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Original Article

Kaempferia parviflora rhizome extract and *Myristica fragrans* volatile oil increase the levels of monoamine neurotransmitters and impact the proteomic profiles in the rat hippocampus: Mechanistic insights into their neuroprotective effects

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ABSTRACT

Potentially useful in the treatment of neurodegenerative disorders, *Kaempferia parviflora* and *Myristica fragrans* have been shown to possess a wide spectrum of neuropharmacological activities and neuroprotective effects *in vivo* and *in vitro*. In this study, we determined whether and how *K. parviflora* ethanolic extract and *M. fragrans* volatile oil could influence the levels of neurotransmitters and the whole proteomic profile in the hippocampus of Sprague Dawley (SD) rats. The effects of *K. parviflora* and *M. fragrans* on protein changes were analyzed by two-dimensional gel electrophoresis (2D-gel), and proteins were identified by liquid chromatography tandem mass spectrometry (LC-MS/MS). The target proteins were then confirmed by Western blot. The levels of neurotransmitters were evaluated by reversed-phase high-performance liquid chromatography (RP-HPLC). The results showed that *K. parviflora*, *M. fragrans* and fluoxetine (the control drug for this study) increased serotonin, norepinephrine and dopamine in the rat hippocampus compared to that of the vehicle-treated group. Our proteomic data showed that 37 proteins in the *K. parviflora* group were up-regulated, while 14 were down-regulated, and 27 proteins in the *M. fragrans* group were up-regulated, while 16 were down-regulated. In the fluoxetine treatment group, we found 29 proteins up-regulated, whereas 14 proteins were down-regulated. In line with the proteomic data, the levels of GFAP, PDIA3, DPYSL2 and p-DPYSL2 were modified in the SD rat groups treated with *K. parviflora*, *M. fragrans* and fluoxetine as confirmed by Western blot. *K. parviflora* and *M. fragrans* mediated not only the levels of monoamine neurotransmitters but also the proteomic profiles in the rat hippocampus, thus shedding light on the mechanisms targeting neurodegenerative diseases.

1. Introduction

The World Health Organization (WHO) predicted that by 2040, neurodegenerative diseases (NDs) will become the second leading cause of death in the world.[1–4] NDs, such as Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington disease (HD), belong to a heterogeneous group of disorders that are characterized by progressive degeneration of the structure and function of the central nervous system or peripheral nervous system. Common NDs include psychiatric disorders such as depression and bipolar

Abbreviations: GFAP, glial fibrillary acidic protein; PDIA3, protein disulfide-isomerase A3; DPYSL2, dihydropyrimidinase-related protein 2; p-DPYSL2, dihydropyrimidinase-related protein 2; NE, norepinephrine; DA, dopamine; 5-HT, serotonin.

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disorder. In addition, depression is the most prevalent symptom in PD and HD, occurring in approximately 40–60% of patients.[5] The hippocampus is considered to be one of the most important brain regions for mood regulation. The monoamine neurotransmitters, including dopamine (DA), norepinephrine (NE), epinephrine (E), and serotonin (5-hydroxytryptamine, 5-HT) are produced from neurons both in the brain and peripheral nervous system.[6,7] The functions of monoamine neurotransmitters are considered to play crucial roles in arousal, emotion, and cognition. Drugs that augment the effects of monoamines on their target tissue are used to treat psychiatric disorders, including depression, anxiety, and schizophrenia.[8,9] For these reasons, the measurement of monoamine neurotransmitters in the rat hippocampus is important for understanding the effects of herbal treatments on the secretion of neurotransmitters. Recently, proteomics is an important tool for identifying the expression of proteins that are important for a comprehensive understanding of the pharmacological role of a drug.[10–12] Many medicinal plants have been identified and used for neuroprotective and neurotrophic agents that promote neuronal survival, differentiation, neuritogenesis and synaptic plasticity, both in *in vitro* and *in vivo* models.[13–15] *Kaempferia parviflora* Wall Ex. Baker, or black ginger with the local name of "Kra-chai-dam", is a plant from the family Zingiberaceae used for health promotion in traditional Thai medicine. The phytochemical studies revealed that the rhizomes of *K. parviflora* contained volatile oil,[16] chalcones,[17] phenolic glycosides[18] and many flavonoids such as 5-hydroxy-7-methoxyflavone, 5,7-dimethoxyflavone and 3,5,7-trimethoxyflavone.[19,20] 5,7-Dimethoxyflavone is a major active constituent of *K. parviflora*[20] that showed high potential inhibitory activity toward acetylcholinesterase (AChE) and butyrylcholinesterase (BChE).[21,22] Moreover, polymethoxyflavones from black ginger (*K. parviflora*) were discovered to be potential inhibitors of β-secretase (BACE1).[23] The rhizomes of this plant have been traditionally used for leucorrhea, oral diseases, abdominal pain, and health promotion as well as an aphrodisiac.[24] In addition, the ethanolic extract of *K. parviflora* has been shown to induce relaxation of both the aortic rings and the ileum precontracted with phenylephrine and acetylcholine.[25] It has been reported that the alcoholic extract of *K. parviflora* rhizomes contained numerous flavonoids[20] that were previously reported to possess antioxidant activity and neuroprotective and cognitive enhancing effects.[26] A recent finding showed that the alcoholic extract of *K. parviflora* rhizome could mitigate depression-like behavior in aged rats[27] and displays antidepressant activity in aged rats. We hypothesized that alcoholic extract of *K. parviflora* rhizome might also possess other neuropharmacological activities. *Myristica fragrans* Houtt. (nutmeg, a tropical evergreen dioecious tree with a narrow range of distribution) is a source of high-value medicinal spices, nutmeg (endosperm) and mace (the reddish aril) with immense phytochemical diversity.[28] It contains volatile oils that include myristicin, elemicin, eugenol, isoeugenol, geraniol, pinese, cineole, borneol, and safrole. Its reported antibacterial, antiviral, antidiabetic, and antileukemic effects as well as its other biological activities[28–30] indicate its enormous therapeutic potential. Medicinally, eugenol and isoeugenol from nutmeg are known for their anti-inflammatory and antithrombotic,[31] as well as anti-rheumatic, carminative and stimulant properties.[32] The psychoactive effects of nutmeg have been reported to cause hallucinations, feelings of unreality, euphoria, and delusions. Both myristicin and elemicin have been shown to be metabolized into amphetamine-related compounds that have effects on the serotonergic systems and possibly an antidepressant effect,[33] but this has not yet been verified. These compounds were shown to have an antidepressant effect in the forced swimming test in male rats.[34] The main bioactive compounds of *M. fragrans* seed essential oil were identified as including camphene, elemicin, eugenol, isoelemicin, isoeugenol and methoxyeugenol.[29] Sabinene, α-pinene, β-pinene, terpine-4-ol, limonene, safrole and myristicin were also reported to be included in the essential oil of nutmeg.[35,36] In this study, we aim to investigate the effects of *K. parviflora* and *M. fragrans* on the levels of monoamine neurotransmitters (norepinephrine, serotonin, and dopamine), as well as on the proteomic profiles in the rat hippocampus. Discoveries from this study could help us to better understand the molecular mechanisms of these compounds.

2. Material and methods

2.1. Plant material and extraction

K. parviflora was collected from the Loei province in northeast Thailand, and *M. fragrans* was from the Nakhonpathom province in central Thailand. Plants were identified by an expert Ass. Prof. Dr. Nijsiri Ruangrungsi. *K. parviflora* rhizomes were shade dried and agitated by using a blender, and the powder was extracted with 95% ethanol by maceration for 4 days (1 kg sample: 3 L) with occasional stirring. The extract was dissolved in 2% Tween-80 in water to a final concentration of 200 mg/ml.[17] Crude extracts of *K. parviflora* were standardized by reversed-phase HPLC (Agilent 1260 Infinity, Austria). 5,7-Dimethoxyflavone was used for the standard. For *M. fragrans*, 1 kg of powder from dry seeds was extracted by stream distillation for 12 h. Then, the volatile oil was dissolved in 2% Tween-80 in water to a final concentration 300 mg/ml.[34] The *M. fragrans* nutmeg volatile oil was analyzed by GC-MS on gas chromatograph with a model 7890A GC and 5978C MSD mass selective detector (EIMS, electron energy, 70 eV) and an Agilent ChemStation data system. Fluoxetine (20 mg tablet) was dissolved in 2% Tween-80 in water.[17,34]

2.2. Experimental protocol for animals

Two-month-old healthy male Sprague Dawley rats (300–400 g) were obtained from and approved by the National Laboratory Animal Center Animal Care and Use Committee (NLAC-ACUC), Mahidol University, Salaya, Nakornpathom, Thailand. Rats were housed individually (one per cage) in standard conditions (NLAC-MU; SOP-VM.VCP-01.10.) at 22 ± 1 °C on a standard fluorescent 12:12 h light:dark cycle, with changing of bedding once a week. Rats were given access to a standard diet and water (hyperchorinate 10–12 ppm) ad libitum.

All rats were randomly divided into 4 groups, each group containing 6 rats.

Group 1: The control group or vehicle-treated group was treated with 2% Tween-80, 1 mL/kg BW/day via oral route for 12 days.

Group 2: The fluoxetine group was treated with 20 mg/kg BW via oral route for 12 days.

Group 3: The *K. parviflora* group was treated with ethanolic extract (200 mg/kg BW via oral route once per day for 12 days).

Group 4: The *M. fragrans* group was treated with seed oil (300 mg/kg BW via oral route once per day for 12 days).

2.3. Tissue preparation and protein extraction

Dissected brains were frozen in liquid nitrogen at −80 °C until extraction. Hippocampal tissue was powderized in liquid nitrogen, and proteins were extracted with buffer containing 40 mM Tris, 7 M Urea, 2 M Thiourea, 4% CHAPS, 65 mM DTT, 0.3 mg/ml EDTA, 35 μg/

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