

Neutralization Effect of a Locally Sourced Lipid Solvent on Mouse Adapted Influenza “A” (A/PR/6/32) Virus: A Review Paper from a Combined Class Semester Term MPH Project, Imo State University Owerri

Ajobiewe OJ^{1,2,3*}, Adedamola OA², Umeji L², Madukwe J², Ajobiewe HF³, and Odunze²

¹National Hospital Abuja, Plot 132 Garki Central District, Nigeria

²Imo state University Owerri, Nigeria

³Bingham University Karu Nasarawa State of Nigeria

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*Corresponding author: Ajobiewe Olu Joseph

Abstract

Original Research Article

This work was aimed at testing the effectiveness of a locally sourced and fortified lipid solvent, on *Influenza A* Disease virus in some infected poultry birds at the National Veterinary Research Institute, Vom (NVRI) Plateau State of Nigeria. The research was designed by randomly blocking (during lipid dilution process) thirty five eggs (35) of 9 (nine) day old Hen embryonated eggs into groups of seven (7) based on lipid dilutions viz; six (6) lipid dilution groups and the control group. Dilutions from 10^{-5} to 10^{-10} were used for the assay. 0.1ml of the respective dilutions was transferred to sterile Bijou bottles labelled with the corresponding lipid solvent dilution. Thereafter, equal volumes of 4HA unit concentration of neat suspension of influenza virus strain (A/PR/6/32) was added to each bottle. Five eggs were labelled for each lipid solvent dilution. 0.1ml lipid/virus mixtures were randomly inoculated into five (5) eggs bearing the respective lipid dilutions. Five eggs were inoculated with 0.1ml each of the virus suspension to serve as virus control. Eggs were incubated at 37°C for 72 hours. Each egg was cut open after incubation and allantoic fluid tested for Hemagglutination reaction using 10% chicken RBC. The results revealed that the locally sourced lipid solvent has neutralizing effects on influenza virus by the Hemagglutination inhibition technique (HAI). Therefore Neutralization titre = $10^{-5.6 \text{ influ.50}}/0.1\text{mL}$. This study findings suggest that the locally sourced lipid solvent is naturally microcidal (killing harmful bacteria, yeast, fungus, and viruses).

Keywords: Hemagglutination HA, *Influenza* virus, Hemagglutination inhibition HAI, Lipid Solvent, NVRI, Neutralization Assay, Fertilized Egg.

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STUDY BACKGROUND

Influenza viruses belong to the family of Orthomyxoviridae. There are different genus under this family. These include Influenza virus A, Influenza virus B, in fluenza virus C, Influenza virus D, and the unnamed Thogoto-like viruses. Virions are pleomorphic, often spherical, 80- 120 nm in diameter. Filamentous forms several micrometer in length also occur [1]. Virions consist of a lipid –containing envelope, with large peplomers 10^{-14} nm in length and 4-6 nm in diameter, representing trimetric hem agglutinin and tetrameric neuraminidase structures. Within the envelope there are helically symmetrical nucleocapsids of different size classes, 150-130 nm in length with a loop at one end. Virion Mr is 250×10^6 ; buoyant density is 1.19g/cm^3 in sucrose. The genome

consists of eight, seven, or six molecules of linear, negative sense, single stranded RNA, 10 to 13.6Kb in overall size. Segment lengths range from 900 to 2350 nucleotides [2].

Influenza, commonly known as "the flu", is an infectious disease caused by an influenza virus [1]. Symptoms can be mild to severe [5]. The most common symptoms include: high fever, runny nose, sore throat, muscle and joint pain, headache, coughing, and feeling tired [1]. These symptoms typically begin two days after exposure to the virus and most last less than a week [1]. The cough, however, may last for more than two weeks [1]. In children, there may be diarrhea and vomiting, but these are not common in adults [6]. Diarrhea and vomiting occur more commonly in gastroenteritis, which is an unrelated disease and sometimes

inaccurately referred to as "stomach flu" or the "24-hour flu" [6]. Complications of influenza may include viral pneumonia, secondary bacterial pneumonia, sinus infections, and worsening of previous health problems such as asthma or heart failure [2, 5].

Three of the four types of influenza viruses affect humans: Type A, Type B, and Type C [2, 7]. Type D has not been known to infect humans, but is believed to have the potential to do so [7, 8]. Usually, the virus is spread through the air from coughs or sneezes [1]. This is believed to occur mostly over relatively short distances [9]. It can also be spread by touching surfaces contaminated by the virus and then touching the eyes, nose, or mouth [5, 9, 10]. A person may be infectious to others both before and during the time they are showing symptoms [5]. The infection may be confirmed by testing the throat, sputum, or nose for the virus [2]. A number of rapid tests are available; however, people may still have the infection even if the results are negative [2]. A type of polymerase chain reaction that detects the virus's RNA is more accurate [2].

Frequent hand washing reduces the risk of viral spread, as does wearing a surgical mask [3]. Yearly vaccinations against influenza are recommended by the World Health Organization (WHO) for those at high risk [1], and by the Centers for Disease Control and Prevention (CDC) for those six months of age and older [11]. The vaccine is usually effective against three or four types of influenza [1]. It is usually well tolerated.¹ A vaccine made for one year may not be useful in the following year, since the virus evolves rapidly [1]. Antiviral medications such as the neuraminidase inhibitor oseltamivir, among others, have been used to treat influenza.¹ The benefit of antiviral medications in those who are otherwise healthy do not appear to be greater than their risks [12]. No benefit has been found in those with other health problems [12, 13].

Influenza spreads around the world in yearly outbreaks, resulting in about three to five million cases of severe illness and about 290,000 to 650,000 deaths [1, 4]. About 20% of unvaccinated children and 10% of unvaccinated adults are infected each year [14]. In the northern and southern parts of the world, outbreaks occur mainly in the winter, while around the equator, outbreaks may occur at any time of the year [1]. Death occurs mostly in high risk groups—the young, the old, and those with other health problems [1]. Larger outbreaks known as pandemics are less frequent [2]. In the 20th century, three influenza pandemics occurred: Spanish influenza in 1918 (17–100 million deaths), Asian influenza in 1957 (two million deaths), and Hong Kong influenza in 1968 (one million deaths) [15-17]. The World Health Organization declared an outbreak of a new type of influenza A/H1N1 to be a pandemic in

June 2009 [18]. Influenza may also affect other animals, including pigs, horses, and birds [19].

HYPOTHESES

Null Hypothesis (H₀)

Locally sourced lipid solvent has no neutralizing effect on the INFLUENZA "A" (A/PR/6/32) Virus and as such the neutralizing dose is not significant.

Alternate Hypothesis (H_a)

Locally Sourced Lipid Solvent has a neutralizing effect on the INFLUENZA "A" Virus and as such the neutralizing dose is significant.

METHOD

The students shared responsibility towards making the semester project for the award of an MPH in public health of Imo state University Owerri a huge success. They were randomly distributed into three planktons, viz; - Plankton A, those to source for particular animal strain of the virus in which the final choice was the "Mouse adapted influenza "A" (A/PR/6/32) Virus";

Plankton B, those to look for the ethical clearance, as most of them were staff of research institutions/or had close affiliates in these research institutions engaged in vaccine production and monitoring in Nigeria:

Plankton C, those to prepare the reagents, get ready the needed materials for egg technique, hemagglutination inhibition technique e.t.c. /result collation and report writing for evaluation and final assessment by me. When the stage was fully set, the following steps were followed:

Thirty five eggs (35) of 9 (nine) day old hen's embryonated eggs were obtained from the poultry unit of the National Veterinary Research Institute, Vom, Jos. Plateau State, Nigeria. The eggs were candled to ensure they were alive and fit for the study.

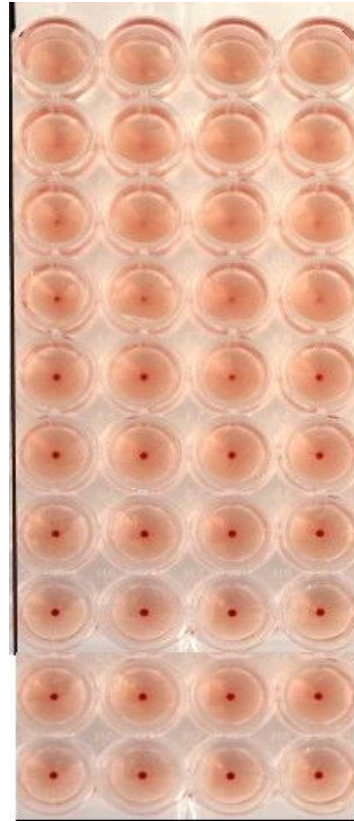
GROUPING OF EGGS

The eggs were grouped into seven (7) based on drug dilutions viz;

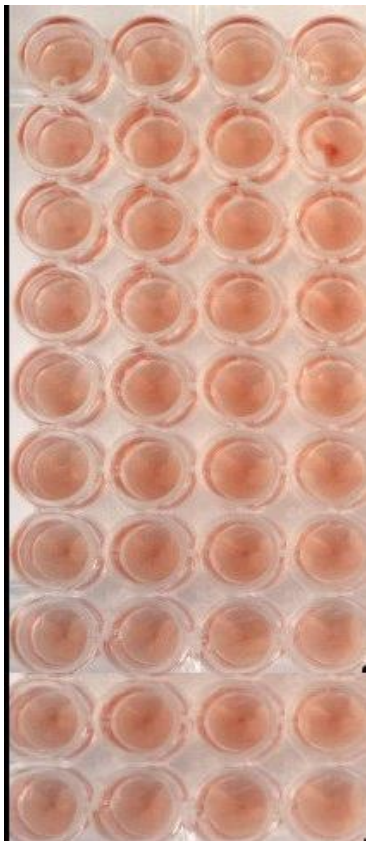
- Group one (1): The control group
- Group two (2): The group with lipid solvent dilution of 10⁻⁵
- Group three (3): The group with lipid solvent dilution of 10⁻⁶
- Group four (4): The group with lipid solvent dilution of 10⁻⁷
- Group five (5): The group with lipid solvent dilution of 10⁻⁸
- Group six (6): The group with lipid solvent dilution of 10⁻⁹

Group seven (7): The group with lipid solvent dilution of 10^{-10}

Each group consists of five (5) eggs respectively. Each egg was cut open after incubation and allantoic fluid tested for Hemagglutination reaction using 10% chicken RBC (Red Blood Cell). A total number of thirty five eggs were used for this study. They were divided into seven (7) groups, i.e., six (6) lipid solvent dilution groups and the control group. A ten-fold serial dilution of the lipid solvent was tested for neutralization i.e. a 10^{-1} to 10^{-10} using absolute alcohol as diluents. Dilutions from 10^{-5} to 10^{-10} were used for the assay. 0.1ml of the respective dilutions was transferred to sterile bijou bottles labelled with the corresponding lipid solvent dilution. Thereafter, equal volumes of 4HA unit concentration of neat suspension of INFLUENZA "A" (A/PR/6/32) Virus strain was added to each bottle. Bottles were gently agitated to mix their contents and kept on ice ready for inoculation. Egg inoculation Thirty (30) 9 (Nine) day old Hen's embryonated eggs were prepared for inoculation and five eggs were labelled for each lipid solvent dilution. 0.1mL lipid/virus mixtures were inoculated into five (5) eggs bearing the respective drug dilutions. Five eggs were inoculated with 0.1ml each of the virus suspension to serve as virus control. Eggs were incubated at 37°C for 72 hour.



RESULTS



Each egg was cut open after incubation and allantoic fluid tested for Hemagglutination reaction using 10% chicken RBC. All virus control eggs tested

positive for Hemagglutination reaction, indicating the presence of Influenza virus. Where allantoic fluid in the egg agglutinated RBC, it indicated that the Lipid failed to neutralize the virus. Where the fluid did not agglutinate RBCs, it showed that the lipid has neutralized the virus.

$$\text{Neutralization Index} = \{ \% \text{ Neutralized at dilution immediately above } 50\% - 50\% \} / \{ \% \text{ Neutralized at dilution immediately above } 50\% - \% \text{ Neutralized at dilution immediately below } 50\% \}.$$

Table-1

Dilution of 10^{-5} % Neutralized above 50% = 71.4%
Dilution of 10^{-6} % Neutralized below 50% = 37.5%
Neutralization index = $71.4\% - 50\% / 71.4\% - 37.5\% = 21.4/33.9 = 0.6$
The Neutralization index is then applied to the dilution that produced the percentage neutralized immediately above 50%, which is 10^{-5}
The index of 0.6 is applied to this dilution = $10^{-5-0.6}$ or 1 neutralized dose (Influ.50) is $10^{-5.6}$ Therefore Neutralization titre = $10^{-5.6}$ Infl. 50 / 0.1 ml
{Stating how to calculate the Neutralization indices}

DISCUSSION

The study showed that lipid solvent had a neutralizing effect on the Influenza Virus. This supports the conclusions made that the locally sourced lipid solvent is naturally antimicrobial (killing harmful bacteria, yeast, fungus, viruses). The results obtained revealed that, the higher the dilutions, the greater the neutralizing effect of lipid solvent on influenza virus. All eggs were cut open and the allantoic fluid tested for neutralization activities by the Hemagglutination Inhibition test. From the Table-1, results showed that Lipid solvent had 71.4% (above 50%) neutralization effect on influenza virus at the dilution of 10^{-5} and 37.5% at dilution of 10^{-6} . At dilution of 10^{-7} , lipid solvent had 10% neutralization effect on the Influenza virus. Therefore we have no basis to reject the researcher's alternate hypothesis, H_a , which stated that lipid solvent has a neutralization effect on the Influenza virus, thus this was retained. While we have no enough evidence to retain the null hypothesis which stated that the lipid solvent had no neutralizing effect on the Influenza A Virus and as such, we could not retain this hypothesis, hence it was rejected, or simply put, the Neutralizing dose was not significant". The dilution index of the Lipid solvent was 0.6 and when applied to the dilution that produced the percentage neutralized immediately above 50% which was 10^{-5} , it resulted in a 1 neutralized dose of (Influ. A 50) of $10^{-5.6}$. Therefore the Neutralization titre was $10^{-5.6}$ Infl. 50 / 0.1ml. The control eggs were all intact and were all positive to Infl. A by the HA technique. This helped validate the experiment.

CONCLUSION

The locally sourced lipid solvent effectively neutralized the influenza A virus Strain used in this assay as evidenced from the hemagglutination inhibition test result. This suggest it could be an excellent antiviral agent during flu or corona /common cold epidemics or pandemics. Likewise, the property of the lipid solvent as a relieving oxidative stress agent further makes it a candidate of choice at combating the

chronic pneumonia that sometimes makes this disease fatal.

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