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Essential Oil from Poly-phytopharm Herbs Attenuate Manifestations of Influenza Illness Mice Model

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Abstract: Influenza caused by influenza a virus, is an acute self-limited respiratory illness, its severe form is characterized by an intense inflammatory response, pulmonary infiltrates, vasodilator shock and high mortality. Treatment of disease is mainly focus to the use of a limited set of anti-viral drugs. To search for the development of new potential drugs for the treatment of influenza disease, here we report the therapeutic effects of an essential oil obtained from a poli-phytopharmacological formulation (PEO), based on cinnamon, guava, propolis, and menthol on influenza A murine model. Animals administered prophylactically with PEO showed less severe clinical symptoms. Histophatologically, there were no evidence of pulmonary parenchymal consolidation and in contrast to non-treated animals, the lung inflammatory process was very limited.

Key words: Influenza A, poli-phytopharmacological formulation, anti-inflamatory effect, murine model.

INTRODUCTION

Influenza is an acute respiratory illness that resolves in almost five days; however infection can cause severe systemic inflammatory response, pulmonary infiltrates, vasodilator shock and high mortality[1]. The emerge of the 2009 H1N1 pandemic swine influenza A virus has prompted new global efforts to find other ways to reduce the prevalence of influenza infection. For example, *Echinacea purpurea*, *Eucalyptus globulus* and *Pinuspinaster*have been used to treat viral respiratory diseases[2]. Pleschka et al in 2009 showed that the commercial extract of Echinacea purpurea (Echinaforce ®, EF) inactivates influenza viral strains H1N1, H5N1 and H7N7 in cell culture.

The objective of this works was to study the therapeutic effects on an influenza A murine model of an essential oil obtained from a poli-phytopharmacological formulation (PEO), based on cinnamon, guava, propolis and menthol.

METHODS

Mice: Six female Balb/C mice were infected intranasally with Influenza A (H1N1) previously adapted to mouse animals according the methodology described by Galabov *et al*.[3]. Epidemic strain was provided by ENCB-IPN, México.

Essential oil extraction of the Poli-phytopharmacological formulation (PEO): The formulation was prepared by mixing homogeneously the powders of propolis (2 kg), camphor (2 kg) and cinnamon (2 kg) obtained from (PROQAVIF S.A. de C.V) and 8 kg of guavas purchased in local market in 5 liters of water. Next PEO was obtained by hydrodistillation using the methodology described previously[4].

Therapeutic administration of PEO: For therapeutic administration 6 animals were anesthetized and instilled with: 16.3x 10^{-3} ml/g mouse/day of PEO diluted in 5% Tween 80 for four days. As controls infected and without infection animals were instilled with 80 µl of 5% Tween 80 saline solution. Animals were sacrificed 7 days post-infection, and lung tissue was obtained from each animal, fixed in 10% formaldehyde or 4% paraformaldehyde for histopathological analysis.

RESULTS

Here, to characterize the effect of PEO *in vivo*, we used a mouse model of H1N1 influenza A infection. First we showed that H1N1 human influenza virus was adapted to mice, displayed clinical and histopathological manifestations; the body mass decreased on average 21% post-infection, showing ruffling, lethargy and an important difficulties in breathing.

Histologically, lungs showed a very important distortion of normal architecture with significant thickening of the interstitium, abundant chronic inflammation, pneumocyte hyperplasia and fibroplasia with reduced airspaces (Figure 1, B).

The mice infection using human influenza virus showed the clinical effects reported (weight loss)[5]. Some reports have showed that the histopathology of primary pneumonia caused by influenza in humans is characterized by vascular congestion, dilation and thickening of the alveolar septum. Within the alveoli also is described the presence of neutrophils, alveolar macrophages and erythrocytes scarce,

appearing wall of the alveoli lined by hyaline membranes and thick eosinophilic[6]. In our study, we observed histopathological similarities, showing a higher degree of damage, suggesting that the virus adapted to mice is highly virulent.

After therapeutic administration of PEO, the important pneumonic process observed infection mice were decreased. Lung tissue from non-infected mice (Figure 2, A) showed architecture of normal tissue, constitute of alveoli with thin walls formed by pneumocytes type I and II; no evidence of inflammation, nor alveolar damage were found. Animals intranasal instilled with 5% tween 80 saline solution were used as control for the excipient effect. Results showed in Figure 2, B a similar architecture to normal tissue, with small areas (less than 20%) of alveoli effect, some of them confluent. In counterpart, as we showed before, influenza infected mice instilled with 5% tween 80 saline solution presented the severe form of murine influenza illness, characterized by an intense inflammatory response.

The structure forming the alveoli was replaced by inflammatory tissue in more than 80% of its area, becoming confluent alveoli with thickened walls (Figure 2, C). In tissue from infected animals treated therapeutically with intranasal PEO (Figure 2, D), although an important area of the parenchyma was preserved, the alveoli in some areas converge between themselves and an important number of inflammatory cells were still present.

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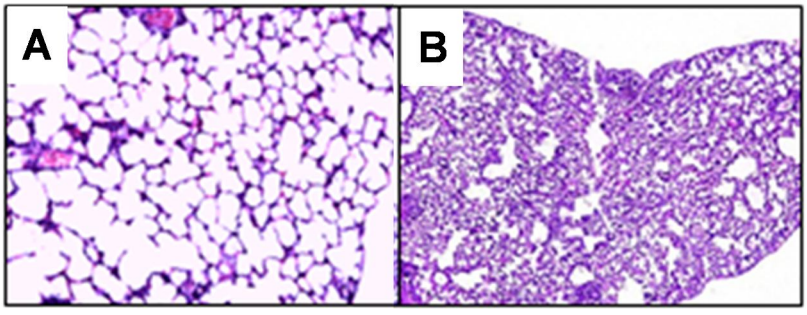

Figure 1: Histophatological lung alterations of H1N1 infection trough viral passage. Lung sections from: non-infected animals (A) and from (B) viral passage, respectively.

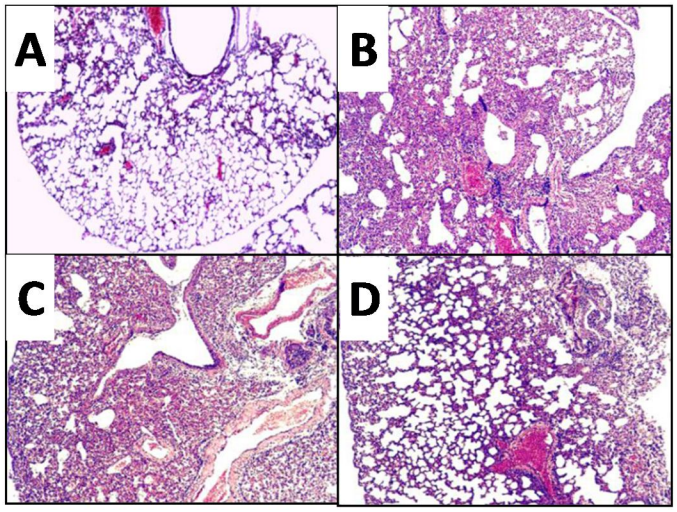

Figure 2: Pulmonary histopathology of Lung. A. non-infected mice, B. non-infected mice intranasal instilled with 5% tween 80 saline solution, C. infected mice intranasal instilled with 5% tween 80 saline solution. D. infected mice treated therapeutically with intranasal PEO

CONCLUSIONS

Our results indicate that PEO modifies influenza-related inflammatory response decreasing pulmonary infiltrate. With more understanding of their antiviral mechanisms, more traditional medicines will be utilized for clinical pharmaceutical purposes and novel drug discovery.

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