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Edible bird's nest: extraction and pharmacological

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Abstract

Edible bird's nests (EBNs) are nests built with saliva and feather by swiftlets (*Apodidiae*) in the breeding season. It is one of the precious Chinese traditional medicines that are reported to have high economic and medicinal values. Biological activity and

pharmacological effects of EBN has attracted more attention with the consumption of EBN increasing in recent years. we summarized and analyzed the extraction and pharmacological effects of the edible bird's nest in recent 5 years. To provide the basis for further study on mechanism of pharmacological effects of edible bird's nest. The edible bird's nest contains protein, amino acids, carbohydrates and minerals. The different extraction in the edible bird's nest is not the same. EBN not only can be used to anti-aging, antioxidant, anti-virus, anti-inflammatory, immune promoting, regulation of intestinal flora. but also can be used to prevent osteoarthritis, improve bone strength in postmenopausal women, improve corneal injury, improve cell apoptosis, prevent high fat diet induced insulin resistance. This article reviewedthe extraction and pharmacological effects of the edible bird's nest. At last, this article prospected research directions in order to provide a basis for further study on EBN.

Keywords： edible bird's nest, extraction , pharmacological effects.

INTRODUCTION

Edible bird’s nests (EBNs) are produced by certain swiftlets of the genera *Aerodramus*, *Apus*, and *Collocalia*. EBNs are formed by swallowed sea fish, silkworm spiral algae, and other small creatures. Secreted gastric juice after digestion and plumule mix, and the resulting substance condenses on cliffs (Wong, 2013). Various sources of EBNs have been reported in different monographs. EBNs can be formed by swiftlets and swifts. The swiftlet has eight species, e.g. the Himalayan swiftlet with three subspecies, namely, the Sichuan, Tibet, and Yunnan subspecies. Swifts have two species (Yu, 2014; Nakagawa, 2007;Tukiran, 2015). The different EBNs sources are shown in Table I. Different EBNs have distinct origins, and the primary origins of EBNare shown in Table II (Chua, 2015).

TABLE I - The sources of bird's nest

Genus	Species	Latin name
Swiftlet genus	White-bellied Swiftlet	*Collocaliaesculenta*
	Brown-rumped Swiftlet	*Collocaliavestita*
	Gray-rumpedSwiftlet	*Collocalialinchiaffinis*
	The South China Sea swiftlet	*Collocaliainexpectata*
	Aerodramusfuciphagus	*Collocaliafuciphaga*
	Monochrome swiftlets	*Collocalia unicolor Jordan*
	Himalayan Swiftlet	*Collocaliabrevirostris*
	Brown back of swiftlets	*CollocaliainopinaThayeretBangs*
Swift grnus	Pacific Swift	*Apus pacificus*
	South China Pacific Swift	*Apus pacificuskanoi*

TABLE II - The main origin of the bird's nest

Distribution	Place of Origin
Southeast Asian countries	Indonesia
	Malaysia
	Thailand
	Vietnam
	Philippines
	Japan
	Others
China	Yan Yan, Huaiji County, Zhaoqing City,
	Guangdong Province
	Jianshui county of Honghe prefecture of Yunnan Province
	Hainan Continent Island
	Fujian
	In the southeast of Tibet

The EBN, which is rich in protein, amino acids, carbohydrates, and mineral, is beneficial for filling gas, nourishing Yin, moistening dryness, relieving spasms, and so on (Yang, 2014). The market and demand for EBN have been increasing. Thus, various EBN products are available, and prices remarkably vary. The authenticity of EBN identification has been reported more frequently in China than the extraction and pharmacological action of EBNs. The extraction of EBN and its pharmacological effects were reviewed in this study to provide a basis for further evaluation of its constituents and pharmacological effects.

EBN EXTRACTION

The EBN exhibits different pharmacological effects, and its extraction is summarized in Table Ⅲ.

EBN protein extraction

An EBN contains 40%–60% dried protein. Numerous studies have proven that the EBN protein exhibits various pharmacological effects, which may be the material basis for its efficacy.(Liu, 2012) used two-dimensional electrophoresis (2-DE) to extract the water-soluble protein of an EBN. The results showed that the extractions for 6 h at 60 ℃ and for 12 h overnight at 4 ℃ showed similar effects. Moreover, the amount of extracted high-molecular weight protein component significantly increased. This result indicated that the high temperature of 60 ℃ not only damaged the protein structure of EBN, but also greatly improved the protein extraction rate and shortened the extraction time.(Hou, 2015).Liquid-phase isoelectric focusing (LIEF) was applied to purify the proteins extracted from the EBN. Protein samples were prepared by water extraction or acetone precipitation, and the latter process was shown to be more effective(Norhayati, 2010). The proteins from EBNs were well separated using LIEF combined with 2-DE. LIEF could effectively remove acidic mucopolysaccharidein the sample protein of EBN. A 2-DE mapping method with better quality protein of EBN was obtained,after the purified protein samples were separated by 2-DE(Xian, 2010; Liu, 2013).The proteins

in the EBN were extracted using multiple extractions and then digested by PNgase F and trypsin. The digested mixture was separated with HPLC, and the peptides were identified based on MS/MS data searching. The results indicated that 79.7% of the total protein in the EBN had been extracted.

EBN DNA extraction

DNA barcoding technique was used to isolate the total genomic DNA in the EBN samples (Goh, 2000).*Cytb* gene sequences were amplified and sequenced by PCR. The experiment was 100% successful (Wang, 2013).A collagenase method was established to extract genomic DNA from rudimental bird feather of EBN, which was harvested from a swiftlet cave. Collagenase was also used in addition to protease K which could substantially increase the DNA yield. This method can be applied to identify the species types in biological products, especially for animal tissue materials rich in collagen. Alkaline lysis, phenol, high salt, and low pH methods were compared using kit method (Chen, 2015). The EBN sample was lysed using SDS with high NaCl concentrations. Chloroform and CTAB were used to eliminate proteins, and cold isopropyl alcohol was used to precipitate DNA (Nakagawa, 2007). A method for extracting the EBN DNA was established and improved on the basis of Ref. (Chen, 2015). We applied kit method, improved CTAB lysis solution method, and improved kit method to extract the total DNA to study their diversity. The result showed that the improved kit method was the most suitable for extracting EBN DNA. This observation could be an effective reference for related advanced research.

EBN sialic acid extraction

The rates for extracting sialic acid from EBN as detected by water extraction, microwave-assisted enzymatic extraction, and papain enzymatic extraction were 9.08%±0.12%,12.58%,and 9.98%±0.05%, respectively. The microwave-assisted enzymatic method showed the highest extraction of EBN among the three different methods(Yagi, 2008; Zhang, 2012; Chen, 2016).

EBN mineral extraction

TheICP-MS method was performed to analyze quantitatively the 20 inorganic elements in the samples of 25 batches of EBN (Zhao, 2015). The EBN consisted of Na, K, Ca, Mn, 57Fe, Co, Zn, Se, and Rb. The average contents of the EBN inorganic elements from high to low were Na > Ca > Mg > K > Al > Sr.

TABLE Ⅲ-The edible bird's nest extraction are summarized

name	extractive	method	extraction solvent	optimal condition/extraction rate	reference
Indonesia YellowNest	protein	2-DE	Ultra pure water	extraction of 6h in 60℃	7
White Nest Bloody Nest	protein	LIEF	Water extraction / acetone precipitation	acetone precipitation	8
White Nest	protein	multiple extraction	Phosphate buffer Ultra purewater	79. 7%	9
White Nest	protein	LIEF	TritonX-100 Mercaptoethanol	To identify	10
YellowNest White Nest	DNA	kit method	Amplification of Cytb gene 1143bp	100%	11
Bloody Nest	DNA	kit method	---	Modified kit	12
Yellow Nest		Modified kit method	---	method	
		Modified CTAB lysis	---		
Collocalia nest White Nest Bloody Nest	DNA	method kit method Alkaline lysis	Digestion Buffer Ethanol	chloroform and CTAB to eliminate proteins,and cold	13

		method	Isopropanol, ethanol	isopropyl alcohol to	
		Phenol method	Isopropanol, ethanol	precipitate	
		High salt and		substantively	
		low pH method		increase the DNA	
				yield	
Swiftlet edible	DNA	Collagenase	Protease K	(9.08±0.12) %	14
bird's nest		method	collagenase		
Malaysia EBN	sialicacid	Water	Ultra pure water	12.58%	15
		extraction			
Malaysia	sialicacid	microwave	Papaya protease	(9.98±0.05) %	16
EBNMalaysia	sialicacid	assisted enzyme	Papaya protease		17
EBN		papain	Ultra pure water		
		enzymatic			
		extraction			
		methods			
White Nest	trace element	ICP-MS	Nitric acid,perchlorate	Na ＞ Ca ＞ Mg	18
				＞ K ＞ Al ＞Sr	

EBN PHARMACOLOGICAL EFFECTS

Anti-aging and antioxidant activities

(Hou, 2015).Study on anti-oxidant effect of EBN in the liver of ovariectomized rats，through the experiment, found the EBN can improve the level of SOD and CAT, improve the ratio of SOD and CAT, and decreased the MDA level significantly. q RT-PCR results show that the EBN can be raised SOD1 mRNA、SOD2 mRNA、SOD3 mRNA、PARP1 mRNA relative expression, so the EBN can regulate the expression of liver cell anti-oxidation pathway related genes.

(Ryu, 2014).Study on the EBN attenuated the oxidative stress-induced matrix metalloproteinase-1 mechanism in human HaCaT keratinocyte. The results show that

EBN can down-regulation ERK/JKN，inhibited the expression of c-Fos and phospho c-Jun .the ERK/JKN is located in the upstream of matrix metalloproteinases-1 gene, c-Fos and phospho c-Jun are an important part of AP - 1 pathway. AP - 1 transcription activity can influence the expression of matrix metalloproteinases-1 promoter. These data indicate that the anti-aging properties of EBN involve the inhibition of MMP-1 expression by downregulating the ERK/JNK and AP-1 pathway.

Study on lactoferrin and ovotransferrin contribute toward EBN to against oxidation. The results show that EBN can reduce the toxicity of H2O2 - induced, increase removal activity to reduced radical oxygen species (RSO). LF, OVF and EBN can affect the hydrogen peroxide - induced oxidative stress related gene transcription, to realize the antioxidant effect (Hou, 2015).

Study on antioxidant properties of EBN in vitro. The experimental results show that the EBN can significantly enhance the antioxidant activity, and no toxic effect on HEPG2 cells, it is likely that EBN bioactive substances release matrix in intestinal digestion, and absorbed in the gut through the passive transport, to exert their effects (Zhang, 2014).

Cell proliferation

Study on the effects of EBN on the transformation of lymphocyte of Con A-induced rats. Study varieties of EBN and adulterants on lymphocyte transformation function. This study showed that EBN did not directly stimulate the transformation of the lymphocytes of rats, but with induction of low concentration of Con A (Zheng,2016).

Study on the EBN induced human adipose stem cells proliferation mechanism.The results show that IL-6 and VEGF can be through the p44 / 42 MAPK and p38 MAPK adjustment of NF-B and AP-1 activity, and the EBN can be upregulated the expression of IL-6 and VEGF gene to realize cell proliferation (Roh, 2012).

EBN anti-virus and anti-inflammatory activities

Study on EBN extract inhibited influenza virus infection. The results show that EBN can neutralize MDCK cells infected influenza virus and inhibit influenza virus

erythrocyte aggregation to achieve inhibition of influenza virus infection, but could not inhibit influenza virus sialidase activity. Fluorescence method showed that molecular of sialic acid in EBNis mainly composed of N-acetylneuraminic acid（Guo, 2006）.

Study on the effects of EBN on tumour necrosis factor-alpha secretion, nitric oxide production and cell viability of lipopolysaccharide-stimulatedRAW 264.7 macrophages. The results show that sialoglycoprotein of the EBN can inhibit the production of TNF-α and NO by 58% and 63%, respectively. Thus, the sialoglycoprotein has an anti-inflammatory effect (Vimala, 2012).

Study on EBN attenuates high-fat diet-induced oxidative stress and inflammation by regulating hepatic antioxidant and inflammatory genes .The results showed that the EBN can increase the oxidative stress reaction and improve the inflammatory markers in HFD rats. By reducing the antioxidant gene expression and increase inflammation gene expression to realize oxidative stress and inflammation (Zhang, 2015).

Osteoarthritis prevention

EBN extract in vitro exhibited protective effects on cartilage cells, which were isolated from knee joints(Chua, 2013; Nakagawa, 2007). Osteoarthritis is caused mainly by the degeneration of articular cartilage. The metal protease is the proinflammatory cytokine and the decomposition medium, sialoglycoprotein of EBN, is a cartilage matrix material. MTT assay, real-time PCR, and ELISA were performed to monitor the decomposition and synthesis of chondrocytes. The result shows that the EBN extract can control osteoarthritis progression and promote the regeneration of cartilage cells. The EBN is expected to become an effective drug to treat arthritis.

Improvement of bone strength in postmenopausal women

The ovaries of rats were removed and these rats were used as research objects. The bone strength and dermal thickness were improved due to dietary EBN extract. The result shows that EBN extract can increase the thighbone and serum phosphorus concentrations which can increase the strength of resectioned bone in ovariectomized rats. The EBN extract can increase the average thickness of collagen fibrils that can

enhance the dermal thickness in ovariectomized rats. EBN extract has been inferred to improve bone strength and increase dermal thickness in postmenopausal women (Matsukawa, 2011).

Immune promotion

The effect of EBN on lymphocyte proliferation stimulated by Con A on rat was determined. MTT assay was used to detect lymphocyte transformation in rats. Low concentration (2 g/ml ConA) under EBN stimulation can promote the lymphocyte transformation in rats (Zhao, 2016).

The immunity regulation of white EBN in Indonesia was investigated for immunocompromised rats (Haghani, 2016). A hydrocortisone low-immunity model of rats was constructed. Four different concentrations of white EBN in Indonesia were administered to rats for 28 days. The experimental results showed that the spleen and thymus indices of white EBN in Indonesia were significantly improved. The allergic reaction of delaying type in rats was improved. The content of serum hemolysin in rats and the phagocytosis and phagocytic indices in the red muscle cells in the peritoneal macrophages of rats were also enhanced. Thus, the white EBN in Indonesia affects humoral immunity and cellular immunity.

Regulation of intestinal flora

The effect of EBN on the regulation of intestinal flora in normal rats was observed (Zainal, 2011). The experimental results show that EBN may enhance intestinal bacteria and inhibit harmful bacteria to regulate the intestinal flora.

Aid in corneal healing

The effect of EBN on the rabbit corneal stroma in vitro was investigated. The EBN extract was added to the serum culture medium of corneal cells. Gene expression was determined by RT-PCR to observe the morphological changes. The results show that the EBN may aid in the rabbit corneal cell division and promote regeneration, which are beneficial to corneal healing process (Zainal, 2014).

Improvement of cell apoptosis

The EBN ameliorates oxidative stress-induced apoptosis in SH-SY5Y human neuroblastoma cells. In vitro PD model induced by neurotoxin was used to study the neuroprotective effects of crude and water extracts. The results show that EBN extracts might exhibit neuroprotective effects against 6-OHDA -induced dopaminergic neuronal degeneration. Thus, EBN may improve cell apoptosis (Yew, 2014).

Prevention of high-fat diet-induced insulin resistance and attenuation of procoagulation

Study on EBN prevents high-fat diet-induced insulin resistance in rats. Simvastatin or EBN extract was administered for 12 weeks to high-fat diet (HFD) rats. The results show that the HFD can aggravate metabolic index and induce insulin resistance through insulin signal transcription gene, EBN can prevent a HFD rats metabolic deterioration and regulate insulin signal transcription gene, improve the HFD rats of insulin resistance (Zhang, 2015).

Study on EBN attenuates the procoagulation effects of HFD in rats. The results show that the EBN can reduce high blood cholesterol and blood clotting by regulating transcription of coagulation - related gene. Achieve a reduction procoagulant effect in rats (Zhang, 2015).

Effect on ovariectomized rats

Study on the effect of EBN on hippocampus and cortex of neurodegenerative disease in ovariectomizedrats , the results show that the EBN can significantly reduce the estrogen deficiency caused by the increase of serum advanced glycosylation product, through the MDA content and superoxide dismutase markers to change redox state, in addition to EBN can downregulate related gene ofneurodegenerative diseases and cell apoptosis of the hippocampus and frontal cortex (Hou, 2015).

Study on EBN to nutritional effects of insulin signal transduction in ovariectomized rats. Ovaries removed rats will worsen metabolism, interfere with the normal mode of hepatic insulin signaling gene transcription. EBN can improve metabolic index and

increase insulin sensitivity, glucose and lipid homeostasis to changehepatic insulin signal transduction in gene transcription. Show that EBN can improve the metabolic disorders of ovariectomized rats caused by a lack of estrogen (Hou, 2015).

The pharmacological effects of EBN are shown in TableⅣ.

TABLE Ⅳ-Pharmacological effects of edible bird's nest

Pharmacological effects	Main Source	Mechanism	Refs
Anti-aging And antioxidant	Malaysia	Upregulated SOD1 mRNA and PARP1 mRNA expression	(19)
	China	Inhibition of MMP-1 expression via down regulation of the ERK /JNK and AP-1 pathway	(20)
	Malaysia	Attenuated H_2O_2-induced cytotoxicity,and decreased ROS through increased	(21)
	Malaysia	Scavenging activity protected HEPG2 cells from hydrogen peroxide induced-toxicity	(22)
Cell proliferation	Philippines China	Con A induced lymphocyttransformation of rats and its promoting effect stimulated by Edible birds' nest	（23）
	China	Increased expression of IL-6 and VEGF genes, which is mediated by the activation of NFκB and AP-1through p44/42 MAPK and p38 MAPK	(24)
Anti-virus and Anti-inflammator	Indonesia	EBN extract could neutralize the infection of MDCK cells with influenza viruses and inhibit hemagglutination of influenza viruses	(25)

Pharmacological effects	Main Source	Mechanism	Refs
		to erythrocytes,	
	Malaysia	Inhibit production of	(26)
		TNF-alpha and NO	
	Malaysia	Attenuated the HFD-induced	(27)
		inflammation.Attenuation of	
		antioxidant gene expression and	
		potentiation of inflammatory gene	
		expression	
Preventive osteoarthritis	Malaysia	Promoted HACs proliferation reduce	(28)
		the catabolic genes' expression	
	China	EBN were rich in a PG	(29)
Improving bone strength	China	Increase the thighbone and serum	(30)
in postmenopausal women		phosphorus concentrations.	
		Increase the average thickness of	
		collagen fibrils	
Immune promoting	Philippines	ConA induced lymphocyt China	(31)
		transformation of rats and its	
		promoting effect stimulated by	
		Edible birds' nest	
	Indonesia	The spleen index and thymus index	(32)
		phagocytosis and phagocytic index	
		were significantly improved	
Regulation of intestinal flora	Vietnam	Foster intestinal bacteria and inhibit	(33)
		harmful bacteria	
Favorable corneal injury	Malaysia	Low concentration of EBN could	(34)
		synergistically induce cell proliferation	
Improving cell apoptosis	Malaysia	Confer neuroprotective effect against	(35)
		6-OHDA-induced degeneration of	

Pharmacological effects	Main Source	Mechanism	Refs
		dopaminergic neurons,particularly through inhibition of apoptosis	
Preventing high fat diet induced insulin resistance and attenuates procoagulation	Malaysia	Prevented the worsening of metabolic indices and transcriptional changes in insulin signaling genes	(36)
	Malaysia	Attenuate HFD-induced hypercholesterolemia and coagulation similar to simvastatin, partly through transcriptional regulation of coagulation-related genes	(37)
In ovariectomized rats	Malaysia	Ecreased estrogen deficiency-associated serum elevation of advanced glycation end-products (AGEs), and they changed redox status as evidenced by oxidative damage (malondialdehyde content) and enzymatic antioxidant defense (superoxide dismutase and catalase) markers	(38
	Malaysia	Improved the metabolic indices and also produced transcriptional changes in hepatic insulin signaling genes that tended toward enhanced insulin sensitivity, and glucose and lipid homeostasis, even better than estrogen	(39)

CONCLUSIONS

The edible bird's nest contains protein, amino acids, carbohydrates and minerals. The different extraction in the edible bird's nest is not the same. EBN was extracted and separated using different methods to obtain the different EBN components. The EBN samples can be swollen and have high carbohydrate content. The extraction efficiency of the EBN protein is not very ideal. Sialic acid is the primary effective component in the EBN. Additional studies on the extraction and separation of sialic acid has been performed in recent years. However, the active ingredient of sialic acid remains unknown. The extraction of the EBN DNA provides an effective theoretical basis to identify EBN.

EBN not only can be used to anti-aging, antioxidant, anti-virus, anti-inflammatory, immune promoting, regulation of intestinal flora, but also can be used to prevent osteoarthritis, improve bone strength in postmenopausal women, improve corneal injury, improve cell apoptosis, prevent high fat diet induced insulin resistance.The authenticity of EBN identification has been reported more frequently in China than the extraction and pharmacological action of EBNs.

The bioactive constituents were isolated and purified from EBN using to determine the biological activity of EBN. However, the separation and purification methods are not perfect, but they remarkably influence the study of subsequent pharmacological effects. Therefore, an advanced technology and measures should be introduced to improve the separation and purification of the bioactive components of the EBN.

The pharmacological effects of EBN were examined. In the experimental design on the pharmacological effects of EBN, the mechanism of the experimental model,such as the anti-aging property of EBN, should be elucidated. Thus, the mechanism of aging should be understood. If the aging mechanism is not clear, the EBN with aging effect conclusion will not be convincing. The pharmacological effects of sialic acid and glycosaminoglycans of EBN were investigated. However, the majorityof these studies only focused on the pharmacological effects of EBN. The pharmacological mechanism

of sialic acid and glycosaminoglycans remains unclear. Future studies on EBN will focus on the origin, composition, and efficacy of EBN. Whether the origin, composition, and efficacy of EBN are correlated is worthy of discussion.

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