

In Vivo, in Vitro Evaluation of the Effect of Mixed Plant Extract Used in the Prevention of the Reproduction & Transmission of Corona Virus inside Cells

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Abstract

It has become vital to look for innovative antiviral medications from plants and other natural sources as alternatives for prophylaxis during the novel coronavirus disease 2019 (COVID-19) pandemic. The antiviral potential of mixed extract (artemisinin and linen seed) is reviewed in this study for the treatment and prophylaxis of coronavirus disease. Based on their origin, native area, utilized plant parts, and antiviral potentials, therapeutic herbs and crops have been described in detail. It has been detailed how plant-derived natural antiviral chemicals may play a part in the development of plant-based coronavirus medications. Objective: This study highlights the effect of the mixed extract (artemisinin and linen seed) as potential preventative agents and therapeutic treatments for SARS-CoV-2 at early stages of infection. Material & Methods: 18 mice was selected in this study & divided into 3 group: Group A: Containing 6 mice were only fed without any medication or virus administrated to be a control group. Group B: Containing 6 mice injected by COVID-19 without the administration of the treatment. Group C: Containing 6 mice injected by COVID-19 and after 5 days of injection, the extract was administrated. Result: we can concluded that the mixture prevent the virus to inter the cell without any effect on the human cell especially if we know the human body can remove all the part of mixture within one to two days. Conclusion: The active ingredient(s) in the extracts from *A. annua* are probably other than artemisinin or a mixture of ingredients that impede viral infection at a stage downstream of viral entry. Additional research will look at in vivo activity to see if *A. annua* can cure SARS-CoV-2 infections at a reasonable price.

Keywords: Artemisia annua, Antivirals, COVID-19, Covid-organics, SARSCoV

INTRODUCTION

All corona virus family including COVID-19 composed from circular virus particle in diameter for 80-160 nm. In the middle of virus genetic material was present which is an RNA which is covered by N-protein for protection, both of composed of central cover that is present in the middle of virus particular [1]. The central core was surrounded by double layer membrane composed from lipid, this lipid membrane composed of four types of protein which located to outside in different space.

The most important protein is surface protein (protein S), protein M, protein G and protein H5. Those protein consider as skeleton protein [1]. Virus composed of single RNA strand which can transcription itself inside of cell after entry in it without need to transcriptase enzyme. One of the most important protein which is produce by hereditary material is S protein which can attached with cell receptors and invading occur after that specially ACE2 receptor which present in respiratory system.

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Viral protein S composed from three units which spread in three dimension manner and it is consider a strong antigen and can activate furan enzyme in the cell which can increase speed of concentration of viral with cell receptors. This virus has unusual features as some of the extensions of the hereditary material of the virus are similar to HIV (AIDS) [2-3]. Scientists found that amino acid can induce the connection of virus with cell receptor using S protein from virus with ACE2 receptor in cell which consider as another evidence that the virus is natural. This virus considered as highly spreading virus in comparison with other type of virus from the same family which helped in the ease of transmission of the virus between cities and countries overall the world [3]. The biochemical nature of the new virus indicate that the virus was evolved into its current form (COVID-19) through natural selection using a host animal which mean this virus was produced via the aid of nature not through laboratory procedures and the nature give the virus all of it's a strong character [2]. A pandemic of coronavirus infection 2019 (COVID-19) [4-6] brought by the severe acute respiratory syndrome coronavirus 2 (SARSCoV-2) [7] had caused a rising mortality toll as well as substantial economic and societal repercussions. In record time, several vaccines were created and approved, and they are now being distributed as soon as feasible [8-9]. The antimalarial drug artemisinin is generated by the natural herb *Artemisia annua* L. It is a sesquiterpene lactone that is created & kept in glandular trichomes present on the plant's shoots, mainly the leaves and flowers. Over 2,000 years ago, the plant & its active ingredient, artemisinin, were safely used in the treatment of a number of fever-related illnesses, particularly malaria [10]. High bioavailability of artemisinin after the ingestion of *A. annua* leaf powder allows for rapid distribution through peripheral circulation to a variety of organs, such as liver, lungs, heart, & brain [11]. Moreover, in vivo inflammatory cytokines level like interleukin-6 and TNF- α are decreased by the administration of the plant *A. annua* & artemisinin [11-13], when numerous SARS-CoV-2 patients experience a "cytokine storm" these effector molecules can be a concern [14] moreover, artemisinin lessens fibrosis [15-16]. Other plant used in this study is *Linum usitatissimum* commonly known as flaxseed is one of the oldest crops traditionally cultivated mainly for its oil purposes. Flaxseed is widely known for its rich source of nutritive and bioactive compounds it has gained considerable interest due to the potential health benefits attributed to its component of metabolites. The benefits of lignin are frequently explained by their antioxidant ability, and they may be used to treat cancer, diabetes, thrombosis, viral infections, and obesity [17].

MATERIALS & METHODS

Plant Materials, Extract preparations

Extraction method of artemisinin from *Artemisia*

In the beginning we mix all the part of the plant except the roots and blinding these parts by using an electrical grinder

until we get a fine powder. Taking 10 gram of plant powder and dispersed it with 200 ml of boiling distilled water and mix these dispersion using a mixture for 15 minute, leave the mixture for 24 hrs at room temperature in glass container. Filtrate the mixture using a gauze (multiple layer) to remove all unwanted material found in mixture. Centrifuge the mixture using a centrifuge with speed 3000 rpm for 10 min. Take the supernatant and spread it in a metal clean sterile dishes. During the extract of artemisinin by using an oven at 40 C.

Extract of lignin from *linum usitatissimum*

Collect the seeds of linum and clean it in propose way. Soak the seeds in warm water for 24 h in closed container, filtrate the solution and take the filtrate, centrifuge it for 15 min. at 3000 rpm, add ethyl alcohol (70 %) for drying the filtrate and extraction of lignin through separation technique by using an oven at 50 C to get a pure lignin.

Preparation of the extract

The mixing of both extract in ratio of (2:1) (artemisinin: lignan) and administration of 10 gram of those mixture to the infected mice and culture cell shows the effect of these extract is very powerful. Through the effect of these extract on the protein receptors on the cell specially ACE-2 protein receptors and blocking the effect of S protein without any adverse effect on the infected cell itself and consequently prevent S protein division to S1 and S2 and prevent the attachment & infusion of the virus to cell membrane, so prevent the entry of the virus or its hereditary material inside cell and the production of new viruses. The virus cover itself by greasy bubble, this mixture can dissolve these bubble which is cover the virus and hence prevent the virus to infuse with cell membrane.

Probable properties of dual extract

It can be classified as protein blocking agent.

The mixture is composed from extract of two plant.

Can be administrated orally.

It have a simple side effect.

The mixture play a role in destruction of glycan cover of S protein which make the virus uncovered to body immune system.

The mixture have the ability to prevent the reproduction of viral RNA inside of the cell.

The mixture can highly broke and rearranged the nucleotide sequences in viral RNA and decrease the viral ability to cell entry.

Laboratory experiment

18 mice were selected in this study & divided into 3 group:

Group A: Containing 6 mice were only fed without any medication or virus administrated to be a control group.

Group B: Containing 6 mice injected by COVID-19 without the administration of the treatment.

Group C: Containing 6 mice injected by COVID-19 and after 5 days of injection, the extract was administrated.

After 15 days of administration of COVID-19 injection & the prepared plant extract, we conclude that:

All mice in the group A are healthy with good movement and feeding.

All mice in the group B are lethargic with inability to movement and feeding.

While all mice in the group C started lethargic not being able to move and feed, and after giving them the plant extract, their movement gradually improved, and their feeding ability started to improve.

After killing one mouse from each group, tissue culture was taken, stained, and investigated using light microscope, we note the following:

Cells from group A were containing all cell content including RNA, DNA and the proper nucleotide arrangement.

Cells from group B were suffering from physiological changes and after using PCR technique we demonstrated the viral RNA was duplicated more than 500 time.

Cells from group C were suffering from physiological changes and the replication of the viral RNA, and after giving the plant extract, these cells began to improve physiologically with a decrease in the number of viral RNA in the infected cells

Tissue Culture

Tissue culture of mice lung was done after isolation of lung cells through mechanical shredder, after that cells were collected, washed and cultured in petri dish. We inject one of the prepared culture with COVID-19 virus (we inject one cell of the cell culture) & let the culture to grow in optimum condition, after few days we observe the spread of virus to all cells (healthy cells) in the culture. While, when we inject another prepared culture with COVID-19 & inject the infected cell by the plant extract, via microscopic examination of the culture we found there is no spreading of virus to other cells in the culture as well as the replication of the viral RNA in the infected cell was stopped which mean the extract prevent the spread of the virus to other cells by preventing the viral spike from attachment to the other cell receptors.

RESULTS

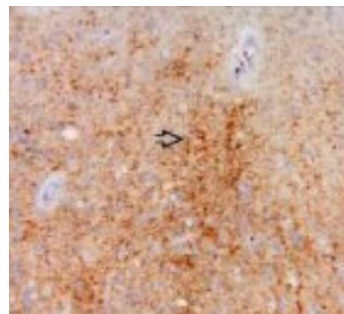


Fig.1: Lung tissue of a mouse infected with the COVID-19

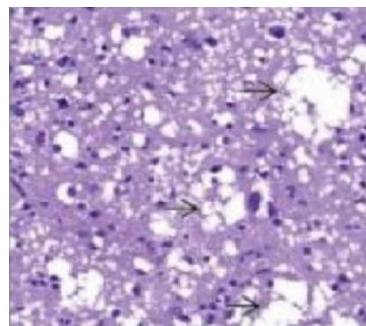


Fig.2: Mouse lung tissue in which viral RNA is replicated

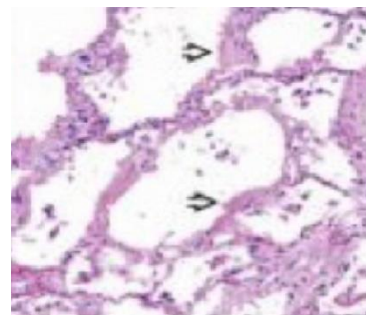


Fig.3: Mouse lung tissue in which infection with the COVID-19 decreases

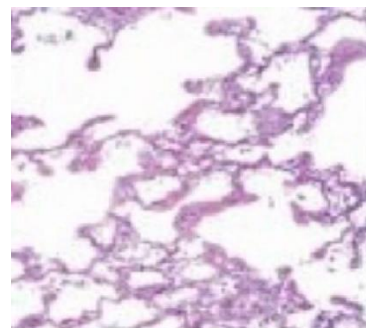


Fig.4: Healthy lung tissue in infected mice after treatment

of the plant extract to inhibit the TMPRSS2 enzyme which is required for entry into epithelial cells. Our results are consistent with the results from other researchers [18-19], but extensive research on human cells are required to

confirm the efficacy & safety of the plant extract on human.

CONCLUSION

The active ingredient(s) in the extracts from linen seed & *A. annua* are probably other than artemisinin or a mixture of ingredients that impede viral infection at a stage downstream of viral entry. Additional research will look at in vivo activity to see if the extract from linen seed & *A. annua* can cure SARS-CoV-2 infections at a reasonable price. From all of the above examination through cell culture & infected mice, we can conclude that the mixture of plant extract will prevent the virus entry to the cell without any side effect on the cell itself especially if we know the body can remove all of the mixture within one to two days.

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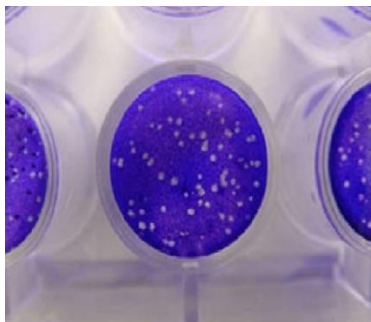


Fig.5: Cell culture infected with COVID-19

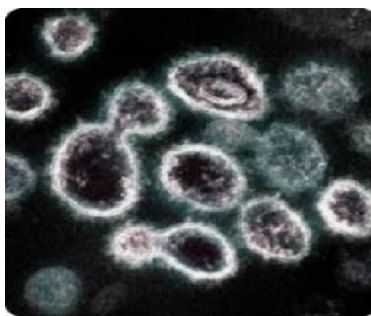


Fig.6: Cell culture showing the mechanism of transmission of the virus from one cell to another

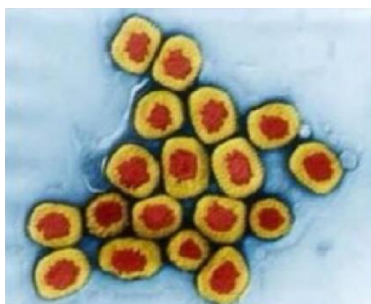


Fig.7: Cell culture of healthy cells after the administration of plant extract

DISCUSSION

From all studies and laboratory work which is done on the mice & cell culture, we got a positive results. Whereas, the results obtained from cell cultures were almost identical with the results obtained by administering the plant extract to infected mice. The plant extract produce its effect though decrease the ability of virus to attached with the cell membrane also decrease the entrance of virus inside cell. This action is done through the prevention of the virus RBD receptor from the attachment to the ACE2 located on the cell surface By changing the structure of the S protein and changing its shape to prevent it from binding to the receptor of cells. In addition, the extract have ability to break down the glycan which cover the virus S protein on the surface & make the virus vulnerable to the immune system. Also through the laboratory analysis we demonstrate the ability

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