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Anti-depression effect of *Chimonanthus salicifolicus* essential oil in chronic stressed rats

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The aim of this study was to investigate the interventional effect of the *Chimonanthus salicifolicus* essential oil on murine chronic stress-induced anxiety. Rats model of uncertainty empty bottle was established to induce anxiety. The exploratory change, grooming and attack behaviors were observed. After 7 days training period followed by continuous 14 days' stimulation, rats serum level of glucose (GLU), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) were detected. The results showed that rat model of uncertainty empty bottle induced anxiety well. *C. salicifolicus* essential oil smelling could significantly appease the anxiety of rat which include the reduction of attacking behaviors. It also showed a reduced effect to the level of TC and GLU. The result also indicated that *C. salicifolicus* essential oil might have an anti-anxiety effect on rat uncertainty empty bottle model and could be used as a potential.

Key words: Anxiolytics, *Chimonanthus salicifolicus* essential oil, chronic emotional stress.

INTRODUCTION

The plants of calycanthaceae have a special odor and contain aroma compositions which can be extracted as essential oils. The *Chimonanthus salicifolicus* S. Y. Hu is a kind of calycanthaceae plant that is distributed mainly in Anhui, Zhejiang and Jiangxi Province, China. It is often used to treat cough, asthma and expectorant. It has been used for a long time by one of the Chinese minorities called She. The *C. salicifolius* S. Y. Hu oil mainly consists of 1,8-Cinele, myrcene, limonene, linalool, linalooloxide and pinocamphone (Li et al., 2008). It has been suspected to have an effect on releasing emotions because of its special aroma.

Anxiety is being explained as a psychological and physiological state marked by cognitive, somatic, emotional and behavioral elements. Together, these components provoke a disagreeable emotion associated with fear, worry, as well as uncomfortableness. Anxiety can appear suddenly without any triggering stimulus. Therefore, it can be an obstacle in everyday life. In a state of anxiety, the body prepares to cope with a menace. As a result, the symptoms are heart palpitations, tension, nausea, chest pain, shortness of breath, sweating, trembling, pale skin, papillary dilatation and so on. At this moment, the body is in "fight or flight" response. This can be experienced as panic in severe situations. Generalized and persistent anxiety often leads to the usage

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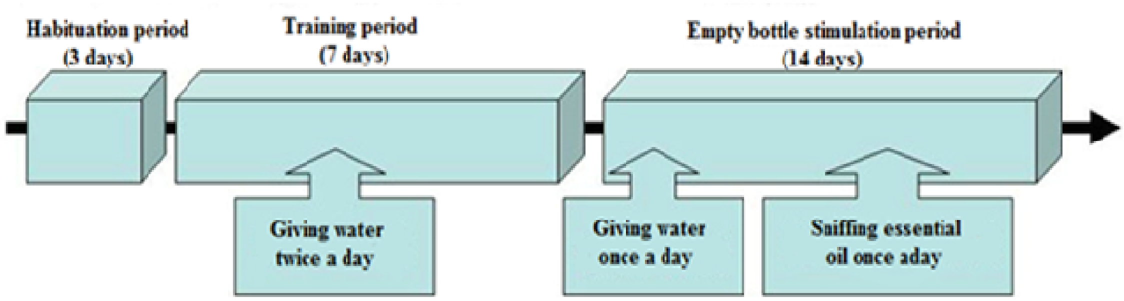

Figure 1. The process of establishing uncertainty empty bottle stimulation model.

of benzodiazepine, even these medications have several side effects, such as sedation. People who suffer from generalized and persistent anxiety often have high potential for drug abuse. Therefore, alternatives such as aromatherapy are needed (Woelk et al., 2010).

There are many medicinal plants with calmative effects on the central nervous system. In traditional Chinese medicine, the calycanthaceae plant has been recorded to have the function of relieving depression etc. Citrus is a popular anxiolytic oil which can help people in mitigating their anxiety and calming themselves down (Leite et al., 2008; Faturi et al., 2010; Giacinto et al., 2010; Lehrner et al., 2005). Lavender remedies are the most frequently used physiotherapeutic medications in the treatment of anxiety (Lehrner et al., 2005; Xu et al, 2008; Cline et al., 2008; Bradley et al., 2009; Braden et al., 2009; Kritsidima et al., 2010; Komori et al., 1995). Taking all of these into consideration, the aim of the presented work was to evaluate the potential anxiolytic effect of inhaled *C. salicifolius* S. Y. Hu essential oil on rats that were submitted to the uncertainty empty bottle by using the lavender and citrus essential oils as positive control.

MATERIALS AND METHODS

Subjects

Male Sprague Dawley rats, aged 35 days and weighing 120 to 130 g, were purchased from the Sino-British Sippr/BK lab. Animal Ltd. company. Upon arrival, the rats were weighed and maintained on a 12/12 h light/dark cycle (lights on 07.00 h) through artificial illumination. The rats were given food and water *ad libitum* except during the testing periods.

Plant collection

C. salicifolius were provided by the Institute of Chinese Medicine, Lishui of Zhejiang. Jade grape fruits were provided by Lishui, Zhejiang Academy of experimental base. Gannan navel oranges were bought from the market.

Extraction of essential oil

Leaves of plant were triturated in a common blender and placed in boiling water (ratio 1 L water/100 g leaves).The essential oil was obtained by means of steam distillation. The oil was stored in the dark in a tightly closed bottle until used in the assays with animals.

Drugs

Lavender essential oil was purchased from Shanghai Apple Spice Co., Ltd.

Enzyme linked immunosorbent assay kit

The glucose (GLU), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) enzyme linked immunosorbent assay kits were purchased from Shanghai Langdun Biotechnology company.

Habituation and training

Rats were habituated to the laboratory environment for 3 days and being gently handled each day. After the habituation period, the rats were trained to drink water at 10.00 to 10.20 h and 20.00 to 20.20 h by allowing them to access water bottles only during these time periods for 1 week. The fluid intakes of the rats were measured during each time period (20 min).

Experimental procedure

From the day of stimulation (day 0), the rats were randomly assigned to one of seven groups (n = 10 for each group): control model group (M), physiological stress group (PS), blank emotional stress group (ES-M) and four essential oil sniffing groups-lavender, grapefruit, orange and *C. salicifolicus* groups (ES-L, ES-G, ES-O and ES-C). Rats in the M group were given water twice a day in the experimental procedure. Rats in the five ES groups (ES-M, ES-L, ES-G, ES-O, ES-C) were irregularly given empty water bottles during one of the two watering periods for 2 weeks to induce emotional stress. Rats in the PS group were given neither empty water bottles nor water during the same watering periods as for ES groups. This group was used to control the possible effect that an animal, which is trained to receive water at a particular time of day, is also emotionally stressed if it does not receive water at this time (Table 1). Rats in the ES-M groups sniffed the same dose water when the ES-L, ES-G, ES-O and ES-C groups sniffed essential oils. ES-L, ES-G, ES-O were positive controls; *C. salicifolicus* group (ES-C) was experimental group. According to the volume of the cage, a dose of 100 μl essential oils/water was added for every cage. The rats of experimental groups sniffed odor at 14:00 to

14:30. The rats of ES-L, ES-G, ES-O and ES-C group sniffed lavender oil, grapefruit oil, orange oil and *C. salicifolicus* oil, respectively at the same time period. Rats in ES-M group sniffed water as another control model. The process of establishing uncertainty empty bottle stimulation model is performed as Figure 1.

Behavior observation

The behaviors of all the rats in the seven groups during the experimental session of 20 min were observed. Fluid intakes of the rats were also evaluated during 20 min access to water. The behaviors observed included attacking (biting empty water bottles and cage shed), exploring (rearing and looking outside from the cage) and grooming (self-grooming, washing and scratching). According to the intensity of activity, the evaluation of each behavior item was recorded as a score of 0, 1, 2, 3 or 4, respectively, and a mean of all scores over the 14 days was used for statistical analysis.

Serology detection

After the completion of 14 experimental sessions, all rats were weighed and obtained a blood sample from the rats' tails. Blood was centrifuged to separate serum and plasma. Serum levels of TC, GLU, HDL-C and LDL-C were determined by enzyme-linked immunosorbent assay (ELISA).

Statistical analyses

All data were expressed as mean ± standard deviation (SD). For statistical evaluation of these results, a one-way analysis of variance (ANOVA) was used. If a significant main effect of a group was identified ($p < 0.05$), posthoc comparisons between groups were performed using the least significant difference (LSD) test. $p < 0.05$ was considered statistically significant.

RESULTS

The changes in fluid intake and body weight of the rats

During training procedure, the fluid intakes of the rats for each day were not significantly different among the three groups ($p > 0.05$). During experimental procedure, the fluid intakes of the rats in ES and PS groups reduced significantly for each day comparing to that of the rats in M group. However, there was no significant difference between the ES groups and PS group ($p > 0.05$). At the time of arrival and after training, there was no significant difference in body weight among the three groups. After experimental procedure, the body weights of the rats in ES and PS groups reduced significantly comparing to that in the M group (Figure 2).

The effect of emotional stress on the behavior of rats

From day 1 to day 14, attacking behaviors were exhibited strongly in the rats of ES-P group. However, this phenomenon did not appear among the rats of M and PS groups. This result indicated that the rats in the ES-P group were experiencing more stress than the other two groups (Table 2). Compared to the ES-P group, attacking behaviors from the other two groups were reduced significantly ($p < 0.05$) by sniffing either lavender oil or *C. salicifolicus* oil. The ES-O group reduced extremely significantly ($p < 0.01$). However, there was no difference in exploring and grooming behaviors between the rats in the ES groups and PS group.

The effect of emotional stress on the serology markers of rats

The TC and GLU levels increased significantly in the rats of the ES-M group compared to that in rats of the PS group ($p < 0.05$) (Table 3). However, compared to the ES-M group, only the group sniffing *C. salicifolicus* oil decreased significantly in TC and GLU ($p < 0.01$). There was no significant difference in the HDL-C and LDL-C levels among all the groups

DISCUSSION

The administration route of plant extracts is an important part of animal research. Most of the studies employ intraperitoneal administration route. However, the major method of aromatherapy in reducing levels of anxiety is by inhaling the essential oil. Considering that the inhalation-drug-deliver method allows a closer approach to the aromatherapy in use of essential oil, it is employed in this study to evaluate anxiety effects of essential oil.

The effect of emotional stress on the behavior of rats

The anxiolytic effects reflected by attacking behaviors were clearly shown in Table 2. In order to examine whether emotional stress influences the anxiety, a reliable animal model of emotional stress should be established in prior. The stimulation of uncertainty empty bottle model has a good physiological effect. It is commonly used in the studies of the mechanism of stress on ethological changes (Shao et al., 2003). According to the behavior observations, rats in the ES-M group that were irregularly given empty water bottles expressed significant attacking behaviors, such as biting bottles and cage shed, whereas rats in the M and PS groups did not. These behavioral results indicated that rats in the ES group were experiencing significant emotional stress during the stress procedure and rats in the PS group were less stressed comparatively. Combining the results of exploring and grooming behaviors as well as the results of the fluid intake and change of body weight, it was proven that the desperate dehydration had influences on both behaviors. The results indicated that bottle attacking behaviors could be a creditable standard to examine

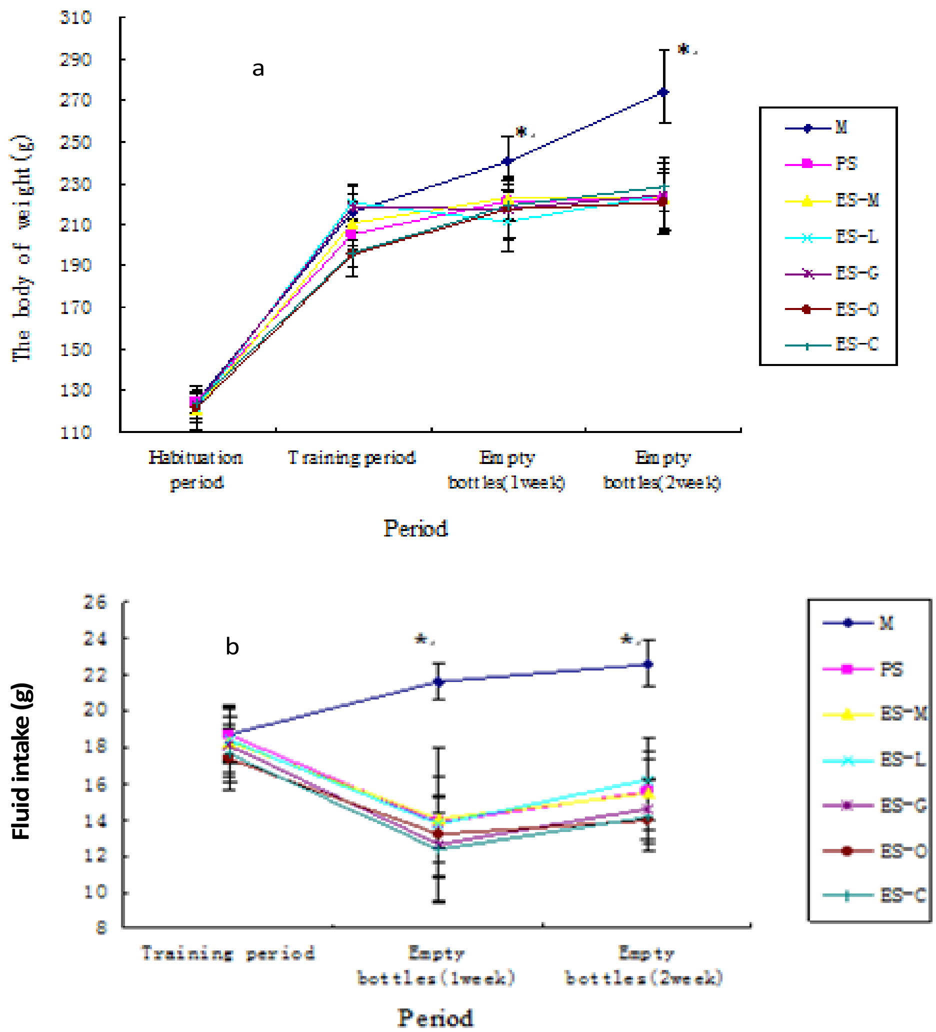

Figure 2. The body weight of the rats in every period (A) .The fluid intake of the rats for each day (B).
*p<0.05 compared with ES-M. M is the control model group, PS is the physiological stress group, ES-M is blank emotional stress group and ES-L, ES-G, ES-O and ES-C represent the four essential oil (lavender, grapefruit, orange and *C. salicifolicus*) sniffing groups, respectively.

anxiety.

The changes in fluid intake and body weight of the rats

According to the results, no difference among groups was detected when all rats received similar amount of fluid intake in two water access sessions in the training period. During the experimental period, rats in the ES and PS groups only randomly received water in one of the two watering periods everyday. Therefore, fluid intake in ES and PS groups were reduced significantly compared to the M group. However, there was no difference between all ES groups and PS group. This result indicated that rats in all the ES groups had the same dehydration as the PS group. Fluid intake is associated with food intake, which can directly influence the body weight (Reed et al., 2004). During habituation and training periods, there was no difference in the changes of body weight among all the groups. However, in the experimental period, the body weight of rats in the ES and PS groups increased slower than the rats in the M group. These results demonstrated that the change in body weight of rats in the ES groups was similar to that in the PS group. The PS group had no difference when compared with ES-M group. Considering the results of the fluid intake, the desperate dehydration had limited the addition of body weight. There were no difference in all the inhaled odor experimental groups on both the fluid intake and body weight. It demonstrated that inhaling these essential oils had no influence to the fluid intake and change of body weight.

Table 1. Stress procedure.

Time	1	2	3	4	5	6	7
10:00-10:20	ES	ES	N	ES	ES	ES	N
20:10-20:20	N	N	ES	N	N	N	ES
Time	**8**	**9**	**10**	**11**	**12**	**13**	**14**
10:00-10:20	N	ES	N	N	N	ES	ES
20:10-20:20	ES	N	ES	ES	ES	N	N

ES, give empty water bottle; N, drink water at trained time.

Table 2. The effect of emotional stress on the behaviour of rats.

Parameter	Attacking behavior	Exploring behavior	Grooming behavior
M	0.08±0.214*	0.253±0.25*	0.287±0.487
PS	0.047±0.074*	1.52±0.493	0.273±0.153
ES-M	0.827±0.339	1.64±0.329	0.213±0.151
ES-L	0.44±0.272*	1.487±0.497	0.28±0.286
ES-G	0.613±0.416	1.38±0.598	0.32±0.254
ES-O	0.375±0.294**	1.727±0.663	0.341±0.366
ES-C	0.518±0.373*	1.437±0.566	0.279±0.233

*p < 0.05, compare with ES-M; p < 0.01, compared with ES-M.

The effect of emotional stress on the serology markers of rats

In terms of the results, inhaling lavender oil, orange oil and *C. salicifolicus* oil could reduce attacking behaviors significantly compared to the ES-P group, especially the orange oil. The results of serology detection also demonstrated that only the inhaling the *C. salicifolicus* essential oil could decrease the level of TC and GLU because of the anxiety caused by a stimulus bottles. There were many studies which proved that diabetes and depression negatively interact, in that depression leads to poor metabolic control and hyperglycemia exacerbates depression. The researchers found that depression by reducing adherence to treatment is partly related to a poor glycemic control (Talbot et al., 1999). It have been reported that some medicine have both effects of antianxiety and hypoglycemic like *Clonorchis sinensis*. *C. sinensis* has an antidepressant-like activity and they can attenuate the diabetes-induced increasing in blood glucose concentration (Guo et al., 2010). In this article, it is suggested that the *C. salicifolicus* may be a potential strategy for contemporary treatment of anxiety and hyperglycemia.

Anxiety may affect lipid metabolism and induce coronary atherosclerosis. Anxiety not only can influence the level of blood glucose, but also the TC, LDL-C, HDL-C and TG. Cholesterol levels and cardiovascular responses to emotionally arousing stimuli were examined in 60 healthy African males and females (Clark et al., 1998). The results indicated that the total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and triglycerides (TG) levels of plasma rose after acute stress. The study of McCann et al. (1999) found that short-term high-intensity workload stress make plasma apolipoprotein (apoB) and TG increase. In this ariticle, the results indicated that only the *C. salicifolicus* essential oil had effect on decreasing the level of triglycerides (TG) caused by anxiety. However, inhaling these kinds of essential oils had no influence on the level of HDL-C and LDL-C in stressed rats. The results indicated that the role of these essential oils was in different metabolic pathways.

All the findings represent the first evaluation of the traditional medicinal potential of *C. salicifolicus*, which is widely distributed in Zhejiang province and have a very special odor. Based on these results, the *C. salicifolicus* essential oils not only had an importance, per se, in treating antianxiety, but also considering its possible use as precursors in the development of new drugs as a contemporary treatment of anxiety, hyperglycemia and hypercholesteremia. The study proved that the administration of *C. salicifolicus* essential oil to Wistar rats reduces anxiety and verifies the anxiolytic effects of lavender and orange essential oils. The results provide a direction to extraction and separation of active ingredients. It is worth to be reminded that because of the reduction effect on glucose and total cholesterol, further research of *C. salicifolicus* ought to be launch on this aspect.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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