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Anxiolytic-like effects of *Portulaca oleraceae* L. using the elevated plus-maze in mice

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SUMMARY

The purpose of this study was to characterize the putative anxiolytic-like effects of the 70% ethanol extract of *Portulaca oleracea* (EPO) using an elevated plus maze (EPM) in mice. The EPO was orally administered at 50, 100, 200 or 400 mg/kg to ICR mice, 1 h before the behavioral evaluation in the EPM, respectively. Control mice were treated with an equal volume of 10% tween 80, and positive control mice with diazepam (1 mg/kg). Single treatments of the EPO significantly increased the percentage of time spent and arm entries into the open arms of the EPM versus controls ($P < 0.05$). Moreover, there were no changes in the locomotor activity and myorelaxant effects in any group compared with the saline controls. In addition, the anxiolytic-like effects of the EPO were blocked by flumazenil (10 mg/kg, i.p), a $GABA_A$ antagonist not by WAY 100635 (0.3 mg/kg, i.p), a 5-HT_{1A} receptor antagonist. These results indicate that *P. oleracea* is an effective anxiolytic agent, and suggest that the anxiolytic-like effects of *P. oleracea* is mediated via the GABAergic nervous system.

Key words: Anxiety; *Portulaca oleracea*; Elevated plus-maze; WAY 100635; Flumazenil

INTRODUCTION

Anxiety is broadly defined as a state of unwarranted or inappropriate worry, often accompanied by restlessness, tension, distraction, irritability, and sleep disturbances. This disproportionate response to environmental stimuli can hyperactivate the hypothalamic–pituitary–adrenal axis and the autonomic nervous system, resulting in the somatic manifestations of anxiety, including shortness of breath, sweating, nausea, rapid heartbeat, and elevated blood pressure (Sandford *et al*., 2000). The Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV, 2000) has classified anxiety disorders into multiple distinct conditions, including generalized anxiety disorder, acute stress disorder, obsessive–compulsive disorder, panic disorder, posttraumatic stress disorder, social and other specific phobias alone or in combination with the above disorders, as well as substance-induced anxiety disorders. Generalized anxiety disorder is the most common of the anxiety disorders, with a lifetime prevalence of approximately 5%

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(Wittchen and Hoyer, 2001). Therefore, anxiety disorders represent not only a significant public health issue, but place a substantial economic burden on society.

Benzodiazepines remain the mainstay of drug treatment in anxiety disorders. However, their side-effects are prominent, such as sedation, myorelaxation, ataxia, amnesia, and pharmacological dependence (Lader and Morton, 1991). Recently, research has been conducted to investigate safer and more specific, and perhaps lower cost therapies. Natural anxiolytic agents feature in such research because herbs have been used to treat psychiatric disorders and generally have fewer harmful effects (Carlini, 2003). The potential clinical benefits of some herbal remedies commonly used in psychiatric practice have been addressed in earlier reviews (Fugh-Berman and Cott, 1999; Walter and Rey, 1999; Beaubrun and Gray, 2000; Lake, 2000; Linde *et al*., 2001a,b; Desai and Grossberg, 2003).

Portulaca oleracea L. (Portulacaceae) is widely used not only as an edible plant, but also as a traditional Chinese herbal medicine. It has been used for the treating dysentery with bloody stools, and externally for boils and sores, eczema, erysipelas, and snake and insect-bite. Phytochemical studies reavealed that *P. oleracea* contains flavonoids, coumarines, monoterpene glycoside, several nitrogenous compounds such as *N-trans*-feruloyltyramine, dopamine, dopa, and a high concentration of norepinephrine, in addition to several alkaloidal compounds (Sakai *et al*., 1996; Xiang *et al*., 2005). Recent research revealed a wide range of biological activities such as antifungal effect against dermatophytes of the genera Trichophyton, antinociceptive, antioxidant, and wound-healing activity. A bronchodilatory effect in asthmatic patients, skeletal muscle relaxant, and antifertility effect were also reported for *P. oleracea* (Elkhayat *et al*., 2008). Until now, however, there were no pharmacological studies upon the anxiolytic-like effects of *P. oleracea.*

The purpose of this study was to characterize the anxiolytic-like activity of the 70% ethanol extract of *P. oleracea* (EPO). Its anxiolytic effects were examined using the elevated plus-maze (EPM) in mice. And we examined the myorelaxant effects using a horizontal wire test in mice. In addition, this study also investigated which nervous systems are involved in the anxiolytic-like effects of the EPO through the co-administration of EPO and either flumazenil or WAY 100635.

MATERIALS AND METHODS

Materials

WAY 100635, flumazenil, were obtained from the Sigma Chemical Co. Diazepam was supplied by the local pharmaceutical company (Daewon pharm Co., LTD, Seoul). *P. oleracea* were obtained from a herbalist supplier in Seoul, Korea, and voucher specimens (DHUHMP2006-04) were maintained. The material was authenticated by one of author Prof. YH Kim. All other materials were of the highest grade and were obtained from standard commercial sources.

Animals

Male ICR mice, weighing 25 - 30 g, were purchased from the Orient Co., Ltd. of the Charles River branch (Seoul, Korea). The animals were housed 5 or 6 per cage, allowed access to water and food ad libitum, and maintained under a constant temperature (23 ± 1 °C) and humidity (60 ± 10%) under a 12-h light/dark cycle (light on 07.30 - 19.30 h). Animal treatment and maintenance were carried out in accordance with the Principle of Laboratory Animal Care (NIH publication No. 85-23, revised 1985) and the Animal Care and Use Guidelines of Daegu Haany University, Korea.

Sample preparation

The ethanol extract of the *P. oleracea* was prepared with 70% ethanol solution under a sonicator (5 × 51, 25 °C) for 1 h and then concentrated under a vacuum. Then the extract solution obtained was filtered, concentrated on a water bath under vacuo,

frozen and lyophilized (Eyela, model FDU-2000, Japan) to yield ethanol extracts (yield 37.4%), which were then stored at –20 until required. EPO and flumazenil were suspended with a 10% aqueous solution of Tween-80 for the orally and intraperitoneally administered, respectively. Diazepam and WAY 100635 were dissolved in saline.

Elevated plus-maze test

The EPM for mice consisted of two perpendicular open arms (30 × 5 cm) and two enclosed arms (30 × 5 cm) with 20 cm high walls, extending from the central platform (5 × 5 cm). The open and closed arms were connected by a central square, 5 × 5 cm, to give an apparatus of a plus sign appearance. The floor and walls of the maze were constructed from the dark opaque polyvinylplastic. The maze was raised to a height of 50 cm above the floor level in a dimly lit room (20 Lux) and a video camera was suspended above the maze to record the movements for analysis (Pellow *et al*., 1986; Lister, 1987). Each mouse was placed at the center of the platform, its head facing an open arm. The animals were tested individually and only once for 5 min. The maze was cleaned after each trial so as to remove any residue or odors. The following measurements were taken and analyzed using the video-based Ethovision System: the number of entries into the open or closed arms, the time spent in each arm, and the total distance moved in the EPM. All the experiments were carried out between 10:00 and 16:00 o'clock.

One hour after the EPO treatment (50, 100, 200 and 400 mg/kg, p.o.), the mice were placed in the EPM. The mice in the control group were given the vehicle solvent only, and the animals were tested individually once only for 5 min.

In a separate antagonism study, the mice were subjected to the co-administration of EPO (400 mg/kg, p.o.) and either WAY 100635 (0.3 mg/kg, i.p.) or flumazenil (10 mg/kg, i.p.) 1 h and 30 min prior to testing, respectively. The mice were treated with diazepam (1 mg/kg, i.p.) 1h before EPM test and used as the positive controls.

Horizontal wire test

A horizontal wire test was carried out by treating the mice with EPO (50, 100, 200 and 400 mg/kg, p.o.) according to a slight modification of the method reported by Bonetti *et al*. (1982). Briefly, the mice were lifted by the tail and allowed to grasp a horizontally strung wire (1 mm diameter, 15 cm long and placed 20 cm above the table) with their forepaws, after which they were then released. The number of mice from each treatment group that did not grasp the wire with their forepaws or actively grasped the wire with at least one hind paw within a 10 s period was recorded. A myorelaxant drug would impair the ability of the mice to grasp the wire, and muscle relaxation is commonly associated with sedation.

Spontaneous behavior in the open field test

Testing was carried out in clear black Plexiglas boxes (40 × 40 × 40 cm) equipped with the video-based Ethovision System (Noldus, Wageningen, The Netherlands). The mice were placed in the center of the apparatus to evaluate horizontal locomotor activity 1 h after being treated with EPO (50, 100, 200, and 400 mg/kg) and video-recorded for 5 min. Horizontal locomotor activity was expressed as total ambulatory distance and the frequency of rearing.

Statistics

Values are expressed as mean ± S.E.M. Data were analyzed by a one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls test for multiple comparisons. Statistical significance was set at $P < 0.05$.

RESULTS

Effect of EPO treatment in the elevated plus-maze

The mice in the vehicle-treated group typically avoided spending time on or entering into the

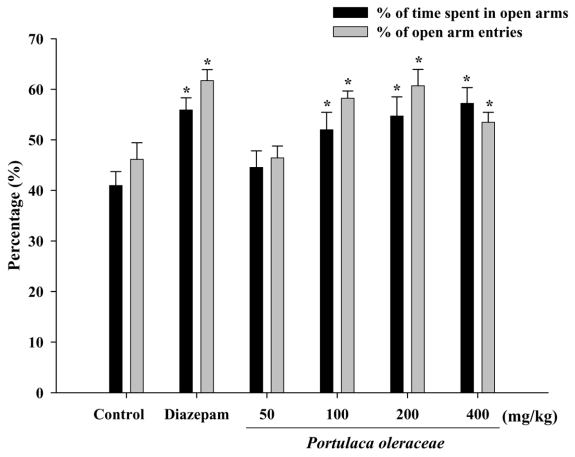

Fig. 1. Effect of a single treatment of the 70% ethanol extract of *Portulaca oleracea* on the percentage of the time spent in and the number of entries into the open arms of the elevated plus-maze over a 5 min test period in the mice. Each bar represents mean ± S.E.M. of 8-10 mice. Mice in the control group were given 10% tween 80 orally. *P* values for the group comparisons were obtained by one way ANOVA followed by Student-Newman-Keuls test ($^{*}P$<0.05 as compared with the control group).

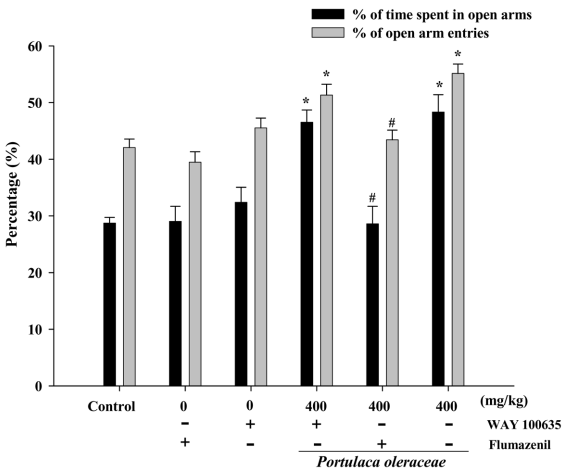

Fig. 2. Anxiolytic-like effects of 70% ethanol extract of *Portulaca oleracea* were blocked by Flumazenil but not WAY 100635. The data were expressed as the mean ± S.E.M. of the percentage of the time spent in and the number of entries into the open arms of the elevated plus-maze, 1 h after the oral administration of EPO (400 mg/kg), EPO (400 mg/kg) + WAY 100635 (0.3 mg/kg) or flumazenil (10 mg/kg) (30 min prior testing, i.p.), or 10% tween 80; n = 8 - 10 mice per group. *P* values for the group comparisons were obtained by one way ANOVA followed by Student Newman-Keuls test ($^{*}P$ < 0.05 versus the vehicle-treated control, $^{\#}P$ < 0.05 as compared with the EPO-treated group).

open arms. The percentage of time spent in the open arms was significantly increased in the EPO-treated mice (100, 200 and 400 mg/kg) compared with vehicle treated group (Fig. 1; $P < 0.05$). In addition, there was also significantly increased in the percentage of open arm entries in the EPO-treated mice (100, 200 and 400 mg/kg) compared with vehicle treated group (Fig. 1; $P < 0.05$). However, no significant change was observed in terms of percentage of time spent or the open arm entries at doses of 50 mg/kg of EPO. The diazepam-treated (1 mg/kg) group, as a positive control, the percentage of time spent and arm entries into the open arms were significantly increased compared with the vehicle-treated group ($P < 0.05$).

Effect of WAY 100635 and flumazenil on the anxiolytic-like activity of EPO

In order to determine if the anxiolytic-like effect of EPO is exerted via the serotonergic or GABAergic nervous system, EPO (400 mg/kg) treated mice were subjected to a co-treatment with either WAY 100635, a 5-HT_{1A} receptor antagonist, or flumazenil, a $GABA_A$ receptor antagonist. As shown in Fig. 2, the anxiolytic-like effects of EPO were abolished by flumazenil (10 mg/kg) but not by WAY 100635 (0.3 mg/kg). Also, there were no significant differences compared with control group in the WAY 100635-treated or flumazenil-treated group.

Horizontal wire test

At 5 mg/kg, diazepam significantly decreased the percentage of mice grasping the wire (Fig. 3). In contrast, EPO (50, 100, 200 and 400 mg/kg) did not compromise the mice grasping the wire compared with saline control group, indicating a lack of myorelaxation at these doses.

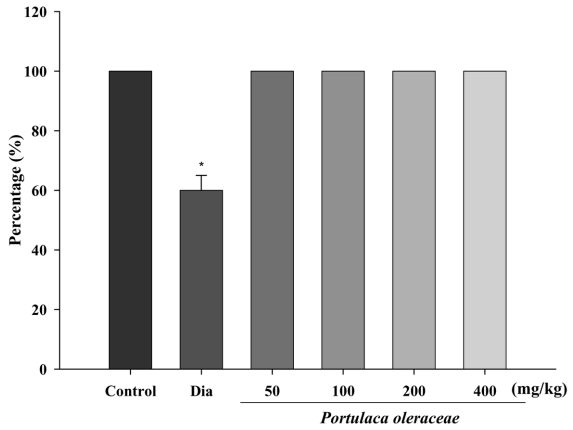

Fig. 3. Performance of mice in the horizontal wire test 1 h after oral administration of control, diazepam (Dia, 5 mg/kg), or EPO. Data represent percentage of mice grasping the wire after administration of EPO. Each bar represents the mean ± S.E.M. of 10 mice. *P* values for group comparisons were obtained by one way ANOVA followed by Student Newman-Keuls test ($^{*}P < 0.05$ versus the vehicle-treated control).

Table 1. Locomotor activity of control and *Portulaca oleracea* (EPO) in open field test

Drugs	Dose (mg/kg)	Locomotor activity (cm)
Control		2543.67 ± 138.20
EPO	50	2445.69 ± 153.69
EPO	100	2330.13 ± 134.53
EPO	200	2392.98 ± 116.25
EPO	400	2412.13 ± 94.91

Data are expressed as means ± S.E.M. (n = 10 per group)

Effect on the locomotor activity test

A locomotor activity test was performed to differentiate between the possible stimulatory effects of the tested drugs on the modulation of exploratory behavior. EPO (50, 100, 200, and 400 mg/kg) caused no significant changes in either the total ambulatory distances or rearing frequencies compared with the vehicle control group (Table 1).

DISCUSSION

An anxiolytic agent increases the frequency of entries into the open arms and increases the time spent in open arms of the EPM. The EPM is considered to be an etiologically valid animal model of anxiety because it uses natural stimuli, such as a fear of a new, brightly lit open space and a fear of balancing on a relatively narrow raised surface (Dawson and Tricklebank, 1995). In this study, the diazepam treatment prolonged the percentage of time spent in the open arms and the percentage of open arm entries (Fig. 1). The EPO treatment also prolonged the percentage of time spent in the open arms as well as the percentage of open arm entries without altering the spontaneous behavior at the chosen dose regimen. The total distances of movement on the EPM were also unchanged by the EPO treatment versus the vehicle controls (data not shown). In addition, the horizontal wire test, no significant myorelaxant effect was observed after administering the EPO (Fig. 3). These observations suggest that the anxiolytic-like effect of EPO is selective, and not the result of either a general stimulation of the locomotor activity or an exploratory behavior consequent to exposure to a novel environment.

The various parts of the *Portulaca oleracea* L. have been applied in the clinical treatment of various diseases in Oriental medicine. Radhakrishnan *et al*. (2001) have recently reported that *P. oleracea* possesses varied effects on the nervous system as it showed a reduction in locomotor activity, anti-convulsant activity, inhibition of electrically stimulated contractions of nerve-muscle preparation, muscle relaxant activity in conscious rats. In additional, several reports showed that flavonoids, coumarins, terpenoids, alkaloids, and fatty acids were isolated from *P. oleracea* (Awad, 1994 and Sakai *et al*., 1996; Liu *et al*., 2000). Xu *et al*. (2006) reported that five flavonoids (kaempferol, apigenin, myricetin, quercetin and luteolin) were isolated from *P. oleracea* by capillary electrophoresis with electrochemical detection. Flavonoids, as a class of naturally occurring compounds found in most vascular plants, have been demonstrated by a number of groups to be centrally active, possessing efficacies for a number of receptor systems in the CNS. Ligands upon

binding at the BDZ-S on the $GABA_A$ receptor complex exert pharmacologically and clinically important profiles including anxiolysis, anti-convulsion, muscle-relaxation, and sedation. Seveal researches demonstrated that some naturally occurring flavonoids (for example, chrysin and apigenin) are the ligands for the benzodiazepine binding sites and have anxiolytic effects (Medina *et al*., 1997). Liao *et al*. also reported that baicalein and baicalin isolated from the *Scutellaria baicalensis* activated the benzodiazepine binding site of the $GABA_A$ receptors (Liao *et al*., 2003). Thus, if the active principle in EPO is a flavone derivative, the anxiolytic-like effects of EPO may be mediated by the activation of benzodiazepine binding sites. However, to our knowledge no study has investigated the anxiolytic effects of EPO or determinted which neuronal mechanism is primarily involved in.

Benzodiazepines remain widely used for the treatment of anxiety disorders despite significant limiting side effects including sedation, muscle relaxation, and ataxia (Stahl, 2002; Atack, 2003). This spectrum of pharmacological actions is produced by augmenting the actions of the inhibitory neurotransmitter GABA through an allosteric modulation of $GABA_A$ receptors, a family of heteropentameric, ligand-gated ion channels (Barnard *et al*., 1998). Although there are at least seven subunit classes, the majority of $GABA_A$ receptors are composed of α-, β-, and γ-subunits, with multiple subtypes of each that can assemble to form GABA-gated ion channels (Korpi *et al*., 2002). This subunit repertoire allows for remarkable receptor diversity, and >12 distinct $GABA_A$ receptor isoforms may be present in the mammalian nervous system (Mckernan *et al*., 1996). We observed that EPO (400 mg/kg) treatment produced good anxiolytic-like activities by EPM. Furthermore, these anxiolytic-like behaviors were completely blocked by flumazenil, a $GABA_A$ receptor antagonist (Fig. 2). It is generally accepted that 5-HT_{1A} agonist also has anxiolytic properties. However, the anxiolytic-like effect of EPO was not blocked by WAY 100635, a 5-HT_{1A} receptor antagonist. Previously, we observed that an herbal materials containly rich flavonoids have the anxiolytic effects. Moreover, those effects were mediated by GABAergic. Thus, we concluded that the anxiolytic-like activity of EPO was mediated via the activation of the $GABA_A$ receptor and that EPO could be useful as a $GABA_A$ receptor agonist.

In summary, the present results demonstrate that the *Portulaca oleracea* has an anxiolytic-like effect, and suggest that this effect may be mediated the GABAergic nervous system. However, the nature of its underlying mode of action remains to be elucidated. Although the findings of herb effects may not in general provide clinically useful outcomes in patients or in normal humans, the findings of this study may be important because they confirm the validity of *P. oleracea* as a medicinal plant.

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