PROPHYLACTIC POTENCY OF METHANOLIC EXTRACT OF *Momordica balsamina L.* AGAINST AVIAN PARAMYXOVIRUS-1 INFECTION IN BROILER CHICKENS

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SUMMARY

The potency of Momordica balsamina extract against avian paramyxovirus-1 (Newcastle Disease Virus) (Kudu 113) was investigated in this study. Thirty, 5-week-old broiler birds were used for the experiment and were grouped in a completely randomised design with each group consisting of 5 birds. Group, I was a positive control, and group II was the negative control. The extract was administered prophylactically at doses of 200, 250, 300 and 350 mg/kg bw to groups III to VI respectively. Thereafter, all the groups were challenged with the strain of ND (Kudu 113) virus on seventh week to induce infection. The birds were monitored for 21 days for the symptoms of Newcastle Disease. No hemorrhagic lesion on the liver of all the groups administered with the extract was observed except group III which was administered 200 mg/kg BW of the extract. The mortality rate was 20% in group VI and 40% in group V. The clinical sign score was between 1 and 2 in groups III to VI and there was a significant difference (p<0.05) in the level of cholesterol and Alkaline Phosphatase (ALP) in the groups administered with the extract and the positive control (Group I) though there were no significant differences (p>0.05) in the level of Aspartate transaminase (AST). Low levels of serum ALP and Alanine transferase (ALT) were also observed in the groups administered with the various doses of the extract compared to the positive control. The study demonstrated the potency of Momordica balsamina extract against the avian paramyxovirus -1 in the experiment.

Keywords: Avian paramyxovirus-1, Broiler chicken, Methanolic extract, Momordica balsamina, Vaccine

INTRODUCTION

Newcastle disease has been one of the major important viral diseases of poultry which has caused huge economic losses to farmers (Muhammad *et al.*, 2015). Newcastle disease is a transmissible and pandemic genic disease that has the potential for very rapid spread. Loss of, or reduced international trade of animals and animal products is the major socioeconomic consequence of this disease (Mustafa and Ali, 2005 and Cheema *et al.*, 2011). This disease is caused by Avian-paramyxovirus-1 (APMV-1) which is a single-stranded, non-segmented, negative-sense RNA virus (Okwor *et al.*, 2007). Irregularities in the respiratory, nervous and digestive systems are the most common symptoms exhibited by birds affected by this disease (Okwor *et al.*, 2007).

The strain of the causative organism (virus) determines to a great extent, the rate of mortality due to this disease while the severity of the disease to a greater extent is determined by the age, immune status and the ability of bird to resist the disease (Okwor and Eze, 2010). Among the avian species, chickens are the most susceptible to Newcastle disease (Vyslouzil and Dhonal, 1988). Direct transmission from diseased to healthy birds through oral and respiratory routes is the most common mode

of transmission of the virus. The spread of this virus in high occurrences is mostly through the faecal droppings, nasal discharge, lacrimal discharge and exhaled air of the infected birds (Nawanta *et al.*, 2008).

High mortality and morbidity, decreased egg production from layer and/or production of eggs of low quality from breeder flocks are some of the economic losses due to Newcastle disease. Extra management of poultry flocks along with the treatment cost during the course of the disease contributes largely to the economic losses incurred (Siddique *et al.*, 2012).

Several diseases in animals such as diarrhoea, mange, retained placenta, coccidiosis, Newcastle diseases, gastroenteritis, foot and mouth diseases, and Fowl pus among others are healed with the aid of medicinal plants. The presence of some chemical substances called bioactive molecules or ingredients from plants extract confers on them their values as potential remedies to disease infections (Olanipekun, 2014; Olanipekun and Tedela, 2013). Okwu (1999; 2001), reported on the predominance reliance of about 80% of the world's population on plants and plant extracts for healthcare. More so, of the top 150 proprietary drugs used in USA, 57% contain at least one major active compound currently or once derived

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from plants. Renewed interest in the use of medicinal plants in the treatment of diseases has been advocated in recent times, as the health care system increasingly utilized herbal preparations to treat various animal diseases especially poultry (Olanipekun, 2014).

Momordica balsamina L. (Cucurbitaceae) is popularly known as Balsamina Apple in the tropical regions of Africa. It is locally known as Balsamina apple (English), Garahuni (Hausa), Akbon-ndewe (Igbo) and Ejirin (Yoruba) (Burkil, 1985). It is also known as Laloentjie (Afr.); Mohodu (Sotho); Nkaka (Thonga); etc. M. balsamina is a climber or trailer with stems attaining 4-5mm in length (Burkill, 1985 and Olanipekun, 2014). The climbing stem is a glabrous to slightly hairy, tendril-bearing annual or perennial climber (10- 12" tall) (Hutchinson and Dalziel, 1954) while seeds are embedded into a sweet edible red fleshy pulp test like water-melon (Welman, 2004). The Balsamina Pear flowers and fruits flourish throughout the year, but mainly from October to May (Northern Hemisphere) (Trivedi and Rov. 1972).

The focus of this study is, therefore, to explore the alternative means of managing Newcastle disease in broiler chickens using the most available, easily accessible and readily affordable floral and faunal resources that abound in the environment in most of the sub-Saharan Africa. In addition to that, the probability of introducing a potential new drug to which the microbes have not developed resistance is very likely.

MATERIALS AND METHODS

Experimental Site:

The research was conducted at the Teaching and Research Farm Unit of Federal College of Wildlife Management, New Bussa, Niger State, Nigeria. The experimental station sits at 9°53'N, 9.88°N and 4°31'E, 4.52°E (NIPOST Archives, 2009). The research work was carried out between the Months of May to July (the early part of the rainy season).

Experimental animals:

Forty, broiler day-old chicks were purchased and raised under good and hygienic management under a deep litter system with a floor space of 0.14 sqm2/ bird. The chickens were brooded together for five weeks to enable them to obtain a source of heat. The heat was provided using two (2) 200-watt electric bulbs. On the 14th day of the arrival, the birds were vaccinated orally against infectious bursal disease (Gumboro vaccine) using an intermediate strain (Biovac®) but not against Newcastle disease and they were managed on broiler starter diet feed and water was supplied *ad-libitum* until they attain five weeks of age before the commencement of the experiment.

Plant Materials:

The plant (*Balsamina* apple) was made available from the surrounding Federal College of Wildlife Management, New Bussa, and the surrounding villages and it was identified with the aid of keys described by (Burkill, 1985; Olanipekun, 2014) and was confirmed in the Herbarium of Forestry Research Institute of Nigeria (FRIN).

Preparation of plant extract:

The whole plant sample was sliced and chopped into pieces to hasten to dry. It was then pulverized using Pestle and Mortar. Cold extraction was performed using methanol as solvent. The preparation of the crude extract was based on the method described by Garba *et al.* (2018): Fifty grams (50g) of the dried sample was pulverized to powdered form and cold extracted in 700 ml of methanol. Extraction lasted for 48 hours. The extract was filtered using a muslin cloth and the solvent was removed and recovered using a rotary evaporator. The extract was then transferred into a sterile universal bottle and stored at 4°C until required for use. The yield of the extract was 30g of sample.

Phytochemical analysis:

The phytochemical analysis (Resin, alkaloids, phenols, tannins, terpenoids, saponin, flavonoids, anthraquinone, steroid, glycosides, and carbohydrates) of the extract of *Momordica balsamina* was carried out based on colouration and precipitation test as described in the methods of Sofowora (1982) and Trease and Evans (2002).

Standard Drug:

The standard (Newcastle Disease) ND vaccine (LaSota) under the trade name Biovac[®] and live strain velogenic NDV strain of Kudu 113 were purchased from State Veterinary Clinic, Bosso, Minna.

Cyclic Prophylaxis and infection of animals:

Thirty (30) healthy broiler birds were selected at the fifth week of age and randomly grouped into six (n =5) (i.e. I-VI). Group I was the positive control (vaccinated through oral administration of 0.2 ml of the La Sota strain (Biovac®) of ND vaccine to each bird, group II was the negative control (neither vaccinated with the LaSota nor administered with the extract) while Group III-VI were administered the methanol extract of *M. balsamina* at 200, 250, 300 and 350 mg/kg body weight per bird, respectively, at 48 hours intervals for six consecutive days:

Group I Positive (vaccinated with LaSota strain (Biovac®)

Group II Negative control (unvaccinated)

Group III 200 mg/kg BW

Group IV 250 mg/kg BW

Group V 300 mg/kg BW

Group VI 350 mg/kg BW

Thereafter, all the groups were then challenged with the strain of ND (Kudu 113) virus on seventh week and the experimental birds in group I-VI were routinely monitored for the manifestation of any of the symptoms as specified in the clinical signs score scale (Table -1) (Gaymary, 2013).

Score	Clinical signs
1 – Normal	No sign (healthy chickens)
2 - Mild	Depression, anorexia (reduced feed intake)
3 – Moderate	Depression, anorexia, greenish diarrhoea
4 – Severe	All signs mentioned above together with anorexia, greenish diarrhoea, difficult breathing (dyspnoea), raised body temperature (> 42° C) and
	loss of bodyweight emaciation and/or death

Table 1. Clinical signs score scale

Source: Gaymary, (2013)

The observation and scoring were done between the first week of trial to the third week and finally one-week post-treatment.

Post-mortem pathological examination of some key organs:

On day 29th of the trial, the final mean weight of the animals in each group was determined, while two animals from each treatment group were randomly selected and sacrificed. They were de-feathered and carefully dissected under the strict instruction and guidance of a veterinary doctor. The weights and lesion characteristics of some vital organs such as the heart, spleen, liver, lungs, intestine, thigh, proventriculus, abdomen and breast muscle were documented and compared with the positive and negative controls.

Blood Biochemical analysis:

Blood samples were collected via wing vein of birds into tubes without anticoagulant and the serum analysis including blood glucose, alkaline phosphatase (ALP), Serum albumin, total protein, cholesterol and blood urea was carried out according to the methods described by Tietz *et al.* (1995). The activities of transaminase (AST and ALT) were determined as described by Reitman and Frankel (1957) while the level of serum total proteins was estimated using biuret in accordance with the method described by Burtis *et al.* (1999). The level of serum urea was assayed as described by Veniamin and Vakirtzi (1970).

Statistical analysis:

Values were calculated for each analysis as mean \pm SEM where significant differences between the groups were determined using analysis of variance (ANOVA), while post-test analysis was conducted with Duncan's multiple comparison tests. Values were regarded as statistically significant when p < 0.05 (Snedecor and William, 1994).

RESULTS

Phytochemical composition of M. balsamina:

The phytochemicals from methanolic extract of the *M. balsamina* plant appeared to contain all the tested polar substances while the non-polar substances such as anthraquinones, steroids and terpenes either are absent or present in low amounts (Table 2).

 Table 2. Phytochemical composition of Methanolic extract of Momordica balsamina

Compound	Present
Resins	+
Alkaloids	+
Tannin	++
Flavonoids	+
Saponin	+ +
Anthraquinone	Trace
Glycosides	+
Carbohydrate	+ +
Steroid	Trace
Terpenes	Trace

Key: + = Low amount; ++ = Moderate; +++ = Abundant

Clinical observations:

All chickens were monitored daily for appearance and disappearance of clinical symptoms of ND. Clinical signs were assessed using a body condition score scale of 4 points in which 1 indicated normal healthy chickens and 4 indicated severely affected chickens as previously presented in Table 1. Despite the observed lesions on some visceral organs due to the administration of the extract, the mortality rate was found to be 60%, 40% and 20% mortality was recorded in group II as well as the groups administered V (300 mg/kg BW) and VI (350 mg/ kg BW), respectively while no mortality was recorded in the remaining groups including positive group (Table 3).

Treatment	Body condition scores	Chickens in the treatment	Mortality rate
Positive control	1	n =5	0%
Negative control	3	n =5	60%
III (200 mg/Kgbw)	1	n =5	0%
IV (250 mg/Kgbw)	1	n =5	0%
V (300 mg/Kgbw)	2	n =5	40%
VI (350 mg/Kgbw)	1	n =5	20%

Table 3. Body condition scores and mortality rates of chickens with experimental ND infection after 14 days post-treatment with methanolic extract of *M. balsamina*

Body weight observed in the experimental birds administered various doses of Momordica balsamina methanolic extract:

The administration of various doses of the extract showed a significant (p<0.05) weight gain for experimental birds in groups III and VI when initial

and final growth were compared (Table 4). There were no significant weight gains in groups IV and V. Loss of weight was further observed in some birds in group V as a final weight of 0.64 ± 0.03 was recorded against the initial weight of 0.77 ± 0.04 .

Table 4. Body weight observed in the experimental birds administered various doses of *Momordica* balsamina methanolic extract

Treatment	Initial body weight (kg)	Final body weight (kg)
Group I (Positive Control)	$0.75^{a}\pm0.03$	$0.94^{b}\pm0.04$
Group II (Negative Control)	$0.72^{a}\pm0.07$	$0.77^{a}\pm0.04$
III (200 mg/Kgbw)	$0.77^{a}\pm0.04$	$0.89^{b}\pm0.06$
IV (250 mg/Kgbw)	$0.78^{a}\pm0.05$	$0.83^{a}\pm0.09$
V (300 mg/Kgbw)	$0.62^{a}\pm0.02$	$0.64^{a}\pm0.03$
VI (350 mg/Kgbw)	$0.76^{a}\pm0.04$	$0.87^{b}\pm0.02$

Note: Mean \pm SD with the same superscripts within each row indicate no significant variations among treatments, otherwise it differs at (p<0.05).

Changes in the organ's weight across a different group of the experimental animals:

The weight of the heart, spleen and liver of the experimental birds in group III is comparatively smaller than all other groups with groups I (positive control) and VI having the biggest hearts. There was no significant difference (p<0.05) in the heart weight of animals in group II and V. Spleen weight in group

I, IV and V were not significantly different (p<0.05) while liver weight in all groups also showed no significant variation (though enlarged liver was observed in group IV). Gizzards of the experimental birds show increasing correlations to the increasing doses of *M. balsamina* methanolic extract (see Table 5).

Organs	Group I (Positive	Group II (Negative control)	Group III	Group IV	Group V	Group VI
	control)					
Heart	$3.20^{\circ} \pm 0.60$	$2.60^{bc} \pm 0.30$	$1.80^{a} \pm 0.10$	2.30 ^{ab} ±0.60	$2.70^{bc} \pm 0.20$	3.00° ±0.13
Spleen	$0.40^{b} \pm 0.01$	$0.90^{d} \pm 0.03$	$0.20^{a} \pm 0.01$	$0.40^{b} \pm 0.03$	$0.40^{b} \pm 0.02$	$0.70^{\circ} \pm 0.01$
Liver	$10.30^{a}\pm1.02$	9.90 ^a ±0.21	$8.80^{a} \pm 0.14$	$10.60^{a} \pm 1.21$	$9.3^{a} \pm 1.02$	$9.9^{a} \pm 0.97$
Gizzard	$31.00^{a} \pm 4.20$	$30.20^{a} \pm 3.23$	$33.00^{a} \pm 2.45$	$42.2^{b} \pm 3.65$	$40.2^{b} \pm 2.18$	$46.5^{b}\pm3.75$

Note: Mean \pm SD with the same superscripts within each row indicate no significant variations among treatments, otherwise, it differs at (p<0.05).

Post-mortem examination of experimental animals administered methanolic extract M. balsamina:

It was grossly observed from the study that group I (the positive control) birds were observed to have a normal spleen and uncongested lungs while no haemorrhage on the heart, liver, thigh and proventricular though pinpoint haemorrhage was seen along the intestine.

Group VI with 350 mg/kg BW doses of methanolic extract of *M. balsamina* brings the most similar results to the positive control in group I with

no hemorrhagic lesion seen in the heart, liver, proventricular and intestines though pale spleen and congested lungs were observed in the experimental birds in the group.

Administration of the extract at a dosage of 200 mg/ kg BW (group III) showed haemorrhageic lesions with a pale appearance of the liver and also hemorrhage in the heart and a few areas of pinpoint haemorrhage along the intestine. Congestion of the lungs in groups II and IV has been recorded (Table 6).

Duisamina						
Organs	Groups					
	I (Positive control)	II (Negative control)	III	IV	V	VI
Heart	No hemorrhagic lesion	Hemorrhagic lesion seen	No hemorrhagic lesion	Fat deposition around the heart	Fat deposition around the heart.	No fat deposition or Hemorrhagic lesion seen
Spleen Liver	Normal spleen No haemorrhagic lesion	Pale spleen Haemorrhagic lesions saw	Normal spleen Haemorrhagic lesion and looks pale	Normal spleen Enlarged and pale	Normal spleen No Hemorrhagic lesions seen	Pale spleen No Hemorrhagic lesions seen.
Lungs	Uncongested lungs	Congested lungs	Uncongested lungs	Congested lungs	Uncongested lungs	Uncongested lungs
Intestines	Pinpoint hemorrhage along the intestine	Pinpoint hemorrhage along the intestine	Pinpoint hemorrhage along the intestine	Pinpoint hemorrhage along the intestine	No hemorrhage along the intestine	No hemorrhage along the intestine
Thigh	No hemorrhage	Pinpoint hemorrhage around the thigh muscle	No hemorrhage	No hemorrhage	Pinpoint hemorrhage around the thigh muscle	No hemorrhage
Proventriculus	No proventricular hemorrhage	No proventricular hemorrhage	No proventricular hemorrhage	No proventricular hemorrhage	No proventricular hemorrhage	No proventricular hemorrhage

Table 6. Post-mortem examination of Group III - IV animals administered methanolic extract of *M. balsamina*

Serum Biochemical components of birds administered various doses of M. balsamina methanolic extract:

In the negative control group (Group I), there were increases in serum biochemicals. There was a significant difference in the level of cholesterol and Alkaline phosphatase (ALP) in the groups supplemented with the extract and the positive control (Group I) though there were no significant differences in the level of Aspartate transaminase (AST). Low levels of serum Alanine transferase (ALT) were also observed in the supplemented groups with the various doses of the extract vs. the positive control group (p<0.05).



DISCUSSION

Over many years it has been known that Newcastle Disease has deferred all control measures and has remained a major infectious disease threatening the poultry industry (Shittu *et al.*, 2015). The result from this study indicated that administration of methanolic extract of *Momordica balsamina* at 200, 250, 300 and 350 mg/kgw portray tremendous pharmacological activities as shown on the body scale scores when compared with Gaymary's (2013) standard. No experimental extract was observed to go beyond scores 1 and 2 and the mortality rate was found to be 40% and 20% for 300 and 350 mg/kgw dosages, respectively. Though, not as efficacious as the standard drugs have proven to significantly reduce the effect of the ND virus and thus could be packaged as phytomedicines that can readily be available, accessible and affordable for rural farmers whose ability to come across standard drugs could be challenging (Ibrahim and Tanya, 2001). The ND vaccine (standard drug such as "Lasota") is seriously limited due to financial constraints among rural farming households (Abdul *et al.*, 2005).

The high mortality recorded for experimental birds in group V can be confidently attributed to acute toxicity of the extract considering the fact birds in the 350 mg/kg BW recorded only 20% mortality. However, inappropriate handling and uncertain environmental factors could be contributing factors (Van Limbergen *et al.*, 2020).

Administration of various plant extracts has shown no significant difference in the body weight of experimental birds in groups IV and V contrary to the significant growth observed in groups III and VI which compared well with both group I (positive control) and group II (negative control) (p>0.05). The increase in the body weight recorded in III and VI might be connected to the high total protein content in the serum of the experimental birds in these groups (Fig. 1). This is in consonance with Shams-ul-Hayat *et al.* (2022) who reported that increasing total serum protein is directly proportional to increased weight gain in broiler fed organic supplement.

There seem to be changed in the weight of some vital organs of the birds administered the methanolic extract of *M. balsamina* as there were significant differences between the gizzard and heart weight in group I (positive control) and the experimental groups. There was no significant variation between the weight of the spleen in group I experimental birds in group IV and V administered 250mg/ kg BW and 300mg/kg BW, respectively while variation was significant (p<0.05) in group II, III and VI (p<0.05). This is an indication of immune stability with the corresponding increase in dosage concentration.

Obtained results are clearly showing that the extract does not compromise the integrity of the hepatocytes (liver cells) as there has not been any significant difference between the liver weight of birds in the positive control and all other groups except II (Negative control), this can most likely be attributed to the physiology and genetic makeup of the animal since other higher concentration did not display such trends (p<0.05). This development has been clearly asserted by Gaymary (2013) that Newcastle Disease infection if untreated comes with pathological consequences. Post-mortem examination of the visceral organs revealed that the infected and untreated birds in group II had pathologically alteration on the liver while other organs such as the spleen, intestine and lungs are also affected though all doses of *M* balsamina also portray slight pathological effect on the organs. This post-mortem observation is similar to reports of Didacu et al. (2012) and Agang (2014) whose studies established that Newcastle Disease Virus (Kudu 113) strains are typically associated with hemorrhagic organs including liver, heart and intestine. However, the acute administration of M. balsamina at 350 mg/kg BW (group VI) appears not to considerably cause pathological injuries to these organs.

An observation was made in relation to the plasma protein concentration due to administration of this extract is the significant increase across all the groups administered the extract compared with the negative control (Figure 1) (p>0.05). Since the experimental animal was allowed access to water *ad*-*libitum*, this plasma protein increase may not be

attributed to high dehydration but rather hypperproteinaemia that may probably be induced by the extract as a result of possible properties they may have to induce B-lymphocyte or ability to produce monoclonal protein such as IgG, IgA, IgE and IgD (Shahzad *et al.*, 2011 and Shahen *et al.*, 2005). It is pertinent to state the non-existence of a significant difference (p<0.05) between cholesterol and ALP in the positive control and the groups administered the extract clearly show the relative safety of the subchronic administration of this extract.

It is noteworthy that there was a non-significant (p<0.05) difference in the concentration of AST in both the positive and all the groups administered the extracts. This is a clear indication that the extract causes very minimal (if any) injury or damage to the heart myocardial cells. An unusual increase in this enzyme is known to be a major sign of injury (Gao et al., 2015 and OIE, 2009). However, since a higher concentration of ALT and ALP is an indication of inflammation of the hepatocyte (Gao et al., 2015), the seemingly reduced value of these enzymes obtained is an indication of improved integrity of the hepatocyte (Iram et al., 2014). Though it cannot be said with certainty the precise mode of action of this extract, qualitative analysis of this extract shows the presence of flavonols, tannin among other phytochemicals (Table 2) and one of the flavons called naringin has been shown to play a significant role as an ND-viral agent (Bot et al., 2007). It was also observed by Arunkumar and Muthuselvam, (2009) that the narigin is converted in monogastric to naringenin which was found to be a potent ND-viral aglutinator. The presence of such compound in the flavonols found to be present in this plant might be attributed to the observed anti-NDV effect.

CONCLUSION

t suffices to state from the observation thus made, that, *Momordica balsamina* methanolic extract has the potential to be employed as a safe drug candidate, for ethnopharmacological interventions against the Newcastle Disease. Its availability, affordability and accessibility qualify further.

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Ethical approval:

Authors hereby declare that "Principles of Laboratory Animal Care" (NIH publication no. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate institutional ethics committee.

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فاعلية المعالجة الوقائية لمستخلص الميثانول من مومورديكا بلسمينا ضد الإصابة بفيروس باراميكس -١ الطيرى في دجاج التسمين

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تم التحقيق فى فاعلية مستخلص بلسم المومورديكا ضد فيروس بار اميسكو الطيور - ١ (فيروس مرض النيوكاسل) (كودو ١٢٣). فى هذه الدراسة تم إستخدام ثلاثين من طيور اللحم ذات عمر خمسة أسابيع، وتم تجميعها فى تصميم عشوائى كامل مع كل مجموعة مكونة من خمسة طيور. إستخدمت المجموعة الأولى كمجموعة ضابطه سلبية. تم إعطاء المستخلص بشكل وقائى بجر عات ٢٠٠، ٢٥٠، ٣٠٠، ٣٥٠ مجم/كجم من وزن الجسم للمجموعات من الثالثة حتى السادسة على التوالى. بعد ذلك تم تعريض جميع المجموعات لسلالة الفيروس المسبب لمرض النيوكاسل (كودو ١٢٠) فى منه من رون الجسم للمجموعات من الثالثة حتى السادسة على التوالى. بعد ذلك تم تعريض جميع المجموعات لسلالة الفيروس المسبب لمرض النيوكاسل (كودو ١٢٣) فى الأسبوع الساب لمرض النيوكاسل (كودو ١٢٠) فى الأسبوع السابع للحث على إحداث الإصابة. تم مراقبة الطيور لمدة ٢١ يوماً بحثاً عن أعراض مرض النيوكاسل. لم تكن هذاك آفه (كودو ١٢٣) فى الأسبوع السابع للحث على إحداث الإصابة. تم مراقبة الطيور لمدة ٢١ يوماً بحثاً عن أعراض مرض النيوكاسل. لم تكن هذاك آفه (ضرر) نزفية فى الكبد فى جميع المجموعات الني كامل مرض النيوكاسل. لم تكن هذاك آفه (كودو ١٢٣) فى الأسبوع السابع للحث على إحداث الإصابة. تم مراقبة الطيور لمدة ٢١ يوماً بحثاً عن أعراض مرض النيوكاسل. لم تكن هذاك آفه (ضرر) نزفية فى الكبد فى جميع المجموعات التى أعطيت المستخلص باستثناء المجموعة الثالثة التى تم إعطاؤها ٢٠٠ مجم/كجم من وزن الجسم من المستخلص. كان معدل الوفيات ٢٠٪ فى المجموعة السادسة، ٢٠ % فى المجموعة المحموعة المجموعة المحموعة المحموعات من الثالثة التى تم عدل الوفيات ٢٠٪ فى المجموعة المادسة، ٢٠ % فى المجموعة الكولسترول والفوسفاتيز القلوى (ALP) فى المجموعات من الثالثة التى ألمولى في مرامي والى محمولية الولى على المحموعات من الثالثة التى تربية معدل الوفيات ٢٠٪ فى المجموعة الأولى على الرغم من عدم وجود فروق ذات معرل (ALP) فى المحموعة الولى على الرغم من عدم وجود فروق ذات المحموعات المحموعات من الثالثة حتى المحموعة الولى على الرغم من عدم وجود فروق ذات معاول فى المحموعات المحموعة الولى على الرغم من عدم وجود فروق ذات معاولي فى الحمو مى مستويات الوسفاتيز القادى ويول ألى المحموم ما مع ملومو فى محاولي فى الح