical signs observed in ND infection, NDV strains have been classified into four pathotypes, starting from the most to the least virulent: velogenic, mesogenic, lentogenic and asymptomatic enteric (Cattoli *et al.,* 2011). The velogenic pathotype causes the most severe form of the disease, and constitutes a serious problem to poultry production in many parts of the world (Okechukwu *et al.,* 2020). Chickens are the most susceptible. Virulent NDV strains can replicate in the central nervous system (CNS), causing various degrees of non-suppurative encephalitis and severe neurological signs (Cattoli *et al*., 2011; Omeke *et al.,* 2021). In response to the threat posed by ND, several attempts have been made to put in place vaccination programmes to prevent epidemics of disease (Spradbrow, 1993). These measures have not been quite effective in the control of ND and outbreaks of ND in vaccinated chicken populations still occur (Okwor *et al.,* 2010).

Plants serve as sources of food and medicine for man and animals. Interest in medicinal plants has increased over the decades together with the number of investigations into their biological effects on animals and human beings (Veiga *et al.,* 2005). The medicinal use of *Anacardium occidentale* (AO), commonly known as cashew has been reported worldwide. The leaves and /or the bark extracts have been used in different parts of the world for treatment of dyspepsia, genital problems and venereal diseases, as well as for impotence, bronchitis, cough, intestinal colic and gastric ulceration (Arekemase *et al.,* 2011; Onoja *et al.,* 2019). Leaf extracts of AO possesses phytochemical constituents such as saponins, tannins, and flavonoids, which have been reported to exert antioxidant activities (Jaiswal *et al.,* 2010). Our earlier studies showed that low doses of AO methanolic extract significantly ameliorated lesions of ND and reduced mortality in infected chickens (Omeke, 2021), but there are no reports in available literature on the effects of the prophylactic administration of AO extracts on the serum biochemistry of chickens infected with ND. The aim of this present study was to evaluate the effects of prophylactic treatment with methanolic leaf extract of AO on the serum biochemical parameters of chickens infected with vNDV.

Materials and Methods

Collection and identification of the AO leaves: Fresh cashew leaves used for the study were collected from Nguru Cashew Plantation in Nsukka Local Government Area, Enugu State of Southeast Nigeria in January, 2018. The plant was identified by a taxonomist in Herbarium Section of the Department of Plant Science and Biotechnology, University of Nigeria Nsukka UNN. Samples of the leaves were kept in the departmental herbarium (UNN|H.AO|2018.1)

Plant leaf preparation and extraction: The AO leaves were sorted and chopped into small bits and dried at room temperature at Drug Discovery Laboratory, Department of Veterinary Physiology and Pharmacology University of Nigeria Nsukka (UNN). The dried leaves were then pulverized into powder using a hammer mill and extracted by cold maceration: 1000 g of the AO dried powder was soaked in 3 litres of 70% hydro-methanol for 48 hours with intermittent shaking. The extract was filtered

using No. 1 Whatman® filter paper, and the filtrate was concentrated to dryness in an evaporator (Buchi, Switzerland). The percentage yield of the extraction was calculated, and the extract was stored in refrigerator at 4°C until the time of use.

Brine shrimp lethality and acute toxicity tests: The brine shrimp lethality and acute toxicity studies on the extract have been described in an earlier publication (Omeke *et al*., 2018*b*).

Experimental animals and Design: One hundred and twenty five cockerels were obtained at one day old for this experiment from a commercial hatchery, Zartech Hatchery, Ibadan Oyo State, Nigeria. They were kept in the isolation facility at Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, University of Nigeria Nsukka. Brooding was done on deep litter. Feed and water were supplied *ad libitum.* At six weeks of age the chickens were randomly assigned to five groups (A – E) of 25 chickens each. Blood samples (2 ml) were collected from five chickens in each group to determine the baseline (day 0) values of the serum biochemical parameters. Afterwards, Groups A, B and C chickens were drenched daily with 300, 150, 75 mg of the AO extract per kg body weight, respectively for 7 days. After the 7-day administration of the extract, blood samples were again collected from five chickens in each group to determine their day 7 post-prophylactic treatment (PT) serum biochemical values. Thereafter each chicken in groups A, B, C and D was infected with 0.1ml of the vNDV inoculum intramuscularly (IM). The group D chickens thus became the untreated infected control, while the group E was the untreated uninfected control.

The velogenic viscerotropic NDV (duck/Nigeria/Plateau/Kuru/113/1992), characterized by Echeonwu *et al.* (1993), was used for the infection. It was obtained from the National Veterinary Research Institute, Vom, Nigeria. The virus belongs to NDV class II, genotype XVII (Shittu *et al.,* 2016). The inoculum had a median embryo infective dose (EID_{50}) of $10^{6.46}$ per ml.

The experimental chickens were observed for clinical signs of ND and blood samples were again collected from five chickens in each group for serum biochemical determinations on days 3 and 6 post-infection with vNDV. The clinical signs, lesions, virus isolation, serology and haematology were also studied, and had been presented in separate publications.

Serum biochemical determinations: At each time of collection, the blood samples were allowed to clot for 45 minutes, and were centrifuged at 3,000 revolutions per minute. The serum supernatant was carefully aspirated and transferred to labeled Eppendorf tubes (1.5 ml capacity) and used immediately for the biochemical determinations.

The serum alanine aminotransferase (ALT) and aspartate aminotransfrase (AST) activities were assayed based on the Reitman-Frankel colorimetric method (Reitman and Frankel, 1957; Colville, 2002), using commercially available ALT and AST test kits sourced from Quimica Clinica Aplicada (QCA), Spain. The serum alkaline phosphatase (ALP) activity was determined by the phenolphthalein monophosphate method (Babson *et al.,* 1966; Colville, 2002), using the ALP test kit (QCA, Spain). Serum total protein levels were assayed by direct Biuret method (Johnson, 2008), while the serum albumin level was determined based on the bromocresol green method (Doumas *et al.,* 1971; Johnson, 2008), using total protein and albumin test kits (QCA, Spain), respectively. Serum globulin was obtained by subtracting the serum albumin from the total protein value (Johnson, 2008). The serum levels of calcium was determined by the ortho-cresolphthalein method (Kessler and Wolfman, 1964), while the serum phosphorus determination was based on the Fiske-SubbaRow method (Fiske and SubbaRow, 1925; Goodwin, 1970), using the calcium and phosphorus test kits (QCA, Spain), respectively. Serum levels of uric acid were determined by the enzymatic colorimetric method (Fossati *et al.,* 1980), using the uric acid test kit (Dialab, Austria), while the serum creatinine was determined by the modified Jaffe method (Blass *et al.*, 1974) using creatinine test

kit (QCA, Spain). All the biochemical determinations were done with a Diatek® semi-automated clinical chemistry analyzer (Diatek Instruments Co. Ltd., Wuxi, China).

Approval for the use of the chickens for the study: The principles governing the humane use and conduct of experiments with animals were strictly observed during this study, and the experimental protocol was approved by the Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine, (IACUC, FVM), with Approval Reference Number: FVM-UNN-IACUC-2019-0446.

Data Analysis: Data generated in the study were subjected to one-way analysis of variance (ANOVA) using SPSS statistical package version 15.0 software. Variant means were separated post-hoc using the least significant difference methods. The level of significance was accepted at $p < 0.05$. Summary of the data from all groups were presented as mean ± SEM in tables.

Results

The AST activity of all the groups did not significantly ($p > 0.05$) vary on day 0, but on day 7 of PT, the AST activity of group C was significantly lower than those of groups A, D and E (Figure 1). On day 3 PI, the AST activity of groups A and B were significantly ($p < 0.05$) lower than that of the group E , while on day 6 PI, the AST activity of groups A and B were significantly ($p < 0.05$) higher than those of groups C, D and E (Figure 1). There were no significant ($p > 0.05$) variation among the groups in their serum ALT activity on day 0, but on day 7 of PT, the serum ALT activity of group A was significantly ($p < 0.05$) lower than those of groups C and D (Figure 2). The day 3 PI values of the ALT activity of group A was also significantly ($p < 0.05$) lower than those of groups C and E, while on day 6 PI only the group D ALT activity was significantly ($p < 0.05$) higher than those of groups A, B and E (Figure 2). There were no significant variations ($p > 0.05$) in the serum ALP activity on day 0 (baseline) and on day 3 PI, but the values recorded for group A on day 7 of PT was significantly lower than those of all other groups, while on day 6 PI, the ALP activities of all the infected groups (A, B, C and D) were significantly lower than that of the untreated uninfected control group E (Figure 3).

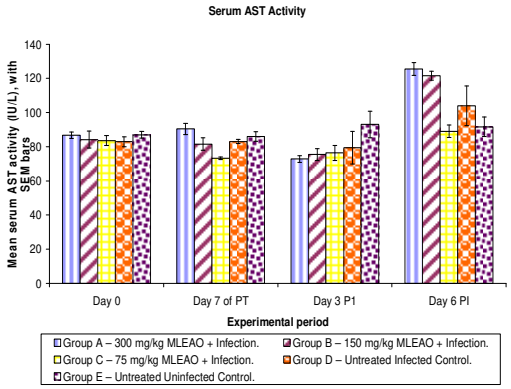

Figure 1. The serum aspartate aminotransferase (AST) activity of chicken groups treated with varied prophylactic doses of methanol leaf extract of *Anarcadium occidentale* (MLEAO) for seven days and infected with velogenic Newcastle disease virus (vNDV). [PT = prophylactic treatment. PI = post infection]

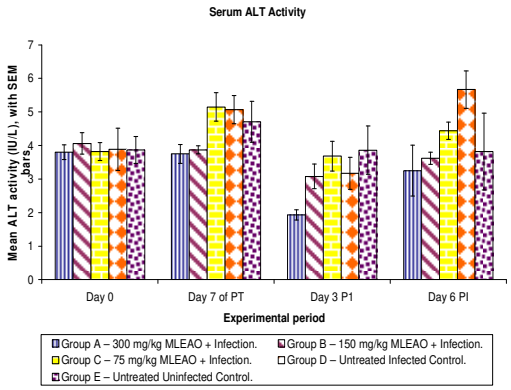

Figure 2. The serum alanine aminotransferase (ALT) activity of chicken groups treated with varied prophylactic doses of methanol leaf extract of *Anarcadium occidentale* (MLEAO) for seven days extract of *Anarcadium occidentale* (MLEAO) for seven days and infected with velogenic Newcastle disease virus (vNDV). [PT = prophylactic treatment. PI = post infection]

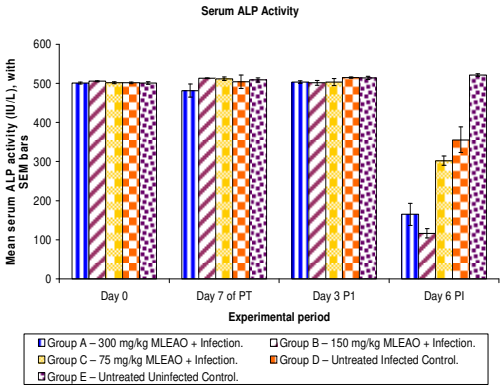

Figure 3. The serum alkaline phosphatase (ALP) activity of chicken groups treated with varied prophylactic doses of methanol leaf extract of *Anarcadium occidentale* (MLEAO) for seven days and infected with velogenic Newcastle disease virus (vNDV). [PT = prophylactic treatment. PI = post infection]

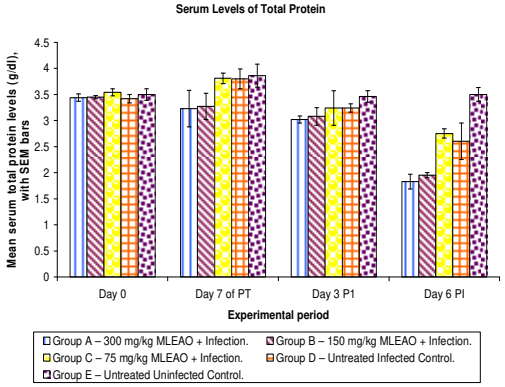

Figure 4. The serum total protein levels of chicken groups treated with varied prophylactic doses of methanol leaf extract of *Anarcadium occidentale* (MLEAO) for seven days and infected with velogenic Newcastle disease virus (vNDV). [PT = prophylactic treatment. PI = post infection]

Though the serum total protein levels on day 0 did not significantly ($p > 0.05$) vary, the day 7 of PT values for groups A and B were significantly lower ($p < 0.05$) than that of group E (Figure 4). On day 3 PI however, only the group A serum total protein value was significantly lower ($p < 0.05$) than that of the group E, but on day 6 PI, the serum total protein values of all the infected groups were significantly ($p < 0.05$) lower than that of group E, though those of group A and B were further significantly ($p < 0.05$) lower than those of groups C and D (Figure 4). The values recorded for the serum levels of albumin and globulin are presented on Figures 5 and 6, respectively. The serum albumin levels on day 0 did not significantly ($p < 0.05$) vary, but on day 7 of PT, the group A serum albumin levels was significantly ($p < 0.05$) lower than those of groups C, D and E (Figure 5). The group B values for serum albumin at day 3 PI was significantly ($p < 0.05$) lower than that of group C, but on day 6 PI the serum albumin levels of groups A and B were significantly ($p < 0.05$) lower than those of groups D and E (Figure 5). The globulin levels did not significantly vary ($p > 0.05$) among the groups on day 0, day 7 of PT and on day 3 PI, but on day 6 PI, the serum globulin levels of group A, B and D were significantly ($p < 0.05$) lower than those of groups C and E (Figure 6).

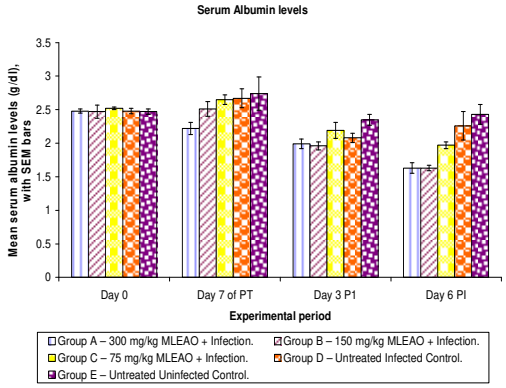

Figure 5. The serum albumin levels of chicken groups treated with varied prophylactic doses of methanol leaf extract of *Anarcadium occidentale* (MLEAO) for seven days and infected with velogenic Newcastle disease virus (vNDV). [PT = prophylactic treatment. PI = post infection]

The serum uric acid levels did not significantly ($p > 0.05$) vary among the groups on day 0 and day 7 of PT, but on day 3 PI, the serum uric acid levels of groups C and D were significantly ($p < 0.05$) lower than that of group B, while on day 6 PI, the serum uric acid levels of all the infected groups (A, B, C and D) were significantly ($p < 0.05$) higher than that of the group E, with those

of groups A and B being further significantly ($p < 0.05$) higher than those of groups C and D (Table 1). The serum creatinine levels of all the groups did not significantly vary ($p > 0.05$) all through the study (Table 2).

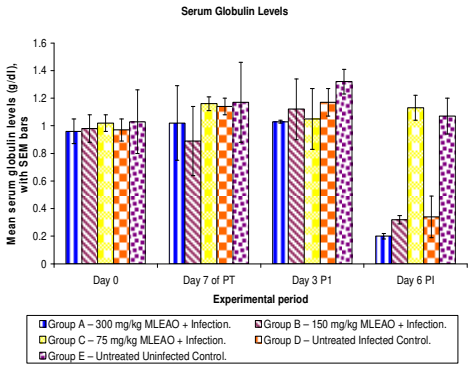

Figure 6. The serum globulin levels of chicken groups treated with varied prophylactic doses of methanol leaf extract of *Anarcadium occidentale* (MLEAO) for seven days and infected with velogenic Newcastle disease virus (vNDV). [PT = prophylactic treatment. PI = post infection]

The values for serum levels of calcium and phosphorus are presented on Tables 3 and 4, respectively. There were no significant ($p > 0.05$) variations in the serum calcium levels among the groups on day 0 and day 3 PI, but on day 7 of PT the serum calcium level of group A was significantly ($p < 0.05$) lower than those of groups C, D and E, while on day 6 PI, group A calcium levels was only significantly ($p < 0.05$) lower than that of group E (Table 3). The serum phosphorus levels on day 0 and day 7 PT did no significantly ($p > 0.05$) vary among the groups, but that of group A was significantly lower than group E on day 3 PI, while those of groups B and C were significantly lower than that of group D on day 6 PI (Table 4).

Table 1. The serum uric acid levels of chicken groups* treated with varied prophylactic doses of methanol leaf extract of *Anarcadium occidentale* (MLEAO) for seven days and infected with velogenic Newcastle disease virus (vNDV). [PT = prophylactic treatment. PI = post infection]

Groups	Means of serum uric acid levels (g/dl) ± standard error			
	Day 0	**Day 7 of PT**	**Day 3 P1**	**Day 6 PI**
Group A	3.82 ± 0.38	4.81 ± 0.30	4.51 ± 0.79 ^{ab}	13.57 ± 0.31 ^{a}
Group B	3.97 ± 0.26	5.46 ± 0.75	5.86 ± 0.82 ^{b}	11.71 ± 1.49 ^{a}
Group C	3.86 ± 0.29	4.78 ± 0.69	3.09 ± 0.28 ^{a}	5.90 ± 0.40 ^{b}
Group D	4.11 ± 0.71	4.91 ± 0.04	3.98 ± 0.66 ^{a}	6.66 ± 1.96 ^{b}
Group E	3.71 ± 0.37	3.87 ± 0.22	4.41 ± 0.32 ^{ab}	3.53 ± 0.30 ^{c}

$^{a, b, c,}$ Different alphabetical superscripts in a column indicate significant differences between the mean uric acid levels of the groups, $p < 0.05$

*** Groups:** Group A – 300 mg/kg MLEAO + Infection; Group B – 150 mg/kg MLEAO + Infection; Group C – 75 mg/kg MLEAO + Infection; Group D – Untreated Infected Control; Group E – Untreated Uninfected Control.

Table 2: Serum creatinine levels of chicken groups* treated with varied prophylactic doses of methanol leaf extract of *Anarcadium occidentale* (MLEAO) for seven days and infected with velogenic Newcastle disease virus (vNDV). [PT = prophylactic treatment. PI = post infection]

Groups	Means of serum creatinine levels (g/dl) ± standard error			
	Day 0	Day 7 of PT	Day 3 P1	Day 6 PI
Group A	0.28 ± 0.04	0.29 ± 0.03	0.33 ± 0.02	0.22 ± 0.04
Group B	0.28 ± 0.03	0.31 ± 0.04	0.35 ± 0.01	0.27 ± 0.07
Group C	0.29 ± 0.04	0.30 ± 0.03	0.33 ± 0.06	0.30 ± 0.06
Group D	0.28 ± 0.04	0.29 ± 0.03	0.28 ± 0.04	0.23 ± 0.06
Group E	0.27 ± 0.03	0.30 ± 0.03	0.27 ± 0.01	0.27 ± 0.09

No significant differences between the mean serum creatinine levels of the groups, $p > 0.05$

*** Groups:** Group A – 300 mg/kg MLEAO + Infection; Group B – 150 mg/kg MLEAO + Infection; Group C – 75 mg/kg MLEAO + Infection; Group D – Untreated Infected Control; Group E – Untreated Uninfected Control.

Table 3: Serum calcium levels of chicken groups* treated with varied prophylactic doses of methanol leaf extract of *Anarcadium occidentale* (MLEAO) for seven days and infected with velogenic Newcastle disease virus (vNDV). [PT = post prophylactic treatment. PI = post infection]

Groups	Means of serum calcium levels (g/dl) ± standard error			
	Day 0	Day 7 of PT	Day 3 P1	Day 6 PI
Group A	9.19 ± 0.08	7.81 ± 0.30 ^{a}	8.70 ± 0.65	8.32 ± 0.61 ^{a}
Group B	8.96 ± 0.11	8.69 ± 0.25 ^{abc}	8.84 ± 0.21	8.76 ± 0.13 ^{ab}
Group C	9.28 ± 0.13	9.50 ± 0.25 ^{bc}	8.66 ± 0.23	9.00 ± 0.43 ^{ab}
Group D	8.98 ± 0.63	9.11 ± 0.76 ^{bc}	9.00 ± 0.67	9.25 ± 0.42 ^{ab}
Group E	9.10 ± 0.08	9.81 ± 0.24 ^{c}	9.50 ± 0.23	9.74 ± 0.19 ^{b}

$^{a, b, c,}$ Different alphabetical superscripts in a column indicate significant differences between the mean calcium levels of the groups, $p < 0.05$

*** Groups:** Group A – 300 mg/kg MLEAO + Infection; Group B – 150 mg/kg MLEAO + Infection; Group C – 75 mg/kg MLEAO + Infection; Group D – Untreated Infected Control; Group E – Untreated Uninfected Control.

Table 4: Serum phosphorus levels of chicken groups* treated with varied prophylactic doses of methanol leaf extract of *Anarcadium occidentale* (MLEAO) for seven days and infected with velogenic Newcastle disease virus (vNDV). [PT = post prophylactic treatment. PI = post infection]

Groups	Means of serum phosphorus levels (g/dl) ± standard error			
	Day 0	Day 7 of PT	Day 3 P1	Day 6 PI
Group A	6.41 ± 0.16	5.78 ± 1.10	4.53 ± 0.36 [a]	6.91 ± 0.39 [ab]
Group B	5.99 ± 0.07	5.34 ± 0.77	5.10 ± 0.32 [ab]	5.55 ± 0.41 [a]
Group C	6.52 ± 0.15	5.62 ± 0.48	5.46 ± 0.80 [ab]	5.32 ± 0.34 [a]
Group D	6.31 ± 0.72	6.54 ± 0.68	5.52 ± 0.97 [ab]	8.00 ± 0.63 [b]
Group E	6.23 ± 0.24	6.64 ± 1.75	6.17 ± 0.38 [b]	6.25 ± 0.36 [ab]

[a, b,] Different alphabetical superscripts in a column indicate significant differences between the mean phosphorus levels of the groups, $p < 0.05$

*** Groups:** Group A – 300 mg/kg MLEAO + Infection; Group B – 150 mg/kg MLEAO + Infection; Group C – 75 mg/kg MLEAO + Infection; Group D – Untreated Infected Control; Group E – Untreated Uninfected Control.

Discussion and Conclusion

Serum biochemical parameters are well known indicators of the cellular integrity and markers of vital organ function in the body (Burtis *et al.,* 2008; Kaneko *et al.,* 2008). The significantly higher serum AST activity recorded for chickens in groups A and B on days 6 PI (compared to Group E) indicates that the administration of high doses (300 and 150 mg/kg) of the extract in combination with the virus challenge adversely affected the integrity of either the liver and/or the muscles, which are the principal organs with high levels of AST activity (Stockham and Scott, 2008), while the significantly lower activity of this enzyme (AST) in the group C chickens (75 mg/kg) at both day 7 of PT and day 6 PI are indicators that at this dosage MLEAO has a membrane-stabilizing effect (Stockham and Scott, 2008). The effects recorded for the chicken groups given high dose of MLEAO as per serum AST activity in this study is in agreement with the reports of Okonkwo *et al.* (2010) on albino rats given high doses of cashew stem bark extracts and the reports of Iyare *et al.* (2017) and Okonkwo *et al*. (2018) both of whom reported that high doses of leaf extract of *A. occidentale* led to significantly elevation of serum AST activity in rats. The membrane stabilizing effect recorded in this study for the group C given 75 mg/kg concurs with what had been reported to occur on administration of some drugs and nutraceutical substances (Waner *et al*., 1990; Ochiogu *et al.,* 2014).

The significantly lower serum ALT activity recorded for group A chickens on day 3 PI when compared to Group E is a pointer that this high dose MLEAO (300 mg/kg) may have a specific membrane stabilizing effect of the hepatocytes or that the combination of the high dose and the viral challenge may be depressing ALT activity in the hepatocytes, since serum ALT activity is nearly liver-specific (Stockham and Scott, 2008). This finding in the present study of a significantly lower serum ALT activity in the group given the highest dose contrasts with the reports of Okonkwo *et al.* (2010), Iyare *et al*. (2017), and Okonkwo *et al.* (2018) who reported elevations of ALT activity in serum when high doses of *A.*

occidentale extract was given, but is in agreement with the reports of Tedong *et al.* (2007) who reported significantly lower ALT activity in mice given high doses of hexane leaf extract of *A. occidentale*.

Alkaline phosphatase in serum is derived from hepatobiliary epithelium and the bones (Yong, 1967; Sharma *et al*., 2014). The significantly lower serum ALP activity in group A chickens on day 7 of PT and in groups A, B, C and D on day 6 PI suggests that the administration of the extract alone at 300 mg/kg and its interaction with the infection on day 6 PI had an adverse effect on the biliary epithelium and/or bone sources of ALP, because in contrast to the transaminases, ALP is an inducible enzyme produced actively by cells rather than leaked out as a result of membrane permeability factors (Burtis *et al*., 2008; Stockham and Scott, 2008).

Serum levels of total proteins and albumins are markers of hepatic synthetic function/ability and renal glomerular barrier function (Burtis *et al.,* 2008; Kaneko *et al.,* 2008). The significantly lower serum total protein and albumin levels recorded in this study for chickens in groups A and B given 300 and 150 mg/kg of MLEAO, respectively suggests that administration of the extract at these doses negatively affected hepatic protein synthesis and/or renal glomerular barrier function (Burtis *et al*., 2008; Kaneko *et al.,* 2008). The finding in this study of a significantly lower serum total protein in chickens given high doses of MLEAO concurs with the reports of Tedong *et al.* (2007) on mice given higher doses of hexane leaf extract of *A. occidentale*, though Okonkwo *et al.* (2010) reported no significant effects on serum proteins in rats given stem bark extracts of *A. occidentale*.

The level of globulins in serum is a reflection of mainly the levels of antibodies in blood, because most of the globulin in blood is immunoglobulin (Burtis *et al.,* 2008; Kaneko *et al.,* 2008). The significantly lower globulin levels recorded for chickens in groups A, B and D on day 6 PI suggests that the interaction of the extracts at high doses with the infection and the infection itself led to significantly severe depreciation of globulins, but the significantly higher globulin levels recorded for chickens in group C, which compares favorably with that of the untreated uninfected group E implies that at this dose (75 mg/kg), globulin synthesis was enhanced enough to overcome the depreciation that occurred in the infected untreated group. There are no reports in available literature on the effects of *A. occidentale* extracts on globulin levels of treated animals or humans.

Serum uric acid and creatinine levels are used as markers of kidney function in avian species (Hochleithner, 1994). The significantly higher serum uric acid levels in groups A, B, C and D, which was dose dependent in the treated groups suggests that the treatment with the extract had an adverse effect on the kidney function of the birds, especially at the higher doses of 300 and 150 mg/kg which were further significantly higher than that recorded for groups C (75mg/kg) and D (untreated infected control). This finding does not concur with the reports of Edet *et al.* (2013) and Iyare *et al*. (2017) both of whom reported no effects of administration of *A. occidentale* extracts on the histology of the kidney. It should however be noted that adverse effects on organ function had been reported to occur without any observable histopathological alterations (Burtis *et al.,* 2008; Kaneko *et al.,* 2008). The lack of significant variations in the serum creatinine levels all through the study may not be unrelated to the reported relative insensitivity of serum creatinine levels as marker of kidney injury in avian species (Hochleithner, 1994).

The significantly lower serum calcium and phosphorus levels recorded for chickens in group A on day 7 of PT and significantly lower serum calcium levels on day 6 PI implies that treatment at this higher dose (300 mg/kg) depressed serum calcium and phosphorus levels. Although there is a report in available literature on the significant lowering of serum phosphorus levels following experimental Newcastle disease virus infection in chickens (Igwe *et al*., 2018) which is in agreement with the finding in this present study in the infected groups, there are however no reports on the effects of *A.*

occidentale extract administration on serum calcium and phosphorus levels.

Based on the results of this study, it was concluded that treatment of chickens with MLEAO at a higher dose of 300 mg/kg and to a lesser extent at 150 mg/kg led to adverse serum biochemical outcomes such as significant elevation of serum AST activity and levels of serum uric acid, and depression of serum ALP activity and levels of serum proteins, albumins, globulin, calcium and phosphorus, while its administration at the low dose of 75 mg/kg produced positively stabilized membrane permeability and enhanced total protein and globulin synthesis. The use of MLEAO at higher doses for prophylactic therapy is not recommended.

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Conflict of Interest

The authors declare no conflict of interest

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