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RESEARCH ARTICLE

# *Kaempferia parviflora* extract ameliorates the cognitive impairments and the reduction in cell proliferation induced by valproic acid treatment in rats

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ABSTRACT

*Kaempferia parviflora* is a herbal plant whose rhizomes are used in traditional medicine. Investigations of this plant have shown it to have antidepressant activity and to improve learning and memory in animal models. The aim of the present investigation was to determine whether *K. parviflora* could protect the brain from the impairments in cognition and hippocampal neurogenesis which are caused by valproic acid (VPA).

Male Sprague Dawley rats (180–200 g) were given once daily *K. parviflora* extract (100 mg/kg) *via* oral gavage for 21 days. Rats received twice daily intraperitoneal injections of valproic acid (300 mg/kg) from days 8 to 21 of the experiment. Spatial memory was tested using the novel object location (NOL) test five days after the end of treatment. Cell proliferation in the sub granular zone (SGZ) of the dentate gyrus was quantified by immunohistochemistry and levels of doublecortin (DCX) were determined by Western blotting.

Co-treatment of VPA and *K. parviflora* prevented the cognitive decline and reduction in proliferating cells caused by VPA. Furthermore, co-treatment significantly increased DCX protein levels within the hippocampus.

These findings demonstrate that *K. parviflora* is able to prevent the brain from VPA-induced the impairments of spatial memory and proliferating cells within the SGZ.



## 1. Introduction

Valproic acid (VPA) is clinically used as a long-term anti-epileptic drug to prevent seizures in both adults and children (Buckley, 2008). It is thought that VPA acts by blocking sodium and calcium channels to reduce neuronal activity. Consequently, GABA and aspartate levels within the brain are elevated and reduced, respectively (Kwan et al., 2001). VPA also functions as a histone deacetylase inhibitor by affecting chromatin modification, which results in a decrease in cell proliferation and an increase in neuronal differentiation in the cells involved in neurogenesis (Hsieh et al., 2004; Kostrouchova et al., 2007). VPA treatment reduces cell proliferation within the sub granular zone (SGZ) of the dentate gyrus (DG) and so inhibits hippocampal neurogenesis (Umka et al., 2010). Several studies have reported that patients treated with VPA develop mild to moderate cognitive deficits (Hommet et al., 2007; Mula and Trimble, 2009). In an animal model, VPA has been shown to reduce performance in a spatial memory test and this behavioral change was associated with a reduction in cell proliferation required for hippocampal neurogenesis (Umka et al., 2010). Although, VPA has been generally considered to have a good safety profile, the possibility of drug-induced cognitive dysfunction must be considered in all patients treated with this drug.

Adult neurogenesis, the process by which neural stem cells continuously generate new neurons throughout life, has been detected in all mammals, including the brains of rodents and humans

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(Ehninger and Kempermann, 2008; Eriksson et al., 1998). The SGZ of the hippocampal dentate gyrus is one of two main regions where adult neurogenesis takes place. In this location, neural stem and progenitor cells proliferate to generate new dentate granule neurons which are incorporated into the circuitry of the hippocampus (Abrous et al., 2005; Ehninger and Kempermann, 2008). In rodents, approximately 9000 new cells are produced each day and this process is required for hippocampal dependent learning (Cameron and McKay, 2001). As neural progenitors differentiate into mature neurons, they transiently express the microtubule associated protein doublecortin (DCX), levels of which provide an additional measure of the rate neurogenesis (Couillard-Despres et al., 2005). Brain-derived neurotropic factor (BDNF), a polypeptide growth factor, is produced from granule cells in the hippocampal dentate gyrus (Conner et al., 1997; Wetmore et al., 1990) and functions to up-regulate neuronal cell proliferation and survival (Linnarsson et al., 2000). Expression of BDNF Levels is associated with learning and adult neurogenesis (Nibuya et al., 1995; Shirayama et al., 2002). Antidepressants increase the level of BDNF expression that leads to induction of adult hippocampal neurogenesis (Duman et al., 2001). Adult hippocampal neurogenesis is thought to provide additional plasticity within the hippocampus, which is required in the process of memory consolidation (Ramirez-Amaya et al., 2006). Neurogenesis in the hippocampus can be influenced by many factors, including drugs and environmental stimuli (Kempermaan, 2006). It has recently been shown that antidepressants play a significant role as a modulator of neurogenesis in the adult hippocampus (Duman et al., 2001). Chronic (>2 weeks) antidepressant administration promotes adult hippocampal neurogenesis, a response which may be essential for the mood altering and cognitive effects of these drugs (Santarelli et al., 2003). For example, antidepressants counteract both the decrease in hippocampal neurogenesis and the behavioral changes caused by stress (Kodama et al., 2004) and chemotherapeutic drugs (Lyons et al., 2012).

*Kaempferia parviflora*, locally called Krachai Dam, is a plant originally found in the North and Northeast of Thailand and is classified in the family Zingiberaceae. Rhizomes of *K. parviflora* are utilized as a traditional medicine for a variety of illnesses, including peptic ulcers and inflammation. Extracts have also been shown to have anti-mutagenic, antibacterial, anti-fungal, anti-allergic and anti-viral properties (Mekjaruskul et al., 2012). *K. parviflora* at a dose of 100 mg/kg has been shown to have antidepressant-like effects (Wattanathorn et al., 2007). If *K. parviflora* increases cell proliferation, and this is correlated with improved spatial memory, this would suggest that it is acting in the same way as conventional antidepressants (Surget et al., 2008). It is, however, unknown whether *K. parviflora* increases hippocampal neurogenesis and cognition in the same way as other antidepressants. Therefore, the first objective of this study was to investigate the effect of *K. parviflora* on cell proliferation and neuronal differentiation in the SGZ of the hippocampal dentate gyrus and whether this was correlated with changes in performance in a hippocampus-dependent spatial memory test in rats. Following this, the plant extract was assessed for its ability to ameliorate the impact of VPA on cell proliferation in the SGZ and spatial memory.

## 2. Materials and methods

### 2.1. Plant material and extraction

*K. parviflora* rhizomes were obtained from Loei province, Thailand and authenticated. The voucher specimen was deposited at the Center for Research and Development of Herbal Health Products, Khon Kaen University, Thailand. The dried plant powder was extracted by maceration in 95% ethanol as previously reported (Mekjaruskul et al., 2012). As detected by HPLC method, the extract contained three major methoxyflavones that are 3,5,7,3′,4′-pentamethoxflavone, 5,7,4′-trimethoxyflavone and 5,7-dimethoxyflavone at concentration of 23.32, 31.06 and 21.10 mg/g, respectively.

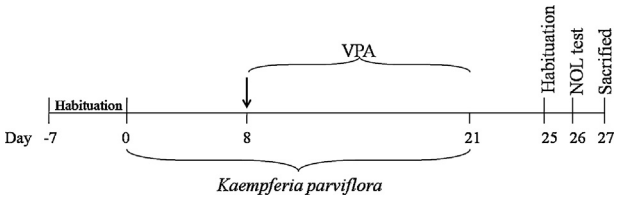


**Fig. 1.** Timeline of drug administration and behavioral testing. Brackets show the period that rats received VPA and *K. parviflora*. Animals were put down one day after the Novel object location (NOL) test.

### 2.2. Animals

Male Sprague Dawley rats (the National Laboratory Animal Center, Mahidol University, Salaya, Nakornpathom) weighing 180–200 g were housed in cages of 3 or 4 and maintained with 12 h light/dark cycle (7:00/19:00 h). Food and water were provided *ad libitum*. Principles of laboratory animal care in this animal study were in accordance with the Ethics of the Animal Experiment of National Research Council of Thailand and with approval from the Animal Ethics Committee of Khon Kaen University under permit number AEKKU 49/2556.

### 2.3. Drug treatment protocols

Rats were weighed daily from arrival and allowed to habituate for 1 week prior to drug administration. Forty rats were randomly allocated to vehicle, *K. parviflora*, fluoxetine, VPA and *K. parviflora* plus VPA groups. The vehicle group received 0.5% carboxymethylcellulose (Fluka, USA) by gavage for 21 days and received 0.9% sterile saline by intraperitoneal (i.p.) injection twice daily for 14 days from days 8 to 21. The *K. parviflora* groups received *K. parviflora* (100 mg/kg, dissolved in 0.5% carboxymethycellulose) by gavage for 21 days. The VPA groups were given two daily i.p. injections of VPA (300 mg/kg dissolved in 0.9% saline; Sigma–Aldrich, USA) for 14 days from days 8 to 21 (Fig. 1). This VPA treatment procedure significantly decreases seizure incidence in epileptic rats (Nissinen and Pitkanen, 2007).

### 2.4. Behavioral analysis (novel object location (NOL) spatial memory task)

Five days after drug or vehicle administration, rats were tested using the NOL test, adapted from Dix and Aggleton (1999). The experiments were conducted at an illumination of 350–400 lx between 09:00 and 16:00 h. The procedure consisted of habituating the animals for 30 min to the arena (36 cm × 50 cm × 30 cm) 24 h prior to testing and for a further 3 min, 5 min prior to familiarization trial. During the familiarization trial, two objects were randomly placed in separate locations in the arena and the rats were allowed to explore for 3 min. Then, the rats were returned to their home cages for a 15 min (inter-trial interval) during which the arena and the object were cleaned with 20% ethanol. In the choice trial, the rats were returned to the arena for 3 min where one object was returned to the same location while the other was moved to a new position (novel location). Exploration was recorded with a video camera mounted directly above each test arena. Exploration was defined as when the rat sniffed, licked, chewed or directed their nose at a distance less than 2 cm from the object (Dix and Aggleton,