Effects of Inhaled the *Cyperi rhizoma* and *Perillae herba* Essential Oil on Emotional States, Autonomic Nervous System and Salivary Biomarker

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Abstract

It is well-known that odor has a good influence on a human mind and body. In this study, we investigated the effect of the essential oil inhalation of *Cyperi rhizoma* and *Perillae herba* which were main aromatic herbs ingredient of the Kososan, to psychological condition, autonomic nerve activity and salivary biomarker. In addition, we examined influence by the favorite difference in odor. 25 healthy female volunteers (control group: 5 people; *Cyperi rhizoma* group: 10 people; *Perillae herba* group: 10 people) were examined. POMS was examined at PRE and POST the experiment. Pulse analyser was examined and saliva samples were collected before the inhalation, just after the inhalation, 10 minutes and 20 minutes later, respectively. Saliva samples analyzed for levels of α-amylase and cortisol. At the examination for POMS after the experiment, the preference for the odor was confirmed. About both *Cyperi rhizoma* and *Perillae herba*, by the essential oil inhalation, mental stability and relaxation effect were provided in psychological and physiological evaluation. In the “like” group, relaxation was greater than “unlike” group. However, about *Cyperi rhizoma*, the stress was relaxed regardless of the favorite difference in odor, and a physical relaxation effect was shown. In addition, about the autonomic nerve activity, the parasympathetic nerve was not activated, and it was suggested that it was made and stabilized the balance of the autonomic nerve.

1. Introduction

It is well-known that fragrance has a good influence on a human mind and body. The effect of fragrance handed down for a long time has been inspected and advanced to help medical care based on scientific grounds. The fragrance ingredient of the plant including Kampo medicine treatment and forest bathing has been used for treatment as well as aromatherapy widely [1]-[3]. Kampo medicine and crude drugs have unique fragrance, but there are few studies about the fragrances. Kososan is composed of five dried medicinal herbs, *Cyperi rhizoma*, *Perillae herba*, *Aurantii Nobilis Pericarpium*, *Glycyrrhizae Radix*, and *Zingiberis rhizoma*. It was reported that Kososan is effective not only for an easy case of a cold of the weak constitution that participated in stress [4], but also for a tendency to depression such as insomnia and the nervous breakdown [5] [6]. This is representative prescription of the autonomic nerve disease to be used for mental depression with the neurosis [7]. It has been already reported that sleeping time is prolonged only by inhalation of the Kososan for sleep disorder of the isolated stress mouse [8].

Physiologic stress effects are regulated by top-down central nervous system processes, as well as by subcortical processes within the limbic system. Both areas forward their messages via neuronal pathways to a central control system, the hypothalamus. The hypothalamus is closely intertwined with two major stress systems, the hypothalamus-pituitary-adrenal (HPA) axis [9] and the sympathetic nervous-adrenal medullary (SAM) axis [10]. The main effector of the HPA axis is cortisol. Its concentration is measured and evaluated to have an index for HPA axis activation. Salivary α-amylase is novel biochemical index for SAM axis activity. Both parameters can conveniently be assessed in saliva.

In this study, we investigated the effect of the essential oil inhalation of *Cyperi rhizoma* and *Perillae herba* which were main aromatic herbs of the Kososan, to psychological condition, autonomic nerve activity and relaxation effect. We defined relaxation as a condition eased stress and a calm state of mind and body, and there were not stress and anxiety. The favorite difference in fragrance is highly individual. It has been reported that brain waves [11] [12], an autonomic nerve activity [12]-[14] and the reaction of the surprise reflection [15] vary according to the difference. In addition, we examined influence by the favorite difference in fragrances.

2. Materials and Methods

2.1. Subjects

The subjects were 25 healthy female volunteers between the ages of 20 and 40 (33.8 ± 1.04 years) obtained consent by a purpose of this study at the Hiroo prime dermatology. The subjects were divided a randomized into three groups. In the *Cyperi rhizoma* group and *Perillae herba* group assumed 10 people, respectively. In the control group assumed 5 people. All subjects were asked to refrain from eating, drinking except water, smoking and exercise for 1 hour before its investigation. All subjects provided informed consent for participation.

2.2. Experimental Essential Oil

The *Perillae herba* essential oil and olive squalan oil as a control were obtained from Tree of Life Co., Ltd (Tokyo, Japan). The *Cyperi rhizoma* essential oil was obtained from Phytoaroma Research (Yokohama, Japan). The essential oil added 50 μl in a blotter card (Mouillettes; Grasse, France) and inhaled it.

2.3. Experimental Design

All subjects were examined on each separate occasion. The experiments were performed with the subjects sitting position in an air-conditioned (temperature 22˚C - 26˚C, humidity 45% - 55%) room. At the beginning of the experiments, subjects were given 30 min. to adapt to the room temperature. Before measurements were taken, at first the subjects filled out the Profile of Mood states (POMS) and were instructed to relax quietly and com-
fortably for 5 minutes, in seated position while equipped with Plus analyzer. In the interval, saliva samples were collected using saliva collection test tubes (Salimetrics Co., Ltd, USA). The Pulse analyzer was recorded 5 min before inhalation of the scent. Each subjects inhaled for 2 min and 30 seconds. We then measured the Pulse analyzer for 5 min and collected saliva samples at 0, 10 and 20 min after inhalation. After all measured, the subjects filled out the POMS test and questionnaire whether the fragrance was liked or not.

2.4. Measurements of POMS-Psychological Index

The Profile of Mood State (POMS) questionnaire is a well established, factor-analytically derived measure of psychological distress, such as mood, for which high levels of reliability and validity have been documented [16]. We used the short-form POMS, which consists of thirty adjectives rated on a 0-4 scale that can be consolidated into six mood scales: “tension-anxiety (T-A)”, “depression-dejection (D)”, “anger-hostility (A-H)”, “vigor (V)”, “fatigue (F)” and “confusion (C)”. The questionnaire was administered at PRE and POST the experiment. And at POST the test, it was added to questionnaire whether its fragrance was liked or not, specifically 5 scale “liked”, “if anything, liked”, “neither liked or hated”, “if anything, hated” and “hated”. About the favorite difference in fragrances, we divided two groups. “Liked”, and “if anything, liked”, were in one group “like” group. “if anything, hated” and “hated” were in “unlike” group.

2.5. Measurements of Autonomic Nervous Activities

Autonomic nervous activities were assessed using Pulse Analyzer Plus TAS9 (YKC Corporation, Tokyo, Japan) [17]. For the measuring finger tip pulse, HRV (the autonomic nerve balance) analysis program was used. Heart rate was measured by conducted on it. A frequency level from 0.04 to 0.15 Hz was classified as Low Frequency (LF), and a frequency level from 0.15 to 0.40 Hz was classified as High Frequency (HF). HF was considered as an indicator for parasympathetic nerve activity. The ratio between LF and HF (LF/HF) showed the overall balance of sympathetic and/or parasympathetic nerve, it is proportional to the degree of sympathetic nerve activity and inversely proportional to parasympathetic nerve activity [18]. TP expressed the general activity of the autonomic nerve.

2.6. Measurements of Salivary Components

Saliva samples were collected using the tasteless and odorless oral swabs (Salimetrics Co., Ltd, USA) retained in the mouth for 5 min in a seated position, and the collected saliva was centrifuged at 1500 rpm for 40 min at 4°C. The Salivary α-amylase Assay Kit (Salimetrics Co., Ltd, USA) was used to determined the α-amylase activity whose activity level was corrected for using salivary protein concentrations. The salivary protein concentrations were measured with the Pierce BCA Protein Assay Kit (Thermo Scientific, USA). The cortisol levels were measured with the Salivary Cortisol Enzyme Immunoassay Kit (Salimetrics Co., Ltd, USA).

2.7. Statistical Analysis

Values are expressed as mean ± SEM. Statistical significance of these data was evaluated by one-way analysis of variance (ANOVA), followed by Bonferroni multiple comparison procedure. p values of <0.05 were defined as statistically significant.

3. Results

3.1. POMS-Psychological Index

The score of three items, tension-anxiety (T-A), anger-hostility (A-H) and fatigue (F) significantly decreased in control group. The results might be by mental stability from 30-minute rest. Both in the Cyperi rhizoma group and Perillae herba group decreased the score of all items. So these three items excluded, we examined the scores of depression-dejection (D), vigor (V) and confusion (C).

The Cyperi rhizoma group significantly decreased the score of D. (from 48.5 ± 3.0 to 42.3 ± 5.6). The Cyperi rhizoma group (from 49.5 ± 3.1 to 39.9 ± 2.4) and Perillae herba group (from 45.3 ± 3.0 to 38.1 ± 1.6) significantly decreased the score of C, respectively. In addition, the Perillae herba group (from 44 ± 3.2 to 35.8 ± 2.4)
significantly decreased the score of V (Figure 1).

About *Cyperi rhizoma* group, “like” group had six subjects, “unlike” group had three subjects. About *Perillae herba* group, “like” group had four subjects, “unlike” group had three subjects.

In “unlike” group of the *Cyperi rhizoma*, there was no significant difference between before and after inhalation about all items. However, “like” group of the *Cyperi rhizoma* significantly decreased the scores of five items except for V. In “unlike” group of the *Perillae herba*, there was no significant difference between before and after inhalation about C. However, “like” group of *Perillae herba* significantly increased the score of C. In addition, “like” group and “unlike” group of *Perillae herba* significantly decreased the scores of V (Table 1).

### 3.2. The Autonomic Nervous Activities

TP indicate that expressed the general activity of the autonomic nerve. In the *Cyperi rhizoma* group, TP significantly increased comparison to before inhalation at 10 minutes and 20 minutes later (Figure 2(a)). HF indicate that a parasympathetic nerve activity index. Both in *Cyperi rhizoma* and *Perillae herba* group, HF was no apparent difference (data not shown here). LF/HF indicate that overall balance of sympathetic and parasympathetic nerve. The *Cyperi rhizoma* group significantly increased at 10 minutes later comparison to before inhalation. The *Perillae herba* significantly increased at 20 minutes later comparison to before inhalation (Figure 2(b)).

![Figure 1. Change of POMS before and after the experiment. (a) Control; (b) *Cyperi rhizome*; (c) *Perillae herba*. Mean ± S.E, control n = 5, *Cyperi rhizoma* n = 10, *Perillae herba* n = 10, *p < 0.05, **p < 0.01 vs pre.](image)

![Table 1. Change of POMS-comparison of the favorite difference in odor. *Cyperi rhizoma* like n = 6 unlike n = 3, *Perillae herba* like n = 4 unlike n = 3. Mean ± S.E, *p < 0.05 vs pre, **p < 0.01 vs pre.](table)

<table>
<thead>
<tr>
<th>Odor</th>
<th>TA</th>
<th>D</th>
<th>AH</th>
<th>V</th>
<th>F</th>
<th>C</th>
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<td><strong>Cyperi rhizoma</strong> like</td>
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<td>52.2 ± 4.6</td>
<td>44.3 ± 2.8</td>
<td>39.5 ± 3.9</td>
<td>52.7 ± 3.6</td>
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<tr>
<td>Like post</td>
<td>37.2 ± 1.5</td>
<td>44 ± 2.8</td>
<td>37</td>
<td>37.7 ± 5.2</td>
<td>38.7 ± 1.2</td>
<td>41.5 ± 3.9</td>
</tr>
<tr>
<td>p</td>
<td>0.029 ± *</td>
<td>0.036 ± *</td>
<td>0.045 ± *</td>
<td>0.058</td>
<td>0.0108 ± *</td>
<td>0.0429 ± *</td>
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<tr>
<td>Unlike pre</td>
<td>42.3 ± 1.8</td>
<td>43.3 ± 0.7</td>
<td>40.3 ± 1.7</td>
<td>46.7 ± 8.1</td>
<td>52.3 ± 4.4</td>
<td>45 ± 3</td>
</tr>
<tr>
<td>Unlike post</td>
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<td>40 ± 1</td>
<td>37</td>
<td>33.7 ± 3.7</td>
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<td>0.0634</td>
<td>0.183</td>
<td>0.142</td>
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<td>0.0941</td>
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<tr>
<td><strong>Perillae herba</strong></td>
<td>TA</td>
<td>D</td>
<td>AH</td>
<td>V</td>
<td>F</td>
<td>C</td>
</tr>
<tr>
<td>Like pre</td>
<td>42.5 ± 3.0</td>
<td>45.3 ± 2.8</td>
<td>42.3 ± 3.2</td>
<td>53.8 ± 3.9</td>
<td>47 ± 3.4</td>
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<tr>
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<td>41.8 ± 1.9</td>
<td>37</td>
<td>41.3 ± 4.8</td>
<td>41.5 ± 1.7</td>
<td>39.8 ± 3.1</td>
</tr>
<tr>
<td>p</td>
<td>0.0892</td>
<td>0.31</td>
<td>0.199</td>
<td>0.0115 ± *</td>
<td>0.0622</td>
<td>0.0052 ± **</td>
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<tr>
<td>Unlike pre</td>
<td>43.3 ± 2.3</td>
<td>41.7 ± 2.7</td>
<td>38 ± 1</td>
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<td>47 ± 2</td>
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<td>Unlike post</td>
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<td>p</td>
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<td>0.422</td>
<td>0.046 ± *</td>
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Figure 2. Change of the autonomic nerve activity before and after the essential oil inhalation. (a) TP; (b) LF/HF. Mean ± S.E, control n = 5, Cypri rhizoma n = 10, Perillae herba n = 10, *p < 0.05, **p < 0.01 vs pre.

In addition, there was no significant difference between “like” group and “unlike” group of the essential oils about autonomic nerves activity (data not shown here).

3.3. The Salivary Components

The control group increased α-amylase activity after inhalation. The α-amylase activity in the Cypri rhizoma group was significantly inhibited compared with that of control group at just after inhalation and 20 minutes later, respectively. The Perillae herba group had no significant effect on α-amylase activity (Figure 3).

The control group increased the cortisol levels just after inhalation. The Cypri rhizoma and Perillae herba group were inhibited compared with that of control group at just after inhalation. In addition, the Perillae herba group significantly decreased compared with that of before inhalation at just after inhalation and 10 minutes later, respectively (Figure 4).

About the favorite difference in fragrances, there was no significant difference in the Perillae herba group (data not shown here). The “like” group in the Cypri rhizoma had no significant effect on α-amylase activity. The “unlike” group in the Cypri rhizoma significantly increased α-amylase activity at 10 minutes later (Figure 5(a)). The “like” group in Cypri rhizoma had no significant effect on cortisol levels. However, the “unlike” group cortisol levels significantly decreased 10 minutes later (Figure 5(b)).

4. Discussion

In the present study, as for the Cypri rhizoma essential oil, mental stable relaxation was obtained in a psychological evaluation, the salivary physiological evaluation by the inhalation of the essential oil. And it was shown that the inhalation of the Cypri rhizoma essential oil activated autonomic nerve activity. It means increase of the ability for stress coping [19]. Some studies were reported that the essential oil told having relaxation effect inhalation made parasympathetic nerve predominant [20] [21]. In addition, there are the findings which do not accord [22]. In this study, it was not the same result and worked in sympathetic nerve predominance after the Cypri rhizoma essential oil inhalation. Kososan and Hangekoubokuto are representative prescription of the autonomic nerve disease to be used for mental depression with neurosis and the depressed mental state. It is suggested that Kososan promotes the sympathetic nerve activity of a case aggravating parasympathetic nerve whereas Hangekoubokuto suppress the sympathetic nerve activity of the case aggravating sympathetic nerve [7]. In this study, we examined the effect only for essential oil inhalation. The standard value of LF/HF changes by individual difference or measurement condition. We divided two groups. A value of LF/HF before the inhalation defined less than 1 as parasympathetic nerve activated group and 1 or more as sympathetic activated group. In parasympathetic activated group of the Cypri rhizoma, the value of LF/HF increased after inhalation and 10 minutes later. In addition, there was significant difference between two groups (Figure 6(b)). In sympathetic
Figure 3. Change of α-amylase before and after the essential oil inhalation. Mean ± S.E, control n = 5, *Cyperi rhizoma n = 10, Perillae herba n = 10, #p < 0.05 vs control.

Figure 4. Change of cortisol before and after the essential oil inhalation. Mean ± S.E, control n = 5, *Cyperi rhizoma n = 10, Perillae herba n = 10; *p < 0.05, **p < 0.01 vs pre, #p < 0.05, ##p < 0.01 vs control.

Figure 5. Change of α-Amylase and cortisol in Cyperi rhizoma -comparison of the favorite difference in odor. (a) α-Amylase; (b) Cortisol. Mean ± S.E, like n = 6 unlike n = 3, *p < 0.05 vs pre.
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Figure 6. Change of LF/HF-comparison of sympathetic and parasympathetic nerve activated groups. (a) Perillae herba sympathetic nerve activated group n = 6, parasympathetic nerve activated group n = 4; (b) Cyperi rhizoma sympathetic nerve activated group n = 6, parasympathetic nerve activated group n = 4; Mean ± S.E., **p < 0.01 vs pre.

activated group of the Cyperi rhizoma, the value of LF/HF did not changed. In the Perillae herba essential oil, the value of LF/HF in parasympathetic activated group increased after inhalation in comparison to sympathetic activated group. However, there was no significant difference (Figure 6(a)). LF/HF is an index of the balance of sympathetic nerve and the parasympathetic nerve. In other words, a parasympathetic nerve activated group, these results were suggested in possibility that the Cyperi rhizoma essential oil fixed the balance by activating sympathetic nerve. However, a further study is necessary to conclude it because there are few subjects. At least, these results were suggested that autonomic nerve activity was not activated influence of the parasympathetic nerve, and it was made and stabilized the balance of the autonomic nerve.

About the Cyperi rhizoma essential oil, mental stability was provided in the “like” group greater than “unlike” group in psychological evaluation. In “unlike” group, α-amylase activity increased at 10 minutes later and cortisol levels decreased clearly. Tornhage reported, the relationship of salivation speed and density of α-amylase, though the density of α-amylase decreased in two minutes from just after the stimulation and increase 5 minutes later [10]. Though the response speed of α-amylase secretion is quick about several minutes, α-amylase did not increase just after inhalation in “unlike” group. So it is thought that stress was controlled by the Cyperi rhizoma essential oil just after inhalation, but the effect decreased as the odor faded and a stress symptom appeared with time. Then α-amylase activity increased 10 minutes later. Cortisol levels is said to take more time for a response than α-amylase activity [9]. The cortisol levels in “unlike” group of the Cyperi rhizoma decreased 10 minutes later. It is thought because the response of the relaxation effect with essential oil is late, and it appeared. It increased again 20 minutes later. In contrast, both in α-amylase activity and cortisol levels in “like” group kept low value, and relaxation state was maintained. The reaction for the essential oil in “unlike” group was stronger than “like” group, but the continuation time of the effect was shorter. These results were supposed that the person who liked odor could continue a relaxation effect.

The fragrances to like made felt relaxation effect more about subjective symptoms by POMS. In this study, POMS was examined at PRE and POST the experiment. The second one was not the evaluation from just after inhalation to 10 minutes later when the response of α-amylase activity and cortisol levels was remarkable about the Cyperi rhizoma essential oil. So it will be necessary to subdivide examinations of POMS to consider the correlation of awareness and physiological reaction of the stress. At least, in this study, it was suggested that physical relaxation effect was provided by the inhalation of the Cyperi rhizoma essential oil regardless of the taste for the odor, and the fragrance liked made felt relaxation effect more than unlike at 20 minutes later.

As for the Perillae herba essential oil as well as the Cyperi rhizoma essential oil, mental stability and relaxation were shown in a psychological evaluation and the physiological one of saliva by inhalation of the essential
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oil. The score of “Vigor” and “Confusion” decreased by inhalation of the Perillae herba essential oil. In “like” group, the score of “Confusion” decreased more than “unlike” group, but “Vigor” significantly decreased regardless of taste of the fragrance. Vigor is related with the awakening. In this study, there were subjects who fell asleep during the examination. In the previous study reported that perillaldehyde which is main aromatic oil manufacture ingredient of the Perillae herba prolonged sleeping time and have sedation [23]. There were reports that perillaldehyde prolonged pentobarbital-induced sleeping time [5] [6] [23] [24]. In addition, about action for the central nervous system, it has reported that the Perillae herba and perillaldehyde have antidepressant effect [7] [25]. The effect was shown Perillaldehyde had antidepressant effect through the sense of olfactory nervous system [24]. In the present study reported that citrus have shown antidepressant effect through the sense of olfactory system of odor [26]. Moreover, it was reported that the hot water extract of the Cyperi rhizoma shows an antidepressant effect in forced swimming test, but the mechanism was not yet clear [27]. The main aromatic essential oil ingredients of the Cyperi rhizoma are cyperene, α-cyperone, isolongifolen-5-one, rotundene and cyperorotundene [28]. It was reported cyperene had anti-inflammatory activity [29]. However, there is not yet the report through the sense of olfactory system.

5. Conclusion

Both in the Cyperi rhizoma and Perillae herba essential oil, mental stability and relaxation effect were shown in psychological and physiological evaluation by the essential oil inhalation. In the “like” group, relaxation was greater than “unlike” group. However, about the Cyperi rhizoma essential oil, the stress was relaxed regardless of the favorite difference in odor, and a physical relaxation effect was shown. In addition, about the autonomic nerve activity of the Cyperi rhizoma essential oil, the parasympathetic nerve was not activated, and it was suggested that it was made and stabilized the balance of the autonomic nerve.

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