Contents lists available at ScienceDirect



Short communication

Contemporary Clinical Trials Communications

journal homepage: http://www.elsevier.com/locate/conctc



Endocrinological effect of lavender aromatherapy on stressful visual stimuli

Masahiro Toda^{a,**}, Ryoichi Matsuse^b

^a Graduate School of Human Life Sciences, Notre Dame Seishin University, Okayama, Japan
^b Department of Social and Environmental Medicine, Osaka University Graduate School of Medicine, Osaka, Japan

ARTICLE INFO

Keywords: Aromatherapy Chromogranin A (CgA) Human saliva Lavender Stress

ABSTRACT

Introduction: Aromatherapy is prominent in complementary and alternative medicine. Little endocrinological evidence, however, of the effects of aromatherapy has yet been presented. We used salivary stress marker chromogranin A (CgA) to examine the effects of lavender aroma on women watching a stressful video. *Methods:* Healthy female university students (n = 23) aged 20–22 years old were randomly assigned to two groups: an aroma group exposed to lavender and an unexposed control group. Both groups watched a stressful video for 10 min. During the protocol, the aroma group was exposed to lavender aroma. Samples of salivary chromogranin A (CgA) were collected immediately before and after watching the video, and at 5 and 10 min after that.

Results: In the aroma group, the levels of CgA statistically significantly decreased throughout the experimental period. In the control group, there was no such change.

Conclusion: The findings suggest that lavender aroma may reduce the stress effects of watching a stressful video.

1. Introduction

Empirical evidence has shown that aromatherapy with essential oils has some efficacy and it has recently become one of the major complementary and alternative therapy. Numerous aromatherapy studies have been conducted, in particular, for the essential oil of lavender (*Lavendula*). Belonging to the family *Lamiaceae*, lavender is a herbaceous plant native to the Mediterranean area and is widely cultivated [1]. It has been suggested that lavender aroma may be associated with improved mood, reduced mental stress and anxiety, sedation, and good sleep [2–7]. In addition, it has been reported that topical application of lavender cream (containing 1.25% essential oils) reduces stress, anxiety, and depression in pregnant women [8]. These studies have added to increasing evidence of the effectiveness of lavender aromatherapy. Meanwhile, Ahmad et al. found some effects of lavender aromatherapy on exam stress for pharmacy students, but these were no more effective than placebo [9].

Understood as the physiological demand placed on the body when one must adapt, cope, or adjust [10], stress responses are triggered by the autonomic nervous system, which influences internal organs, heart rate, respiratory rate, blood vessel, galvanic skin response, and so on [11]. Lavender aroma may reduce stress and produce relaxation via the limbic system, particularly the amygdala and hippocampus [1]. In a previous study, by measuring salivary endocrinological stress markers, we found that lavender aroma can relieve stress caused by performing mental arithmetic calculations [12]. Even so, little endocrinological evidence of the effects of aromatherapy has yet been presented. To examine the effects of lavender aroma on women watching a stressful video, we used salivary stress marker chromogranin A (CgA).

CgA is an acidic glucoprotein that is released along with catecholamines from the adrenal medulla and the sympathetic nerve endings [13–15]. A recent study has reported that CgA is produced by human submandibular glands and secreted into saliva [16]. Salivary CgA reflects the sympatho-adrenomedullary (SAM) system and has gained attention as a novel stress marker [17,18]. Incidentally, for evaluating effects of lavender aroma, in a previous study, we found that salivary CgA is a better stress marker than cortisol via the hypothalamus-pituitary-adrenocortical (HPA) axis [12].

2. Methods

For the study, approved by the Ethics Committee of Notre Dame Seishin University, we recruited 23 healthy female university students aged 20–22 years old. None were receiving any medication. Prior to the study, informed consent was received from each participant. The women were randomly assigned to two groups: aroma group (n = 12) and

https://doi.org/10.1016/j.conctc.2020.100547

Received 8 July 2019; Received in revised form 14 February 2020; Accepted 22 February 2020 Available online 25 February 2020 2451 8554 (© 2020 Published by Electric Inc. This is an open access article under the CC BY NC ND license (http://www.commonstance.com/com

2451-8654/© 2020 Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

^{*} Corresponding author. Graduate School of Human Life Sciences, Notre Dame Seishin University, 2-16-9 Ifuku-cho, Kita-ku, Okayama, 700-8516, Japan. *E-mail address:* mt@post.ndsu.ac.jp (M. Toda).



Fig. 2. Changes in mean values (±SD) for salivary chromogranin A (CgA) levels before and after watching a stressful video in a lavender aroma group (\bullet , n = 12) and a control group (\circ , n = 11). *Statistically significant difference compared with preintervention; p < 0.05 (ANOVA with repeated measures and Dunnett's test).

control group (n = 11). To avoid aroma contamination of the control group, protocols for each group were conducted on different days.

On a 23-inch TV monitor placed at eye level approximately 100 cm away from the faces of the viewers, both groups watched a 10 min ophthalmic surgery video. A previous study has found that this kind of stressful video activates the SAM system [19]. Saliva samples were collected immediately before and after watching the video, and at 5 and 10 min after the video ended (Fig. 1). At each sampling point, subjective stress was also evaluated using a 10-division visual analog scale. After the first saliva collection, to the end of the protocol, at a comfortable distance (approximately 10 cm from the nose), the aroma group was exposed to organic essential oil of lavender (Saiseisha Co. Ltd., Shiga, Japan) that had been infiltrated into filter paper (150 μ L). There was no air current in the room.

Saliva samples were collected using the Salivette system (Sarstedt Co. Ltd., Nümbrecht, Germany). In this method, by centrifuging (at 3000 rpm for 15 min), saliva samples are extracted from cotton wads that subjects had held in their mouths (for 2 min). To minimize the effects of food and drink on the levels of salivary CgA, participants were asked to abstain from food or drink, other than mineral water, for 2 h before saliva sampling [20]. The samples were stored at -30 °C until the

Fig. 3. Changes in mean values (±SD) for subjective stress (visual analog scale score) before and after watching a stressful video in a lavender aroma group (\bullet , n = 12) and a control group (\circ , n = 11). *Statistically significant difference compared with preintervention; p < 0.05 (ANOVA with repeated measures and Dunnett's test).

assay. Using a previously described method [21], levels of salivary CgA were evaluated using enzyme-linked immunosorbent assay (ELISA).

All results are displayed as mean values \pm standard deviation. ANOVA with repeated measures was performed to detect time-related differences. Dunnett's test was used for multiple comparisons. Values are considered to be statistically significantly different if p < 0.05.

3. Results

Immediately after watching the video, somewhat lower levels of salivary CgA was found in samples from the aroma group (Fig. 2). Then, at 5 and 10 min after watching the video, we detected statistically significantly lower levels of salivary CgA than in those taken preintervention (*F*[3, 33] = 4.38, p < 0.05). In the control group, while levels slightly increased after viewing, throughout the protocol, no statistically significant changes in levels of salivary CgA was detected.

Immediately after watching the video, evaluated by visual analog scale, subjective perceptions of stress were statistically significantly higher than at preintervention in both the aroma (*F*[3, 33] = 27.17, p < 0.01) and the control group, (*F*[3, 30] = 51.81, p < 0.01) (Fig. 3). At 5 and 10 min after watching the video, still higher levels were scored by



Fig. 4. Changes in mean values (±SD) for salivary chromogranin A (CgA) levels during the lavender aroma protocol after watching a stressful video in a group with a reported liking of lavender aroma (\blacksquare , n = 9) and a group with a reported a dislike of lavender aroma (\square , n = 3). *Statistically significant difference compared with preintervention; p < 0.05 (ANOVA with repeated measures and Dunnett's test).



Fig. 5. Changes in mean values (±SD) for subjective stress (visual analog scale score) during the lavender aroma protocol after watching a stressful video in a group with a reported liking of lavender aroma (\square , n = 9) and a group with a reported disliking of lavender aroma (\square , n = 3). *Statistically significant difference compared with preintervention; p < 0.05 (ANOVA with repeated measures and Dunnett's test).

the control group. Meanwhile, the aroma group showed a quick return to preintervention scores.

Fig. 4 shows changes, during the aroma protocol, in the mean levels of CgA for participants grouped according to whether they reported liking or disliking lavender aroma. While those who liked lavender aroma immediately, and at 5 min, and 10 min after watching the video, showed statistically significantly lower CgA levels (F [3,24] = 4.17,

p < 0.05), those who did not like lavender aroma showed no such change.

In both groups, irrespective of liking lavender aroma, statistically significantly higher levels of subjective perceptions of stress were detected immediately after watching the video (F [3,24] = 21.89, p < 0.01 and F [3,6] = 12.96, p < 0.01, respectively) (Fig. 5). At 5 min after watching the video, still higher levels were detected in the group who did not like lavender aroma. Meanwhile, the group who liked lavender aroma showed a quick return to preintervention levels.

4. Discussion

In the present study, we used an ophthalmic surgery video as a stressor. A previous study found statistically significantly increased levels of salivary α -amylase, a stress marker of the SAM system, after watching this kind of video [19]. We failed to detect any such difference in salivary CgA in either the aroma or the control group. This may have resulted from a difference in the type of participants or from advance knowledge: all participants were non-medical students and had never seen such a video; in addition, as part of informed consent, they were notified of the contents of the video. Immediately before watching the video, therefore, their sympathetic nervous activity may have already reached higher levels. Even so, in both the aroma and the control group, subjective perceptions of stress statistically significantly increased after watching the video. Such a finding may indicate a difference between subjective and objective stress reactions.

In a previous study, several minutes after the inhalation of the lavender aroma, levels of CgA that had been elevated at the end of a mental arithmetic task were statistically significantly lower [12]. Similarly, in the present study, in the aroma group, levels of CgA decreased throughout the experimental period. These findings suggest that lavender aroma is able to relieve various types of stress. This conjecture is supported by results of subjective perceptions of stress. The aroma group reported recovering from stress faster than the control group. This finding is in line with our previous results [12].

When the results were analyzed according to like or dislike of lavender aroma, only the group who liked lavender aroma showed a statistically significant decrease in CgA levels during the protocol. The group of dislikers showed no statistically significant change in salivary CgA. These findings suggest that the effects of lavender aroma may differ according to individual aroma preference. Volatile compounds of organic essential oils may diffuse into the olfactory nerve and pass to the limbic system. In the central nervous system, main constituent of lavender linalool may inhibit NMDA receptor binding of glutamate, a major excitatory neurotransmitter [22,23]. Individual aroma preference, therefore, may influence the sensitivity of the receptor. Further studies are needed to confirm this speculation. Incidentally, visual analog scale results suggest that individual aroma preference may also influence objective stress reactions.

In this study, we found that lavender aroma can relieve the stress of watching a stressful video, particularly for individuals who report liking lavender aroma. This research, however, has several limitations. All the participants were healthy female university students aged 20–22 years old, a restricted cohort that cannot be representative of the general population. Furthermore, the sample size was too small to provide conclusive results. Further examination of larger and more varied populations is required. Elsewhere, the effect of aromatherapy using essential oils other than lavender, such as rosemary or peppermint has been investigated [2,24]. We are planning further investigations with different kinds of aroma.

5. Conclusions

Our evidence suggests that lavender aroma is capable of relieving stress. Furthermore, individual preference for this particular aroma may contribute to this effect.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

References

- P.H. Koulivand, M.G. Khaleghi, A. Gorji, Lavender and the nervous system, Evid. base Compl. Alternative Med. 2013 (2013) 681304.
- [2] M. Moss, J. Cook, K. Wesnes, P. Duckett, Aromas of rosemary and lavender essential oils differentially affect cognition and mood in healthy adults, Int. J. Neurosci. 113 (1) (2003) 15–38.
- [3] J. Lehrner, G. Marwinski, S. Lehr, P. Johren, L. Deecke, Ambient odors of orange and lavender reduce anxiety and improve mood in a dental office, Physiol. Behav. 86 (1–2) (2005) 92–95.
- [4] R. McCaffrey, D.J. Thomas, A.O. Kinzelman, The effects of lavender and rosemary essential oils on test-taking anxiety among graduate nursing students, Holist. Nurs. Pract. 23 (2) (2009) 88–93.
- [5] N. Motomura, A. Sakurai, Y. Yotsuya, Reduction of mental stress with lavender odorant, Percept. Mot. Skills 93 (3) (2001) 713–718.
- [6] S. Kim, H.J. Kim, J.S. Yeo, S.J. Hong, J.M. Lee, Y. Jeon, The effect of lavender oil on stress, bispectral index values, and needle insertion pain in volunteers, J. Alternative Compl. Med. 17 (9) (2011) 823–826.
- [7] N. Goel, H. Kim, R.P. Lao, An olfactory stimulus modifies nighttime sleep in young men and women, Chronobiol. Int. 22 (5) (2005) 889–904.
- [8] F. Effati-Daryani, S. Mohammad-Alizadeh-Charandabi, M. Mirghafourvand, M. Taghizadeh, A. Mohammadi, Effect of lavender cream with or without foot-bath on anxiety, stress and depression in pregnancy: a randomized placebo-controlled trial, J. Caring Sci. 4 (1) (2015) 63–73.
- [9] R. Ahmad, A.A. Naqvi, H.M. Al-Bukhaytan, A.H. Al-Nasser, A.H. Baqer Al-Ebrahim, Evaluation of aromatherapy with lavender oil on academic stress: a randomized placebo controlled clinical trial, Contemp. Clin. Trials Commun. 14 (2019) 100346.
- [10] J. Nevid, S. Rathus, Psychology and the Challenges of Life: Adjustments in the New Millennium, Wiley, Hoboken NJ, 2003.
- [11] C.T. Li, J. Cao, T.M.H. Li, Eustress or distress: an empirical study of perceived stress in everyday college life, in: UbiComp '16, 2016, pp. 1209–1217.

- [12] M. Toda, K. Morimoto, Effect of lavender aroma on salivary endocrinological stress markers, Arch. Oral Biol. 53 (10) (2008) 964–968.
- [13] A.D. Smith, H. Winkler, Purification and properties of an acidic protein from chromaffin granules of bovine adrenal medulla, Biochem. J. 103 (2) (1967) 483–492.
- [14] W.J. Smith, N. Kirshner, A specific soluble protein from the catecholamine storage vesicles of bovine adrenal medulla, Mol. Pharmacol. 3 (1) (1967) 52–62.
- [15] H. Winkler, R. Fischer-Colbrie, The chromogranins A and B: the first 25 years and future perspectives, Neuroscience 49 (3) (1992) 497–528.
- [16] J. Saruta, K. Tsukinoki, K. Sasaguri, H. Ishii, M. Yasuda, Y.R. Osamura, Y. Watanabe, S. Sato, Expression and localization of chromogranin A gene and protein in human submandibular gland, Cells Tissues Organs 180 (4) (2005) 237–244.
- [17] H. Nakane, O. Asami, Y. Yamada, T. Harada, N. Matsui, T. Kanno, N. Yanaihara, Salivary chromogranin A as an index of psychosomatic stress response, Biomed. Res. 19 (6) (1998) 401–406.
- [18] H. Nakane, O. Asami, Y. Yamada, H. Ohira, Effect of negative air ions on computer operation, anxiety, and salivary chromogranin A-like immunoreactivity, Int. J. Psychophysiol. 46 (1) (2002) 85–89.
- [19] N. Takai, M. Yamaguchi, T. Aragaki, K. Eto, K. Uchihashi, Y. Nishikawa, Effect of psychological stress on the salivary cortisol and amylase levels in healthy young adults, Arch. Oral Biol. 49 (12) (2004) 963–968.
- [20] M. Toda, K. Morimoto, S. Nagasawa, K. Kitamura, Effect of snack eating on sensitive salivary stress markers cortisol and chromogranin A, Environ. Health Prev. Med. 9 (1) (2004) 27–29.
- [21] S. Nagasawa, Y. Nishikawa, L. Jun, Y. Futai, T. Kanno, K. Iguchi, T. Mochizuki, M. Hoshino, C. Yanaihara, N. Yanaihara, Simple enzyme immunoassay for the measurement of immunoreactive chromogranin A in human plasma, urine and saliva, Biomed. Res. 19 (6) (1998) 407–410.
- [22] E. Elisabetsky, J. Marschner, D.O. Souza, Effects of linalool on glutamatergic system in the rat cerebral cortex, Neurochem. Res. 20 (4) (1995) 461–465.
- [23] E. Elisabetsky, L.F. Brum, D.O. Souza, Anticonvulsant properties of linalool in glutamate-related seizure models, Phytomedicine 6 (2) (1999) 107–113.
- [24] M. Toda, K. Morimoto, Evaluation of effects of lavender and peppermint aromatherapy using sensitive salivary endocrinological stress markers, Stress Health 27 (5) (2011) 430–435.