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Antioxidant effect of *Lepidium meyenii* (Maca) root on testis and spinal cord tissues of avian influenza virus-infected chicken embryo

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ABSTRACT

This paper focuses on the antioxidant effect of powder extracts from Maca on the testis and spinal cord tissues of chicken embryo infected with the avian influenza virus. An experiment was conducted on 500 embryos; 300 eggs were used as a control, and each of the remaining 100 eggs was infected with the avian influenza virus. One of the powder extracts was also injected. After an incubation period of 19 days, the embryos were studied through histopathology, and different histopathological changes recorded. The paper then discusses the various morphological patterns seen in the specimens. The avian influenza virus causes cytopathic effects in the cells of the embryo that manifest as edema, necrosis, and fibrosis. The antioxidant effects of the Maca plant extract protect the cells from these changes in the group of embryos injected with powder. We found that Maca plant can inhibit avian influenza from in vitro replication. It also protects body cells from the oxidative effects of resulting inflammation.

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INTRODUCTION

Avian influenza, deadly influenza infecting birds, can get transmitted to humans through contact with infected birds/animals. This may result in severe pneumonia along with fever and cough, poising a greater mortality rate among the elderly population (WHO 2018). In recent years, the world has witnessed the pandemic effect of avian influenza that affected millions of people. Due to the higher infection rates and rapid progression of the disease with a shorter incubation period, the outbreak has been reported multiple times. According to research done in 16 countries, the case fatality rate of the disease is nearly 33% in the year 2017 (WHO 2017). Various guidelines and steps have been taken to control the viral illness; however, because of the increasing mutation of the virus, treatment research for viral infections is still of scientific importance.

Avian influenza is a type A (Flu-A) category, which is further categorized into subtypes based on two surface antigens: hemagglutinin and neuraminidase (Spackman 2008). Researchers have reported variation in the antigen structure of the virus (Alexander 2006). In the current practice, vaccination remains the primary method for the prevention of influenza. However, the rapid mutation of the virus challenges the efficacy of vaccination, mainly in people over 65 years of age who are 'at-risk' population for the disease owing to their poor immunity (Khanna *et al.,* 2014). The role of vaccine among birds is controversial and reportedly has many challenges (CDC 2018, Suarez *et al.,* 2016). Another research says that vaccination among the bird population is the cause of the spread of the disease (Parry 2005). In the current knowledge, where the complete types of avian influenza have not been understood (Kaleta *et al.,* 2005), exploring alternative methods for controlling the disease could be useful.

A systematic review done by Wang *et al.,* revealed the beneficial effect of traditional Chinese medicine in treating avian influenza (Wang *et al.,* 2014). Thus, the present study aims to evaluate in vitro the antiviral activity of *Lepidium meyenii* (*L. meyenii*) (Maca) against avian influenza. *L. meyenii* (Walpers), commonly known as Maca, is a perennial herbaceous plant abundantly found in the Peruvian forest. Due to the presence of carbohydrates, proteins, vitamins, and minerals, it is considered as one of the most nutritional element and used in the treatment of undernourishment (Gustavo 2012). Besides, it is also used in the treatment of cancer and reproductive diseases (Gonzales *et al.,* 2009, Chung *et al.,* 2005). Another recently published article has reported the efficacy of Maca in human influenza (Del Valle Mendoza *et al.,* 2014).

MATERIALS AND METHODS

In this experiment, 500 fertilized chicken eggs were used. The eggs were categorized into three different groups and were given three different types of intervention (Distilled water; Influenza; and Influenza + maca). The eggs were kept under observation for 19 days of incubation for the extraction of embryos. Extracted tissues were studied under a microscope for histopathological changes and abnormalities in testis and spinal cord.

Study design

Five hundred eggs were randomly divided into three experimental groups (i) control (C) (ii) influenza-infected eggs without any intervention (IF), and (iii) infected eggs with intervention (IF+M). The control group had 300 eggs, whereas the other two groups had 100 eggs each.

Chickens were obtained from avian influenza-infected flocks that were in the process of being depopulated. These chickens belonged to the Al-Watania poultry company in Al-Qassim (Buridah) of Saudi Arabia and were declared to be infected based on clinicopathological findings and history of mortality. Chickens 1 to 5 came from a flock of 45,000 seven-week-old broilers which had experienced 35% mortality over the past six days. Depression, ataxia, and swelling of the combs and wattles were prominent signs in the flock. The presence of influenza virus was determined using a standard technique involving the inoculation of chick embryos (eggs).

Intervention

Control group eggs (group-I) were injected with distilled water (0.1 ml/ egg) through the air space on the 5th day of incubation (first week). Then the embryos were extracted at 19 days of incubation to study the histological changes and structure of the testis and spinal cord. Group-II (IF) contained fertile eggs (100) infected with avian influenza virus (IF); the embryos were extracted at 19 days of incubation to study histopathological changes in the testis and spinal cord. Group-III (IF+M) has 100 avian influenza-infected eggs, which were injected with powdered Maca root obtained from the iHerb company, America (USA). The standard dose for Maca ranges from 1,500 – 3,000 mg daily, and it is typically split into 3 servings (Gonzales *et al.,* 2002). Obtained average 2,250 mg/ per weight 60 kg of normal human), then took (1 capsule) diluted in 5 ml of distilled water, then obtained of solution (2 mg/ per Wight 60,000 mg of egg) extract of powder Lepidium meyenii (maca) root (M), took of this solution by syringe, injected with (0.2 ml / egg) into the air space (Levinson 2016), according to the standard techniques (Tavakkoli and Salandari 2014). It was injected in the first week of the 5th day of incubation, and the embryos were extracted at 19 days of incubation.

Sample collection

All fertilized chicken eggs were incubated at 37.5°C and 55% relative humidity and were opened and extracted on the 19th day of incubation. A specimen of testes and spinal cord was dissected out following the scarification of chicken. All the specimens were processed for histopathological examination.

Microscopic examination

Specimens were immediately fixed in 10% formaldehyde. The specimens were cleared and conventionally dehydrated with graded ethanol in an ascending manner. These dehydrated specimens were cleaned with xylene and embedded in paraffin wax. Thin section of size 5 µm was collected and stained with Hematoxylin-Eosin stain (H&E) for microscopic readings. The tissues were studied under the light microscope.

RESULTS

The histopathological findings of the testes specimens are given in Figure 1 while Figure 2 depicts the findings for the spinal cord.

DISCUSSION

The Avian Influenza

The avian influenza virus primarily affects birds, particularly chickens, and has not been known to cause human disease. However, there is evidence of person-to-person transmission, which until now has remained a significant concern in case gene reassortment occurs and the virus adapts to the human body. Several outbreaks of the avian influenza infections have occurred in the past with the most recent being reported between 2003 and February 2009. The first reported case of bird influenza was

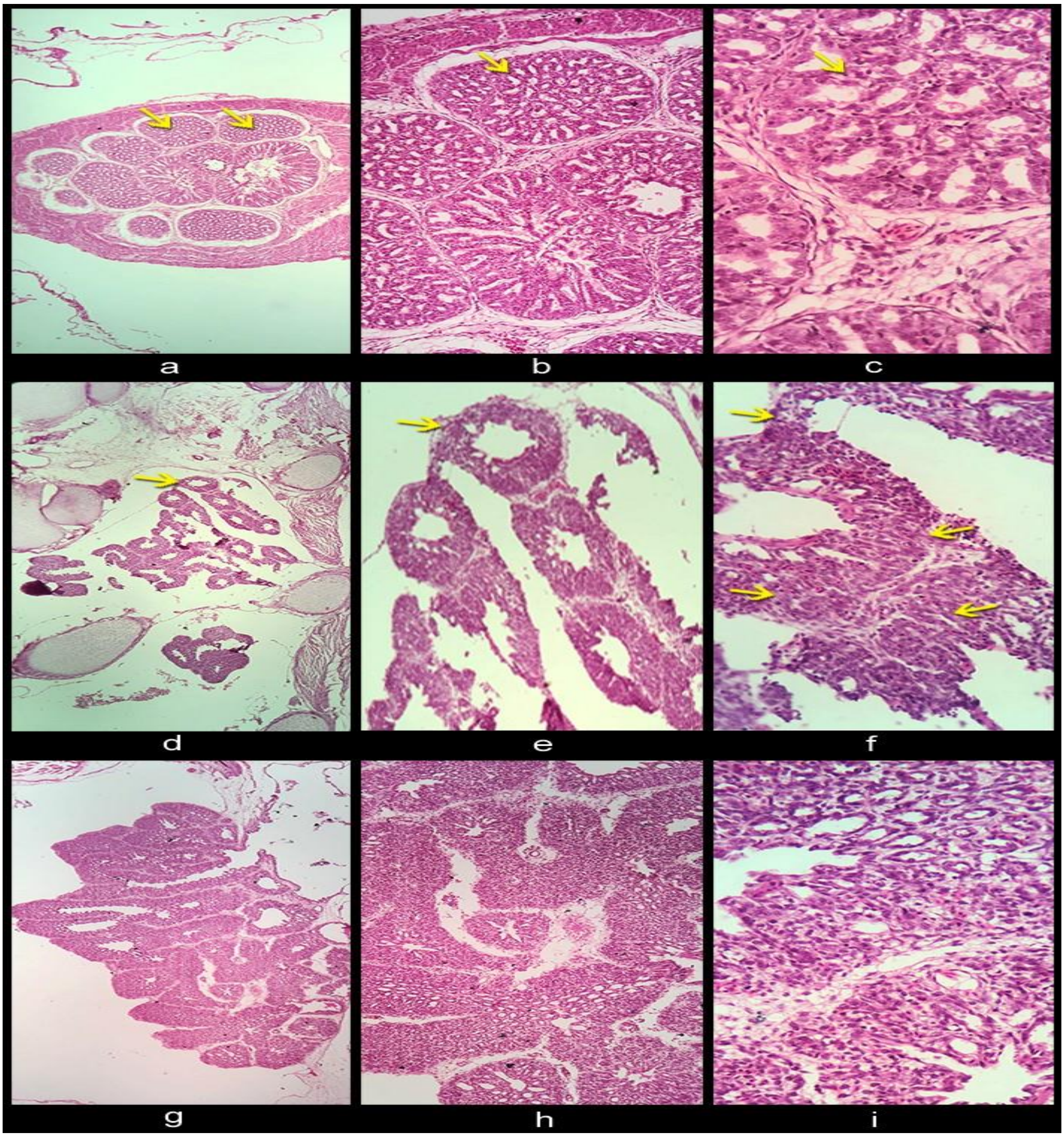

Figure 1: The testes specimen tissue (group-I) in the control group was normal and showed a similar histological structure of germinal epithelium of the seminiferous tubules (↑) (Fig.1a, b & c). Whereas, the testes of group-II showed thinner germinal epithelium of the seminiferous tubules in places along with other abnormalities such as atrophy, shrunken and vacuolar degeneration (➢). Nuclear membrane arrangement and irregularities observed such as edema, vacuolization, and hemorrhage (✶). Along with that, patchy necrotic cells (◊) (pyknotic nuclei and eosinophilic cytoplasm) were also found in some specimens, and reduced seminiferous epithelial layers were found in numerous tubules (Fig. 1d, e & f). Samples from Group-III showed the normal histological structure of most of the seminiferous tubules (Figure 1g, h & i). Magnification: (Fig.1 a x40, b x100, c x400), (Fig.1 d x40, e x100, f x400), (Fig.1 g x40, h x100, i x400).

in 1997 when the H5N1 strain of Influenza A caused high mortality rates in Hong Kong (Ip *et al.,* 2015). The infection was spread primarily by chickens and caused a very aggressive form of the disease. In 2003-2004, another outbreak caused by a similar strain emerged in the Asian countries, and thousands of chickens were killed to eliminate the disease. No case of an outbreak caused by the H5N1 strain has been reported in the United States.

The H5N1 strain of influenza virus is well known to infect chickens more efficiently than humans mainly because the chicken's upper respiratory tract harbors a particular kind of viral receptor that is important in the pathogenesis of the strain (Hamburger 1942). In humans, this receptor is

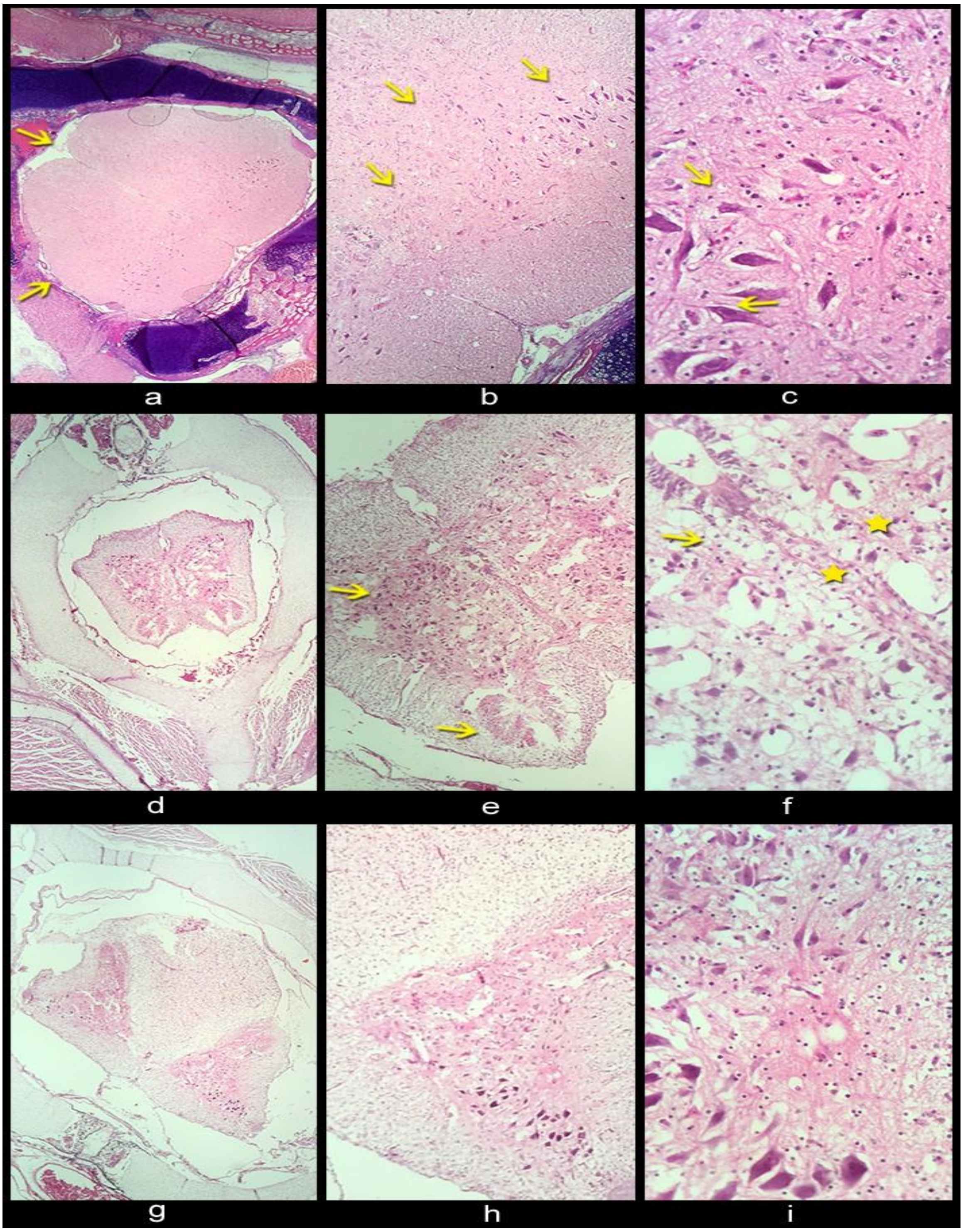

Figure 2: The control group (group-I) showed normal histological structure (↑) (Fig. 2 a, b & c). In group-II, the specimens revealed thinner (↑), marked vacuolar degeneration (↑), fibrosis, edema in most of the white matter and grey matter cells (➢). Along with it, damage and loss were present in most of the neuron cell bodies in grey matter (✶). Axons were seen to have karyolitic and neuron necrosis with most of the nuclear displacement. Disturbed central canal (◊) and intercellular spaces were also found in some specimens (Fig. 2 d, e & f). Samples from group-III with sections of the spinal cord with *L. meyenii* (Maca) showed normal histopathological structure for most of the tissues (Fig. 2 g, h & i). Magnification: (Fig.2 ax40, bx100, cx400), (Fig.2 dx40, ex100, fx400), (Fig.2 gx40, hx100, ix400).

only found in the lower respiratory tract, mainly in the alveoli. Thus, humans are rarely infected by the strain. Another critical factor in the pathogenesis of avian influenza is that it has a higher virulence factor than the human influenza viruses' strains such as HINI and H3N2. The H5NI strain also has shown resistance to interferon treatment used in the human influenza strains and characteristically induces increased production of cytokines, especially TNF (Ip *et al.,* 2015). These cytokines are responsible for the disease manifestations of pneumonia and acute respiratory distress syndrome.

Cytopathic effects of Avian Influenza

Research on the pathogenesis and cytopathic effects of the avian influenza virus is less compared to research done on human influenza viruses. It is not known whether the results seen in the human cells infected with the influenza virus differ from those seen in infected avian chicken. However, it is postulated that the viral infection induces the production of pro-inflammatory cytokines such as IL-10, IL-6, TNF-alpha and IFN-gamma, which are

involved in the disease pathogenesis. The pathological changes were seen in avian influenza, however, depending on the pathogenicity of the strain.

There are two strains, namely low pathogenicity avian influenza (LPAI) and high pathogenicity avian influenza (HPAI). In the LPAI, lesions in the chicken are mostly found in the respiratory tract (Dimitrov *et al.,* 2016). Other organs involved are the reproductive system, which includes the ovaries, oviduct, testis, and yolk sac.

The HPAI histopathological alterations have been well studied and analyzed in different strains. In the acute form of the disease, there are no gross morphological changes. However, infected chickens show hemorrhagic patches and mucus congestion in the trachea. There is also the presence of serious exudates in various body cavities, edema, and petechial hemorrhages. Microscopic identification of the virus first in the endothelial cell is characteristic, and later, virus-infected cells are found in the pancreas, heart muscle and adrenal glands (Kim *et al.,* 2015). The central nervous system is also involved in the disease process as neurons as well as glial cells also become infected. Non-suppurative brain lesion and encephalitis are characteristic of avian influenza infections. The most common cytopathic effects in the central nervous system include multifocal neuronal necrosis, edema vacuolar degeneration, and thinning of the cortex (Kim *et al.,* 2015). Similar features of necrosis and edema are evident in other organs such as the lungs and pancreas. Infiltration of the virus in the blood vessels, especially the capillaries, is probably the mechanism responsible for atrophy and degeneration of the organs and brain substance.

From the experiment conducted on the chicken embryos, cytopathic effects are demonstrated in the slides containing the avian influenza virus while normal histology is seen in the control specimens. The spinal cord sections showed features of necrosis and edema. There was noticeable thinning of the cord, vacuolar degeneration, fibrosis, edema in the grey and white matter and axons. These changes reflect an acute immunological response probably mediated by induced cytokine release by the virus. Edema and inflammation are early changes, which are then followed by healing with fibrosis. This inflammatory response leads to characteristic nuclear changes such as the dissolution of the chromatin termed karyolitic degeneration and nuclear necrosis. The central core of the spinal cord is also affected. Similar cytopathic effects were observed with standard slides containing the specimens from the control group. The germinal epithelium of the seminiferous tubules was thinner in some areas, and there was atrophy and shrinking of the nucleus termed pyknosis, vacuolar degeneration and oedematous. The nuclear membrane showed irregularities, hemorrhage, and evidence of necrosis in some cells. The nuclei were stained with typical eosinophilic cytoplasm. Therefore, the experiment was successful in demonstrating the specific cytopathogenic effects of avian influenza.

Antioxidant Effect of Lepidium Meyenii (Maca)

Maca (*L. Meyenii*) is a plant grown in the Andean region of Peru. The primary source of the extract is the tuberous root, which acts as the storage for its food. Maca is widely cultivated because of its nutritional benefits and medical applications. In the recent years, this plant has become more common as studies have shown that it has potent fertility enhancing properties, helps in the inhibition of benign prostatic hyperplasia, improves immune function, and reduces fatigue among others (Wang *et al.,* 2017). These effects are thought to be due to the presence of biologically active chemicals namely isothiocyanates and other phytochemical components such as essential fatty acids. Recent research studies have demonstrated that extracts from Maca have antioxidant and antiviral effects. These extracts scavenge the reactive oxygen species and radicals and thus, protect the cell from oxidative stress (Rodríguez-Huamán *et al.,* 2017). The mechanism involved has been demonstrated in a study done on rats; it has been reported that injection of Maca caused an increase in the antioxidants levels in the blood and organs such as superoxide dismutase in the liver, glutathione peroxidase in blood, and glutathione levels. It has been even postulated that the various nutritional and pharmacological effects of Maca are due to the antioxidant effects. It is hypothesized that these effects also result in the inhibition of lipid peroxidation, increase in antioxidant enzymes, and scavenging of radicals.

Maca extracts contain a variety of primary chemical compounds including fatty acids, vitamins, amino acids, and carbohydrates. However, most of the antioxidant effects are thought to come from secondary metabolites such as alkaloids like macamides, phenols, sterols, tannins, and glucosinolates (Caicai *et al.,* 2018). The alkaloids and the phenols are the most important chemicals that contribute to antioxidant effects (Rodríguez-Huamán *et al.,* 2017). Further studies have also shown that the combined use of phenols and alkaloids results in synergistic antioxidant effects. Tannins have been reported to inhibit many enveloped and non-enveloped viruses including the influenza family. The methanol extract from Maca can impede influenza replication and has a higher therapeutic index than most of the antiviral drugs. It is worth noting that the antiviral effects of Maca have been demonstrated through the interference of viral attachment, prevention of fusion, and reducing viral load in influenza infection. These mechanisms can all be a possible explanation for the mechanism of antioxidant and antiviral effects of the plant extract. In the central nervous system, oxidative stress is postulated to be the leading cause of degeneration and neuronal stress. Alkaloids and phenols have a neuroprotective function that helps prevent the cytopathic effect of the virus.

From the experiment conducted in the study, specimen slides from group III showed normal histological pattern and structure without any cytopathological changes as seen in the previous group II. It is, therefore, evident that the Maca powder extract can inhibit viral replication of avian influenza in the tissues of the chicken embryo and protect most of the cells from the resulting oxidative stress. The spinal cord slides showed a normal histopathological structure in most of the tissues. There were no edema and no vacuolar degeneration, and the neurons were well preserved. The testes, on the other hand, showed the normal histopathological structure in the seminiferous tubules.

The avian influenza virus is a well-known virus that primarily affects the birds. Many outbreaks have been witnessed in the recent past, and studies directed towards the understanding of its pathogenicity and effective ways to control the infection are on the rise. The primary strain implicated in the recent outbreaks is the H5N1 serotype, which has also shown resistance to interferons. The main cytopathic effects of avian influenza virus have been identified using bird study models, which have demonstrated edema, necrosis, and fibrosis. The virus has a preponderance to infect body cavities and organs such as the brain where it causes neuronal degeneration and damage. The Maca plant has shown adequate antiviral and antioxidant effects against most viruses. Our results showed that this plant could inhibit avian influenza from in vitro replication and protects body cells from the oxidative effects of resulting inflammation.

REFERENCES

Alexander DJ. An overview of the epidemiology of avian influenza. Vaccine, vol. 25, 2006 pp. 5637-5644.

Caicai K, Limin H, Liming Z, Zhiqiang Z, Yongwu Y. Isolation, purification and antioxidant activity of polysaccharides from the leaves of maca (Lepidium Meyenii). Int J Biol Macromol, vol. 107, 2018 pp. 2611-2619.

CDC. Prevention and Treatment of Avian Influenza A Viruses in People. Avian Influenza (Flu). [Online], Available: https://www.cdc.gov/flu/avianflu/prevention.htm [Accessed in 2018]

Chung F, Rubio J, Gonzales C, Gasco M, Gonzales GF. Dose-response effects of Lepidium meyenii (Maca) aqueous extract on testicular function and weight of different organs in adult rats. J Ethnopharmacol, vol. 98, 2005 pp. 143-147.

Del Valle Mendoza J, Pumarola T, Gonzales LA, Del Valle LJ. Antiviral activity of maca (Lepidium meyenii) against human influenza virus. Asian Pac J Trop Med, vol. 7, 2014 pp. S415-420.

Dimitrov KM, Zarkov IS, Dinev I, Goujgoulova GV, Miller PJ, Suarez DL. Histopathologic Characterization and Shedding Dynamics of Guineafowl (Numidameleagris) Intravenously Infected with an H6N2 Low Pathogenicity Avian Influenza Virus. Avian dis, vol. 60, 2016 pp. 279-285.

Gonzales GF, Córdova A, Vega K, Chung A, Villena A, *et al.,* Effect of Lepidium meyenii (MACA) on sexual desire and its absent relationship with serum testosterone levels in healthy adult men. Andrologia, vol. 34, 2002 pp. 367-372.

Gonzales GF, Gonzales C, Gonzales-Castañeda C. Lepidium meyenii (Maca): a plant from the highlands of Peru--from tradition to science. Forsch Komplementmed, vol. 16, 2009 pp. 373-380.

Gustavo GF. Ethnobiology and ethnopharmacology of Lepidium meyenii (Maca), a plant from the Peruvian highlands. Evid Based Complement Alternat Med 2012, Article ID 193496, 10 pages.

Hamburger V. A manual of experimental embryology. University of Chicago Press, Chicago, 1942.

Human cases of influenza at the human-animal interface, January 2015-April 2017-Cas humans de grippe a l'interfacehomme-animal, Janvier 2015-Avril 2017. Mondial de la Sante, Organisation and the World Health Organization. 33, s.l. World Health Organization= Organisation mondiale de la Sante, 2017, Weekly Epidemiological Record, Vol. 92.

Ip H, Torchetti MK, Crespo R, Kohrs P, DeBruyn P, Mansfield KG. Novel Eurasian highly pathogenic avian influenza A H5 viruses in wild birds, Washington, USA, 2014. Emerging infect dis, vol. 21, 2015 pp. 886.

Kaleta EF, Hergarten G, Yilmaz A. Avian influenza A viruses in birds--an ecological, ornithological and virological view. Dtsch Tierarztl Wochenschr, vol. 112, 2005 pp. 448-456.

Khanna M, Sharma S, Kumar B, Rajput R. Protective immunity based on the conserved hemagglutinin stalk domain and its prospects for universal influenza vaccine development. Bio Med Res Int 2014, Article ID 546274, 7 pages.

Kim HR, Kwon YK, Jang I, Lee YJ, Lee EK, Song BM. Pathologic changes in wild birds infected with highly pathogenic avian influenza A (H5N8) viruses, South Korea, 2014. Emerg Infect Dis, vol. 21, 2015 pp. 775-780.

Levinson WE. Review of medical microbiology and immunology. McGraw Hill Professional, New York, 2016.

Parry J. Vaccinating poultry against avian flu is contributing to spread. British Medical Journal Publishing Group, London, 2005.

Rodríguez-Huamán Á, Casimiro-Gonzales S, Chávez-Pérez JA, Gonzales-Arimborgo C, Cisneros-Fernández R, *et al.,* Antioxidant and neuroprotector effect of Lepidium meyenii (maca) methanol leaf extract against 6-hydroxydopamine (6-OHDA)-induced toxicity in PC12 cells. Toxicol Mech Methods, vol. 27, 2017 pp. 279-285.

Spackman E. A brief introduction to the avian influenza virus. Springer, Berlin, 2008 pp. 1-6.

Suarez DL, Lee CW, Swayne DE. Avian influenza vaccination in North America: strategies and difficulties. Dev Biol (Basel), vol. 124, 2016 pp. 117-124.

Tavakkoli H, Salandari S. Pathological Evaluation of Essential Amino Acid Injection for Chicken Embryonated Eggs. Int. J. Adv. Biol. Biom. Res, vol. 2, 2014 pp. 2874-2879.

Wang C, Wang H, Liu X, Xu D, Tang Y, Luo P. Traditional Chinese Medicine for the treatment of influenza: a systematic review and Meta-analysis of randomized controlled trials. J Traditional Chinese Med, vol. 34, 2014 pp. 527-531

Wang, Xin, *et al.,* Study on Antioxidant Activity of Ethanol Extract of Maca. Evaluation and Analysis of Drug-Use in Hospitals of China, vol. 9, 2017 pp. 8.

WHO. Avian and another zoonotic influenza. WHO. [Online], Available: http://www.who.int/influenza/human_animal_interface/en/ [Accessed in 2018].

