

Antiviral Activity of the Fruit Extract of *Cucumis metuliferus* E. Meyer (Cucurbitaceae) in Chicks

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Abstract: *Aim of Study:* Antiviral property of the alkaloidal components of fruit extract of *Cucumis metuliferus* E. Meyer (100-600 mg/kg) was evaluated in chicks infected with newcastle disease. *Materials and Method:* The alkaloidal component of the fruit extract was investigated for antiviral properties in chicks. The birds were infected with a virulent strain of newcastle disease virus and the following were examined: physical signs of clinical illness, time of death and histopathological examination of the culprit organs of infection (proventriculus, ventriculus and caecum) and compared with control groups. *Results:* The result showed that the alkaloids isolated from the plant material were capable of reducing and/or reversing the effects of the virus in chicks. *Conclusion:* The study showed that the alkaloidal component of this fruit is a potent antiviral agent since it prevented and reversed virus induced manifestations in infected chicks.

Key words: Alkaloids • *Cucumis metuliferus* • Newcastle disease • Antiviral property

INTRODUCTION

The annual climber *Cucumis metuliferus* E. Meyer belongs to the gourd family cucurbitaceae, it is a monoecious, climbing, annual herb commonly found in the semi arid regions of South Africa and the guinea savanna belt of Nigeria. The plant bears fruits, which are bright yellow–reddish orange in colour. The fruit is shaped like a short stout cucumber with many thorns on its surface, thus, the common name: “horned cucumber”. The seeds are embedded in the mesocarp, which is emerald green and consists of juicy, bland tasting tissue. The herb flowers from July to September and the fruits ripen from October to December [1].

Cucumis metuliferus grows usually in Northern Nigerian especially in Jos, Plateau State, where it has been variously claimed to protect viral and bacterial infections. The fruit pulp of this plant has also been claimed by herbalists in Jos, Plateau state, Nigeria, to cure diabetes, peptic ulcer disease, hepatitis and HIV/AIDS [2]. Local poultry farmers also administer the fruit juice of *C. metuliferus* in drinking water to poultry and claim that the flocks were protected against new castle disease. Similarly, the people of Vel-Pankshin area council, Nigeria,

have used the fruit in the treatment of hepatitis and jaundice and have claimed some level of success [2].

The seed flour is used as a vermifuge [3]. The leaves are employed as a source of vegetable in diets. The melon fruit contains iron, calcium and is particularly rich in ascorbic acid. Cucurbitacine, an alkaloid from the fruit, is a bitter component that is known to be toxic to mammals. Other constituents include lutein, lutein epoxide, 3-epiluttein, myristol, palmitol and dipalmitol lutein [4]. In addition, the plant also contains alkaloids, phenylpropanoids, flavonoids and terpenoids [5].

Newcastle disease (NCD) is a viral infectious disease of chicken and turkeys. The virus belongs to the negative strand RNA paramyxoviridae family and like other members of the family, they possess two surface proteins that are important in the identification and behaviour of the virus [6, 7]. According to Alexander [7], all newcastle virus strains share a common surface antigen regardless of their virulence.

The aim of the present study is to investigate the folkloric use of the fruits of *Cucumis metuliferus* in the management of hepatitis and HIV/AIDS using chicks infected with newcastle disease, as a model of viral diseases.

MATERIALS AND METHODS

Plant Material: Ripe fruits of *Cucumis metuliferus* were collected in October 2008 in Vel, Pankshin Local Government Council, Plateau state, Nigeria. The fruits were authenticated by A. Kareem, Federal School of Forestry, Jos, Nigeria.

Extraction: The ripe fruits were cut open longitudinally with a clean sharp blade. The greenish-yellow pulp and seeds were carefully scooped out of the pericap. The yellowish fibres were separated and dried. The seeds were then blended using a low-speed blender. The resulting blend was sieved to yield a filtrate which was freeze-dried. This dried portion and the yellowish fibre were combined and pounded to a fine powder using a wooden mortar and pestle. This was stored at in an air tight container pending alkaloid isolation.

Isolation of Alkaloids: 100 g of the powdered drugs was dissolved in a 50 ml solution of dilute ammonia. The resulting mixture was filtered and the filtrate was extracted with 10 ml of chloroform. This was subsequently evaporated to dryness. This was further reconstituted in 10 ml diluted hydrochloric acid and the resultant solution basified with 5 ml of dilute ammonia. 50 ml of chloroform was used to extract the alkaloids after which the chloroform layer was decanted and evaporated to dryness. The isolated alkaloid was refrigerated in an air tight container.

Animals: sixty (60) day-old unvaccinated chicks (25-40 g) obtained from ECWA farms, Bukuru, Plateau state, Nigeria were kept in a clean, fumigated and electrically-heated

room screened from insects and rodents. The animals were kept in this condition for 2 weeks before the commencement of the study. They were fed with standard feed (broiler starter: vital feeds, Nigeria) and had access to food and water *ad libitum*.

Newcastle Disease Virus: The virulent strain of the virus was obtained at National Veterinary Research Institute, Vom, Nigeria and reconstituted in 10 ml of phosphate buffer. Birds were infected intramuscularly on the right thigh.

Curative Effect: Birds of 100 g average weight (2 weeks from day of collection) were divided into 6 groups of 5 birds each. The extract (600 mg/kg,i.p) was administered one (1) hour post infection. One group of birds was challenged the virus only, while the other control group were administered normal saline only. The birds were observed six hourly over a period of 72 hours for clinical signs of illness. The groups administered the virus and extract were sacrificed on the 4th day for histological examination of proventriculus, ventriculus and caecum.

RESULTS AND DISCUSSION

Newcastle disease is a haemorrhagic disease of birds that has a predilection for the respiratory, digestive and nervous systems, causing moderate to several deaths in susceptible flock [8]. Nervous signs include twisted necks and paralysis while digestive symptoms include watery-greenish diarrhea. In this study, the extract (100-600 mg/kg,ip) suppressed these clinical signs of viral infection in chicks, with 600 mg/kg being the most potent, while 400 mg/kg did not show much activity against these clinical signs (Table 1).

Table 1: Effect of the alkaloidal component of *Cucumis metuliferus* on clinical signs of chicks infected with newcastle disease virus

Clinical signs	Dose of extract (mg/kg)					
	100	200	400	600	Normal saline	Virus alone
Loss of appetite	+	+	++	+	-	+++
Greenish diarrhoeal stool	+	+	+	+	-	++
Neck twisting	+	+	++	-	-	+++
paralysis	+	+	++	+	-	++

Key+ indicates slightly present ++ indicates moderately present +++ indicates highly present- not present

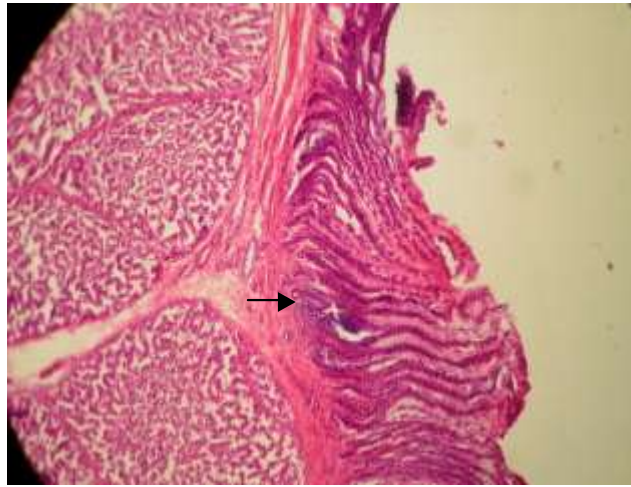


Fig. 1: Proventriculus of animal administered the virus (Severe cellular infiltration into the tunica mucosa with a focus of whorling of the papillae)

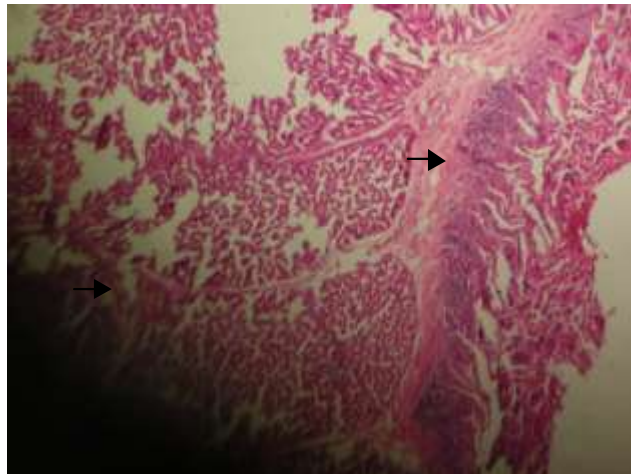


Fig. 2: Proventriculus of animal administered 600 mg/kg of the extract (Mild destruction of the adenomere)

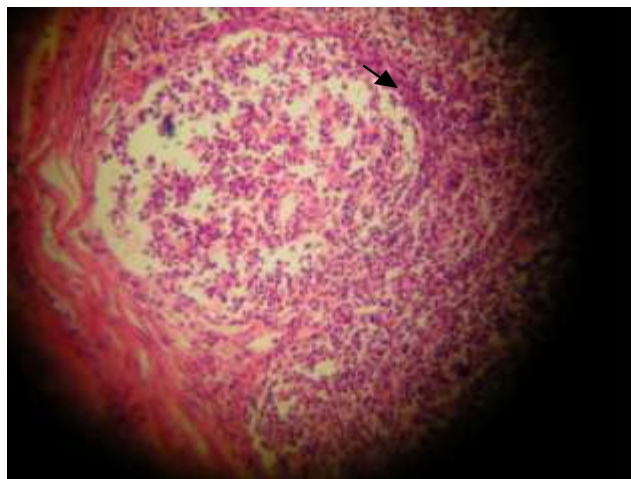


Fig. 3: Ventriculus of animal administered the virus (Massive turnover of underlying epithelium into the cornified layer)

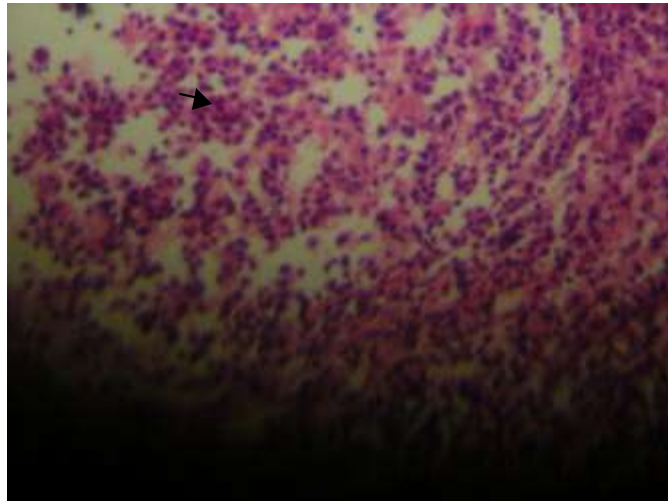


Fig. 4: Ventriculus of animal administered 600 mg/kg of the extract (Mild hyperkeratinization)

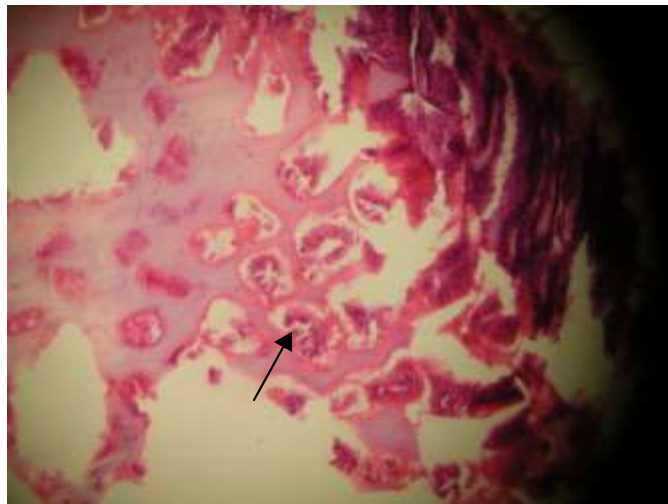


Fig. 5: Ceca of animal administered the virus (Severe mononuclear infiltration into the lamina propria)

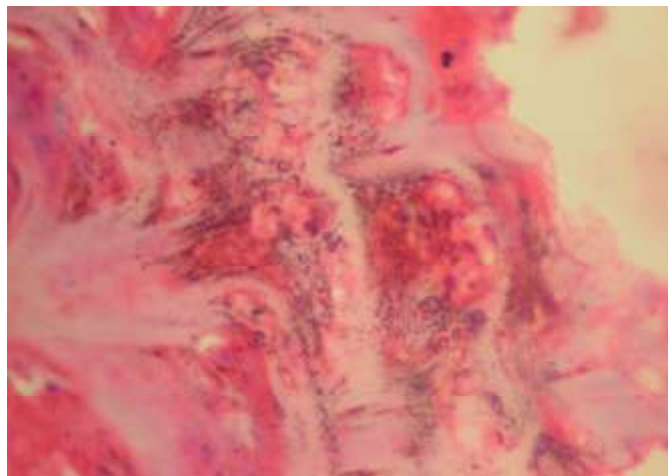


Fig. 6: Ceca of animal administered 600 mg/kg of the extract (No visible lesion)

Histopathological lesions of the proventriculus and ventriculus with associated necrotic haemorrhages of the caecum are common in newcastle disease. This is evident in the destruction of the adenomere and severe cellular infiltration of the proventriculus. Similarly, there is often hyperkeratinization of the ventriculus and the caecum is seen to have severe mononuclear infiltration especially into the lamina propria. The result of this study showed that the extract (600 mg/kg,i.p) reversed the haemorrhagic lesions induced by newcastle disease virus (Figs. 1-6).

CONCLUSION

In the present study, the results provided scientific support for the use of *Cucumis metuliferus* for the management of virus associated illness. Further investigation is ongoing in our laboratory to identify and characterize the molecule responsible for activity and to elucidate the precise mechanism of this effect.

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