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INHIBITORY PROPERTIES OF CRUDE ETHANOLIC EXTRACT OF *GOSSYPIUM HIRTUSUM* LEAVES ON NEWCASTLE DISEASE VIRUS (NDV) IN CHICKS

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Abstract: Newcastle Disease Virus (NDV) causes Newcastle disease in poultry and grows readily in embryonated eggs. Infection of poultry by NDV results in huge economic losses to farmers and prevention is only by means of vaccines which are not assessible and affordable and have also not protected the poultry industry from frequent outbreak of the disease. This necessitated the invention of an alternative control method, thus the antiviral activity of the ethanolic extract of *Gossypium hirsutum* leaves on NDV was studied using chicks. Extract of the plant was obtained by Soxhlet extraction. Antiviral study was carried out with Forty five (45) 8- weeks old chicks arranged into nine groups of five. 0.2ml of 10^{-2} dilution of the LD_{50} concentration ($10^{8.7}$ particles/ml) of the virus (Hertforthshire isolate) was injected into each chick in seven groups. The extract (10mg/ml, 50mg/ml, 100mg/ml, 150mg/ml, 200mg/ml and 250mg/ml) was fed to seven groups by gavaging. Three groups served as virus, extract and negative controls respectively. The chicks were observed for virus and extract activity for fourteen days. One chick died onday one in the group fed with 10mg/ml, while two deaths were observed on day two in the groups fed with 10 and 20mg/ml of extract respectively. No death was observed in any group afterwards. There was no significant difference in protection ($P<0.05$) and antiviral activity across the concentrations. The ethanolic extract of the plant was found to be non toxic ($p< 0.05$) to the chicks. Therefore, the ethanolic extract of *G. hirsutum* has antiviral activity ($p < 0.05$) on Newcastle disease Virus and it is safe for use on chicks. Further studies should be carried on the toxicological effect at other concentrations and the plant should also be screened for antiviral activity on other viral diseases.

Keywords: Inhibitory, *Gossypium Hirsutum,* Ethanolic Extract, Newcastle Disease Virus, Chicks

INTRODUCTION

Gossypium hirsutum (cotton plant) belongs to the family *Malvaceae*. It is a shrub of about 3m in height grown in South America, West Africa and China [1]. The plant requires heavy sunshine and well drained soil consisting of equal parts of loam and sand soil. The plant has many medicinal properties which includes; treatment of hypertension, diarrhoea, dysentery, headache and cancer [2]. Newcastle disease is a contagious and fatal viral disease affecting most species of birds [3]. The effects are most notable in domestic poultry due to their susceptibility. The clinical signs include; gasping, sneezing, nasal discharge and watery diarrhea and also the disease is transmitted by direct contact between healthy birds and infected ones. Newcastle disease has greatly affected the poultry industries in many countries causing huge economic losses or damages to the poultry industries or farmers [3]. There is need to find out the prevention and control since there is no treatment for (NDV) but use of prophylactic vaccines and sanitary measures which have not been very effective preventing and controlling

outbreaks[4]. Therefore, this project determined the inhibitory property and toxic potentiality of crude ethanolic extract of *Gossypium hirsutum* leaves on Newcastle disease virus in Issa brown cockerels.

MATERIAL AND METHODS

STUDY AREA

The study was carried out in the microbiology laboratory of the department of Science Laboratory Technology, Federal Polytechnic, Idah. Idah is the head quarter of Idah local government area of Kogi State. It is located along the Eastern bank of the River Niger, and has a land mass of 38km^2. It lies between latitude 7°56"N and 6°45'0"E. The daily temperature experienced in this region ranges form 20-30°C. The main language of the people is Igala and their major occupations are farming, fishing and petty trading. The soil nature of the land of Idah is mostly a sandy soil.

SAMPLE COLLECTION AND IDENTIFICATION

Fresh leaves of *Gossypium hirsutum* were collected from its natural habitat in Idah in Idah local government area of Kogi State, Nigeria and identified by Prof. C.U. Okeke, a plant taxonomist in the Department of Botany, Nnamdi Azikwe University, Awka, Anambra State, Nigeria. The voucher specimen of the plant was kept in the herbarium of Department Science Laboratory Technology, Federal Polytechnic Idah.

SAMPLE PREPARATION AND EXTRACTION

The sample was air-dried at room temperature for two weeks and pounded to a fine powder using clean mortar and pestle. It was stored in the dark at a temperature of about 28°C until used. About 100g of the plant was soaked in 1 litre of ethanol in a conical flask covered with aluminum foil and allowed to stand in the dark for 72 hours with constant agitation. The extract was filtered through Whattman No. 24 filter paper. The filterate was poured into flat stainless steel plates and dried in the hot air oven at 40°C for three days. The dried solid extract was further broken into fine powder in a clean enamel mortar. Quality control of extract was carried out by mixing it with a little distilled water and plated on blood agar, and incubating at 37°C for 24 hours and observed for microbial growth.

Source of Virus

Newcastle disease virus (Hertz i.e. Herthforthshire) isolate was obtained from the Regional Laboratory for Avian Influenza and other transboundary Avian viral diseases the National Veterinary Research Institute (NVRI), Vom and transported in cold chain to the Microbiology Laboratory of Science Laboratory Technology Department, Federal Polytechnic Idah. It was then stored at freezing temperature of 20°C until used.

Virus Titration

The titration was carried out to determine the LD_{50} (50% Lethal Dose) of the virus by the method of [5].

Antiviral Assay

Forty five (45) birds were grouped into nine of five birds per group. Three (3) groups served as controls (i.e. viral, extract and negative). The group of birds serving as virus control was injected with 0.2ml each of the LD_{50} concentration of the virus, while the group serving as extract control was fed with 250mg/ml of crude ethanolic extract of the sample. The group serving as

negative control was administered with sterile distilled water. The remaining six (6) groups were infected with 0.2ml of LD_{50} concentration of the virus, after which they were fed with different concentrations (10, 50, 100, 150, 200 and 250mg/ml) of the extract respectively. They were observed every 24 hours for 72 hours for mortality. The number of deaths for each extract concentration was recorded as well as the protective property of the extract.

Toxoicological Assay

The birds administered with extract only, and those that served as negative control were observed for signs of toxic potentialities.

Statistical Analysis

Data were analysed using One-way ANOVA at a confidence interval of 95% and a probability value (P value < 0.05).

RESULTS

Virus Titration

The virus titration for the determination of LD_{50} concentration was pre-determined by the Regional Laboratory for Avian Influenza and other trans-boundary Avian viral diseases the National Veterinary Research Institute (NVRI), Vom to be $10^{8.7}$ particles/ml prior to transporting to the Microbiology Laboratory, Science Laboratory Technology Department Federal Polytechnic Idah, Kogi State, where the LD_{50} concentration was determined to be $10^{7.6}$ particles/ml.

MORTALITY RATE OF CHICKS INFECTED WITH NEWCASTLE DISEASE VIRUS (NDV) AND FED WITH DIFFERENT CONCENTRATION OF THE ETHANOLIC EXTRACT OF *Gossypium hirsutum* LEAVES

On the first day after infection of the chicks with the virus, no death was observed in all the groups. One death was observed in only the group fed 10mg/ml 48 and 72 hours post infection. One death was observed 72 hours post infection in the group fed with 50mg/ml. No death was recorded in the groups fed with 100mg/ml, 150mg/ml, 200mg/ml and 250mg/ml respectively during the study period (table1). In extract and negative controls no death was recorded throughout the days of observation. In virus control one died on the first day, one died on the second day, two died on the third day and one died on the fourth day (table 1).

Table 1: Mortality rate of Chicks Infected with NDV and Fed with different Concentration of Ethonolic extract of *Gossypium Hirsutum* Leaves on NDV in Chicks

Concentration (mg/ml)	Day 1	Day 2	Day 3	Day 4	Total	% Protection
10	00	01	01	00	02	60
50	00	00	01	00	01	80
100	00	00	00	00	00	100
150	00	00	00	00	00	100
200	00	00	00	00	00	100
250	00	00	00	00	00	100
Virus control	01	01	02	01	05	00
250 extract control	00	00	00	00	00	100
Negative control	00	00	00	00	00	100
Total dead	**01**	**02**	**04**	**01**	**08**	**82.2**

MORBIDITY PRODUCED IN CHICKS INFECTED WITH NDV

Three (3) chicks lost their appetite, while one (1) chick showed sign of paralysis 24 hours post infection in the group assayed with 10mg/ml. By 48 hours, there was no sign of morbidity in all the chicks. On day three, two (2) chicks showed drooping of wings. On day four (4), one chick was observed with sign of twisting of neck (Table 2).

Table 2: Morbidity Rate Due to NDV Infection in Chicks

Symptoms	Day 1	Day 2	Day 3	Day 4	Total	% symptoms
Drooping of wings	00	00	02	00	02	28.6
Loss of appetite	03	00	00	00	03	42.9
Sneezing	00	00	00	00	00	00
Twisting of neck	00	00	00	01	01	14.3
Paralysis	01	00	00	00	01	14.3
Total	**01**	**00**	**02**	**01**	**07**	

DISCUSSION AND CONCLUSION

DISCUSSION

The observation of 60 – 100% protection of the birds from dying from Newcastle disease is an indication that ethanolic extract of *G. hirsutum* leaves has inhibited Newcastle disease virus from replicating in the tissues of their host (Table 1). There was no significant difference ($p < 0.05$) in the protective property of the extract at the concentration grades administered to the chicks. This therefore confirms that the ethanolic extract of *G. hirsutum* leaves at the concentrations used has antiviral activity ($P < 0.05$) on NDV. This result obtained in this study does not compare with the result obtained by [3] in a similar study. [6] Reported the presence of phytochemicals such as carbohydrates, saponins, steroids, glycosides, phenolic compounds such as tannins, flavonoids and gossypol which have earlier been reported to possess antiviral and antibacterial property [7, 8, 9]. The observation of no death or morbidity with the extract control group (table 1 and 2) showed that the ethanolic extract of *G. hirsutum* leaves does not have toxicological effects on the chicks ($P< 0.05$) at the concentration of 10mg/ml to 250mg/ml. The safety of the extract in tissue culture has been reported by [8] who observed that continuous exposure of the cells to extract for 5 days, resulted in the detection of cytotoxic effects leading to cell death as well as more subtle effects on the cells that may not be deleterious e.g. alteration of cell shape to a more rounded morphology at concentration higher than 0.079mg/ml. This therefore means that the extract could be used with no risk of organs damage. The signs and symptoms observed in this study were in agreement with the signs and symptoms of Newcastle disease [10].

CONCLUSION

The ethanolic extract of *G. hirsutum* L. does not have toxicological effects on chick at the concentration 200mg/ml and 250mg/ml and it also has antiviral activity on Newcastle disease virus. The plant can also serve as potential source of novel molecules for the development of chemotherapeutic agents for the treatment of Newcastle disease and probably other viral diseases. The toxicological effects of ethanolic extract of *G. hirsutum* L. at other concentrations should be studied at lower and higher concentration. Further research should be done on the

root bark and seeds to determine possible antiviral activity. The ethanolic extract of the plant should be screened against other viral disease that has no treatment.

REFERENCES

1. Ahamed, T. Hosssain, K. M., Billah, M. M., Ilam K. M. D., Ahasan, M. M. and Islam, M. E. (2004): Adoption of Newcastle Disease virus (NDV) in tissue culture. *International Journal of Poultry Science*, 3(2): 153 – 156.
2. Chan, B.G. Waiss, A.C. and Lukefahr, M. (2001). Condensed Tannin, an Antibiotic Chemical from *Gossypium Hirsutum. J.Insect Physicol*; 24(2): 113-118.
3. Micheil, V.B., Annemarie, B., Teun, H.F., Elly, K. and Gnus, K. (2008): Herd Immunity to Newcastle Disease Virus in Poultry by Vaccination. J*ournal of Avian Pathology*; 37(1): 1-5.
4. Aldous, E. W. and Alexander, D. Y. (2001): Detect ion and differentiation of Newcastle disease virus (Avian paramyxoviurs type 1) *Avian pathol,* 30: 117 – 28.
5. Reed, L.J. and Muench, H. (1938): A Single Method for Estimating Fifty Percent end Points. *American Journal of Hygiene,* 27 493 – 497.
6. Khaleequr, R., Arshiya, S. and Shafeequr, R. (2012): Gossypium herbaceum Linn: An ethnopharmacological Review. J. pharmaceutical and Scientific Innovation. 1(5): 1-5.
7. Omojasola, P.F. and Awe, S. (2004): Antibacterial activity of the leaf extracts of Anacardium occidentale and Gossypium hirsutum against some selected microorganisms. Bioscience Research Communication. 16(1):
8. Fasola, T. R., Adeyemoa, F. A., Adeniji, J.A. and Okonko, I.O. (2011): Antiviral Potentials of *Gossypium hirsutum* Extracts on Yellow Fever Virus. New York Science Journal. 4(10): 30-35. http://www.sciencepub.net/newyork.
9. Chandrashekar, R., Kumar, A.K., Reddy, Y.R., Chaitanya, P. J., Bhavani, N.L. and Pochampalli, J. (2013): Isolation of Gossypol and Analysis of Phytochemicals in Seed Extract of Bt and Non-Bt Varieties of Cotton. Journal of Pharmacognosy and Phytochemistry. 2(1): 180 – 186.
10. Nelson, C., M. D., Pomeroy, B. S., Schrall, K., Park, W. E., and Lindeman, R. J. (2006): An out break of conjunctivitis due to Newcastle disease virus (NDV) occurring in poultry workers. *A. M. J. Public Health* 42 (6): 672 – 678. [6]

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