DISCOVERY AND CLINICAL EFFECTIVENESS OF A COMPOSITION THAT PROMOTES HAIR GROWTH (PATENT PENDING)

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Contents

1. Development Background
2. Clinical Trial Data
3. Putative Mechanisms of Action
1. Development Background

- Development Background

- Rationale for Selecting Ingredients:
  “Lipopolysaccharide (LPS)” and “pine bark polyphenol”
Development Background

• Modern Pet disease
  – Heart disease
  – Cancer
  – atopic dermatitis and alopecia etc

• The reason of appearance
  – Stress
  – High calorie of pet food and lack of physical exercises
  – Pet clothes
  – Aging etc
Rationale for Selecting Ingredients

- Limit of pharmaceuticals
- Side effects of medicine
- Start caring before occurring a disease

To focus on the action “Immune function”
Hygiene hypothesis

Most significantly different factor is the exposure amount of endotoxin (LPS) in life.

Many brothers and sisters
Country life with animals

Only one child
Urban life

Hygiene hypothesis: a study of canine atopic dermatitis

This study suggests that there is a protective effect of high indoor endotoxin exposure on the development of canine atopic dermatitis.
Although 100% of animals have innate immunity, only 5% of animals have acquired immunity.

Note: counted as animal species
Extensive phylogenetic work: proposal of phagocyte theory

Macrophages play an important role in the body’s defenses (for all multicellular animals)

Dr. Elie Mechnikov: Discoverer of lactobacillus, author of “macrophage”

Dr. Elie Mechnikov
Distribution of tissue macrophages and their physiological roles

Defence against infection
- Removal of amyloid
- Aging red blood cells
- Dead white blood cells

Metabolic control
- Regulation of iron metabolism
- Cholesterol
- Steroid
- Hormone
- Pregnancy

Wound healing
- Reproduction of skin
- Bone
- Liver
- Peripheral nerve
Macrophages are activated in stages

Steady-state macrophage (Resident stage)

Priming stage
An example:
phagocytic capacity $\uparrow$
Preparatory stage for removal of foreign bodies

Activated macrophage (Triggering stage)
An example:
active oxygen $\uparrow$
Inflammatory cytokine $\uparrow$

Interferon

Bacteria
What is LPS?

• LPS is a substance contained in bacteria, which greatly enhances self-healing power.

• LPS is 1,000–10,000 times as effective as β-glucans, which are already known to be effective ingredients in mushrooms.
Utilization of micro-organisms
Past (*lactobacillus*) and future (gram-negative bacteria)

- **Gram-positive bacteria** (bacteria with good images)
  - *Lactobacillus*, *bacillus subtilis*, etc.
  - Yogurt, pickles, natto, probiotics

- **Gram-negative bacteria** (bacteria with bad images)
  - *E. coli*, *Pantoea agglomerans*, *acetic bacteria*, *Xanthomonas*, *Zymomonas*, etc.
  - Kefir yogurt, nata de coco, vinegar, probiotics
Receptor and intracellular signals of LPS

**The amount for macrophages activation**

$\beta$-glucan $1,000 - 10,000 : 1$ LPS

- Dectin
- Receptor TLR-2
- Signal
- Receptor TLR-4
- Signal
- MD2
- LPS bind in protein

**Nucleus**

- NFκB
- Interferon (antiviral activity)
- Macrophage activation (antibacterial activity)

**β-1,3 glucan**

- Peptidoglycan

**LPS**
What is the active substance?

Wheat seeds from Canada, USA, Australia, Japan were analyzed concomitant bacteria.

Lipopolysaccharide (LPS)

Pantoea agglomerans
A Gram-negative bacteria

Water extract of wheat flour contains Macrophage activating substance

1. From foods 2. Waste products

Gluten, starch

Screening macrophage activating substances from foods by oral or percutaneous route

Relative mRNA expression

- Less than 80%
- More than 120%

**IL-4 (Th2 type)**

**IL-12 (Th1 type)**

**IL-10 (Th3, Treg)**

**IL-4 and IL-12:** No correlation with responses

**IL-10:** Increasing mRNA in responded dogs
Pine bark extract

- Extracted from French maritime pines with an average age of 20-25 years from Bordeaux, France.

- Compared with other types of pine, French maritime pine bark is very thick and contains more than 40 kinds of antioxidants polyphenols.
Pine Bark Polyphenol Features

1. Excellent antioxidant action
2. Improves circulation
3. Stabilizes blood sugar
4. Powerful Anti-inflammatory action
5. Enhances the skin
2. Clinical Trial Data

Hair-growth promotion effect on bald spots:
Thirteen animals treated with combination of LPS and pine polyphenol
Details of trials

• **Methods and materials**
  – Evaluation of hair-growth effect on alopecia in dogs, cats, rabbits, and hamsters

• **Test materials**
  – “LPS tablet” and “Pine polyphenol tablet”

• **Test methods and duration**
  – Administration of foods and drugs was not regulated. Combination with other supplements was ruled out. Animals were assigned to receive either an LPS tablet alone, or to receive both LPS and pine polyphenol tablets.
  – Test duration was 30 days (in principle).

• **Test results**

<table>
<thead>
<tr>
<th></th>
<th>Hair growth</th>
<th>Number of animals</th>
<th>Rate of hair growth response</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS alone (n=24)</td>
<td>None</td>
<td>14</td>
<td>58.3%</td>
</tr>
<tr>
<td></td>
<td>Observed</td>
<td>10</td>
<td>41.7%</td>
</tr>
<tr>
<td>LPS + Pine polyphenol (n=13)</td>
<td>None</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>Observed</td>
<td>13</td>
<td>100%</td>
</tr>
</tbody>
</table>
List of 13 animals treated with combination of LPS and pine polyphenol

<table>
<thead>
<tr>
<th></th>
<th>Breed</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Body weight (kg)</th>
<th>Concomitant drugs</th>
<th>Dosage</th>
<th>Pine polyphenol</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M. dachshund</td>
<td>♀</td>
<td>2</td>
<td>3.5</td>
<td>None</td>
<td>2</td>
<td>4</td>
<td>Allergic dermatitis Hair regrew after 12 weeks.</td>
</tr>
<tr>
<td>2</td>
<td>M. dachshund</td>
<td>♀</td>
<td>10</td>
<td>4.2</td>
<td>Used</td>
<td>2</td>
<td>4</td>
<td>Atopic dermatitis Hair regrew after 6 weeks.</td>
</tr>
<tr>
<td>3</td>
<td>Border collie</td>
<td>♀</td>
<td>7</td>
<td>20</td>
<td>Used</td>
<td>4</td>
<td>4</td>
<td>Dry dermatitis (systemic) A steroid drug was used once.</td>
</tr>
<tr>
<td>4</td>
<td>T. Poodle</td>
<td>♀</td>
<td>3</td>
<td>2.5</td>
<td>None</td>
<td>2</td>
<td>2</td>
<td>Allergic dermatitis Deep-colored hair regrew after 7 weeks.</td>
</tr>
<tr>
<td>5</td>
<td>T. Poodle</td>
<td>♀</td>
<td>3</td>
<td>3.1</td>
<td>None</td>
<td>2</td>
<td>2</td>
<td>Stress-induced lick dermatitis Hair regrew fully after 3 weeks.</td>
</tr>
<tr>
<td>6</td>
<td>T. Poodle</td>
<td>♂</td>
<td>11</td>
<td>3.1</td>
<td>None</td>
<td>2</td>
<td>2</td>
<td>Dermatitis due to subcutaneous infection Hair regrew after 4 weeks.</td>
</tr>
<tr>
<td>7</td>
<td>T. Poodle</td>
<td>♂</td>
<td>1</td>
<td>3.9</td>
<td>None</td>
<td>2</td>
<td>2</td>
<td>Stress-induced hair loss Hair regrew after 3 weeks.</td>
</tr>
<tr>
<td>8</td>
<td>Cairn terrier</td>
<td>♂</td>
<td>12</td>
<td>8.2</td>
<td>None</td>
<td>3</td>
<td>3</td>
<td>Unexplained hair loss Itching and hair loss were relieved after 4 weeks.</td>
</tr>
<tr>
<td>9</td>
<td>Chihuahua</td>
<td>♀</td>
<td>5</td>
<td>3.2</td>
<td>Used</td>
<td>2</td>
<td>2</td>
<td>Allergic dermatitis (Convenia was used for pyoderma)</td>
</tr>
<tr>
<td>10</td>
<td>Mix-breed cat</td>
<td>♀</td>
<td>7 (estimated)</td>
<td>2.8</td>
<td>None</td>
<td>1</td>
<td>1</td>
<td>Unexplained hair loss Hair regrew after 4 weeks.</td>
</tr>
<tr>
<td>11</td>
<td>Mix-breed cat</td>
<td>♀</td>
<td>12</td>
<td>2.7</td>
<td>None</td>
<td>2</td>
<td>2</td>
<td>Unexplained hair loss Hair regrew after 4 weeks.</td>
</tr>
<tr>
<td>12</td>
<td>Hamster</td>
<td>♀</td>
<td>1</td>
<td>Not weighed</td>
<td>None</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>Unexplained hair loss Hair regrew after 4 weeks.</td>
</tr>
<tr>
<td>13</td>
<td>Rabbit</td>
<td>♀</td>
<td>5</td>
<td>2.5</td>
<td>None</td>
<td>1</td>
<td>1</td>
<td>Self-inflicted hair loss Hair regrew after 9 weeks.</td>
</tr>
</tbody>
</table>
**Case (ii) Hair loss due to atopic dermatitis**

Miniature dachshund/female/10 years old/4.2 kg

- **Daily doses** 2 LPS tablets / 4 pine polyphenol tablets
- **Administration period** Nov. 2013 – present
- **Concomitant drugs** An immunosuppressive drug

Severe facial hair loss

Hair grew back tidily

Severe Facial hair loss

After 15 weeks

Mar. 8, 2014

Beginning Nov. 6, 2013
Case (vi) Hair loss due to dermatitis caused by infection of subcutaneous tissues

Toypoodle/male/11 years old/3.1 kg

- **Daily doses**: 2 LPS tablets / 2 pine polyphenol tablets
- **Administration period**: Jul. 2014 – present
- **Concomitant drugs**: Not used

A 4cm diameter bald spot on the left loin

Beginning **Jul. 18, 2014**

Hair almost regrew

Sep. 8, 2014

After 7 weeks
Case (viii) Unexplained hair loss

Cairn terrier/male/12 years old/8.2 kg

- **Daily doses** 3 LPS tablets / 3 pine polyphenol tablets
- **Administration period** Apr. 10, 2014 – present
- **Concomitant drugs** Not used

Hair loss in the areas from root of the tail to the perianal area

Itching and hair loss were relieved

Beginning Apr. 25, 2014

May 21, 2014

After 4 weeks
Case (xi) Unexplained hair loss

Mix-breed cat/female/12 years old/2.7 kg

- **Daily doses** 2 LPS tablets / 2 pine polyphenol tablets
- **Administration period** Sep. 30 – Oct. 30, 2014
- **Concomitant drugs** Not used

Hair loss in the areas outside of both ears and above the eyes

Hair growth was observed After 4 weeks

Beginning Sep. 30, 2014

Oct. 30, 2014
Case (xii) Unexplained hair loss

Djungarian hamster/female/1 year & 4 months old

- **Daily doses** 5 LPS and 5 pine polyphenol tablets were mixed and powdered. An earpick of the mixed powder was administered 3 times daily.
- **Administration period** Nov. 26—Dec. 30, 2013
- **Concomitant drugs** Not used

Unexplained hair loss in the abdominal area

Hair regrew in the abdominal area

Beginning Nov. 26, 2013

Dec. 30, 2013

After 4 weeks
Case (xiii) Self-inflicted hair loss

Rabbit (Holland Lop)/female/5 years old/2.5 kg

- **Daily doses**: 1 LPS tablet / 1 pine polyphenol tablet
- **Administration period**: The end of May 2014 – present
- **Concomitant drugs**: Not used

Hair loss on the jowl

- **Beginning**: May 1, 2014
- **Aug. 4, 2014**: Hair regrew in the bald area

After 9 weeks
**Conclusion and discussion**

**Disorders of the 13 animals**
- Atopy, allergy: 31%
- Unexplained disorders: 31%
- Stress, self-infliction: 23%
- Dry dermatitis: 7%
- Subcutaneous infection: 8%

**Use of concomitant drugs**
- Not used (LPS + pine polyphenol alone): 77%
- Used: 23%

**Newly grown hair was typically deep colored and firm, and hair regrew in the bald areas regardless of the presence of inflammation.**
3. Putative Mechanisms of Action
Putative mechanisms of action

- **LPS**
  1. Macrophages are turned into priming stage and inhibit induction of catagen-inducing cytokine.
  2. Combination of mechanisms generates effect.

- **Pine polyphenol**
  1. Antioxidant effect
  2. Blood circulation promoting effect
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