Studies on the Biological Activities of Edible Bird’s Nest (EBN) and its Extracts

Prof Dr Yuen Kah Hay &
Dr Lim Sheau Chin
School of Pharmaceutical Sciences,
Universiti Sains Malaysia
Edible Bird’s nest (EBN)

- EBN is made from the saliva of swiftlets
- An important Chinese cuisine in ancient China (since Tang dynasty)
- Traditionally, EBN has been used for skin beauty and anti-aging, treating dry coughs, alleviating asthma, relieving gastric problems and general weakness of bronchial ailments.
- Despite the long history of EBN consumption, not many scientific research to substantiate its therapeutic benefits
Some scientific publications to date

• Kong et al. (1986) reported that EBN could potentiate the mitogenic response
• Epidermal growth factor (EGF)-like activity was detected from the aqueous extract (Kong et al., 1987)
• EBN extract was found to neutralize influenza virus in MDCK cells (Guo et al., 2006)
• Matsukawa et al. (2011) showed that rats’ bone strength and skin thickness were increased when administered with 100 mg/kg of EBN extract
Challenges in the EBN industry

• Recent regulatory hurdles
  – Nitrite levels, microbial load, heavy metals

• Source of raw material:
  – Contamination with nitrite, heavy metals and microbes
  – Adulteration
  – Inferior quality

• Elaborate cleaning and processing procedures
  – Loss of biomolecules/bioactives

• Consumers better informed: evidence-based

• Still lacking in scientific proof of their benefits of this highly priced product
Our strategies to meet some of these challenges

- Processing method to meet regulatory hurdles
- Standardization and authentication (raw & finished)
- Scientific proof of benefits from taking EBN
- Determine dose regime
(1) Overcoming the increasing regulatory requirements

- With proper scientific research, one can overcome the regulatory requirements which are getting more stringent nowadays
- Eg: Nitrite levels in EBN, microbial load, heavy metals
- Special formulated cleaning method to ensure that nitrite levels remained less than 10 ppm
- At the same time, the biomolecules levels are checked at every critical point of processing to ensure the loss of biomolecules is minimal
- Method to reduce microbial load
## Results: nitrite and heavy metals

### Traditional cleaning method

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Analysis Result</th>
<th>Standard Method/Technique/Equipment Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrite</td>
<td>mg/kg</td>
<td>76.2</td>
<td>IC</td>
</tr>
<tr>
<td>Arsenic</td>
<td>mg/kg</td>
<td>ND(&lt;0.5)</td>
<td>In-house method LWI-TEC-097 based on Pearson's Composition and Analysis of Foods 9th Edition (1991)</td>
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<tr>
<td>Cadmium</td>
<td>mg/kg</td>
<td>ND(&lt;0.1)</td>
<td>In-house method LWI-TEC-100 based on AOAC 973.34, 16th Edition (1997)</td>
</tr>
<tr>
<td>Lead</td>
<td>mg/kg</td>
<td>ND(&lt;1)</td>
<td>In-house method LWI-TEC-098 based on Pearson's Composition and Analysis of Foods 9th Edition (1991)</td>
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<td>Mercury</td>
<td>mg/kg</td>
<td>ND(&lt;0.05)</td>
<td>In-house method LWI-TEC-099 based on Pearson's Composition and Analysis of Foods 9th Edition (1991)</td>
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### Special formulated cleaning method

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<tbody>
<tr>
<td>Nitrite</td>
<td>mg/kg</td>
<td>2.6</td>
<td>IC</td>
</tr>
<tr>
<td>Arsenic</td>
<td>mg/kg</td>
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### Results: Microbial load

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<tr>
<td>Coliform [LST Broth, 35 degrees Celsius, 48h]</td>
<td>MPN/g</td>
<td>49</td>
<td>FDA, BAM Chapter 4</td>
</tr>
<tr>
<td>E. coli [EC Broth, 45.5 degrees Celsius, 48h]</td>
<td>MPN/g</td>
<td>ND (&lt;1.8)</td>
<td>FDA, BAM Chapter 4</td>
</tr>
<tr>
<td>Salmonella in 25 g</td>
<td>Absent</td>
<td>FDA, BAM Chapter 5</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus [BP Agar, 35 degree Celsius, 48h]</td>
<td>CFU/g</td>
<td>ND (&lt;10)</td>
<td>FDA, BAM Chapter 12</td>
</tr>
<tr>
<td>Total Plate Count [PCA,35 degree Celsius, 48h]</td>
<td>CFU/g</td>
<td>1.3 x 10^7</td>
<td>FDA, BAM Chapter 3</td>
</tr>
<tr>
<td>Yeasts and Molds [DG 18, 25 degree Celsius, 7d]</td>
<td>CFU/g</td>
<td>5.2 x 10^3</td>
<td>FDA, BAM Chapter 10</td>
</tr>
</tbody>
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#### After

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<td>CFU/g</td>
<td>&lt;10</td>
<td>FDA, BAM Chapter 18</td>
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• Biomolecules in EBN are similar to drugs, they will degrade if not handled properly
• Source of EBN (eg: different geographical areas) may have different levels of these biomolecules (N-acetylneuraminic acid, N-acetylglucosamine)
• Extensive processing procedures (eg: soaking and washing) may result in loss of biomolecules (highly water soluble)
• Hence, implementation of quantitative standardization method is required to ensure the quality of the EBN product (raw & finished)
Standardization with quantitative LCMS/MS method

Biomolecules standards
(N-acetylneuraminic acid; N-acetylglucosamine)

Sample A – raw
(incoming EBN)

Sample B – cleaned raw
EBN
• Measuring biomarkers in the EBN can be used to:
  – Reject raw (incoming) EBN with low biomarker levels (considered poor quality)
  – Develop processing method with minimal losses (biomarkers before & after)
  – Authenticate EBN products (to differentiate from adulterated EBN products) – a major concern of consumers
(3) Studies on EBN’s biological activities

(a) To provide scientific evidence of their benefits
   – Focused on skin anti-aging properties
(b) To establish dose regime (how much and how often)
(c) To differentiate between quality products from inferior ones (due to over processing/cooking etc)

Studies conducted using various in vitro and in vivo methods may able to answer these issues
What we have done (1)

- In vitro method using Human dermal fibroblasts (HDF)
- EBN extract was found to increase the cell proliferation by 50% when compared to control
- The procollagen I peptide content was increased by approximately 20% when HDF was incubated in EBN extract
- Also found a correlation between biomarker content and activity
- Over processing/cooking lead to lowered/reduced biomarker contents and less effects
What we have done (2)

- C. elegans study
- A nematode with short lifespan
- EBN extract was found to significantly increase the mean lifespan of C. elegans

<table>
<thead>
<tr>
<th>Extract</th>
<th>Mean Lifespan</th>
</tr>
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<tr>
<td>EBN-1</td>
<td>19.8 ± 1.4</td>
</tr>
<tr>
<td>EBN-2</td>
<td>20.4 ± 2.1*</td>
</tr>
<tr>
<td>EBN-3</td>
<td>18.8 ± 0.5</td>
</tr>
<tr>
<td>Control</td>
<td>18.0 ± 1.5</td>
</tr>
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- Follow-up studies: mechanism?
What we have done (3)

• Human studies were carried out to:

(1) Determine whether anti-aging effects could be achieved in humans

(2) Determine the dose (amount) and dosing frequency that could produce these effects

(3) Products prepared with developed processing method (high biomarker contents) were used in studies
(1) Skin collagen content (found to increase)
(2) Fine lines of the skin surface (digital microscope) (also found to reduce the depth of fine lines)
Results from human studies (1)

Note: The amount of collagen denoted by the white (brighter) spots in the image.
Results from human studies (2)

Week 0  Week 4  Week 6

Note: Fine lines of the skin of a subject under magnification (250X)
• Results obtained showed that consuming EBN could result in an increase in skin collagen content
• Also found that amount consumed and frequency of consumption important
• Currently doing further studies to determine the optimal regime
• Product is also important
  – Properly processed to maintain the biomolecules
In summary

• Traditional method of processing EBN cannot meet stringent regulatory requirements
• We have develop a processing method that can meet the requirements and at the same time with minimal loss of biomarkers/biomolecules
• We have also developed an assay method for QC and authentication
• Younger generation may need scientific evidence to be convinced
• Proper studies need to be conducted to provide the evidence
• EBN shown to have skin anti-aging properties
• Proper processing method and dose regime are essential to achieve these effects
This work is carried out in collaboration with FUCIPHAGUS AGRITECH SDN BHD
Thank you

Presented by
Dr Lim Sheau Chin| School of Pharmaceutical Sciences