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Xylocarpin H, a Limonoid of *Xylocarpus granatum*, Produces Antidepressant-Like Activities in Mice

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Abstract

Major depression is a common psychiatric disorder worldwide that imposes a substantial health burden on society. Currently available antidepressants do not meet the clinical needs. Here, we report that Xylocarpin H, a limonoid of *Xylocarpus granatum*, has antidepressant-like effects in mouse forced swimming and tail suspension tests, two validated models of depression. 7-day oral administration of Xylocarpin H resulted in dose-dependent decreases immobility duration within the dose range of 15 - 50 mg/kg. Xylocarpin H dose-dependently increases the time spent in the central zone at doses of 5 - 50 mg/kg in locomotion activity test. In addition, 7-day treatment Xylocarpus H at 15 and 50 mg/kg doses significantly decreases levels of serum corticosterone and adrenocorticotropic hormone (ACTH) following the acute stress of forced swimming test. Furthermore, these effective doses of Xylocarpin H do not affect locomotor activity and levels of serum corticosterone and ACTH in the absence of stress. In summary, the present study, for the first time, demonstrates that Xylocarpin H exerts antidepressant-like effects in mouse behavioral models of depression, likely by inhibiting HPA axis systems. These data provide primarily basis for developing Xylocarpin H as a novel antidepressant candidate for the treatment of depression and stress related disorders.

Keywords

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1. Introduction

Major depression is a common psychiatric disorder that affects about 17% of individuals worldwide. It is estimated that depression causes about 1 million people to commit suicide each year, imposing a major burden on our society [1]. Despite the clinical availability of antidepressants for several decades, most of them are not totally effective. Indeed, only 33% depressed patients are sensitive to the first antidepressant medication. In addition, current antidepressants are associated with serious adverse-effects. Recent research interest has turned progressively to traditional herbal medicines in a quest for new antidepressant drugs [2] [3]. Natural products have attracted increasing attention as a supplemental intervention for prevention and treatment of diseases, including neurodegenerative and psychiatric disorders. A growing number of herbal medicines are being introduced into the psychiatric practice, many of which have comparable efficiency to prescription medications with no or reduced side effects. Herbal therapies are being provided as desirable alternative treatment for severe depression [4]-[6].

The genus *Xylocarpus*, is widely distributed in the coastal areas of South-East Asia, Australia, East Africa, and the Indian Ocean [7]. The mangrove plant *Xylocarpus granatum* Koenig is used in folk medicine for the treatment of diarrhea, cholera, and fever diseases. Multiple compounds, such as limonoids, have been isolated from different tissues (wood, bark, and seeds) of genus *Xylocarpus* [8]. Limonoids, the major components of *Xylocarpus granatum (X. granatum)*, have been classified into phragmalin-, mexicanolide-, abacunol- and andirobin-types [9] [10]. The unique structural patterns and biological activities of limonoids have attracted considerable attention from medicinal chemists and chemical biologists. More than 50 limonoid derivatives have been isolated from *X. granatum*. Limonoids possess a broad range of biological effects [10]. Experimental evidences suggest that limonoids of citrus fruits and juice have cancer chemopreventive properties [9]. The first limonoid, gedunin, is reported to have antifilarial activity towards the human parasite *Brugia malayi*. In addition, a chloroform extract of *X. granatum* fruits shows antimalarial activity against *Plasmodium falciparum* [11] [12]. These effects are ascribed to gedunin and xyloccensin-I. Xylogranatumine F, a limonoid is isolated from the twigs and leaves of the Chinese mangrove; *X. granatum* is exhibited in cytotoxic activity against A549 tumor cells *in vitro* [13]. Xylocarpin H is firstly isolated from *X. granatum* fruits, and represents a mexicanolide modified by ring oxidation and unusual 9, 10-bond cleavage [14]. The potential activity of Xylocarpin H is not well understood. Sarker and colleagues firstly reported that the methanolic extracts from stem and root of *X. granatum* had inhibitory effects on the central nervous systems (CNS), including sleep time prolongation and anxiolytic activity; these findings indicated that some *Xylocarpus granatum*'s active components had potential application in the treatment of psychiatric diseases [15].

It is well known that stress is one of the most important factors, which is responsible for depressive disorders [16]. Maladaptive response to stress causes hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis by stimulating adrenocorticotropic hormone (ACTH) release and subsequent peripheral release of steroids/cortisol from the adrenal grand [17]. Based on the previous findings, the present study aims to examine whether oral administration of Xylocarpin H produces antidepressant-like properties in accepted mouse models of depression. In addition, we assess the HPA activity via measurement of serum corticosterone and ACTH levels in order to clarify the potential mechanism underlying the Xylocarpin H effects.

2. Materials and Methods

2.1. Animals

Male ICR mice (weighing 18 - 22 g upon arrival) were individually housed at a constant temperature (23°C ± 2°C) with 12 h/12h light/dark cycles and free access to food and water. All animal procedures were performed in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*, and approved by the Local Animal Use Committee. All mice were transferred to the experimental room 1 h before behavioral tests and all behavioral tests and drug administration were performed in the dark phase.

2.2. Drugs

Xylocarpin H was isolated from the fruits of *Xylocarpus granatum* (purify > 95%, chemical structure shown in **Figure 1**) and obtained as a gift from Prof. Qinwen Shi (School of Pharmacy of Hebei Medical University, China). Sodium Carboxy Methyl Cellulose (CMC-Na) was obtained from Sinopharm Chemical Reagent Co., Ltd.
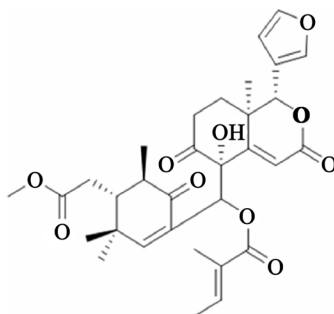
Figure 1. Chemical structure of Xylocarpin H from *Xylocarpus granatum*.

For oral administration, Xylocarpin H was freshly suspended in a solution of 0.5% CMC-Na. Venlafaxine was purchased from Chengdu Daxi'nan Pharmaceutical Co., Ltd (Chengdu, Sichuan Province, China) and was dissolved in saline before the experiment. Xylocarpin H, venlafaxine and saline were given by intragastric administration once daily for 7 days in all experiments. The doses of Xylocarpin H (5, 15 and 50 mg/kg) used were selected according to a pilot study.

2.3. Forced Swimming Test

The forced swimming test was carried out as described previously [18] [19]. Mice were placed into a 20 cm diameter × 35 cm height plastic cylinder filled to 20 cm with 23˚C - 25˚C water. This session was videotaped and the floating time measured. Immobility was defined as the absence of movement except motions required to maintain the animal's head above the water. Results were expressed as time that animals spent immobile in the last 4 min during the 6 min session. Observers were blind to treatment groups.

2.4. Tail Suspension Test

Tail suspension test was carried out according to previous reports [6] [18]. Briefly, mice were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. The session was videotaped and immobility time during 6 min was measured. Immobility was defined as the absence of limb or body movements, except for those caused by respiration when they hung passively and completely motionless. During the test, mice were separated from each other to prevent possible visual and acoustical associations. The number of seconds spent in immobility was scored as measurement parameter. Observers were blind to treatment groups.

2.5. Locomotor Activity

The locomotor activity was tested in an open field by an activity-monitoring system developed by Ji-Liang Co., Ltd. (Shanghai, China) [6] [19]. Each mouse was placed in the center of the apparatus and monitored for 10 mins. The parameters were recorded by video camera and registered in a computer. The total distance traveled by the mouse during the last 6 min was recorded to evaluate the locomotor activity. Meanwhile, the time spent in the central zone of the apparatus was also recorded to reflect the anxiety-like behavior. Upon experiment completion, animals were returned to home cages.

2.6. Serum Corticosterone and ACTH Measurements

Detection of serum corticosterone and ACTH levels was carried out according to our previous study [6]. Briefly, 1 ml of blood was collected from decapitation bleeding immediately after 6 min of forced swimming test (FST). Blood samples were kept at room temperature for 1 h, and centrifuged at 3000 rpm for 10 min. The serum (supernatant fraction) was transferred into a new tube for subsequent assays. Serum corticosterone and ACTH levels were measured with commercially available enzyme immunoassay kits (corticosterone ELISA, ml001959, mlbio, China; ACTH ELISA, ml001895, mlbio, China) according to the manufacturer's instructions. To exclude the potential impact of diurnal rhythm on mouse hormone levels, blood samples were collected in the same time window of 4:00 to 6:00 pm from each mouse immediately after 6 mins of FST. Data were expressed as ng/l for

corticosterone and ACTH levels.

2.7. Experimental Design

Experiment 1: Effects of Xylocarpin H on floating time in the FST

In this experiment, mice were divided into five groups for oral administration of saline, Xylocarpin H (5, 15, and 50 mg/kg) and the positive control venlafaxine (10 mg/kg), respectively, once daily for 7 consecutive days. On day 7, 30 mins after the last treatment, mice were subjected to FST for 6 mins (n = 8 - 12 per group).

Experiment 2: Effects of Xylocarpin H on immobility time in the TST

A separate group of mice was used to examine the antidepressant effect of Xylocarpin H in the TST. Five groups of mice were treated orally with saline, Xylocarpin H (5, 15, and 50 mg/kg) and venlafaxine (10 mg/kg), respectively, once daily for 7 consecutive days. On day 7, 30 mins after the last treatment, mice were subjected to tail suspension test for 6 mins (n = 8 - 10 per group).

Experiment 3: Effects of Xylocarpin H on locomotor activity

In this experiment, mice were divided into five groups to receive oral saline, Xylocarpin H (5, 15, and 50 mg/kg) and venlafaxine (10 mg/kg), respectively, once daily for 7 consecutive days. On day 7, 30 min after the last treatment, total distance traveled and time spent in the central zone were measured during the last 6 mins to reflect the anxiety-like behavior and locomotor activity, respectively (n = 8 - 9 per group).

Experiment 4: Effects of Xylocarpin H on serum corticosterone and ACTH levels after exposure to FST

Immediately after the FST, mice (n = 5 - 7 per group) were killed by decapitation, blood was collected for measurement of corticosterone and ACTH concentrations.

2.8. Data Analysis

Data are expressed as mean ± SEM. Statistical analysis of behavioral and biochemical data in control and drug-treated mice was performed by one-way analysis of variance (ANOVA), followed by a post hoc Dunnett's test. $P < 0.05$ was considered statistically significant.

3. Results

### 3.1. Chronic Xylocarpin H Administration Decreased the Floating Time in Forced Swimming Test

The potential antidepressant-like effects of Xylocarpin H were evaluated in mouse forced swimming test. As shown in **Figure 2(a)**, chronic oral administration of Xylocarpin H resulted in a dose-dependent reduction of immobility time. The classic antidepressant Venlafaxine (10 mg/kg) also significantly reduced the floating time of mice. One way ANOVA revealed a significant effect for Xylocarpin H doses [$F_{(3, 36)}$ = 3.324, P = 0.031 vs. control group]. Post hoc analyses indicated that 15 and 50 mg/kg Xylocarpin H significantly decreased the floating time in mice.

3.2. Chronic Xylocarpin H Administration Reduced Immobility Time in Tail Suspension Test

Tail suspension test results presented in **Figure 2(b)** revealed that treatment with Xylocarpin H for 7 days significantly reduced the immobility time. The positive control venlafaxine (10 mg/kg) significantly reduced the immobility time in TST as expected. One way ANOVA indicated a significant effect for Xylocarpin H doses [$F_{(3, 38)}$ = 3.220, P = 0.034 vs. control group]. Post hoc analyses indicated that 50 mg/kg Xylocarpin H significantly decreased the immobility time in mice.

3.3. Chronic Xylocarpin H Treatment Resulted in Anxiolytic-Like Effects

The potential effect of different Xylocarpin H doses on the general locomotor activity in naïve mice was examined. Results showed that Xylocarpin H did not significantly alter the general locomotor activity in mice for the doses studied. Indeed, one-way ANOVA showed no significant effect for Xylocarpin H doses [$F_{(3, 38)}$ = 2.089, P = 0.117 vs. control group]. In addition, post hoc analysis revealed that no Xylocarpin H dose significantly altered the locomotor activity (**Figure 2(c)**). However, Xylocarpin H had potential anxiolytic effects in
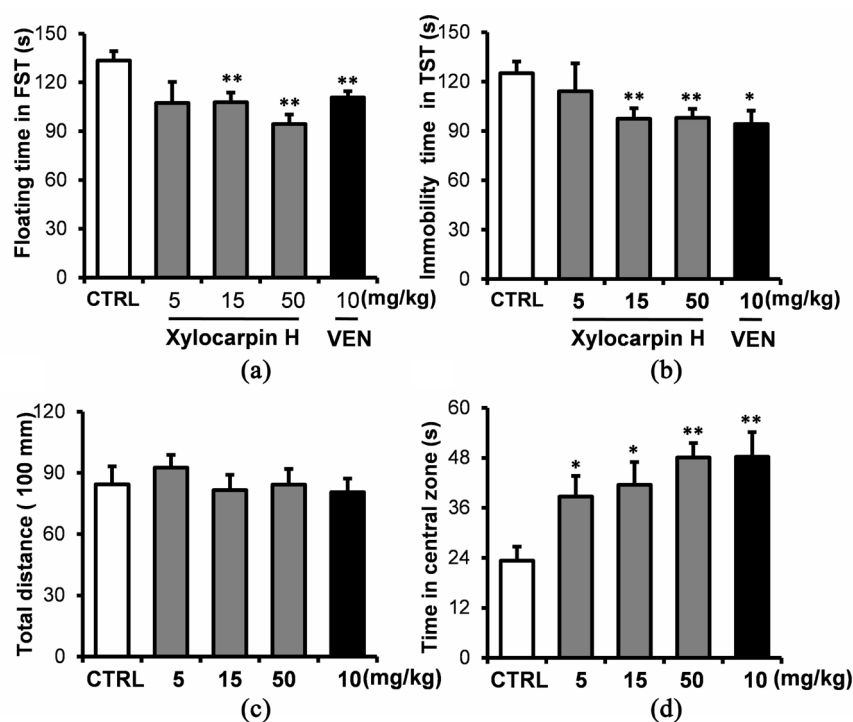
Figure 2. Effects of Xylocarpin H on depressive-like behaviors in mice. Mice were administrated vehicle, Xylocarpin H (5, 15, 50 mg/kg) or venlafaxine (10 mg/kg). 7 days of Xylocarpin H treatment decreased immobility time in forced swimming test (a) and in the tail suspension test (b) and increased the time spent in the central zone (d), but without any effects on total distance in locomotor activity test (c). Data are expressed as mean ± SEM. $^*P < 0.05$, $^{**}P < 0.01$ vs. control group. $n = 9$ -12 per group.

mice. One-way ANOVA revealed a significant effect for Xylocarpin H doses [F (3, 38) = 3.035, $P = 0.042$ vs. control group]. Post hoc analysis indicated that 5, 15 and 50 mg/kg Xylocarpin H significantly increased the time spent in central zone (**Figure 2(d)**).

3.4. Chronic Xylocarpin H Administration Reduced Serum Corticosterone and ACTH Levels in Mice Exposed to FST

In order to determine the potential mechanism underlying the antidepressant-like effects of Xylocarpin H, serum corticosterone and ACTH levels in mice exposed (or not) to FST were measured by ELISA. Treatment with Xylocarpin H for 7 days significantly decreased serum CORT and ACTH levels in mice after FST. One way ANOVA indicated a significant effect for Xylocarpin H doses [CORT: F (3, 26) = 3.084, $P = 0.047$; ACTH: F (3, 26) = 6.300, $P = 0.003$]. Post hoc analyses indicated that 15 and 50 mg/kg Xylocarpin H significantly reduced serum CORT and ACTH levels in mice (**Figure 3(a)** and **Figure 4(a)**). Interestingly, treatment with Xylocarpin H resulted in no significant effect in mice without FST at all doses studied. One-way ANOVA showed no significant effect for Xylocarpin H doses [CORT: F (3, 20) = 1.283, $P = 0.327$; ACTH: F (3, 19) = 0.692, $P = 0.570$]. Post hoc analysis confirmed that no Xylocarpin H dose significantly altered the serum CORT and ACTH levels in mice without FST (**Figure 3(b)** and **Figure 4(b)**).

4. Discussion

In the present study, we demonstrated that Xylocarpin H, one limonoid extracted from the fruits of *Xylocarpus granatum* Koenig (Meliaceae), produced antidepressant-like effects in two well-validated mouse models of depression, namely the forced swimming and tail suspension tests. Preliminary study of pharmacological mechanism also proposed the involvement of the HPA axis in Xylocarpin H's activity. Furthermore, Xylocarpin H treatment significantly increased the time spent in the central zone without any effects on locomotion activity in the LA test. Although *Xylocarpus granatum* Koenig or its extracts had been used to treat various malaises,
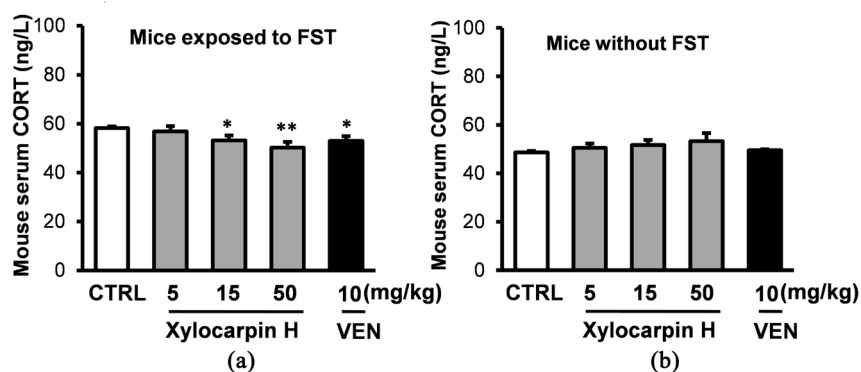
Figure 3. Effects of 7-day Xylocarpin H treatment on serum CORT levels in mice. Xylocarpin H (15, 50 mg/kg) and venlafaxine (VEN 10 mg/kg) administered orally for 7 days. Blood was collected for CORT levels analysis immediately after or without the FST. Serum CORT levels in mice exposed to FST (a) or without exposure to FST (b). Data are expressed as mean ± SEM $^*P < 0.05$, $^{**}P < 0.01$ vs. control group. $n = 5$ - 8 mice per group. CORT, corticosterone.
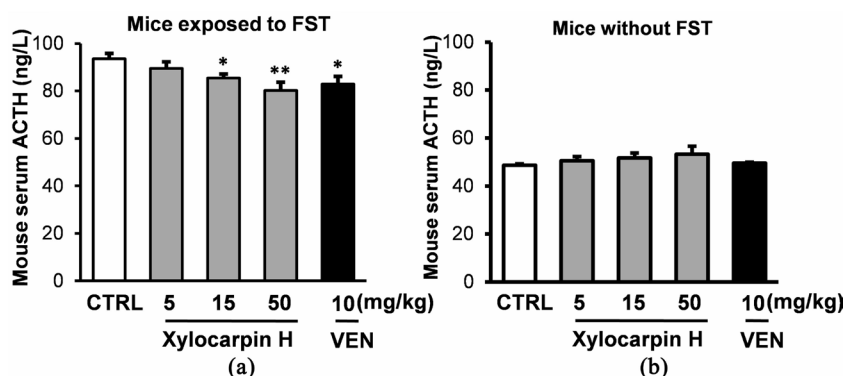
Figure 4. Effects of 7-day Xylocarpin H treatment on serum ACTH levels in mice. Xylocarpin H (15, 50 mg/kg) and venlafaxine (VEN 10 mg/kg) administered orally for 7 days. Blood was collected for ACTH levels analysis immediately after or without the FST. Serum ACTH levels in mice exposed to FST (a) or without exposure to FST (b). Data are expressed as mean ± SEM $^*P < 0.05$, $^{**}P < 0.01$ vs. control group. $n = 5$ - 8 mice per group; ACTH, adrenocorticotropic hormone.

contemporary pharmacological studies only began to discern the active components of this plant that were responsible for its multiple therapeutic actions [20]-[22]. A previous study reported that the methanolic extracts of the barks and pneumatophores of *Xylocarpus moluccensis* produced CNS depressant activity, with the pneumatophore extract being more potent than the bark's [15]. Very interestingly, as a single component of methanolic extracts from *Xylocarpus granatum*'s fruits, Xylocarpin H also significantly increased the time spent in central zone in the LA test, indicating a potential anxiolytic activity for Xylocarpin H. This study presented the first evidence of the potential antidepressant effects of *Xylocarpus granatum* Koenig in widely used mouse models of depression, which called for more detailed pharmacological studies of this new compound for its potential to treat depression and other mental disorders alike.

Forced swimming and tail suspension tests were widely used in pre-clinical antidepressant evaluation and were to provide insights into the neuropathology of depression [18] [19] [23]-[26]; the floating and/or immobility time in these paradigms reflected the antidepressant-like activity [16]. The present study demonstrated that chronic administration of Xylocarpin H at 50 mg/kg dose resulted in significant antidepressant-like effect both in the tail suspension and forced swimming tests in mice. The antidepressant-like activity of Xylocarpin H was largely comparable to that of venlafaxine, a classical antidepressant. A potential anxiolytic activity of Xylocarpin H at 5 mg/kg or higher doses was also observed. Furthermore, chronic Xylocarpin H administration had no significant effect on locomotor activity in mice at doses that significantly improved antidepressant performance. These results clearly demonstrated the antidepressant-like effect of Xylocarpin H in these mouse models of depression.

HPA axis dysfunction, exhibited by elevation in circulating glucocorticoids (corticosterone in rodents; cortisol

in humans), contributed to the development of depression in humans [27] [28]. It was known that most depressive patients produced higher plasma cortisol levels compared with healthy subjects [29]. Moreover, acute restraint stress was shown to induce increased serum corticosterone levels in mice, which was attributed to increased immobility in FST and TST, and associated with depressive-like symptoms [30]. To determine whether HPA axis activity induced by forced swimming test was affected by Xylocarpin H, we measured the serum corticosterone and ACTH levels in mice in response to FST exposure. As shown above, Xylocarpin H reduced serum corticosterone and ACTH levels in mice exposed to forced swimming test, which suggested that the antidepressant action of Xylocarpin H might occur through regulation of HPA homeostasis to increase the ability of mice to cope with stressful conditions. The effects observed for Xylocarpin H and venlafaxine were consistent with previous findings that agents with antidepressant-like efficacy decreased the levels of glucocorticoids like corticosterone [31] [32]. The present study raised the possibility that chronic treatment with Xylocarpin H could reduce the HPA axis activity in response to stress. It had been shown that hyperactivity of the HPA axis decreased the function of the glucocorticoid receptor (GR), particularly in the hippocampus, consequently leading to the impairment of glucocorticoid feedback inhibition. Dysfunction of GR caused reduction of neurogenesis and impairment of neuroplasticity, thereby leading to the development of depressive performance [33]. Effective antidepressants exerted their therapeutic actions in part by modulating the expression of GR, accordingly ameliorating many of the behavioral disturbances in depressive-like behaviors. Considering that GR played a key role in the development and treatment of depression, the regulatory effect of Xylocarpin H on GR expression and the target genes involved in its antidepressant-like activity needed to be addressed in the future.

The unique structural patterns and biological activities of limonoids have attracted considerable attention from medicinal chemists and chemical biologists [10]. In 1989, gedunin, a limonoid, was isolated from *Xylocarpus granatum* Koenig [34]. By now, more than 50 limonoid derivatives have been isolated from *X. granatum* and classified into phragmalin-, mexicanolide-, abacunol- and andirobin-types. Limonoids possess a broad range of biological effects such as antifeedant, antibacterial, antifungal, antiviral, antimalarial, anticancer, and neuroprotective activities [8] [14] [20]. Xylocarpin H was isolated from the fruits of *X. granatum* [14]. Xylogranin B, isolated from the leaves of *X. granatum* (Meliaceae) was shown to inhibit TGF/beta-catenin transcriptional activity and exhibit strong cytotoxicity against colon cancer cell lines [35]. 7-oxo-7-deacetoxygedunin (7-OG), a gedunin type limonoid from seeds of the mangrove *Xylocarpus moluccensis* was reported to be a potent inhibitor of osteoclastogenesis [36]. Treatment with this limonoid suppressed RANKL-induced activation of p38, MAPK and ERK and nuclear localization of NF-κB p65 [36]. Xyloccensins X and Y were found to possess anti-ulcerogenic activity, which might be due to its anti-secretory activity and subsequent strengthening of the defense mechanism [37]. However, the exact mechanism underlying the antidepressant-like activity of Xylocarpus H should be well investigated.

In summary, this study, for the first time, demonstrated that Xylocarpin H, one limonoid from *Xylocarpus granatum*, displayed antidepressant-like activity and potential anxiolytic activity without apparent adverse effects (motor alteration). The finding that the antidepressant-like effects of Xylocarpin H were associated with a normalization of HPA axis dysfunction induced by stress is consistent with a large body of literature. Further research is needed to determine whether the antidepressant effects of Xylocarpin H in mice are applicable to depressed patients. Additionally, investigations for identifying new potential components from *Xylocarpus granatum* Koenig and other herbal plants are still needed.

Acknowledgements

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