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Cananga odorata essential oil reverses the anxiety induced by 1-(3-chlorophenyl) piperazine through regulating the MAPK pathway and serotonin system in mice

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ABSTRACT

Ethnopharmacological relevance: *Cananga odorata* essential oil, known as ylang-ylang essential oil (YYO), was commonly used in the aromatherapy for relaxation and mood adjusting use. In our previous study, YYO played anxiolytic effects on the mice in several behavioral tests that based on the instinctive responses to novel environments.

Aim of the study: To investigate the effects and mechanisms of YYO reversing the anxiety induced by 5-HT2C receptor agonist 1-(3-chlorophenyl) piperazine (m-CPP).

Materials and methods: m-CPP was administrated to the male ICR mice to develop an anxiety model. The anxiolytic effect of YYO (0.1%, 1% and 10%, v/v) was evaluated in the elevated plus maze (EPM) test after odor exposure. Western blot was used to detect the phosphorylation levels of extracellular signal-regulated kinase 1/2 (ERK1/2) and cAMP response element-binding protein (CREB) and the expression of c-Fos in the prefrontal cortex (PFC) and hippocampus after the EPM test. Serotonin and its metabolite change in the brain were detected by liquid chromatogram with an electrochemical detector. The effect of YYO on the plasma corticosterone level was evaluated using enzyme-linked immunosorbent assay (ELISA) after the odor exposure.

Results: The behavior analysis showed that m-CPP (2 mg/kg and 4 mg/kg) could induce anxiety behaviors in the mice while diazepam (2 mg/kg) reversed the anxiety behavior induced by m-CPP. YYO dose-dependently increased the time and number of entries in the open arms ($p < 0.05$) compared to the Tween 80 group. YYO reduced the phosphorylation levels of ERK1/2 ($p < 0.05$) in both PFC and hippocampus. Down-regulations of phosphor-CREB ($p < 0.05$) and c-Fos ($p < 0.05$) were only observed in the hippocampus. YYO also affected the brain serotonin metabolism and reduced the blood plasma corticosterone level of the m-CPP treated mice.

Conclusion: YYO odor exposure could reverse the anxiety behaviors generated by m-CPP. The anxiolytic effect of YYO was associated with the ERK1/2/CREB pathway in the hippocampus and relevant to the serotonin system.

1. Introduction

Anxiety is defined as a physiological and behavioral response to avoid harm and elevate the chances of survival (Gelfuso et al., 2014). Deregulations of this normal response result in anxiety disorder. Anxiety disorders are chronic and give rise to enormous costs on individuals and society (Kessler et al., 2009). Complementary therapies combined with conventional medical treatment could offer an effective and holistic approach for anxiety treatment (Jaruzel and Kelechi, 2016). Application of complementary therapy such as aromatherapy for anxiety relief is popular in many countries (Gnatta et al., 2016). Many essential oils from natural plants like *Lavandula angustifolia*, *Salvia lavandulaefolia* and *Citrus bergamia* were used for this purpose.

Cananga odorata (Lam.) Hook. f. & Thomson, known as "ylang-ylang", is widely distributed in tropical and subtropical regions. Ylang-ylang essential oil (YYO) is extracted from flowers of *Cananga odorata*. It has a long history of fragrance use and has been approved for food use by the US Food and Drug Administration (Burdock and Carabin,

Abbreviations: YYO, ylang-ylang essential oil; m-CPP, 1-(3-chlorophenyl) piperazine; ICR, institute of cancer research; GC/MS, gas chromatograph-mass spectrometer; MAPK, mitogen-activated protein kinase; ERK1/2, extracellular signal-regulated kinase 1/2; CREB, cAMP response element-binding protein; DA, dopamine; 5-HT, 5-hydroxytryptamine; GABA, gamma-aminobutyric acid; SSRIs, selective serotonin reuptake inhibitors; DZP, diazepam; EPM, elevated plus maze; PFC, prefrontal cortex; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; PVDF, polyvinylidene fluoride; i.g., intragastric; i.p., intraperitoneal

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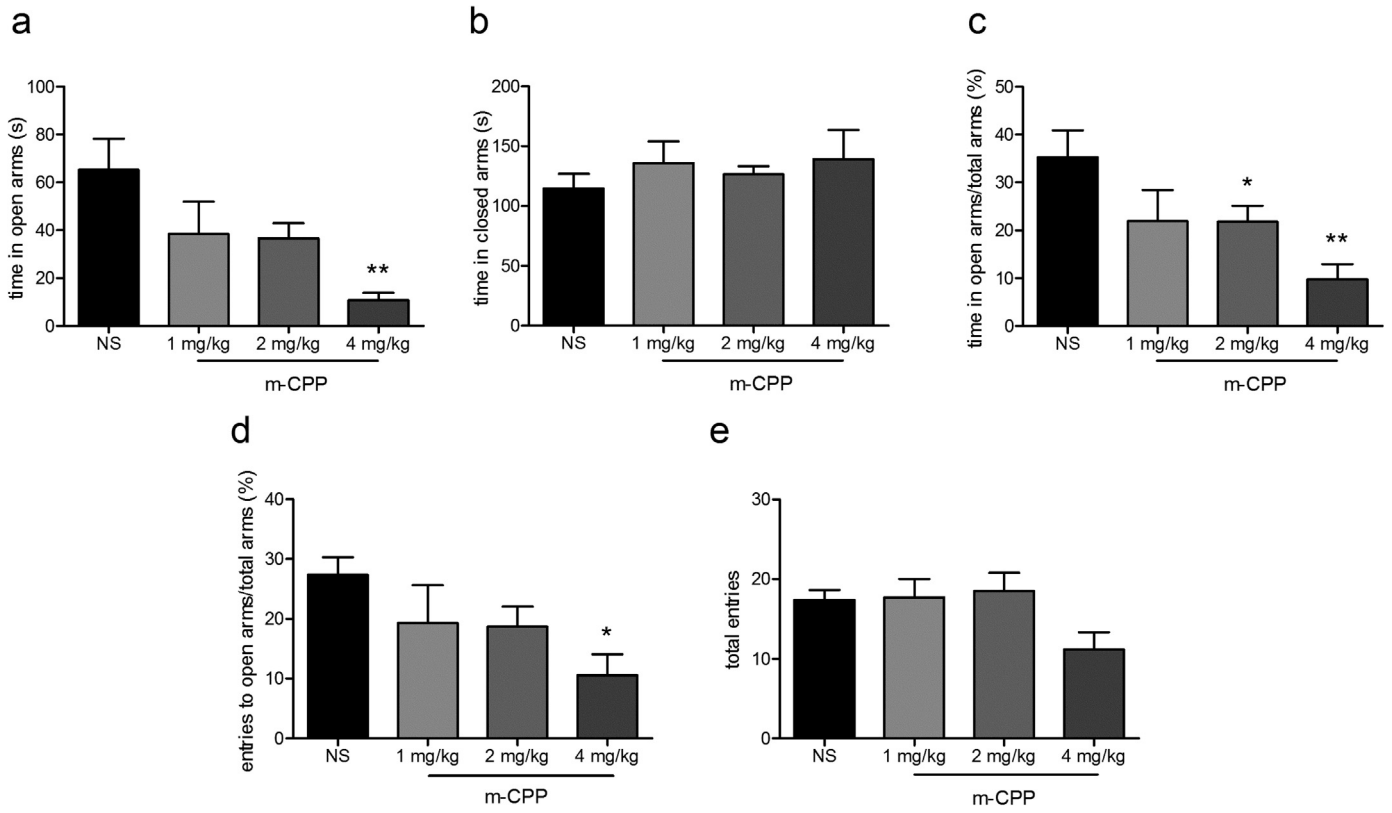

Fig. 1. m-CPP induced anxiety behaviors in the EPM test. a-c, Effects of m-CPP (1–4 mg/kg, i.p.) on the time in open arms (a), time in closed arms (b) and the percentage of time into the open arms (c) in the EPM test (n = 10–12); **d-e,** Effects of m-CPP (1–4 mg/kg, i.p.) on the percentage of entries into the open arms (d) and the total numbers of entries into all the arms (e) in the EPM test (n = 10–12). Values represent the mean ± SEM. $^{*}p < 0.05$ vs. NS group, $^{**}p < 0.01$ vs. NS group. One-way ANOVA followed by a post hoc Duncan test was used.

2008). YYO was commonly used in the aromatherapy through either massage or inhalation ways for relaxation and mood adjusting (Ali et al., 2015; Walsh et al., 2011). It could reduce blood pressure (Hongratanaworakit and Buchbauer, 2004) and improve cognition and mood (Moss et al., 2008) on healthy participants through inhalation way. In our previous study, YYO showed anxiolytic effect on the mice in several behavioral tests that based on the instinctive responses to novel environments (Zhang et al., 2016). However, in clinical practice, anxiolytic agents are usually used for people with pathological anxiety symptoms. Certain compounds could be used to generate anxiety in mice before drug administration for a further study of the effect of YYO on pre-existing anxiety and study the mechanism.

1-(3-Chlorophenyl) piperazine (m-CPP) is the metabolite of antidepressant drugs trazodone (Eriksson et al., 1999) and nefazodone (Barbhaiya et al., 1996). It could induce anxiety in both healthy subjects and panic disorder patients (Charney et al., 1987). The anxiogenic effect of m-CPP was also evaluated in animal models such as social interaction (Bagdy et al., 2001), elevated plus maze (Gibson et al., 1994) and light-dark box (Bilkei-Gorzó et al., 1998).

Mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase 1/2 (ERK1/2) are sensitive to a wide range of behavioral experiences (Alonso et al., 2002). MAPK/ERK1/2 is activated by a large variety of stressful stimuli in neurons of the prefrontal cortex (PFC) and hippocampus (Fu et al., 2009; Gerrits et al., 2006; Tronson et al., 2008). Activation of ERK1/2 activates the cAMP response element-binding protein (CREB), which further increases multiple gene expression such as the immediate early gene *c-fos*. High levels of c-Fos expression in the dorsomedial section of PFC, the dentate gyrus of hippocampus and the median raphe nucleus were observed in the rats with anxiety behaviors (Lehner et al., 2009). m-CPP is the agonist of 5-HT2C receptor, which is a G-protein-coupled receptor that activates the phospholipase C signaling pathway and mediates the ERK1/2 phosphorylation (Santana and Artigas, 2016; Werry et al., 2005). Moreover, blocking of 5-HT2C receptor augments the SSRIs-evoked 5-HT output in the hippocampus and PFC (Gatch, 2003).

In the present study, we explored the effective anxiogenic dose of m-CPP on the ICR mice in the EPM test and investigated whether YYO exposure could reverse the anxiety induced by m-CPP. We further analyzed the phosphorylation levels of ERK1/2 and CREB and the expression of c-Fos in the PFC and hippocampus to investigate whether the MAPK pathway play a role in the anxiolytic effect of YYO. The effect of YYO on the brain serotonin system and the plasma corticosterone level were also evaluated.

2. Materials and methods

2.1. Animals

Male ICR (Institute of Cancer Research) mice were obtained from the Shanghai Experimental Animal Center of Chinese Academy of Sciences (Shanghai, China). Mice were housed under a 12 h/12 h light/dark cycle and controlled temperature (21 ± 2 °C) with free access to food and water. Mice weighing 25–30 g were used in all experiments. All the experimental procedures were performed following the rules of Association for Assessment and Accreditation of Laboratory Animal Care International.

2.2. Chemical and treatment

YYO, which was extracted from flowers of plant *Cananga odorata* (Lam.) Hook. f. & Thomson, was obtained from LiYing Trading Company Limited (Guangzhou, China). The quantitative and qualitative analyses of the essential oil were carried out using GC/MS (PE Clarus 600) in the previous study (Zhang et al., 2016). Three concentrations of