

Cytotoxic Evaluation of Antivir-H and IMB Herbal Supplements Used For Management Of Covid 19

(Laboratory Based Tests)

Amos Lewa Mwavita¹, Athuman Nyae Chiguzo^{2*}

1. Kenya Medical Research Institute (KEMRI), P.O Box 54840-00200, Nairobi, Kenya Email: amoslewa@gmail.com

2. Kenya Medical Research Institute (KEMRI), P.O Box 54840-00200, Nairobi, Kenya Email: athuman.chiguzo@gmail.com

Corresponding Author: * athuman.chiguzo@gmail.com

Abstract

The COVID-19, a new zoonotic coronavirus RNA virus after MERS -CoV, and SARS-CoV pandemic represents the greatest global public health crisis of this generation and scientist all over the world are working around the clock to get products which can be used in the treatment of the disease. The aim of the study was to carry out cytotoxicity screening of the herbal formulations Antivir –H and IMB for safety use. An experimental study to evaluate the cytotoxicity profile of Antivir H and Immune Booster (IMB) herbal combinations of natural products was carried out in a laboratory to assess their toxicity in the use for treatment of COVID -19 and for bolstering immunity respectively. Mixed Herbal powders for the Antivir-H and Immune Booster (IMB) were obtained from a herbal company based at the Kenyan Coast. Cytotoxicity assay was performed to predict toxicity of the herbal extracts on Vero Cells. Two methods were performed. In the first method, a 10mg of herbal extract was dissolved in distilled water. 100µl picked topped up with 900µl PBS to make the working solution. The second method, MTT assay was performed on Plant extract Concentration CC_{50} of the medicinal plant extract on vero cell (lines in µg/ml). The cytotoxicity of the plant extracts on Vero cells was determined by calculation of CC_{50} . The 50% cytotoxic concentration (CC_{50}) was defined as the extracts concentration (µg/mL) required for the reduction of cell viability by 50%. The CC_{50} of the medicinal plant extract on vero cell (lines in µg/ml) was 240.145 and 211.585 for Antivir -H and IMB respectively. In conclusion, the plant extracts are considered NON-TOXIC as the cytotoxic concentration meets the National Cancer Institute recommendations concerning the inhibitory activity of crude extracts. Thus Antivir-H and IMB can safely be used by human beings for the management of COVID - 19.

Key Words: Antivir-H, IMB, Cytotoxicity, COVID - 19, Herbal Supplements

1. INTRODUCTION

The COVID-19 pandemic represents the greatest global public health crisis of this generation and, potentially, since the pandemic influenza outbreak of 1918. The speed and volume of clinical trials launched to investigate potential therapies for COVID-19 highlight both the need and capability to produce high-quality evidence even in the middle of a pandemic. Given the rapid pace of scientific discovery and clinical data generated by the large number of people rapidly infected by SARS-CoV-2, clinicians need accurate evidence regarding effective medical treatments for this infection. The rapidly expanding knowledge regarding SARS-CoV-2 virology provides a significant number of potential drug targets.

COVID-19 is a new zoonotic coronavirus RNA virus after MERS -CoV, and SARS-CoV that is highly infectious and has killed many people; causing anxiety in governing bodies globally. COVID-19 initial attachment is on the pharyngeal regions, Rhinitis channels, sinus glands, tear glands and the Oral-pharyngeal sites. The Virus cultures on the pharyngeal glands for 3 - 4 days resulting into flu-like symptoms, sore throat including fever and coughing. From 5th to 14th day, the viruses descend to the lungs with aggressive abrasions, degenerative effects and oxidative mechanisms resulting to fibrosis, inflammatory responses and fluid accumulation, which brings shortness of breath. What kills patients infected with COVID-19 is Cytokine storm [1], of Acquired Respiratory Disease Syndrome (ARDS), a common immune-pathological event for SAS-CoV-1, SARS-CoV and MERS- CoV infections [2]. The infectivity patterns progresses to the lungs, liver, spleen and Kidneys. In more severe cases, infection can cause pneumonia, severe acute respiratory syndrome, kidney failure and even death.

WHO states that globally, around half of countries are seeing declines in COVID 19 while the other half are experiencing increasing numbers of new cases. It is important to note that WHO states that Americas and Europe account for around 80% of new cases and new deaths reported globally. Many trials for discovery of drugs for COVID-19 and immune boosting are done in Africa. Most initiative trials in Africa focus on Herbal Medicinal Resources. The purpose for this study was to evaluate the cytotoxicity profile in the laboratory to evaluate possible toxicity in the progressive use of Antivir H and IMB, herbal combinations used for bolstering immunity and for treatment of people with COVID -19.

The chance for Kenyan Scientists to produce drugs is primarily innovative: the use of supplement herbal medicines known to have anti-viral, antioxidant potentials, antibacterial, antitussive and mucolytic effects, liver protection, kidney protection, and immune boosting. They enhance resistance to disease; prevent cell damage from free oxygen radicals, provide antiviral and antibiotic properties, fight colds, flu and bronchitis, have anti-inflammatory; anti-allergic, antithrombotic and vaso-protective inhibiting tumours and protective for the gastric mucosa [3].

These herbal supplements are registered by Kenya Pharmacy and Poisons board RG/ Antivir H No.1938/IMB.1939 From these products, the Natural Products Initiative (NPI) through National Museum of Kenya and Primate Research received grant to investigate safety and efficacy in Baboons infected with tumour causing immunological factors.

Study Materials

Antiretroviral (Antivir- H) is composed of *Cetraria e.*, *Glycyrrhizins g.*, *Warbugia u.*, *Zanthoxylums c.*, *Papain*, *Tylosema e.*, *Vernonia a.*, *Oldefildea s.*, and Zinc supplement all formulated and composed and packaged in 500mg tablets. It is used together with immune booster (IMB) for clinical care. The Herbal Immune Booster (IMB) is composed licorice, vincas alkaloids, delonix alkaloids, *Sylibum m.*, Carrot, *Allium.s*, *Moringa. o.*, *Carrisa e.*, *Azadiracta leaf*, *Chanca. p.* and *Nigella. s.*

The Antivir-H components include; *Cetraria islandica* lichen consisting of algae and fungus used for treating irritation of the mouth and throat, loss of appetite, common cold, dry cough, bronchitis, indigestion, fevers, lung disease, kidney and bladder complaints (3). Glycyrrhizin possesses anti-inflammatory and antioxidant activities and can stimulate endogenous production of interferons [4]. It has been shown to down regulate pro-inflammatory mediators in CCl₄-induced hepatotoxicity. Glycyrrhizin produce an inhibitory effect on HBeAg secretion that imparts anti-HBV activity. Anti-fibrotic activity of glycyrrhizin could be attributed to its inhibitory activity on NF- κ B (ibid) showed that glycyrrhizic acid induces inhibitory effects on hepatocyte apoptosis and liver fibrosis, which was associated with down regulation of CTGF, HSC activation, MMP-2 and MMP-9, and collagen type I and III mRNA.

These effects provide potential therapeutic strategies for antifibrosis [5]. *Warburgia u.* has potentials for the treatment of pneumonia, asthma, malaria, candidiasis, skin infections,

human immunodeficiency virus opportunistic infections, diarrhea, and measles given the common use in the management of these diseases [6].

Zanthoxylum.c. has several components, including coumarins, alkaloids, benzenoids, triterpenes, sterols and lignans [7, 8, 9]. *Z. monophyllum*, used in Venezuelan traditional medicine to treat runny nose, jaundice and ophthalmia, in addition to being used as an anaesthetic [10; 1; 11]. Papain used to treat sore throat and throat swelling (pharyngitis). Taking papain by mouth, together with other treatments, relieves sore throat and swelling inflammation of the throat and pharynx, shingles (herpes zoster) symptoms, ongoing diarrhea hay fever, runny nose, and a skin condition called psoriasis. It is used as a digestive aid and for treating parasitic worms. Papain is used for pain and swelling (inflammation) as well as fluid retention following trauma and surgery. Tylosema e. certain protease inhibitors have been shown to impart high anti-enteroviral effects through interference with proteolytic cleavage during the replication process [12].

The antiviral activity of phytosterols is through the blocking effect on immediate-early antigen expression in fibroblast cells and blocking of virus-cell interaction and/or virus multiplication [13]. *Vernonia a.* has two major classes of bioactive compounds: four sesquiterpene lactones and seven new stigmastane-type steroid glucosides. The sesquiterpene lactones are well known for their anthelmintic, antiamoebic, antitumor, and antibiotic properties. Two known sesquiterpene lactones, vernolide and vernodalol isolated from *Vernonia amygdalina* displayed good antimicrobial activity against several bacterial and fungal species [14]. Zinc has effects against viruses. It appears to lessen symptoms of the rhinovirus (common cold). In addition, there is some evidence that zinc has some antiviral activity against the herpes virus. Zinc is needed for the proper growth and maintenance of the human body. It is found in several systems and biological reactions, and it is needed for immune function, wound healing, blood clotting, thyroid function, and much more. Zinc might also have effects against viruses.

Herbal Immune booster (IMB) a combination of Liquorices, Delonix glycosides, Silymarins, Carotenoids, Aloe glycosides, Garlic, and Vinca alkaloids. These multiple active constituents have polyvalent action boosting immune system. These multiple constituents contain bioactive molecules that combine to produce synergism to reduce undesirable effects of any individual constituent. Anthraquinones mucilaginous gel possesses anti-inflammatory properties, gastrointestinal activity, and antidiabetic activity, anticancer, antibacterial activity

and radiobiological protection [15]. Interferon components included as an immunomodulator and for adjuvant therapy and prophylaxis of recurrent infections of the upper respiratory tract and of urogenital tract.

Flavonolignans that exert antihepatotoxic activity preventing galactosamine induction of cell lesions, hence hepatoprotective. The flavonolignans antagonize possible liver degenerative mechanism, diglucopyranisiduronic acid and deoxycorticosterones for treatment of Addison disease and various inflammatory conditions. Flavonoid components possess antimicrobial properties and also exert spasmolytic and anti-ulcerogenic activity [16]. The flavonoids have antiretroviral activity and have been shown to have activity against influenza A virus, HSV-1 and HSV-2 viruses [17].

2. MATERIALS AND METHODS

The study employed experimental laboratory testing design by screening cytotoxicity of Antivir- H and IMB in the Biomedical Research laboratory to evaluate safety. The research materials were obtained from Pona Herbs Ltd Company in the Kenya Coast Kilifi County, registered as a limited company in the Kenyan Coast, Kilifi County, Kibarani estate, Reg /No 415895 whose mandate is to undertake research, manufacture and produce nutraceutical products, herbal and nutrition supplements, natural oils & natural medicinal cosmetics.

Methodology Extract Preparation

Cytotoxicity assay was performed to predict toxicity of the herbal extracts on Vero Cells. Two methods were performed. In the first method, a 10mg of herbal extract was dissolved in distilled water. 100µl picked topped up with 900µl PBS to make the working solution.

MTT Assay

In the second method, MTT reagent (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) was used to convert to an insoluble formazan product. After solubilization of the formazan with the included SDS (sodium dodecyl sulfate) reagent, the concentration of the colorimetric probe is determined by an optical density measurement at 570 nm. In order to evaluate cytotoxicity by the mitochondrial viability parameter, the 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed on Plant extract Concentration CC_{50} of the medicinal plant extract on vero cell (lines in µg/ml).

Upon attainment of confluence, cells are washed with saline phosphate buffer and harvested by trypsinization. The number of viable cells determined using Trypan blue exclusion method (Cell density counting) using haemocytometer. An aliquot of 100ul containing 2×10^5 cells/ml suspension seeded into a 96 –well plate and incubated at 37C for 24 hours at 5% CO₂ for 24hrs. After 24 hrs, 15µl of sample extra at seven different concentrations each serially diluted and added on Row H-B.

Row A, containing media and cells alone served as the negative control experiment done in triplicate. Then 10ul of MTT dye (5mg/ml) added and the plates incubated for 2 hrs at 37C and 5% CO₂. The cells incubated for 48 hrs, then 10ul of MTT dye (5mg/ml) added and the plastic plates incubated for 2 hrs at 37C and 5% CO₂.

Mitochondrial dehydrogenase which is a biomarker of live cells interacts with MTT dye reducing it to insoluble formazan. The formazan formed is directly proportional to the number of the live cells and then solubilized with 50ul of 100% DMSO and optical density (OD) read using a calorimeter reader at 540nm and reference wavelength of 720nm.

The cytotoxicity of the plant extracts on Vero cells was determined by calculation of CC₅₀. The 50% cytotoxic concentration (CC₅₀) was defined as the extracts concentration (µg/mL) required for the reduction of cell viability by 50%.

3. RESULTS AND DISCUSSION

3.1 Results

The cytotoxicity of the plant extracts on Vero cells was determined by calculation of CC₅₀. The 50% cytotoxic concentration (CC₅₀) was defined as the extracts concentration (µg/mL) required for the reduction of cell viability by 50%.

The CC₅₀ of the medicinal plant extract on vero cell (lines in µg/ml) was 240.145 and 211.585 for Antivir -H and IMB respectively (Table 1)

Table 1. Results for cytotoxicity of Antivir H and IMB plant extract concentration

	Plant extract Concentration	CC ₅₀ OF THE MEDICINAL PLANT EXTRACT ON VERO CELL (LINES IN µg/ml)
1	ANTIVIR -H	240.1452
2	IMB	211.585
3	AmiraMesh (AM)	831.746

3.2 Discussion

The CC_{50} of the medicinal plant extract on vero cell (lines in $\mu\text{g/ml}$) was 240.145 and 211.585 for ANTIVIR -H and IMB respectively against the 831.746 AM. The interpretation of these findings is that the plant extracts are considered non - toxic as the cytotoxic concentration meets the National Cancer Institute recommendations concerning the inhibitory activity of crude extracts. Thus these extracts provide scientific based evidence for their safety in human beings. In this regard, the specific herbal materials of the mixtures were registered by the Kenya Pharmacy and Poisons Board RG/ Antivir H No.1938/IMB. These findings are in line with the constitution of Kenya (2010), The Health ACT (2017), the Health Policy (2014-2030), Traditional and Alternative Medicine Policy (2018) and provision of Universal Health Coverage through integration of traditional and alternative medicine into the mainstream healthcare delivery system. Therefore Antivir H and IMB combination provides hope and should be included in the arsenal for the management of Covid 19.

4 CONCLUSION

The results of the study indicate that the plant extracts of ANTIVIR H & IMB are considered non-toxic as the cytotoxic concentration meets the National Cancer Institute recommendations concerning the inhibitory activity of crude extracts. Therefore, it can be concluded that Antivir H and IMB has No toxicity in Vero cells, is safe for human use and should be included in the arsenal for the management of COVID- 19.

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