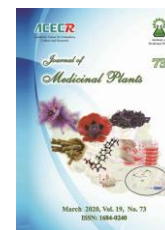




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Research Article

Antidepressant effects of hydroalcoholic extract of *Alpinia officinarum* rhizome on chronic unpredictable stress induced depression in BALB/c mice

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ABSTRACT

Background: *Alpinia officinarum* is an aromatic plant that show some neuroprotective functions in animal models. **Objective:** This study aimed to evaluate the antidepressant effects of hydroalcoholic extract of *A. officinarum* rhizome using two behavioral models. **Methods:** Forty male BALB/c mice were randomized to four groups. All studygroups underwent three weeks of daily chronic unpredictable stress (CUS) sessions. Control group received daily intraperitoneal (i.p.) injection of normal saline 30 min before daily CUS; groups 2, 3 and 4 received i.p. injection of 50, 100, and 150 mg/kg/day of *A. officinarum* extract, respectively. Behavior tests were performed after 21 days of treatment. Serum and brain malondialdehyde (MDA) and total antioxidant capacity (TCA) were also measured. **Results:** Immobility duration in both forced swim test and tail suspension test decreased significantly in the groups treated with *A. officinarum* extract at doses of 50 and 100 mg/kg compared to control group ($P < 0.05$). Extract treatment reduced MDA and increased TCA in both brain and serum ($P < 0.05$). Results demonstrate that the hydroalcoholic extract of *A. officinarum* possesses antidepressant activity in the animal model.

1. Introduction

Depression is one of the most common psychiatric disorders that affects more than 300 million people worldwide [1]. It is considered as

the mother of many illnesses and can influence different aspects of human functioning, including occupational, social, and family [2]. Two third of patients with depression develop suicidal

Abbreviations: MDA, malondialdehyde, CUS, chronic unpredictable stress, TCA, total antioxidant capacity; IP, intraperitoneal

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ideation and around 10-15% of them attempt suicide [3]. Studies have shown that distribution of monoamine neurotransmission, impairment of the hypothalamus-pituitary-adrenal axis, neuroinflammatory process, oxidative and nitrate stress and also neurodegenerative pathway are involved in the pathogenesis of depression [4]. Available pharmacotherapies for the depression include tricyclic antidepressants, selective serotonin reuptake inhibitors, monoamine oxidase inhibitors, and certain new drugs such as nefazodone and bupropion [5]. All available drugs for depression are associated with different, sometimes life-threatening, side effects; it is therefore necessary to seek out effective drugs with the least possible number of side effects [6].

Plant extracts are one of the most valuable sources of novel therapeutic compounds and have been shown to produce promising antidepressant effects both in animal models and clinical studies [2]. *Alpinia officinarum*, known as lesser galangal, is an herbaceous plant in the Zingiberaceae family which distributed throughout Southeast Asia [7]. *A. officinarum* has a reddish-brown tuberous rhizome with an aromatic spicy odor and high pharmaceutical and nutritional value [8]. In Iran, *A. officinarum* rhizome is often used in pickles and grinded to be applied in a variety of stews and as an aromatic spice [9]. In Iranian traditional medicine, it has been suggested to improve sexual desire, relief back and sciatica pain, treat rheumatic diseases, support immune system function and treat digestive disorders and stomach pain [10]. Recent studies indicated its potential as antioxidant [11, 12], antimicrobial [12], anti-inflammatory [13], anticancer [14], anti-obesity Hypolipidemic [15] and anti-diabetic [15] agents.

Neuroprotective functions of the plant

rhizome have been also evaluated in some studies. Lee et al, reported that *A. officinarum* extract show anti-inflammatory, anti-rheumatic and analgesic activities against carrageenan induced inflammation and Freund's adjuvant (CFA) induced pain and arthritis. It also shows anti-psychiatric effects through down regulation of c-Fos expression in the hippocampus of CFA-exposed rats [16]. A diarylheptanoid isolated from *A. officinarum* also demonstrated to show neuroprotection against amyloid- β induced neural toxicity and damage [17]. Anticholinergic effect for the water, ethanol and water/ethanol extracts of *A. officinarum* rhizome was reported [11]. Given the useful effects of *A. officinarum* rhizome on the nervous system, we aimed to investigate the antidepressant effects the hydroalcoholic extract of *A. officinarum* rhizome in mice.

2. Material and Methods

2.1. Preparation of plant extract

A. officinarum was purchased from a reliable grocery and scientifically and systematically approved as *A. officinarum*; then, 1000 g of its dried rhizome was dissolved in 2000 ml of 70% ethanol. After the solution was left at room temperature for 28 hours, it was filtered and its solvent was separated using a rotary evaporator. Then, the resulting extract was completely dried at room temperature and stored at -20 °C until use [16].

2.2. Experimental animal and grouping

Male *BALB/c* mice weighing 25-30 g were used in this study. They were kept in the animal house of Islamic Azad University, Izeh Branch, at 23 \pm 1 °C under a 12:12 h light-dark cycle with *ad libitum*, constant access to standard water and food. The mice were then randomized to four groups of 10 mice. All studies groups underwent

three weeks of daily stress sessions. Control group received daily i.p. injection of normal saline 30 min before the stress session; groups 2, 3 and 4 received i.p. injection of 50, 100, and 150 mg/kg/day of *A. officinarum* extract respectively for 21 days [13]. The study was approved by the Ethics Committee of Islamic Azad University and all animal procedure was based on Guidelines for the Care and Use of Laboratory Animals [18].

2.3. Induction of depression through Chronic Unpredictable Stress (CUS)

In this study, depression was induced by CUS exposure based on the protocol of Kumar et al (Table 1). For this purpose, the mice were daily exposed to one of the stressors for 3 weeks, i.e. swimming in cold (12 °C) water for 5 minutes (A), swimming at room (23 ± 2 °C) temperature for 10 minutes (A₁), swimming at room (23 ± 2 °C) temperature for 15 minutes (A₂), pressing the tail for 30 seconds (B), pressing the tail for 60 seconds (B₂), pressing the tail for 90 seconds (B₃), depriving the water and food for 24 hours (C), keeping waking up overnight, (D), and no stress (E) [19].

Table 1. Exposure of mice to CUS based on the weekly schedule

Friday	Thursday	Wednesday	Tuesday	Monday	Sunday	Saturday	weeks
E	D	C	B	A	B ₂	A ₂	1
E	A ₁	B ₃	D	A	C	A	2
E	A ₂	B ₂	A	D	C	D	3

2.4. Forced swim test (FST)

The forced swim test (FST) is a reliable and common test to study the antidepressant-like effects of drugs. In this test, a glass cylinder (25 × 12 × 15 cm) is filled with 25 °C water and the mouse from a 20-cm distance to the water surface is slowly placed in the water. Animal is allowed to acclimatize for first two minutes. The time of immobility was recorded during the last 4 min of the 6 min tests. All measurements of variables were conducted by one individual [20].

2.5. Tail suspension test (TST)

In tail suspension test, metal bars of 70 cm high are used with a 50-cm rope longitudinally stretched between them. Mouse tail is closed by the rope with the mouse hanged up from its tail. The test begins with jerking mice. Total duration of TST is six minutes, animals were allowed to adapt the apparatus for first two minutes. The immobility time in the following four minutes was counted using a chronometer. All

measurements of variables were conducted by one individual [20].

2.6. Rotarod test

The mice were first placed on the rotarod roller rod and trained to move on it based on the main protocol (speed: 10 rpm and acceleration: 7 rpm²), and the balance test was performed 30 min later. All mice were placed on the rotarod 1 h after injection of the extract. The period when mouse could maintain its balance and resist against the movement of the rotarod was considered resistance time. The maximum test time for each mouse was considered 300 seconds [20].

2.7. Measurement of lipid peroxide levels in serum and brain tissues

200 µl of tissue homogenate/ serum was mixed with 1.5 ml of 20 % acetic acid, 1.5 ml of 0.8 % TBA and 200 µl of 8.1 % SDS. The mixture was made up to 4 ml with distilled water

and heated in a boiling water bath for 60 minutes. After cooling under tap water, 1 ml of distilled water and 5 ml of n-butanol/pyridine were added to the reaction mixtures and shaken vigorously. Then, the resulting solutions were centrifuged at 4000 rpm for 10 min and the optical absorbance of the supernatant at 532 nm was recorded. Lipid peroxide levels were determined using standard calibration curve and expressed as a micromol of malondialdehyde [20].

2.8. Measurement of total antioxidant capacity in serum and brain tissues

Antioxidant capacity of serum and tissue homogenate was measured by using ferric reducing antioxidant power (FRAP) assay. The working FRAP reagent was prepared by mixing 10 ml of 0.25 M acetate buffer (pH 3.6), 2.5 ml of 10 mM TPTZ in 40 mM HCl and 2.5 ml of 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$. 25 μl of tissue homogenate/serum were added to 1.5 ml of working FRAP solution and left at 37 °C for 10

minutes. After incubation, the optical absorbance at 593 nm was recorded [20].

2.9. Statistical analysis

Data were analyzed using SPSS version 20. Analysis of Variance (ANOVA) followed by Duncan's test used to identify statistical differences between means. All data were presented as mean \pm SD and p value less than 0.05 was considered statistically significant.

3. Results

Results demonstrated that 50 and 100 mg/kg body weight (BW) of *A. officinarum* extract could significantly decrease the immobility duration in forced swim test (FST) compared to normal saline (control group) ($P < 0.001$ and $P < 0.05$, Fig. 1).

According to our results, 50 and 100 mg/kg BW of *A. officinarum* extract could significantly decrease the immobility duration in tail suspension test (TST) compared to control group ($P < 0.001$ and $P < 0.05$, Fig. 2).

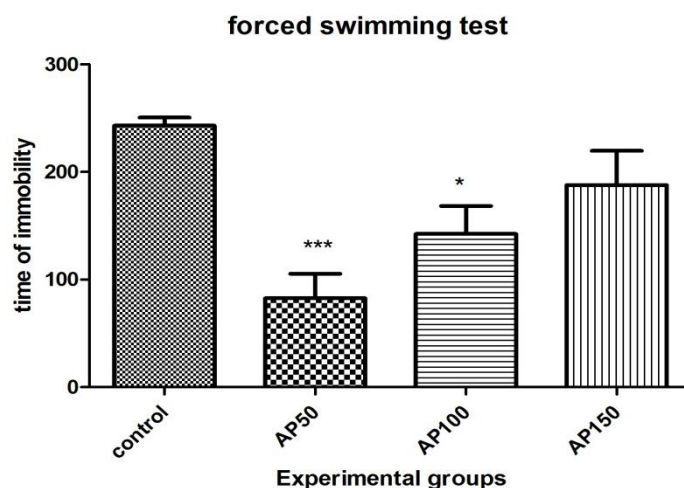


Fig. 1. Immobility duration in forced swim test in different groups; * shows significant differences with control group (* $P < 0.05$ and *** $P < 0.001$). AP: *Alpinia officinarum*

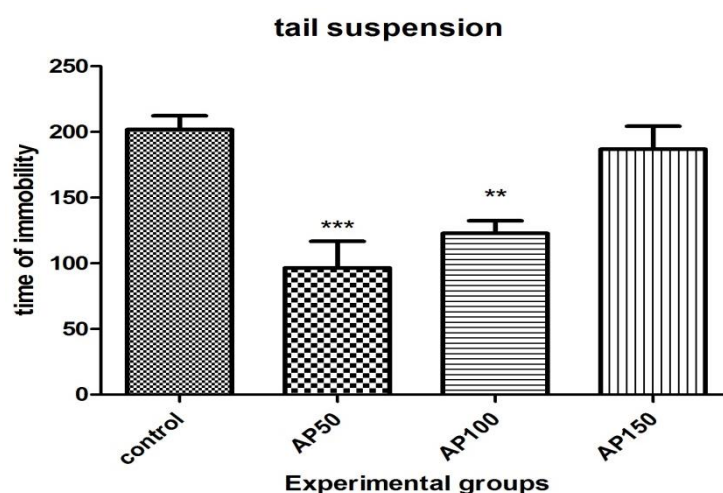


Fig. 2. Immobility duration in tail suspension test in different groups; * shows significant differences with control group (** $P < 0.01$ and *** $P < 0.001$). AP: *Alpinia officinarum*

According to our results, in rotarod test there was no significant difference between the experimental groups ($P > 0.05$, Fig 3).

In this study, we observed that 50, 100, and 150 mg/kg BW of *A. officinarum* extract could significantly increase serum and brain ferric reducing antioxidant power (FRAP) ($P < 0.05$, Fig. 4).

Results also demonstrated that all doses of *A. officinarum* extract significantly decrease malondialdehyde (MDA) levels in serum of experimental groups compared to control group ($P < 0.003$, Fig. 5). Brain MDA level showed significant reduction only in group treated with 150 mg/kg BW of *A. officinarum* extract ($P < 0.001$). ($P < 0.05$, Fig. 5).

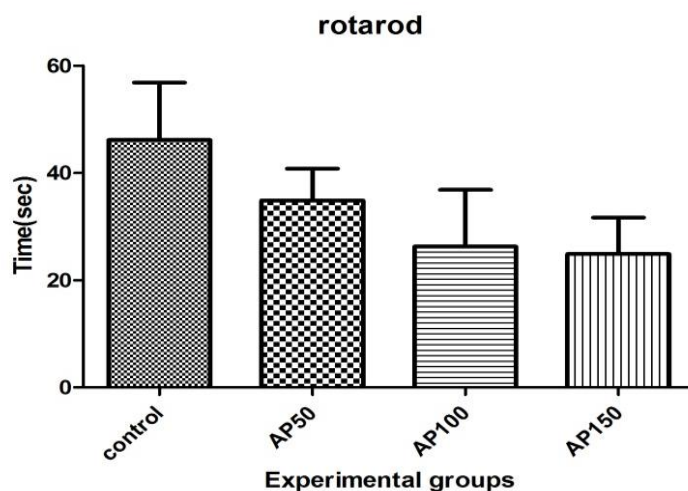


Fig. 3. Duration of time to maintain balance on the rotating rod of rotarod in different groups. AP: *Alpinia officinarum*

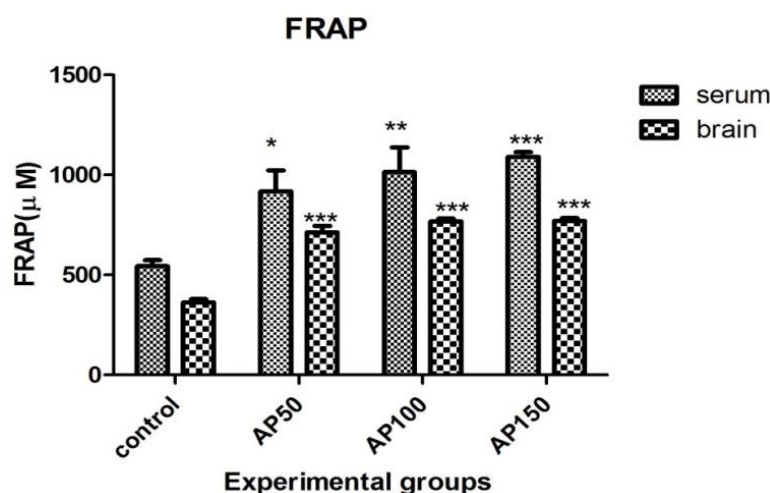


Fig. 4. Comparison of serum and brain ferric reducing antioxidant power (FRAP) among groups; * shows significant differences with control group (* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$); AP: *Alpinia officinarum*.

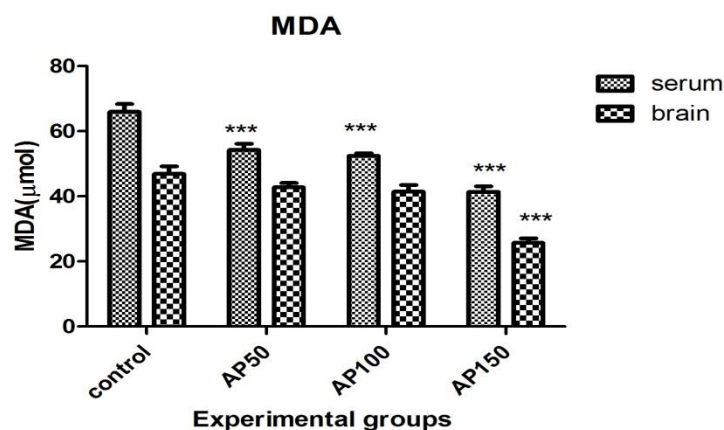


Fig. 5. Comparison of serum and brain malondialdehyde levels among groups; * shows significant differences with control group (*** $P < 0.001$); AP: *Alpinia officinarum*.

4. Discussion

In the present study, the effects of 50, 100, and 150 mg/kg BW of *A. officinarum* extract on symptoms of CUS-induced depression in mouse model were investigated. Besides that, the effects of *A. officinarum* extract on MDA and TCA levels in both serum and brain of CUS treated mice were examined. Results demonstrated that the immobility duration in the FST and the TST were significantly longer in the group under CUS than other groups; and *A. officinarum* extract could significantly decrease the immobility

duration in the mice. It was also found that as the dose of the extract increased, its antidepressant effects decreased, reinforcing the possibility that the observed effects may be due to the non-pharmacological and possibly toxic effects of the extract.

In our study, treatment of mice with *A. officinarum* extract significantly inhibited chronic stress induced lipid peroxidation in both brain and serum and improved their antioxidant capacity. The role of oxidative stress in CUS-induced depression has been reported by a

number of studies. CUS is associated with decreased superoxide dismutase (SOD) and glutathione reductase activities and reduced glutathione level in rat brain [21]. In addition, chronic stress causes significant decrease in glutathione peroxidase (GPX) activity, glutathione content and vitamin C level of brain and increases its lipids peroxidation [22]. Increased protein and lipids peroxidation and reduced SOD activity in the different brain regions of CUS-exposed mouse [23]. CUS-induced depression led to decrease in antioxidant capacity and the activity of GPX, SOD, and catalase in the hippocampus and hypothalamus [24]. So, the antidepressant-like activity observed for *A. officinarum* extract in our study, may be related to its antioxidant action and inhibition of oxidative stress marker in both brain and serum. Our results also demonstrated that *A. officinarum* extract significantly decrease malondialdehyde (MDA) levels in serum and brain in animals. *A. officinarum* extract could significantly increase serum and brain total antioxidant capacity.

Aqueous, ethanolic, and hydroalcoholic extracts of *A. officinarum* rhizome displayed significant antioxidant activity in inhibiting the ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)], DPPH (1,1-diphenyl-2-picrylhydrazyl), superoxide radicals and hydrogen peroxide. These extracts also showed significant effects in chelating and reducing iron and copper ions [11]. Galangin, the main flavonoid compound in *A. officinarum* rhizome, significantly prevented D-galactose induced memory loss and passive avoidance memory. They argued that galangin exerts protective effects on the central nervous system via decreasing oxidative stress parameters [25].

Studies suggest that *neuroinflammatory* process following chronic stress has potential

mechanistic role in the development of depressive-like behavior. A study showed that exposure to CUS caused increase in serum tumor necrosis factor alpha (TNF α) level in mouse brain [21]. The expression of proinflammatory cytokines (TNF α , interleukin 6 (IL-6), and interleukin 1 beta (IL-1 β)) significantly increased in the brain and spleen of the mice under chronic stress, and the expression of anti-inflammatory cytokines (*transforming growth factor beta (TGF- β)* and interleukin 10 (IL-10)) significantly decreased in these mice [26]. Chronic stress-induced depression increased the levels of the inflammatory markers IL-6 and TNF- α [24]. It was reported that celecoxib, an anti-inflammatory drug, shows therapeutic effects on major depression in clinical trial [27]. *A. officinarum* is a medicinal plant known for anti-inflammatory properties. Traditionally it is recommended to treat and *alleviate* the symptoms of rheumatic diseases and *other inflammatory conditions* [10]. The anti-inflammatory effects of *A. officinarum* extract have been examined in a number of studies. *A. officinarum* extract significantly decrease slipopolysaccharide induced nitric oxide generation in mouse macrophage cell and inflammatory cytokines (IL-1 β and TNF α) production in human peripheral blood mononuclear cells [13]. Lee et al. study showed that ethanolic extract of *A. officinarum* rhizome ameliorate inflammatory response in carrageenan-treated rats [16]. It has been reported that the galangin found in *A. officinarum* rhizome exerts anti-inflammatory effects [28].

The Rotarod test was to confirm that the animals had no motor impairment and were not in the forced swimming and tail suspension test immobility was not due to a motor problem. Results demonstrate that the hydroalcoholic extract of *A. officinarum* possesses

antidepressant activity in the animal model. The antidepressant activity observed for *A. officinarum* extract may be related to the *suppression of inflammatory responses and oxidative stress markers*.

5. Conclusion

Administration of depressed mice with *A. officinarum* extract caused antidepressant effects and significantly increased the immobility duration in the FST and TST. In the current study, *A. officinarum* extract at different concentrations caused significant increase in the antioxidant capacity and significant decrease in the brain and serum MDA levels. It seems that the antidepressant effects of this extract are due to antioxidant and anti-inflammatory mechanisms.

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Author contributions

Zahra Rabei conceived and extracted the data, revised the paper; Mahbubeh Setorki designed the study, analyzed the data and wrote the manuscript; Azadeh Salehi done all testes.

Conflict of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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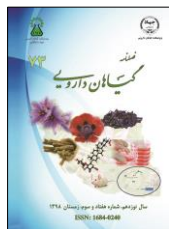
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مقاله تحقیقاتی

اثرات ضد افسردگی عصاره هیدروالکلی ریزوم گیاه خولنجان بر افسردگی ناشی از استرس غیرقابل

پیش‌بینی در موش BALB/c

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اطلاعات مقاله	چکیده
گل‌واژگان:	مقدمه: خولنجان یک گیاه معطر است که برخی از عملکردهای محافظت‌کننده عصبی را در مدل‌های
خولنجان	حیوانی نشان می‌دهد. هدف: هدف این مطالعه ارزیابی اثرات ضد افسردگی عصاره هیدروالکلی ریزوم
عصاره	خولنجان با استفاده از دو مدل رفتاری می‌باشد. روش بررسی: ۴۰ موش نر BALB/c در چهار گروه
افسردگی	تصادفی قرار گرفتند. همه گروه‌های مطالعه سه هفته روزه تحت استرس غیرقابل پیش‌بینی مزمن
تست شنای اجباری	(CUS) قرار گرفتند. گروه کنترل روزانه از طریق تزریق داخل صفاقی (i.p) ۳۰ دقیقه قبل از CUS
	نرمال سالین دریافت کردند. گروه‌های ۲، ۳ و ۴ به روش i.p به ترتیب ۵۰، ۱۰۰ و ۱۵۰ میلی‌گرم /
	کیلوگرم در روز عصاره خولنجان دریافت کردند. تست‌های رفتاری پس از ۲۱ روز درمان انجام شد.
	مالون دی‌آلدهید (MDA) مغز و سرم و ظرفیت آنتی‌اکسیدان کل (TCA) نیز اندازه‌گیری شد. نتایج:
	مدت زمان تحرک در هر دو تست شنا اجباری و تست تعلیق دم در گروه‌های تحت درمان با عصاره
	خولنجان در دوزهای ۵۰ و ۱۰۰ میلی‌گرم بر کیلوگرم نسبت به گروه کنترل به طور معنی‌داری کاهش
	یافت ($P < 0/05$). تیمار عصاره باعث کاهش MDA و افزایش TCA در مغز و سرم شد ($P < 0/05$).
	نتیجه‌گیری: نتایج نشان داد که عصاره هیدروالکلی خولنجان دارای فعالیت ضد افسردگی در مدل حیوانی
	است.

مخفف‌ها: (MDA) malondialdehyde, (CUS) chronic unpredictable stress, (TCA) total antioxidant capacity; (i.p.) intraperitoneal

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