

## THE ROLE OF *Momordica balsamina* FRUIT PULP EXTRACT IN DEVELOPMENT OF IMMUNITY TO AVIAN NEWCASTLE DISEASE VIRUS.

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### SUMMARY

Aqueous fruit pulp extract of *Momordica balsamina* was examined for its role on immune responses to Newcastle disease virus. Sera obtained from birds 10 days post-inoculation with live, inactivated mesogenic Newcastle disease virus strains and treated with the extract (400mg/ml) were compared by haemagglutination inhibition test (HAI). The HAI titres revealed significant difference by ANOVA and chi square statistical analysis ( $P < 0.001$ ). The correlation analysis further revealed significance at values of  $r < 0.50$ . *In vitro* studies showed 100% inhibition of cytopathic effect and a significant reduction in the number of plaques in comparison to untreated controls. The findings showed that the extract has no protective immunogenic effect but rather some potent antiviral tendency. Thus, justifying its use traditionally. In conclusion, the fruit pulp extract of *Momordica balsamina* can indeed be a potential ethno-veterinary antiviral product.

**KEYWORDS:** *Momordica balsamina*, Immune response, Avian NDV

### INTRODUCTION

*Momordica balsamina* (cucurbitaceae) commonly known in English: balsam apple, and French: margose, is a creeping annual vine that grows wild in the drier parts of the tropics. The plant flowers in September in Nigeria (Burkhill, 1985) and the fruit ripens in the harmattan period of November to December. Another species of the plant, which is usually cultivated in India and China, is *Momordica charantia*. The whole plant is used as a bitter stomachic, an infusion, as a wash for fever and for yaws (Dalziel, 1937). Other medicinal properties ascribed to it as reported by Burkhill (1985) include its use as abortifacient and as remedy for urethral discharge.

This study is aimed at verifying the claims of traditional practitioners in some parts of Northern Nigeria, that the fruit pulp is effective against avian Newcastle disease virus- a single stranded genome of negative sense, non- segmented RNA containing virus with helical capsid symmetry (Pringle, 1990), which belongs to the family

Paramyxoviridae and the genus Aulavirus (Mayo, 2002). The disease still remains a serious economic challenge to all segments of the poultry industry (David-West, 1972) because of its contagious and mortality records (Adeboyea, 1999). The only control method so far still remains vaccination, which does not confer 100% immunity in all vaccinated birds (USDA/APHIS, 2003). Thus there is a need to search for an alternative and effective control measure.

### MATERIALS AND METHODS

#### Analytical and laboratory reagents

The reagents used for the phytochemistry of extract (Wagner and Dragendorf reagents) were purchased from ICN Biochemicals Inc., Ohio, U.S.A. Other solvents used were of laboratory reagent grade. Phosphate buffered saline, sodium hydroxide and other laboratory chemicals were purchased from BDH Chemical Company Ltd., Poole, England.

### **Plant Material**

The ripe fruit of *Momordica balsamina* were harvested from Bukuru, Plateau state, Nigeria. They were identified according to description given by Burkhill (1985). Further identification was carried out by comparison to a Voucher specimen (ECN/02F/FCF, Jos) kept in the herbarium of the Federal College of Forestry, Jos. Each fruit was cut open with sharp knife; the pulp was removed and sieved with a 0.05 mm mesh to separate the seed, into a clean beaker. The pulp was freeze-dried (EKAT2000) and stored in a dessicator until use.

### **Experimental Animals, Biological Materials and Media**

Seventy dayold pullets were purchased from Zartec Farms, Sapele, Delta state, Nigeria. The birds were brooded and managed on deep-litter. They were given water and feed ad libitum throughout the period of the experiment. Both the live and inactivated mesogenic strains of the Newcastle disease virus, ND Lasota vaccine and Bovine kidney cells were obtained from the Virology Division, National Veterinary Research Institute (N.V.R.I.) Vom, Nigeria. The cells were used at passage levels (P) of between 51 and 55. The growth medium was Eagles minimum essential medium (MEM) containing 10% foetal calf serum, 1% of 100 g/ml streptomycin-penicillin and Amphotericin-B. The overlay medium consisted of Eagles MEM containing 10 ml per 100 ml of 1% Bato agar (Difco Laboratories, Detroit, Michigan, U.S.A.), 10% foetal calf serum and antibiotics in the same concentration as the growth medium.

### **Vaccination/ Treatment of Birds**

The vaccination of birds as indicated in the experimental design below was carried out by giving orally 0.2 ml of ND Lasota (N.V.R.I, Vom) vaccine to each bird, after reconstitution of one vial in 20 ml of distilled water. While 2 ml of the aqueous extract (400 mg/ml) was administered orally to each bird for treatment.

### **Experimental Design**

The birds were grouped at four weeks into 7 of 10 birds each as a group and kept in separate cages. The groups were treated as follows:

Group A was treated with normal saline, serving as placebo control; Group B was neither vaccinated nor administered the pulp extract, serving as healthy control; Group C was treated orally with the freeze-dried powdered plant extract dissolved in distilled water at 400 mg/ml; Group D was vaccinated, orally with live mesogenic strain of Newcastle disease vaccine only; Group E was vaccinated, orally with live Newcastle disease vaccine and treated 1 hour post-vaccination with aqueous solution of powdered pulp extract at 400 mg/ml; Group F was vaccinated, orally with inactivated Newcastle disease vaccine only, and Group G was vaccinated, orally with inactivated Newcastle disease vaccine and treated 1 hour post-vaccination with aqueous solution of the powdered pulp extract at 400 mg/ml.

### **Collection and storage of Sera**

The blood was collected and sera extracted from birds 10 days post-inoculation with the viral antigens and treatment with the extract. They were collected from five birds, each from every group. The sera were removed into vials and stored overnight at -4 o C before being used for the test.

### **Haemagglutination inhibition**

All sera were titrated for haemagglutination inhibition antibodies (HAI) as described by Office International des Epizootics [OIE] (2000) and recommended by WHO/FAO International Laboratory for Biological Standards using 4HA units of Newcastle disease antigens and 1% chicken erythrocytes. The tests were conducted in V- shaped wells (Linbro Titertek). The sera were serially diluted in PBS, and allowed to settle for about 40 minutes at room temperature before being evaluated.

### **Cytopathic inhibitory assay**

Cytopathic inhibition effect (CPE) of the extract was assayed by reacting in vitro a number of serially diluted mesogenic strains (live and inactivated) of the virus with the extract and viewing the cytopathic effect for each dilution under the light microscope at low magnification (X40).

Monolayers of bovine kidney cells were prepared in 60 mm glass Petri dishes. They were inoculated with 0.2 ml of the virus and treated with the extract (400 mg/ml). The mixtures were incubated at 37°C in air incubator and monitored every 24hrs for a maximum period of 7 days post-treatment.

#### **Antiviral Properties of *Momordica balsamina* Plaque assay**

Plaque forming assay was used to determine the effect of the plant extract on the biological activity of the live and inactivated mesogenic viruses. For each dilution the number of the plaques was counted. The monolayers of BKC were prepared in 60 mm diameter multiwell tissue culture plates (Costar, Broadway Cambridge, Massachusetts, U.S.A.). They were inoculated with 0.2ml of the serial dilution of the virus strains and mixed with 400mg/ml of the extract. The mixtures were incubated in a humidified CO<sub>2</sub> (5%) incubator at 37°C for 1h. After 1h adsorption at 37°C, the monolayers in each plate were overlaid with 1ml of overlay medium and the cultures incubated in 5% CO<sub>2</sub> at 37°C. They were and monitored every 24 hours for 3 days post treatment. On the third day, 1ml of neutral red solution was added to each culture well and incubated at 37°C for 2-3 hrs. The stain was aspirated and plates inverted to allow the plaques to clear. The plaques, which appeared as cleared circles against a red background, were counted. Known negative controls were also incubated. The viral titre was calculated and expressed as plaque forming units (pfu) per ml.

#### **Phytochemistry**

The freeze-dried fruit pulp extract of *Momordica balsamina* was tested for the presence of resins, tannins, alkaloids, glycosides, flavonoids and saponins according to the method of Harbone (1984).

#### **Statistical Analysis**

The mean and standard deviation of the serum antibody level were recorded. The difference between the mean titre values was evaluated by one-way ANOVA and chi square goodness of fit. They were further subjected to correlation statistical analysis.

## **RESULTS**

The results of HAI titres of birds inoculated with live and inactivated viral antigens are shown in Figure 1. The birds in groups D and F vaccinated with live and inactivated mesogenic virus had mean titres of  $28.2 \pm 1.79$  and  $24.0 \pm 2.74$  respectively while group C treated with extract only did not show any inducement of antibody synthesis ( $2.14 \pm 0.89$ ). These results analysed by the ANOVA test ( $P < 0.001$ ) showed a significant increase in the HAI antibodies stimulated by live and inactivated strains over that of the extract. The chi square test at  $P < 0.001$  indicated a significant inhibition of antibody synthesis in birds inoculated with the mixtures of live, inactivated viral strains and the extract with mean titres of  $2.66 \pm 1.52$  and  $21.2 \pm 0.45$ , respectively. The correlation analysis further revealed significance at values of  $r < 0.50$ .

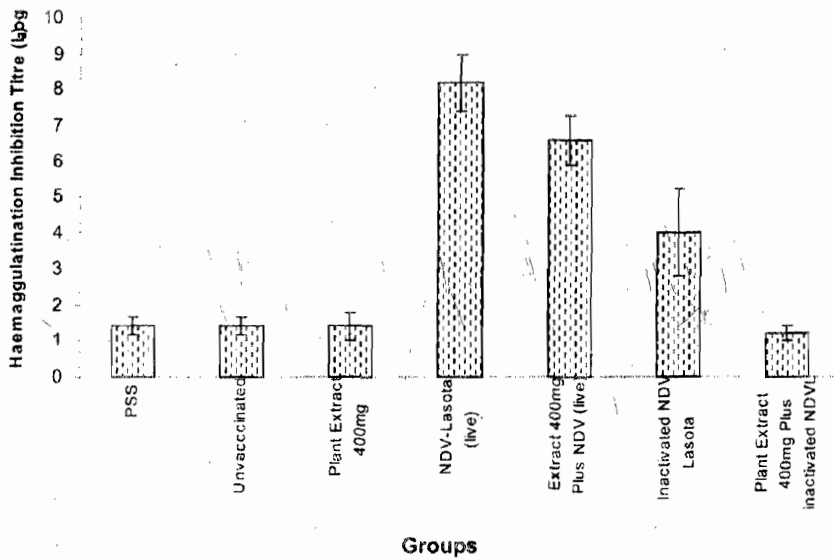
The Cytopathic effect inhibitory assay of the extract showed 100% inhibition of syncytia and giant cells (cytopathic effect) in all cultures as indicated in Table I. However, mesogenic virus groups (live and inactivated) served as positive control. The plaque assay indicated quantitative effect of the extract on the virus, which is illustrated by the reduction in viral (live and inactivated) titres expressed as (pfu/ml) as shown in Figure 2 and 3. Phytochemical analysis of extract showed that alkaloids, saponins, tannins and flavonoids are present in the pulp of *Momordica balsamina*, but resin and glycosides are absent.

TABLE I: Cytopathic inhibition effect of the extract on mesogenic Newcastle disease virus.

| Dilution         | Groups |   |   |   |   |   |   |
|------------------|--------|---|---|---|---|---|---|
|                  | A      | B | C | D | E | F | G |
| 10 <sup>-9</sup> | -      | - | - | + | - | + | - |
| 10 <sup>-8</sup> | -      | - | - | + | - | + | - |
| 10 <sup>-7</sup> | -      | - | - | + | - | + | - |
| 10 <sup>-6</sup> | -      | - | - | + | - | + | - |
| 10 <sup>-5</sup> | -      | - | - | + | - | + | - |
| 10 <sup>-4</sup> | -      | - | - | + | - | + | - |
| 10 <sup>-3</sup> | -      | - | - | + | - | + | - |

Key: + Syncytia and multinucleate cell.

-No effect.



Key: PSS: Physiological saline solution.

NDV: Newcastle disease vaccine.

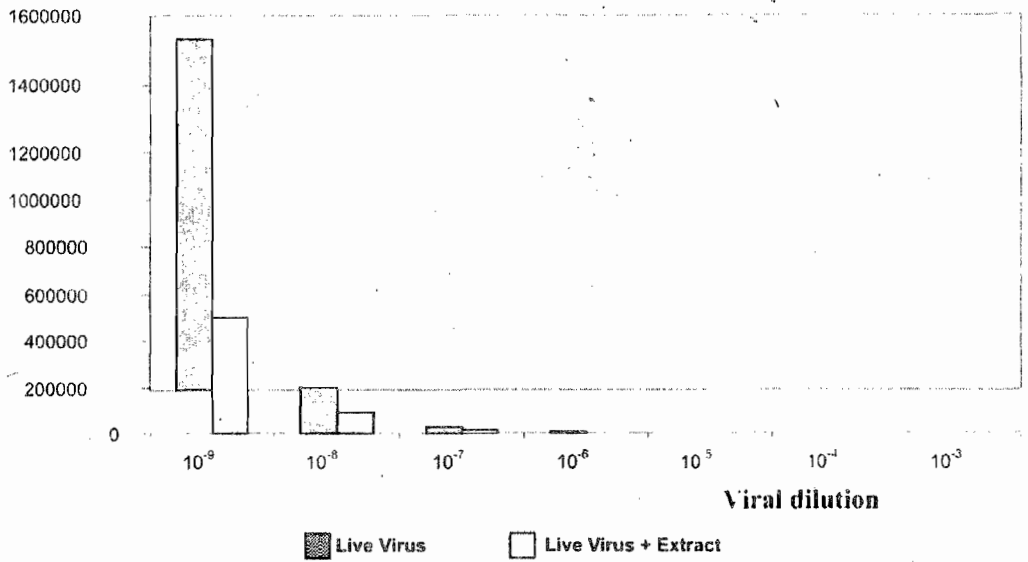


Figure. 2a: The reductive effect of the extract on live viral titres

Pfu/ml

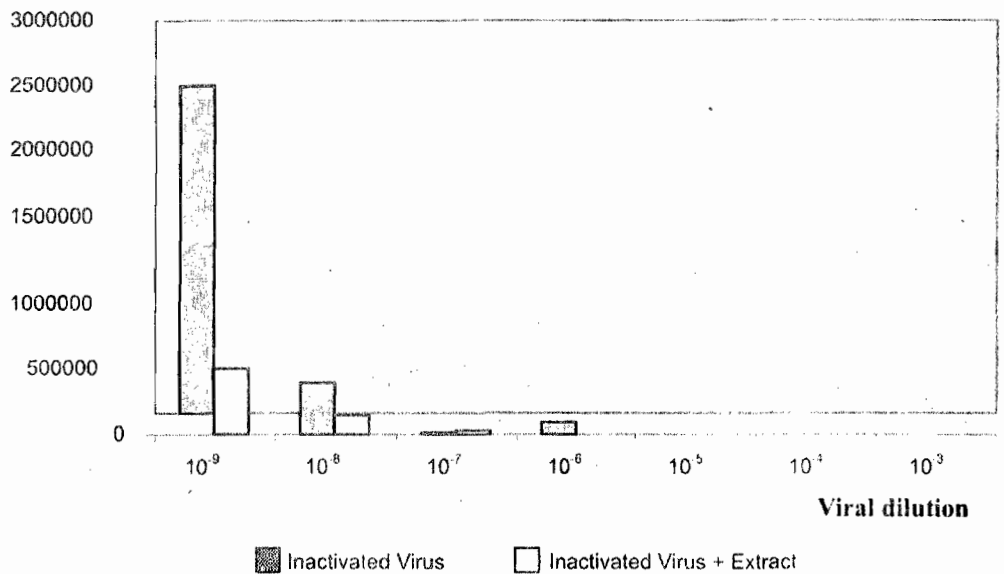


Figure. 2b: Showing the reductive effect of the extract on the inactivated viral titres

## DISCUSSION

The immune responses of different groups of birds tested revealed that antibody could not be induced in birds vaccinated with live and inactivated viral antigens and treated with aqueous pulp extract, suggesting that the pulp extract has an effect either on the physiology of the virus or the host animal. It is possible that the extract prevented the attachment of the virus to the receptor site or unmasked the antigenic determinants on the virus; it may also have altered the chemistry of the fusion protein cleavage site. Thus, the extract reduced the basic amino acid residue in the site, which is associated with virulence (Collins et al., 1993).

In addition, the extract may act on the host protease enzyme preventing the post-translation cleavage of the precursor Fo to yield a disulphide F 1- F 2 complex essential for infectivity (Rott and Klenk, 1988) hence, the obtained resultant in vivo inhibition of serum antibody synthesis.

The In Vitro experiments of cytopathic effect inhibitory study and plaque assay also supported the suggestion that the extract could be acting on the physiology of the virus. The exact mechanism of action and stage of viral replication cycle altered by the extract is not known. However, it is presumed that the extract may act on the neuraminidase activity associated with NDV (Lamb and Kolakofsky, 1996) following the removal of the sialic acid from progeny virus particle. This results in self-agglutination of progeny virus and a resultant none - low infectivity as indicated by the assay.

The observed antiviral activity variously exhibited by the pulp extract was remarkable and could be attributed to the constituent phytochemicals. Alkaloids, saponins, tannins, and flavonoids all of which were present are regarded as novel antiviral agents (Jassim and Naji, 2003), but the specific phytochemical agent responsible for the antiviral activity is not known. It is conceivable that the antiviral activity of the crude extract of *M. balsamina* may arise from interactions between different components, in various combinations culminating in additivity or synergism, rather

than individual effects of the components thus justifying its use traditionally. Therefore, the resultant antiviral activity suggests, that the extract has no protective immunogenic effect. It can be safely concluded that the fruit pulp extract of *Momordica balsamina* can indeed be a potential ethno- veterinary antiviral product.

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