J. Vet. Malaysia (2017) 29 (2):14-19

Review Articles

EDIBLE BIRD'S NEST (EBN) IS A POTENTIAL NATURAL PRODUCT AGAINST INFLUENZA VIRUS INFECTION

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SUMMARY

Edible bird's nest (EBN) is an emergent industry in Malaysia. In 2016, EBN worth RM 1.2 billion with the total production of 228-tonne metrics. EBN is salivary secretion of swiftlets (*Aerodramus spp.*) that contained various nutritive values. Back in Tang Dynasty, it had been recognized as a natural product with broad medicinal effects. Recently, various scientific studies have been done to elucidate the medicinal properties of this precious food. For the past 10 years, antiviral effects of EBN had been explained via *in-vitro* and *in-vivo* well-designed researches, in which generally EBN is exerting a good alternative food for prophylactic and therapeutic agent against Influenza A virus infection in the laboratory setting. The limited study had been done to identify the bioactive ingredient of EBN that have antiviral properties. Nevertheless, based on the previous nutritional studies, some contents of EBN have been hypothesised to serve as an antiviral agent, and comprehensive study is required to explicate those claimed. The aim of this paper is to review on the recent discovery pertaining to the potential antiviral effect of EBN in the cell culture and animal model studies.

Keywords: Edible Bird's Nest (EBN), Antiviral, Influenza A Virus, Bioactive Ingredient

INTRODUCTION

Edible bird's nest (EBN) production is an emerging industry in Malaysia. This industry is worth RM 1.2 billion in 2016 with 105 tan metrics of EBN was exported last year. There are two types of EBN have been produced locally, namely white and black (red) EBN(Isa, 2016). Due to nutritional content, quality and demand, white EBN is the most common type of EBN been produced and marketed in Malaysia (Looi and Omar, 2016). This industry is supported by Department of Veterinary Services (DVS) and parallel with government campaign in promoting EBN soup, instead of shark fin soup in 5-star hotels and restaurants (Isa, 2016). Currently, this industry is expanding dramatically due to high market demand from the local and international consumers (Ma and Liu, 2012b). Therefore, the EBN collectors started to create a swiftlet housing for man-made EBN, instead of natural EBN from the cave, in the paddy and oil palm plantation. As a result, in 2016 the EBN production had been met the local market demand and projected the surplus to be exported to international markets such as Middle East, Europe and even the Republic of China. This remarkable growth has made Malaysia as a second largest EBN producer country in the world, behind our neighbour Indonesia with the total production of 228-tonne metrics in 2016 (Isa, 2016).

The history of this natural product was documented ever since Tang Dynasty (618-907 CE) and frequently consumed by Chinese royal family. Due to their extensive medicinal properties and well-being effects (Marcone, 2005). In general, EBN is mainly composed by glycoprotein conjugated such as sialic acid, glucosamine and galactosamine (Ma and Liu, 2012b). Besides that, it also contained minerals, vitamins and some hormones (Yu-Qin *et al.*, 2000; Ma and Liu, 2012a). This product which derived from the secretion of swiftlets' salivary glands is produced all year round, but predominantly during the breeding season (between September to December). Both sexes are involved in the nest building but mainly the nest was built by the male swiftlet (Looi and Omar, 2016). EBN composed of polymerised salivary secretion with varies the degree of feather and plumage composition that will support the nestling and mothers for up to 40 days (Langham, 1980; Ma and Liu, 2012b). In general, swiftlets (*Aerodramus fuciphagus*) is an aerial insectivore that mainly inhabits in limestone cave situated at more than 1300 meters above the sea level to avoid interspecies competition (Lim *et al.*, 2002). This amazing bird is anatomically different from another common swift. They have shorter metatarsal bones and digits together with underdeveloped (smaller and thinner) caudoproximal of pelvic muscles bundle (biceps femoris, semitendinosus, semimembranosus and gastrocnemius), resulting inability of the swiftlet to perch, walk and stand (Zuki *et al.*, 2012).

In the past decade, several proteomic and metabolic studies have been documented in discovering the bioactive ingredient of EBN, using various modalities and technique (Chua *et al.*, 2014a; Chua *et al.*, 2014b; Zulkefli *et al.*, 2017). Most of the protein of interest are formed in a glycoconjugate sequences with smaller protein size ranged between 37-52 kDa (Zulkefli *et al.*, 2017), compared to China (106-128 kDa) (Liu *et al.*, 2012) and Indonesia (95.5 kDa) (Utomo *et al.*, 2014). Among the highlighted proteins that play important role in preventing infectious agent is acidic chitinase precursor, which involved in protective mechanism against nematodes, fungi and several pathogens (Zulkefli *et al.*, 2017). Besides that, anti-fungicidal such as 3-phenyl-5-ureido-1, 2, 4-trizole and heptadecasphinganine have been

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detected in the EBN (Chua *et al.*, 2014a), and this finding is in concordance with claimed described by Ma and Liu (2012). Thymol-β-D-glucopyranoside was also detected via gas-chromatography mass-spectrometry (GC-MS), which is effective against food-borne microorganism (*Staphylococcus aureus* and *Escherichia coli*) (Chua *et al.*, 2014a). However, up to this point, there is limited study pertaining to the bioactive ingredient that plays a pivotal role in antiviral properties. Hence, in the past two years, several studies have been conducted to explain the possible antiviral effect of the EBN in cell culture and animal model. Thus, in this article, substantial review pertaining antiviral properties and the effects towards Influenza virus will be discussed.

Pandemic Influenza A Virus

There are three subtypes of influenza virus, namely A, B and C. Among those three, only type A is the most common causing pandemic outbreaks. Influenza virus is a family member of *Orthomyxoviridae*, containing eight single-stranded, negative sense, RNA genome in a viral envelope, coated with two major nucleoprotein hemagglutinin (HA) and neuraminidase (NA) (Noda, 2012). The HA and NA are important glycoproteins for fusion of the virus inside the host cell, and release of the new virions from the infected cell, respectively (Ni *et al.*, 2014; Gong *et al.*, 2007). These two surface proteins can be classified into 16 and 9 subtypes accordingly; shifting and drifting of these proteins will lead to the pandemic outbreak of influenza A virus worldwide (Rambaut *et al.*, 2008). This seasonal influenza virus causing death 250, 000-500, 000 people annually with the severe illness about 3-5 million worldwide (Rotrosen and Neuzil, 2017). Therefore, it is a worldwide importance disease for the medical and veterinary field.

The recent epidemic of H1N1 in Malaysia was documented back in June 2017 at Perak and Ministry of Health Malaysia had been released a statement that Malaysia is free from this disease for this time being. Unfortunately, this disease is highly infectious, contagious and zoonotic. High mortality and culling rate recorded in animals particularly in the epidemic area was causing a detrimental impact on the farmers and indirectly been compromising food security for human consumption. In a recent re-emerging H1N1 pandemic outbreak (2009) had caused negative impacts to the farmer which directly linked to the involvement of veterinarian in culling to prevent the disease to spread (Beigent and McCauley, 2003). In fact, domestic pig (*Sus scrofa*) was recognised as the mixing vessels to produce a virulent type of influenza virus, causing interspecies infection and outbreaks; including human (Hab *et al.*, 2011). Therefore, this disease is a public health issue which involving medical practitioners and veterinarians to play a role in preventing the disease to spread.

Prophylactic and Therapeutic Antiviral

Pandemic influenza A virus (IAV) infection was tremendously given an impact to the worldwide population in term of health and economic perspective (Fukuyama and Kawaoka, 2011). Up to this point, there are no effective preventive and therapeutic measures were documented. Several antivirals have been developed in the past few decades, but every single of them possessing either weaknesses or benefits (Mehrbod *et al.*, 2014). All the antiviral that available in the market is primarily struggling to contain this virus via inhibition of their molecular viral replication (Haghani *et al.*, 2017). The dynamic mechanism of cell infection started with the attachment of viral hemagglutinin (HA) protein-bounded lipid envelope with N-acetylneuraminic acid (sialic acid)-receptor on the plasma membrane of the host cell and causing internalization of viral particles by receptor-mediated macropinocytosis. Followed with trafficking the endosomes to the perinuclear region and initiating endosomal membrane fusion, and finally releasing viral ribonucleoprotein complex (vRNP) in the cytosol, before transported into the host nucleus for replication (Edinger *et al.*, 2014). At the same time, influenza virus had started to regulate lysosomes fusion with auto phagosomes, which will accelerate the virus replication, reduce antigenicity of the infected cell and manipulate the process of apoptosis (Zhang *et al.*, 2014). In the recent studies, EBN has been demonstrated scientifically to act as prophylaxis and potential therapeutic agent in managing influenza infection (Hanghani *et al.*, 2017). This statement is reinforced by the *in-vitro* and *in-vivo* study in the past two years as will be discussed in the following paragraphs.

In a simple hemagglutination assay, EBN able to inhibit erythrocytes hemagglutination, indicating a direct interaction between EBN and influenza virus in a dose-dependent manner (Hanghani *et al.*, 2017). By contrast, a recent natural-derived antiviral was unable to inhibit IAV in hemagglutination assay as what been observed in the EBN (Shi *et al.*, 2017). On the other hand, the virus titter in the EBN treated cell showed significant reduction while increasing the cell viability in a post-viral inoculation. Besides that, EBN-treated cell was showing significant antiviral activity by reducing virus titre from 1:128 to 1:10-1:20 with 61% of protection (Haghani *et al.*, 2017), compared to phenanthrenes of *Blettila striata* which can provide protection ranged between 21-76% (Shi *et al.*, 2017). Rab5 and RhoA are the early markers for the viral entry that has been expressed in the development of endosome in EBN treated cell was showing significant protein suppression of these Rab5 and RhoA proteins (Hanghani *et al.*, 2017). As the autophagosomes are accelerating virus replication via serving as the anabolic pool (Zhirnov and Klenk, 2013), the protein marker for autophagosomes development (LC3-II) supposedly high in the infected cell, but in cell treated with EBN that protein expression was reduced significantly, indicating antiviral properties of the EBN. Interestingly, EBN demonstrated cellular shapes normalization and actin filament reorientation upon immunoblotting staining. On the other hand, upon the viral entry, lysosomes only mildly induce due to their inhibition of the virus, when cell treated with EBN, lysosomes densities were increased when stained with immunofluorescence. In a brief conclusion, the authors suggested the pharmacodynamic of EBN in treating IAV infection is could be from early

endosome stimulation and amelioration of lysosomes degradation (Hanghani *et al.*, 2017). In the other paper from the same authors, via *in vitro* study, EBN was suggested to have an antiviral effect via preventing of the virion from the infected cell and contained it in the cytosol. In addition, the authors showed the EBNwas demonstrating cytokines tumour necrosis factor-α (TNF-α) and nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) up-regulation significant (Haghani *et al.*, 2016). TNF-α is important for viral replication inhibition, and NFκB is important in cell survivability and anti-apoptotic (Swardfager *et al.*, 2010). Besides that, EBN-treated cell was down-regulating chemokine (C-C motif) ligand 2 (CCL2). This chemokine will be exuberated by the influenza virus and causing aberrant cytokine production and excessive cell apoptosis by induction of tumour necrosis factor-related apoptosis inducing legend (TRAIL) (Herold *et al.*, 2008). This chemokine has the ability to cause deadly acute encephalopathy associated with influenza A virus due to excessive immune stimulation (Lee *et al.*, 2010) Therefore, reduction of this chemokine is advantageously in human that had been infected by influenza virus (Aldridge *et al.*, 2009). Simultaneously, interleukin-27 (IL-27) was also increased in the EBN treated cell and this chemokine can balance out the excessive innate inflammatory and as anti-pyretic by a synergistically production of interleukin-10 (IL-10) (Sun *et al.*, 2014).

In the *in-vivo* study, EBN treated mice was significantly reduced NA viral copy in the lung. In fact, in the pre-treated mice with IAV showed zero viral loads in the lung indicating EBN efficiently served a prophylactic antiviral. In regard to the immunomodulatory properties, EBN treated mice showed amazing immune chemistry reaction (Hanghani *et al.*, 2016). Interferon-γ (IFN-γ) was the highest cytokines been produced by the EBN treated mice and this cytokine is very important for viral replication inhibition and major histocompatibility type I and II (MHC-I and MHC-II) activation (Haghani *et al.*, 2016; Lee *et al.*, 2010). Other cytokines that increased significantly on the first day post infection was IL-1β (initiate immune response), IL-2 (crucial factor for T cell-dependent IFN-γ), IL-6 (controlling lung damage-induced by influenza virus, viral-induced apoptosis and activating virus-specific antibody production) and TNF-α (antiviral activity, suppress CCL2 and fibrotic growth factor [TGF-b1]). However, IL-1β, IL2, IL-6 and IL-10 were significantly reduced by day 3 post-infection in EBN treated mice; in order, to reduce pulmonary inflammation and lethal effect of these cytokines (Haghani *et al.*, 2016). In fact, the anti-inflammatory and antioxidant properties of EBN have been described in other previous studies and might be linked to the anti-viral properties of EBN (Aswir and Wan Nazaimoon, 2011; Yida *et al.*, 2014; Yida *et al.*, 2015b). In addition, at day 5 of post-infection, there is no viral copy was detected in all mice EBN-treated group (Haghani *et al.*, 2016). Significant of IL-2 and IL-10 reduction by day 10 in the EBN-treated group is important because persistence high secretion of these cytokines will be an open entry for secondary bacterial pneumococcal pneumonia (van der Sluijs *et al.*, 2004). In general, EBN able to activate innate immune system since day one, to inhibit viral infection and indirectly contain the influenza virus pathogenesis (Haghani *et al.*, 2016). Paradoxically, in other natural-based antiviral is exerting their antiviral properties via inhibition of virus replication (Shi *et al.*, 2017).

Antiviral Bioactive Ingredient

In overall, EBN potentially can serve as the prophylactic and therapeutic antiviral against influenza infection by modulating the immune response and improve the adverse effect of influenza disease (Haghani *et al.*, 2016). Nonetheless, the proteomic and metabolomics studies of the EBN should be conducted to identify the signature bioactive ingredient that plays the major role in antiviral properties. However, the authors will discuss the possible bioactive ingredient that might serve as antiviral agent. One previous study had described that sialic acid is the possible component of EBN that have been neutralising the IAV infection (Guo *et al.*, 2006). Sialic acid (SA) or *N*-acetylneuraminic acid is a major glycoprotein conjugated on the oligosaccharide of the EBN (Schauer, 2016). In other two recent studies demonstrated the EBN contained 9-11% sialic acid (Colombo *et al.*, 2003; Yida *et al.*, 2015a), supported by another review paper on sialic acid which mentioned EBN is the highest natural product that contained sialic acid (Schauer, 2016). Sialic acids on the EBN released from the major carbohydrate component via acidic hydrolysis of the digestive enzyme (pancreatic enzyme) (Yagi *et al.*, 2008). In the previous study, Guo and co-researchers (2006) have documented the hydrolysed SA is propitious for the antiviral (Guo *et al.*, 2006). The major type of SA in the EBN is Neu5Ac which composed up to 96.8% with *O*-acetyl sialic acid is the main species of SA (Guo *et al.*, 2006; Yagi *et al.*, 2008).

Once a host gets infected with IAV, viral HA protein will play its major role in the first contact with host cell receptor (Ni *et al.*, 2014). This binding and attachment arespecific to the sialic acid (SA)-containing receptor on the host cell, to allow internalization of viral-receptor complex inside the clathrin-coated endosome (Edinger *et al.*, 2014). As the availability of sialic acid abundant in the extracellular, this sialic acid will act as a decoy and resulting IAV binding, instead of attachment on SA-containing receptor of the host cell. Hence, reducing the incidence of the infected cell with IAV. The previous study had shown hydrolysed SAis more potent to act as decoy antiviral, plus the smaller size of SA will also give more impact as antiviral properties of EBN (Guo *et al.*, 2006).

Other than that, authors also hypothesised the role of lactoferrin as a bioactive ingredient in the EBN that help in its antiviral properties. Lactoferrin (LF) is a family member of transferrin (serum transferrin, melanotransferrin and ovotransferrin) and is formed in a glycoprotein with single polypeptide chain, weighing about 78 kDa (Baker *et al.*, 2000; Lambert *et al.*, 2005). There is no study has been done to measure the size of LF in the bird particularly swiftlet, but then again the structural size of LF in human and bovine milk is 691 and 696 amino acids (Baker *et al.*, 2000; Moore *et al.*, 1997).

Authors believe the size of swiftlet's LF should be around that range, as the LF is a body biological fluid that had been secreted in the milk, saliva and seminal fluid (Cheng *et al.*, 2008); EBN is a polymerised saliva secretion. LF ubiquitously present in human and bovine milk with concentration up to 3.00 and 0.49 g/L respectively (Artym and Zimecki, 2005). In EBN, the LF has contained about 4.68 µg/mg with a combination of another transferrin family such as ovotransferrin (10.23 µg/mg) (Hou *et al.*, 2015). Instead of antiviral properties, LF also serves as antibacterial, antifungal, anti-inflammatory and anti-carcinogen (Wang *et al.*, 2017). In the previous study, LF is might protect the host cell tropism in the early stage of infection, but later they claimed LF might prevent the virus replication after infection of the host cell (Beljaars *et al.*, 2004; Ikeda *et al.*, 2000). LF is effective against both enveloped and non-enveloped virus (Lin *et al.*, 2002; Seganti *et al.*, 2004). In pharmacodynamics wise, a worldwide accepted hypothesis suggested the LF will bind and block the glycosaminoglycans (GAG) viral receptor specifically heparan sulphate. Thus, averting the first contact of viral protein with cell tropism receptor of the host and preventing cell infection (Gonzalez-Chavez *et al.*, 2009). In a recent study, bovine LF was incorporated in the H1N1 vaccine to stimulate the production of antibody against IAV (Sherman *et al.*, 2015). The other proposed mechanism of LF in exerting antiviral properties is by iron transferring (Voswinkel *et al.*, 2016). LF induced and promoted absorption of iron in the body (Paesano *et al.*, 2010). The iron will be released in the GIT for absorption after been acidified in the gastric juice, as the iron will be released up to 40% from the initial structure (Rastogi *et al.*, 2016). In a recent *in-vitro* and *in-vivo* study had demonstrated high concentration of iron been absorbed will inhibit IAV growth and replication (Kumar *et al.*, 2016). Therefore, LF is another potential bioactive component that should be considered as a metabolite that served as an antiviral effect in EBN.

CONCLUSION

Re-emerging of the pandemic IAV particularly Highly-Pathogenic Avian Influenza (HPAI) had drawn attention to the world population. As this disease is infectious, contagious and zoonotic, medical officer and the veterinarian must have a strong networking in preventing the outbreak and spreading of this disease. Even though, the existence of prophylactic and therapeutic agents in the market, the effectiveness of those measures is persistence doubtable. Therefore, a more extensive study must be conducted to give a sight for better treatment. Long-term consumption of chemically derived drugs is believed to give the side effects on the health status. Thus, the emerging of nutraceutical industry will give a new sight for prevention and treatment in future via a holistic, safe and effective approach. EBN is one of the precious Chinese food that is believed to give a well-being effect in many aspects since thousands of years ago. The authors believed the more recent study will be published to enlighten world population on the benefits of EBN. Validation of bioactive ingredient is another prospect that should be considered when claiming the beneficial effect of EBN or other natural product.

CONFLICT OF INTEREST

The authors have agreed, there is no conflict of interestsin writing this review article.

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