

Inhibitory Property of Crude Ethanolic Extract of Harungana Madagascariensis Root on New Castle

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Abstract— It has been observed from the research work that Newcastle disease Virus has no cure and the prophylactic vaccines available are not potent enough to eliminate the Newcastle disease virus, thus, it is pertinent to carry out research on Newcastle disease virus in order to proffer solution to the menace. The aim of this study is designed to; determine the inhibitory property of ethanolic extract of *Harungana madagascariensis* root on Newcastle disease virus in chicks, determine the histopathology of the extract on the chicks and to equally determine the number of organic components of the extract. The inhibitory property of the crude ethanolic extract *Harungana madagascariensis* roots on Newcastle disease virus was studied in two weeks old chicks. 0.2ml of LD50 concentration of the virus was injected into the chicks intramuscularly. An hour later, after the infection of the Newcastle disease virus, they were fed with graded concentration of the extract (10mg/ml, 50mg/ml, 100mg/ml and 250mg/ml) and were observed for inhibitory/antiviral activity for 24-96 hours. Significant inhibitory property was observed ($P < 0.05$) at the concentration of 100mg/ml of the extract. Therefore, the result shows that crude ethanolic extract of *Harungana madagascariensis* has inhibitory property at the concentration of 100mg/ml and the extract did not have any toxicological effect. Further studies should be done on the inhibitory property of the plant at other concentrations and purification of the plant for its chemical constituents should equally be studied.

Index Terms— Crude ethanolic extract, *Harungana madagascariensis*, Inhibitory property, New castle

I. INTRODUCTION

Harungana madagascariensis is a species of flowering plants in the family Clusiaceae and the sole member of the genus *Harungana*. English (orange-milk tree, blood tree); Hausa (alillibar); Igbo (ururtu); Yoruba (elepo). It is found in tropical Africa (including Madagascar) and Mauritius. *H. madagascariensis* occurs at medium top low attitudes in evergreen forest, at forest margins and along river and stream banks^[14]. It is a bushy tree which is 4-7m in height, sometimes reaching 10-25m; it is much branched with a cylindrical trunk. Fruits are berry-like (drupe), 2-4mm in diameter, greenish-orange becoming red when matured^[4]. Leaves are opposite to each other, simple, ovate or ovate elliptic. Artificial propagation is carried out using seedlings and pre-sowing is not necessary. Wildings can also be used, or

seeds can be sown directly at the planting site^[13]. The plant has a red sap which is used as a treatment for ringworm in Liberia^[12]. Different parts of the plant *Harungana madagascariensis* are employed in African folk medicine for the treatment of a wide spectrum of veterinary and human diseases. The red juice from the plant leaves and stem bark are reputed for arresting post-partum or post-abort bleeding in Sierra Leone, while the unopened buds are equally reputed for treating puerperal infection^[7]. In Ghana, the plant stem bark is employed in treating skin diseases and as dressing materials for wound^[12]. Amongst the Yoruba herbalists, the plant which is locally known as “elepo” is equally employed for the treatment of drug related liver and kidney poisonings. The boiled water decoction of the plant root is believed to neutralize the toxic activities of ingested poisons and restores deranged hepatic and renal functions to normal^[1]. However, despite the ancestral use of the plant water decoction in the management of drug-related renal lesion, there is paucity in the scientific validation of this therapeutic usage^[8]. The antimicrobial and antiviral activities of different extracts of *H. madagascariensis* were tested *in vivo* and *in vitro*. The LD50 of the extracts were obtained^[5]. The virus of Newcastle disease is classified within the genus paramyxovirus of the family paramyxoviridae. Members of this family have a single stranded, linear, RNA, with an elliptical symmetry. The total genome is roughly 16,000 nucleotides^[3]. Replication of the virus takes place in the cytoplasm of the host cell. The *in-exact* replication of the RNA will frequently produce variants with differences often subtle differences in phenotype from the parent particle^[6].

Newcastle disease is a serious and commonly fatal disease of chickens caused by Newcastle disease virus. Other avian species are also infected, but usually with less severe consequences^[11]. Both epidemic and endemic forms of Newcastle disease occur. Four broad clinical signs and symptoms (syndromes) are recognized by scientists. They are viscerotropic velogenic, neurotropic velogenic, mesogenic and lentogenic^[6]. Morbidity and mortality rates vary greatly depending on the virulence of the strain and susceptibility of the host. Lentogenic and mesogenic viruses usually kill few birds; in poultry, the mortality rate is approximately 10% for mesogenic strains and negligible with lentogenic strains^[2]. Concurrent illnesses may increase the severity of illness and result in a higher death rate. In contrast, velogenic isolates have morbidity and mortality rates up to 100% in unvaccinated chickens and 30% in vaccinated ones^[10]. Diagnosis of Newcastle disease can be done clinically and in the laboratory using the following test; haemagglutination inhibition and sequence technology, polymerase chain reaction and ELISA^[3]. There is no known treatment for Newcastle disease. Thermo stable Newcastle disease vaccines are now available that can give substantial protection to village flock against the disease. Other preventive measures

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include: Bio-security, proper hygiene and slaughtering of infected ones^[9].

Research hypothesis

1. Crude ethanolic extract of *Harungana madagascariensis* root did not have any inhibitory effect on the chicks.
2. Crude ethanolic extract of *Harungana madagascariensis* root possessed inhibitory activity on the chicks.
3. Crude ethanolic extract of *Harungana madagascariensis* root has antiviral activity on Newcastle disease virus.
4. Crude ethanolic extract of *Harungana madagascariensis* root does not have antiviral activity on Newcastle disease virus.

Purpose of the study

- i. Determine the inhibitory property of crude petroleum ether extract of *Harungana madagascariensis* root on Newcastle disease virus in chicks.
- ii. Determine the histopathology of the extract on chicks
- iii. Determine the number of organic components of the extracts

Materials and methods

Study area

The study was carried out in Microbiology Department of Federal Polytechnic Idah, Nigeria on the Eastern bank of the river Niger in the middle belt region of Nigeria. It lies between latitude 7°5'0"N and 6°45'0"E.

Preparation of plant extract

Freshly roots of *H. madagascariensis* were collected in March, 2011, after harvesting the root of the plant (*H. madagascariensis*) was chopped and air dried at room temperature in the laboratory for three (3) weeks. The dried root was pounded using a clean mortar and pestle. 100g of the plant was soaked in one (1) litre of crude ethanol (solvent) and the conical flask was covered with an aluminum foil and allowed to stand in the dark for 72 hours with constant agitation. The extract is then filtered through whatman number 24 filter papers. The filtrate was allowed to stand for 24 hours for the solute or colloid to settle. The supernatant was decanted and the extract was air dried by exposing it to the fan in a flat stainless steel plate. The dried solid extract was further broken into fine powder in clean enamel mortar.

Quality control test of the extract

The extract was tested for possible microbial contamination. The extract was mixed in small distilled water and plated on blood agar medium which was incubated for 24 hours at 37°C.

Preparation of phosphate buffer saline (pbs)

Phosphate buffer saline was used as a Buffer during virus titration.

The following are the procedure of its preparation.

1. A volume of 800ml of dd H₂O was measured with a graduated cylinder and transferred to a conical flask.
2. 8g of NaCl, 0.2g of KCl, 1.44g of H₂PO₄ and 0.2g of KH₂PO₄ were transferred into the flask.

3. The solutes were allowed to dissolve for 3-4 minutes.
4. The solution was continuously stirred to ensure that there was no particle left undissolved.
5. 1ml of HCL was added slowly drop wise with a transfer pipette and HCL was allowed fully dissolve.
6. The pH of the solution was measured with a calibrated pH meter.
7. The solution was allowed to attain a pH value of 7.4
8. The solution was poured into a fresh graduated cylinder and adjusted to the final volume of 1 litre with ddH₂O.

Virus titration/quantitation

The LD₅₀ titre of the virus was prepared and determined by the Avian Virus Research Unit of National Veterinary Research Institute (NVRI), VOM using Reed and Muench method. The chicks were infected with 0.2ml of 10⁻² dilution of the LD₅₀ concentration (10^{8.7} particles/ml) of the virus. This was injected intramuscularly into each group of the chicks.

Antiviral assay/examination

30 birds (chicks) were used and were grouped into 6 of 5. Three (3) groups served as control.

- a) First group serves as normal control and was fed only with distilled water.
- b) Second group serves as extract control and was fed with 250mg/ml of the extract only.
- c) The third group serves as virus control and was inoculated with the LD₅₀ concentration of the virus.
- d) The remaining groups were fed with 10mg/ml, 50mg/ml, 100mg/ml of the extract respectively and was observed for every 24hrs for 4 days (96hrs). The number of death for each extract concentration as well as its protective/inhibitory property was recorded.

II. TOXICOLOGICAL ASSAY/STUDY

The birds (chicks) administered with extract only and those that served as controls were sacrificed and their internal organs (kidney, liver, lung and parts of intestine) were harvested and preserved in 10% formalin and were sent to National Veterinary Research Institute (NVRI) VOM for histopathological examination.

III. RESULTS

Virus titre: The virus titre of the Newcastle disease virus (Viscerotropic velogenic strain) was determined to be 10^{8.7} particles/ml.

Table 1: Inhibitory property of crude ethanolic extract of *Harungana madagascariensis* root on Newcastle disease virus in chicks. After the infection of the chicks with the virus (exception of those meant for normal control and extract control) and an hour later, they were fed with the extract (exception of those meant for normal control and virus control). Day 1 (24hrs) none died in 10mg/ml of the extract, two (2) died at 48hrs (day 2), one (1) died at 72hrs (day 3) and none died at 96 hrs (Day 4) For that of 50mg/ml, none died on day 1 (24hrs), on the second day, 1 died and the rest of days none died. For 100mg/ml, none died in all the days (day 1, 2,

3 and 4) for the extract control, none died at all. The control no death was recorded on the 1st and 2nd days, but 4 and 1 died on the 3rd and 4th days respectively. Control, none died throughout the whole days

Route: Intramuscular administration..

Age of chicks: 14 days old (two weeks).

TABLE 1:

Time Group	17 th (Day1) 24 hours Mortality ratio	18 th (Day 2) 48 hours Mortality ratio	19 th (Day 3) 72 hours Mortality ratio	20 th (Day 4) 96 hours Mortality ratio	Total Mortality rate
10mg/ml	0/5	2/5	1/3	0/2	3
50mg/ml	0/5	1/5	0/4	0/4	1
100mg/ml	0/5	0/5	0/5	0/5	0
Extract control (250mg/ml)	0/5	0/5	0/5	0/5	0
Virus control	0/5	0/5	4/5	1/1	5
Normal	0/5	0/5	0/5	0/5	0

TABLE 2: EFFECT OF MORBIDITY RATE ON NEWCASTLE DISEASE VIRUS

Time	17 th (Day 1) 24 hours	18 th (Day 2) 48 hours	19 th (Day 3) 72 hours	20 th (Day 4) 96 hours	Total
Symptom	No. of chicks affected				
Dropping wings	0	4	2	1	7
Twisting of head and neck	1	2	5	0	8
Circling	0	4	3	0	7
Nervousness	3	1	2	0	6
Nasal discharge	2	4	3	1	9
Gasping form	1	3	3	1	8
Watery diarrhea	3	4	2	0	9
Muscular tremors	1	2	2	0	5

Base on morbidity rate of Newcastle disease virus, the total number of 7 chicks exhibited dropping of wings, 8 chicks exhibited twisting of head and neck, 7 chicks equally exhibited circling, 6 chicks extract exhibited nervousness, 9 chicks exhibited nasal discharge, 8 chicks exhibited gasping for air, 9 chicks exhibited watery diarrhea and 5 chicks exhibited muscular tremor (table 2).

IV. DISCUSSION

The crude ethanolic of *Harungana madagascariensis* exhibits inhibitory property on Newcastle disease virus on the two weeks old chicks at the concentration of 100mg/ml (table

1). At concentration of 50mg/ml the extract had little or no inhibitory property, at concentration 10mg/ml, there was no inhibitory property. This may be due to there low concentrations. At concentration 250mg/ml, which served as the extract control, no death was recorded ($p < 0.05$). These results showed that the ethanolic extract of *H. madagascariensis* at concentration 100mg/ml has inhibitory property oseeeee4eeeee Newcastle disease virus and has no any toxicological effect on the chicks. The result at concentration 100mg/ml could be compared to similar published work by^[18]. This result shows that the extract has antiviral property on the chicks. The plan can be said to be safe for animal and human consumption at minimal concentration in curing several ailments. Effects of Newcastle virus base on morbidity were observed in the chicks. There were some observable effects in their behaviour and physical appearance of the chicks during the days of observation (i.e. 1-4 days or 24hrs to 96hrs). Some of the chicks developed two (2) or more symptoms of Newcastle disease each ($P < 0.05$). The highest symptoms exhibited by the chicks are nasal discharge and watery diarrhea followed by twisting of head and neck and gasping for air. This is followed by circling and dropping of wings (Table 2). Similar study was carried out by Piacenti *et al.*, (2006) in California.

V. SYMPTOMS

New Castle Disease Virus affects the respiratory nervous and digestive systems. The incubation period for the disease ranges from 2 to 15 days. An infected bird may exhibit the following signs. Respiratory; Sneezing, gasping of air, nasal discharge, Digestives: greenish, watery diarrhea, nervousness, depression, muscular tremors, dropping wings, twisting of head and neck circling, complete paralysis, partial to complete drop in egg production and thin shelled eggs, sudden death (Linchuan and Hong, 1998).²⁴

VI. PREVENTIONS AND CONTROL

There is no known treatment for Newcastle disease. It can be controlled trough the following ways:

1. Good bio security can help to prevent ND in poultry flocks. It includes bird-proofing houses, feed and water supplies, minimizing travel on and off the facility, and disinfecting vehicles and equipment that enter the farm.
2. Vaccines are used in chickens, pheasants and other species to control the disease. This can protect birds from clinical. Signs but does not necessarily prevent virus replication and shedding.
3. Outbreaks are eradicated with quarantines and movement controls.
4. Proper carcass disposal. (Katin, 2005)^[9].

VII. CONCLUSION

The crude ethanolic extract of *Harungana madagascariensis* has inhibitory property/activity against Newcastle disease virus and had no toxicological effect on chicks.

The chicks showed some of the major signs and symptoms of Newcastle disease virus (Vescerotropic Velogenic strain). It can therefore be conclude that the plant has some inhibitory activities or properties on Newcastle disease Virus as

observed in the chicks ($p < 0.05$). Histopathological examination could not be completed due to lack of facilities.

RECOMMENDATIONS

Further research should be done on the leaves and the roots of *Harungama madagascariensis* using different solvents to determine antiviral activities.

The ethanolic extract of the plant should be screened against other viral diseases that have no treatment.

- The toxicology effects of ethanolic extract of the plant at other concentrations should be studied.
- It is therefore recommended for farmers use, either the crude or pure form.
- Purification of its chemical constituents should be investigated with the view of developing novel drug for human consumption.

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