Research Article

Antioxidant and Antifatigue Activities of *Ophiopogon japonicus* Extracts in Exercised Rats

Chi-Ting Horng[1,2], Hui-Yun Wang[2], Yu-Syuan Huang[2], Jai-Sing Yang[3] and Fu-An Chen[2,*]

[1]Department of Ophthalmology, Kaohsiung Armed Force General Hospital, Kaohsiung, Taiwan.
[2]Department of Pharmacy and Graduate Institute of Pharmaceutical Technology, Tajen University, Pingtung, Taiwan.
[3]Departments of Pharmacology, School of Pharmacy, China Medical University, Taichung 404, Taiwan.

ABSTRACT

This study evaluated the effective of antioxidant and antifatigue potential of *Ophiopogon japonicus* extracts (OJE) in exercised Sprague Dawley rats. The OJE was prepared and measured its antioxidant components (total polyphenols, flavonoids and polysaccharides) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity. OJE were administered to endurance exercise rats by gavages for 8 weeks. An exhaustive exercise examination on a treadmill and the measurements of body weight, biochemical parameters (i.e. hepatic glycogen; serum urea nitrogen; lactate) and antioxidant potential including malondialdehyde (MDA), superoxide dilmutase (SOD) and total antioxidant capacity (ABTS: 2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulphonic acid) scavenging activity) related to fatigue that were carried out to investigate the antioxidant and antifatigue effect of OJE. Our results showed that OJE contained polyphenols, flavonoids and polysaccharides in which polysaccharides content had about 17 times more than that of polyphenols and flavonoids. OJE was able to significantly scavenge DPPH radical in a concentration-dependent manner. OJE had no significant effect on the body weight of rats in whole experimental period. In addition, rats in MOJE (middle-dose) and HOJE (high-dose) groups significantly increased the hepatic glycogen to exhaustion when compared with that of the control group. OJE also significantly extend the endurance time of treadmill running to exhaustion, as well as decreased the blood lactate and serum urea nitrogen contents in comparison to that of control group. Finally, MDA level of rats in all OJE treated groups were significantly decreased, while the total antioxidant ability and SOD contents of MOJE and HOJE groups were significantly higher than that of the control group. Our findings indicate that OJE is capable of ameliorating biochemical

parameters which are related to fatigue in the exercised rat model that could be associated with the antioxidant components and with free radical scavenging activities.

Keywords: antioxidant activity, antifatigue; *Ophiopogon japonicus* extracts; exercised rats.

INTRODUCTION

Fatigue is a multifaceted event that can be described as a time-dependent exercise-induced reduction in the maximal force generating capacity of a muscle[1]. Alteration in performance tends to vary across sports that are prejudiced more or less by factors like decreased motor skill performance, decreased endurance and muscular power and mental lapses[2]. The available therapies for fatigue in modern medicine are very limited, and thus potential alternatives from traditional Chinese medicine (TCM) and their respective mechanisms were investigated[2-5].

The genus *Ophiopogo*n (Liliaceae) comprises in the region of species and some varieties distributed in East and South Asia and in China[6]. *Ophiopogon japonicus* is an evergreen perennial and widely distributed in China[6,7]. The tuberous roots of *Ophiopogon japonicus* aponicus (known as Maidong) have been used as traditional Chinese medicine to cure acute and chronic inflammation and cardiovascular diseases for a long time[6]. The previous phytochemical investigations have revealed that *Ophiopogon japonicus* is rich in flavonoids and polysaccharides[8,9]. Scientific literature has indicated that *Ophiopogon japonicus* possesses various pharmacological properties including antithrombotic activity[10], anti-inflammatory activity[11], antimicrobial activity[12], antioxidant ability[9,13], hypoglycemic activity[14], and antitumor activity[15]. However, there is only diminutive available information concerning the antioxidant and its effect on biochemical parameters related to anti-fatigue of *Ophiopogon japonicus* extract (OJE). The present study is to clarify the antioxidant activities of OJE and its effect on biochemical parameters related to laboratory rats involved in the physical exercise experiments.

MATERIALS AND METHODS

MATERIALS

The *Ophiopogon japonicus* were purchased from local herbal medicine store, Pingtung Taiwan. Gallic acid, rutin, 1,1-diphenyl-2-pycrylhydrazyl (DPPH), Folin–Ciocalteu reagent, 2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulphonic acid) (ABTS), galactose and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased form Sigma Chemicals (St. Louis, MO, USA). Commercial diagnostic kits used to determine blood lactate and serum urea nitrogen (SUN) were purchased from Roche Diagnostics (Basel, Switzerland). Superoxide dismutase (SOD) and total antioxidant capacity kit (ABTS scavenging assay) was the product from Randox Laboratories (Antrim, UK). All other chemicals were of analytical reagent grade.

Preparation of *Ophiopogon japonicus* extracts (OJE)

OJE were prepared in our laboratory as

following description. Five hundred grams of *Ophiopogon japonicus* was added to 2000 ml of distilled water and then refluxed for 3 h in a reflux extraction apparatus (Angu, Kaohsiung, Taiwan). After that, the aqueous extract solution was filtered using filter paper and filter funnel. The filtered extract was further lyophilized to obtain aqueous extract of burdock by a freeze dryer (Panchun, Taipei, Taiwan). OJE was stored in an electronic dry cabinet (Komry, Taipei, Taiwan) for following study.

Determination of total polyphenols, flavonoids, polysaccharides and DPPH scavenging activity in OJE

Total polyphenols in OJE were measured spectrophotometrically using the Folin-Ciocalteu reagent based on a colorimetric oxidation/reduction reaction as protocol described previously[16-18]. The amount of total polyphenols was expressed as microgram of the gallic acid equivalent (GAE). Flavonoid content was measured based on the formation of chelatic colorimetrable compounds when reacted with aluminium chloride[19,20]. Briefly, 0.5 mL of diluted OJE solution was prepared in 80 % methanolic solution and then mixed with 0.15 mL of 10% aluminium chloride and 2 mL of 4 % sodium hydroxide, and then stand for 15 min at room temperature. The amount of flavonoids was calculated from the calibration curve of rutin standard solutions at 510 nm with a spectrophotometer (Hitachi, Tokyo, Japan) and expressed in mg/g as rutin equivalent (RE). The content of polysaccharides in OJE was determined by phenol-sulfuric acid method[21,22]. Briefly, 1 mL of OJE solution was mixed with 1 mL of 5 % phenol solution and 5 mL of concentrated sulfuric acids. The absorbance was measured at 490 nm (Hitachi, Tokyo, Japan) after shaking the mixtures in water bath at 30º C for 30 min. The amount of polysaccharides was calculated from the calibration curve of galactose standard solutions. The antioxidant activity of OJE was evaluated using DPPH free radical-scavenging assay as protocol described previously[16,23].

Animal administration

The experimental procedures for our study were approved by the appropriate animal care and use committees (approval #IACUC-100-19, Tajen University, Pingtung County, Taiwan). Total 32 male Sprague-Dawley rats obtained from BioLASCO Taiwan Co. Ltd. were used in this study. They were housed in a light (12 h light: dark cycle) and temperature (22 ± 2 °C) controlled animal facility. Food and water were available *ad libitum*. To determine the bio-activities of OJE in endurance training rats, animals were randomly divided into four groups: control group, and low (200 mg/kg B.W.), middle (400 mg/kg B.W.) and high (800 mg/kg B.W.) doses of OJE-treated groups.

Treadmill exercise, biochemical and antioxidant analysis

The whole experimental period is 8 weeks. All experimental rats were trained on the treadmill (rat/mouse treadmill T306, Singa, Taipei, Taiwan) for twelve days before exhaustive exercise according to the exercise program same as described previously[16,24]. Motivation was provided by an electric shock zone at the

rear of each compartment. On the day of the exhaustive exercise, rats were required to run to exhaustion on the treadmill at a final speed of 24 mph. The point of exhaustion was determined when the rat was unable to right itself when placed on its back. After that, the blood samples from rats were collected from orbital puncture before exercise and after exhaustion by a microcapillary tube with the rat anesthetized. These blood samples were for serum urea nitrogen and lactate test. After exhaustive exercise, the rats were sacrificed by carbon dioxide (CO_2) inhalation. The blood from the hepatic veins of scarified rats was collected for evaluations of MDA, total antioxidant ability (ABTS scavenging assay) and SOD enzyme level. After blood was taken, part of the liver in rats was used for hepatic glycogen test as protocol described previously[16,25]. After experiment, the liver and kidney of the rats were cleaned and then soaked in 10 % neutral formalin solution. The fixed liver and kidney were embedded in paraffin wax and processed in a paraffin tissue processing machine (Leica, Nussloch, Germany). Sections were made at a thickness of 5 µm and stained with hematoxylin and eosin for histopathology assessment. In biochemical and antioxidant analyses, the blood lactate, SUN, SOD and total antioxidant activity (ABTS scavenging assay) were tested according to the recommended procedures provided by the commercial diagnostic kit, while MDA was same as protocol described previously[16,26].

Statistical analysis

All the experimental values are presented in the means ± standard deviation (SD). Statistical comparisons were made by one-way ANOVA and subsequently applying Duncan test was performed using an SPSS statistical software. Statistical significance was defined as $p < 0.05$.

RESULTS AND DISCUSSION

Antioxidant compositions and the free radical scavenging activity of OJE

In preparation of OJE, 33.2 % of recovery was obtained and then subjected to analysis of total polyphenols, flavonoids, polysaccharides and the DPPH free radical scavenging activity. The compositions of OJE can be seen in Table 1. As shown in table 1, polyphenols and flavonoids had similar level in OJE, while polysaccharides content had about 17 times more than that of polyphenols and flavonoids indicating that polysaccharides was abundant in OJE. In case of antioxidant activity of OJE, the free radical scavenging activity of OJE was evaluated using a DPPH free radical scavenging activity. As shown in Table 2, OJE possessed significant DPPH inhibitive activity in a concentration-dependent manner with IC_{50} about 2.5 µg/mL. It has been reported *Ophiopogon japonicas* with antioxidant activity was relative to the flavonoids and polysaccharides[9,13]. The results suggested that OJE with significant antioxidant activity could be associated with antioxidant compositions including total polyphenols, flavonoids and polysaccharides.

Table 1: The antioxidant compositions of OJE

Ingredients	Amount
Polyphenols	17.29±0.69 mg GAE/g
Flavonoids	18.60±0.47 mg RE/g
Polysaccharides	312.65±0.64 mg/g

Data are presented as the mean ± SD (n = 3).

GAE: Gallic acid equivalent; RE: Rutin equivalent

Table 2: Effect of OJE on DPPH free radical scavenging activity

OJE (μg/mL)	DPPH inhibition (%)[a]
5	61.44±0.88
2.5	52.11±1.31
1.25	42.61±0.95
0.625	37.30±0.58
0.312	31.99±1.15

[a]The free radical scavenging activity was evaluated as the DPPH scavenging percentage based on the reduction of the absorbance at 490 nm in the presence of OJE for 30 min. Data are presented as the mean ± SD (n = 3)

Effects of OJE on physical fatigue and its antioxidant and biochemical parameters relative to fatigue in rats

The body weights of the rats were measured after gavage administration with LOJE (low-dose, 200 mg/kg), MOJE (middle-dose, 400 mg/kg) and HOJE (high-dose, 800 mg/kg) for eight weeks. The results in Fig. 1 showed that the increased weights in the experimental groups were no significant difference compared with the control group. Thus, we suggest that OJE had no effect on the body weight of rats.

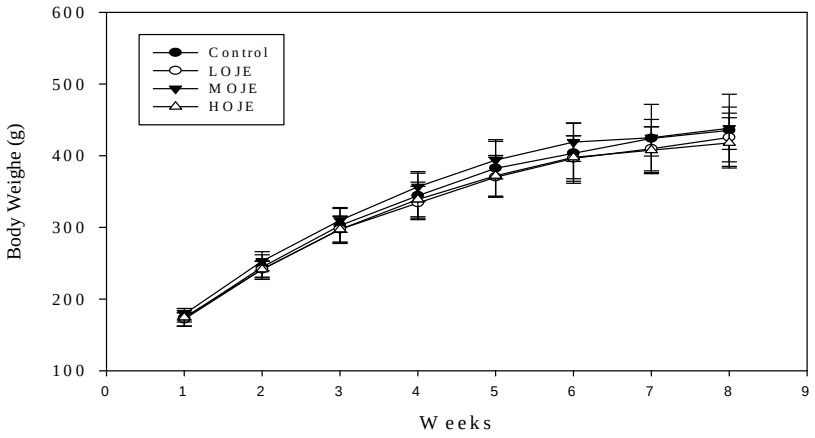


Fig. 1: Effect of OJE on the body weight of rats.
Values represent the means ± SD (n=8). LOJE: low dose (200 mg/kg);
MOJE: middle dose (400 mg/kg); HOJE: high dose (800 mg/kg)

In order to measure the antifatigue effect of OJE, physical fatigue using exhaustive test on the treadmill in rat was carried out. As shown in Fig. 2, the MOJE and HOJE increased significantly the endurance time to exhaustion in the rats when compared with that of the control group ($p < 0.01$). However, endurance time in LOJE group showed no significant difference compared with that of the control group ($p > 0.05$). The exhaustive test on the treadmill has been used broadly for the measurement of the antifatigue property of herbal medicine[27]. Our data showed that OJE was able to extend significantly the endurance time to exhaustion of rats, indicating that OJE had antifatigue activity and could enhance the exercise tolerance.

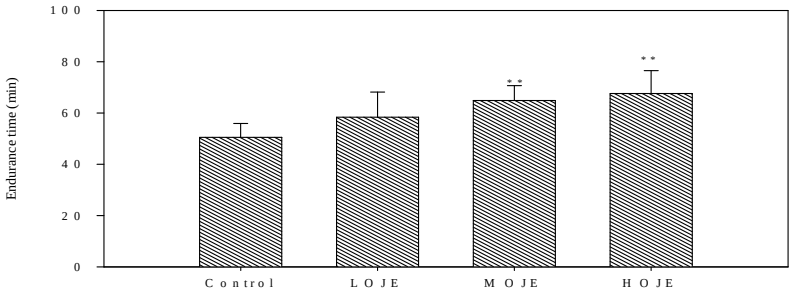
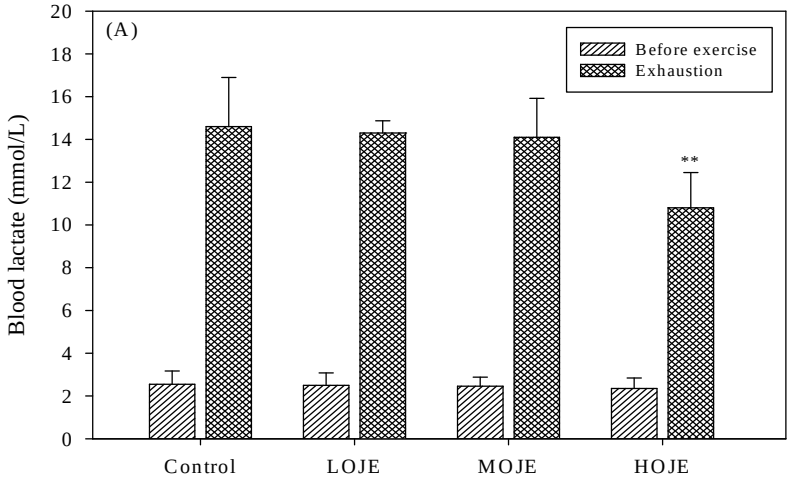


Fig. 2: Effect of OJE on endurance time to exhaustion of rats. Values represent the means ± SD (n = 8). $^{**}p < 0.01$ as compared with control. LOJE: low dose (200 mg/kg); MOJE: middle dose (400 mg/kg); HOJE: high dose (800 mg/kg)

In order to verify the effect of OJE on biochemical parameters relative to fatigue, blood lactate, SUN, and hepatic glycogen were evaluated. As shown in Fig. 3, blood lactate and serum urea nitrogen (SUN) contents showed significant increase after exhaustion in comparison to the control group ($p < 0.01$). HOJE group was able to decrease significantly the blood lactate level after exhaustion compared with that of control group ($p < 0.01$) (Fig. 3A). After exhaustion, serum urea nitrogen (SUN) contents of MOJE and HOJE were significantly lower than that of the control group ($p<0.01$) (Fig. 3B). In case of hepatic glycogen, both MOJE and HOJE treating groups were significantly increased in the hepatic glycogen after exhaustion in comparison to the control group ($p < 0.01$) (Fig. 4). The above results indicated that OJE had antifatigue activity with ameliorating biochemical parameters related to fatigue in the exercised rat. Blood lactate is the glycolysis product of carbohydrate under anaerobic conditions[28]. The accumulation of lactate can interfere with nerve impulses and muscle contraction resulting in fatigue[29]. Urea is synthesized by hepatocytes from ammonia generated by catabolism of protein and amino acids[30]. After a long time of exercise, SUN will obviously increase from a stronger catabolic metabolism when the body cannot obtain enough energy by sugar and fat catabolic metabolism[30]. The accumulation of ammonia is relative to physical fatigue[30]. Decrease of blood lactate and SUN formation is thus beneficial to relieving fatigue. In case of hepatic glycogen, muscle glycogen will be exhausted after strenuous exercise, and later, energy will then derived from circulating glucose released from liver through breakdown of glycogen[31]. The hepatic glycogen is important parameter relative to fatigue. After exhaustive exercise, our results revealed that JOE was able to lower the blood lactate and SUN formation, and increase hepatic glycogen level by reserving glycogen and/or reduction of glycogen consumption, which is beneficial to postpone the appearance of physical fatigue.



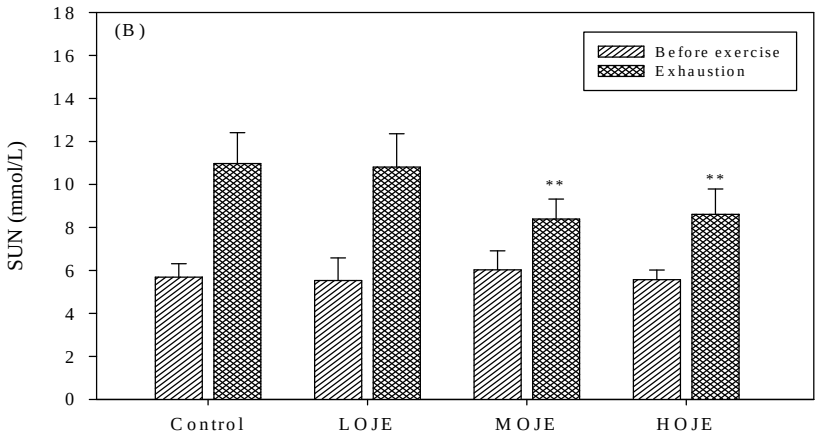


Fig. 3: Effect of OJE on blood lactate and serum urea nitrogen (SUN) of rat. Values represent the means ± SD (n = 8). $^{**}p < 0.01$ as compared with control. LOJE: low dose (200 mg/kg); MOJE: middle dose (400 mg/kg); HOJE: high dose (800 mg/kg)

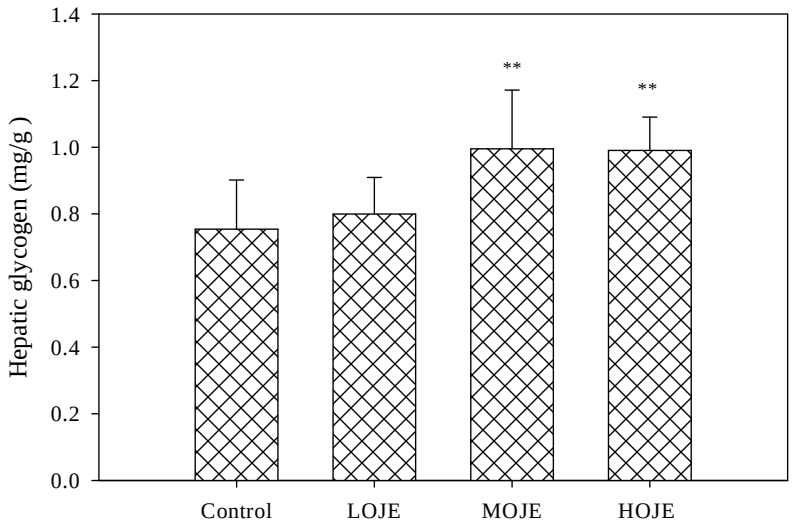


Fig. 4: Effect of OJE on hepatic glycogen of rats. Values represent the means ± SD (n = 8). $^{**}p < 0.01$ as compared with control. LOJE: low dose (200 mg/kg); MOJE: middle dose (400 mg/kg); HOJE: high dose (800 mg/kg)

To evaluate the antioxidant potential of OJE in rats, we conduct the total antioxidant ability (ABTS scavenging assay), MDA and SOD enzyme level relative to fatigue after exhaustive exercise. The ABTS free radical scavenging assay is based on the ability of the

antioxidants to scavenge the long-life radical cation ($ABTS^{\cdot+}$)[32]. The total antioxidant ability was expressed as trolox equivalent antioxidant capacity (TEAC). As shown in Table 3, the TEAC values in MOJE and HOJE groups after exhaustive exercise were significantly enhanced compared with that of control group ($p < 0.01$) indicating JOE can increase the total antioxidant ability in exercise rats. In addition**,** SOD level of MOJE and HOJE groups were significantly higher than that of the control group ($p < 0.01$) after exhaustive exercise. SOD is very important in protecting against oxygen free radical damage that results in direct peroxidative damage to cellular components[27]. Exhaustive exercise can increases the degree of lipid peroxidation and reduces the antioxidant activity[33]. The results indicate that OJE were able to up-regulate antioxidant enzyme activities to protect against oxidative stress induced by exhaustive exercise. In case of MDA, MDA levels in all OJE treating groups revealed significant decrease compared with that of control group after exhaustive exercise ($p<0.05$) . MDA, the end product of lipid peroxidation, is a good marker of free radical radical-mediated damage and oxidative stress[34]. Most studies show that endurance exercise causes an increase in MDA[27]. In this experiment, the MDA level decreased apparently in the all OJE treated groups indicating that OJE had the effective antioxidants preventing from the lipid peroxidation damage.

The mechanism of anti-fatigue of *Ophiopogon japonicus* is still unclear. Different types of hypothesis on fatigue were proposed due to its complex phenomenon and multiple factors involvement[34]. Free radical theory has most attracted much attention in antifatigue study. Free radical production during exercise contributes to fatigue and antioxidant treatment might be a valuable therapeutic approach[35]. It has been reported *Ophiopogon japonicus* with polysaccharides and flavonoids has antioxidant activity[8,9]. In the present study, we demonstrated OJE contain polysaccharides, flavonoids and polyphenols having free radical scavenging activity. The results suggested that the free radical scavenging activity and these antioxidant components of OJE could be involved in ameliorating biochemical parameters related to fatigue. In case of safety evaluation, there are no apparent abnormal histopathology changes in the liver and kidney of rates fed with OJE for 8 weeks (data not shown) indicating that OJE is safe as functional food.

Table 3: Effects of OJE on total antioxidant ability, superoxide dilmutase and malondialdehyde levels in rat after exhaustive exercise

Items	Groups			
	Control	LOJE	MOJE	HOJE
TEAC (mg trolox /mg protein)	0.10±0.01	0.11±0.01	0.13±0.02**	0.16±0.02**
SOD (U/mg protein)	64.45±8.22	71.55±4.03	80.64±8.55**	80.10±10.3**
MDA (nmol/mg protein)	0.15±0.06	0.12±0.03*	0.11±0.02*	0.08±0.02**

*p<0.05 and **p<0.01 as compared with control. Values represent the means ± SD (n=8).

TEAC: trolox equivalent antioxidant ability using ABTS scavenging assay; MDA: malondialdehyde; SOD: superoxide dilmutase. LOJE: low dose (200 mg/kg); MOJE: middle dose (400 mg/kg); HOJE: high dose (800 mg/kg)

In conclusion, we have demonstrated that OJE could extend the endurance time to exhaustion of the rats besides increasing the total antioxidant ability (TEAC values), blood SOD levels and decreasing the blood lactate, SUN contents and MDA levels. The overall results indicate that *Ophiopogon japonicus* is a safe functional food with ameliorating biochemical parameters related to fatigue. OJE with anti-fatigue activity may be associated with its antioxidant components (polysaccharides, flavonoids and polyphenols) and free radical scavenging activity. Further studies are needed to clarify the detailed mechanism(s) involved in the anti-fatigue property of OJE.

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