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**Research Article**

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# *In vitro* comparative studies of antioxidant activities of venlafaxine and *o*-desmethylvenlafaxine

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**ABSTRACT**

*In present investigation, the in vitro antioxidant activities of venlafaxine (VEN) and O-desmethylvenlafaxine (ODV) were studied in DPPH, hydroxyl radical, reducing power, and ferrous ion-chelating models. In DPPH and reducing power model, the phenolic hydroxyl group of ODV contributed to the antioxidant capacities, where the antioxidant activities of ODV was better than that of VEN. In hydroxyl radical and ferrous ion-chelating models, the antioxidant capacities of ODV was equal or somewhat inferior to that of VEN, which indicated that the phenolic hydroxyl contributed a little in these in vitro antioxidant models. The current results might provide certain proofs to obtain the insight of action mechanisms of VEN and ODV.*

**Keywords**: venlafaxine, *O*-desmethylvenlafaxine, antioxidant activities, *in vitro*.

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## INTRODUCTION

Depression is a common mental disease estimated to affect some 350 million people worldwide [1]. In fact, a World Mental Health Survey conducted in 17 countries in 2012 found that about 5% of people reported having an episode of depression in the previous year [2]. The condition displays a high rate of lifetime incidence, early age onset, high chronicity and significant role impairment. Despite a wide range of pharmacotherapeutic options, response to antidepressant medication is subject to delayed onset and is highly variable. It is also not without significant adverse effects. Thus the search for improved antidepressant drugs remains an ongoing concern.

Venlafaxine (VEN) is a bicyclic phenylethylamine-based antidepressant which selectively blocks presynaptic reuptake of norepinephrine (NE) and serotonin (5-HT) without blocking histaminergic, muscarinic or $\alpha_1$-adrenergic receptors [3, 4]. Because of this action as a selective 5-HT-NE reuptake inhibitor (SNRI), VEN has a wide therapeutic index and improved tolerability profile when compared with tricyclic antidepressants (TCAs) [5]. Apart from depression, VEN is also used to treat generalized anxiety, obsessive-compulsive and panic disorder as well as social phobia [6]. Its most common side effects are nausea, somnolence, dizziness, dry mouth, and sweating [7].

*O*-Desmethylvenlafaxine (ODV, also known as desvenlafaxine) is the major (56%) metabolite of VEN (Figure 1) with antidepressant activity similar to that of VEN but with a much longer half-life [11–13]. ODV is mainly metabolized by UDP-glucuronosyltransferase enzymes [14], making it less prone to polymorphic pharmacokinetic and pharmacodynamic variability than VEN [10]. These favorable properties led to ODV being approved by FDA in 2008 for the treatment of major depressive disorder.

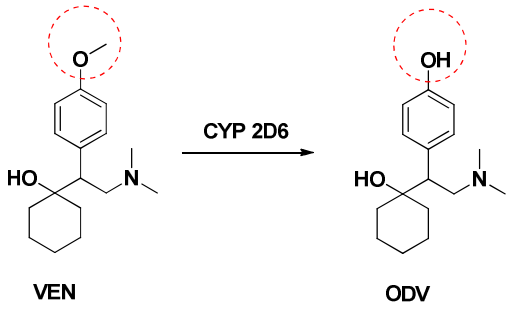


**Figure 1. The conversion of venlafaxine to its principal metabolite *O*-desmethylvenlafaxine by CYP2D6**

Depression is associated with an increase in oxidative stress and a decrease in antioxidant status, which damage neurons and play an important role in the pathophysiology of depression [15]. People who suffer from major depressive disorder display lower serum/plasmatic total antioxidant potentials [16] and reduced brain GSH levels [17] as compared with normal controls. Some phenolic substances, such as vanillin [18], thymol [19], and flavonoids [20], have free radical scavenging activities. In *vivo*, VEN is converted by CYP 2D6 enzyme through demethylation reaction to generate ODV, which is also a phenolic compound. Based on the above metabolic profile, we infer that ODV may have the antioxidant activity, which is beneficial for the treatment of depression. In previous work, the ester prodrugs of ODV were designed and synthesis, and evaluated their pharmacokinetic properties both in rats and in beagle dogs [21].

In present investigation, we focused on the contributions of phenolic hydroxyl group of ODV to the antioxidant activities. Hence, the in *vitro* antioxidant activity of ODV will be tested in four different methods, and compared with those of VEN to obtain the further insight of antidepressant effects.

## EXPERIMENTAL SECTION

### 1. General

ODV (*O*-desmethylvenlafaxine) and VEN (venlafaxine)was purchased from Dingjin Chemical Co., Ltd (Zibo City, China). DPPH (2, 2-diphenyl-1-picrylhydrazyl), tris (tris(hydroxymethyl) aminomethane), ferrozine, and 1,10-phenanthroline monohydrate were purchased from Sigma-Aldrich Chemical Reagent Co., Ltd (Shanghai, China). Ferric chloride, ferrous sulfate, and vitamin C were obtained from Energy Chemical Reagent Co., Ltd (Shanghai, China). Other commercial reagents and solvents were analytical grade and used without further purification. Ultraviolet-visible spectrophotometer 752N was provided by Shanghai Jinjke Industrial Co., Ltd (Shanghai, China).

### 2. 2-Diphenyl-1-picrylhydrazyl (DPPH) Assay

DPPH radical scavenging activity was determined by the method reported according to Wang et al [22]. The absorbance (A) of various concentrations of ODV and VEN (0.2、0.4、0.6、0.8、1.0 mg/mL) were determined at the wavelength of 517 nm respectively, and vitamin C (0.002、0.004、0.006、0.008、0.01 mg/mL) were used as positive control. 2 mL of distilled water was added to 0.1 mmol/L DPPH solution to test the absorbance (Ao), 2 mL of sample solution was added to absolute ethanol to test the absorbance (Aj), and 2 mL of sample solution was added to 0.1 mmol/L DPPH solution to test the absorbance (Ai). The clearance ratio (%) was calculated as the following formula: [1-(Ai - Aj)/Ao] × 100%.

### 3. Hydroxyl Radical-Scavenging Assay

Hydroxyl Radical-Scavenging activity was determined by Fenton method [23]. 1 mL of 1,10-phenanthroline monohydrate (0.75 mmol/L), 2 mL of PBS buffer (pH 7.4), and 1 mL of ferrous sulfate solution (0.75 mmol/L) were added to test tube and mixed, then 1 mL of hydrogen peroxide solution (0.12%) was added. After incubation at 37℃ for 60 min, the absorbance (Ap) was determined at the wavelength of 536 nm. Hydrogen peroxide solution was replaced with distilled water to determine the absorbance (Ab). Distilled water was replaced with various concentrations of ODV and VEN (0.2、0.4、0.6、0.8、1.0 mg/mL) to determine the absorbance (As) respectively, and vitamin C (0.2、0.4、0.6、0.8、1.0 mg/mL) were used as positive control. The clearance ratio (%) was calculated as follows: [(As - Ap)/(Ab - Ap)] × 100%.

### 4. Reducing Power Assay

Reducing power was determined by the method according to Moein et al [24]. 1 mL of various concentration of ODV and VEN (0.2、0.4、0.6、0.8、1.0 mg/mL), 2.5 mL of PBS buffer (pH 6.6), and 2.5 mL of potassium ferricyanide solution (1%) were added to a test tube, mixed and incubated at 50℃ for 20 min. 2.5 mL of

trichloroacetic acid solution (10%) was added to quench the reaction, and centrifugated at 5000 rpm /min for 10 min, then 2.5 mL of supernatant, 2.5 mL of distilled water, and 0.5 mL of ferric chloride solution were added and mixed. After 10 min, the absorbance was measured at the wavelength of 700 nm. vitamin C (0.2、0.4、0.6、0.8、1.0 mg/mL) were used as positive control.

**5. Ferrous Ion-Chelating Assay**

Ferrous ion-chelating capacity was determined using the method reported by Erdogan-Orhan et al [25]. The absorbance (A) of various concentrations of ODV and VEN (0.25、0.5、1.0、2.0、4.0 mg/mL) were determined at the wavelength of 562 nm respectively, and EDTA (0.25、0.5、1.0、2.0、4.0 mg/mL) were used as positive control. 1 mL of distilled water was added to the mixture of ferrous sulfate (0.1mmol/L) and ferrozine (0.25mmol/L) solutions to test absorbance (Ao), distilled water was replaced with1 mL of sample or 1 mL of EDTA to test the absorbance of sample (Ai) or the absorbance of positive control (Aj). The clearance ratio (%) was calculated as the following formula: [Ao-(Ai - Aj) /Ao] × 100%.

## RESULTS AND DISCUSSION

**1. 2-Diphenyl-1-picrylhydrazyl (DPPH) Assay**

As shown in Figure 2, DPPH radical scavenging activities of ODV and VEN were all increased with the elevation of sample concentration, their clearance ratio (CR) reached the peak values at a concentration of 1.0 mg/mL (17.01 *vs.* 11.65 %). The results indicated that DPPH radical scavenging activity of ODV was superior to that of VEN, but inferior to that of vitamin C (0.01 mg/mL, 92.43%). The $IC_{50}$ values also confirmed the above fact (ODV:1.34 mg/mL, VEN: 1.46 mg/mL, 0.013 mg/mL).

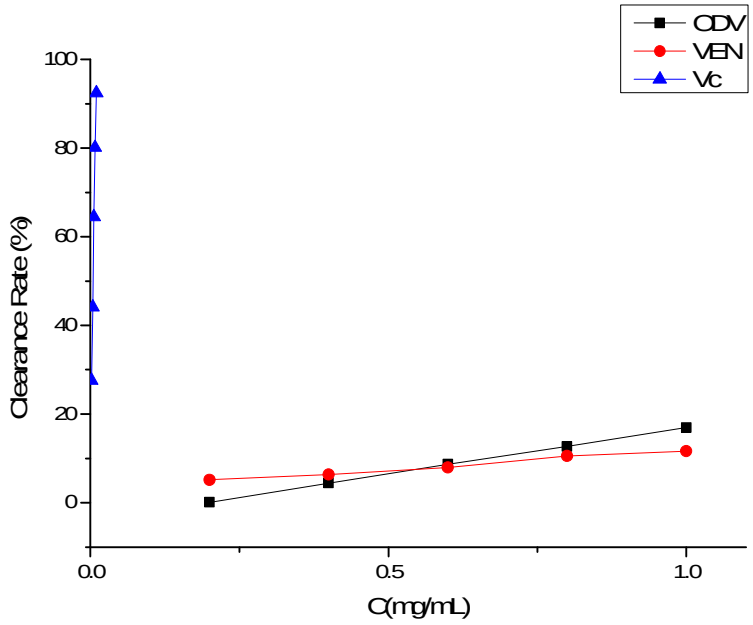


**Figure 2. DPPH radical scavenging activities of ODV and VEN**

**2. Hydroxyl Radical Scavenging Assay**

As shown in Figure 3, hydroxyl radical scavenging activities of ODV and VEN were all increased with the elevation of sample concentration, CR values reached the peak values at a concentration of 1.0 mg/mL (9.92 *vs.* 15.86 %), and the peak CR value of vitamin C was 45.28 %. The corresponding $IC_{50}$ were 1.47 mg/mL, 1.46 mg/mL, and 1.10 mg/mL. These results showed that the hydroxyl radical scavenging capacities of ODV was somewhat inferior to those of VEN and vitamin C.

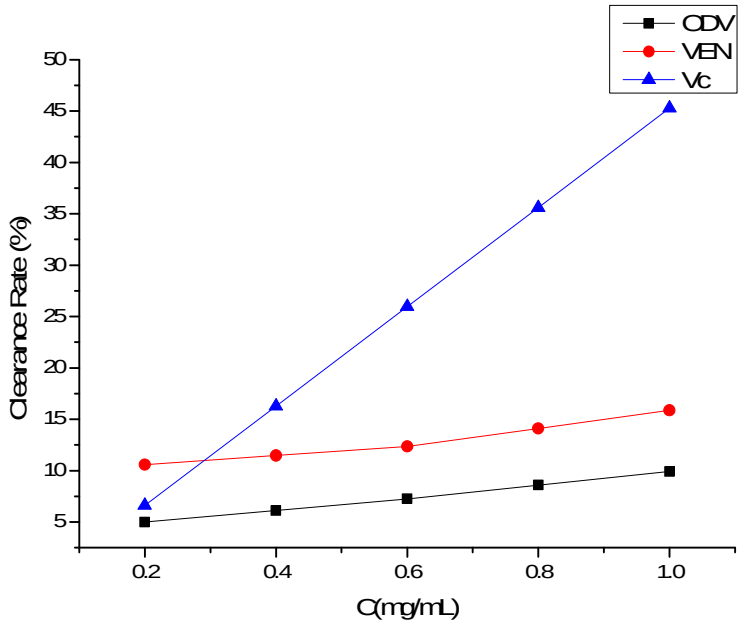


**Figure 3. Hydroxyl radical scavenging activities of ODV and VEN**

### 3. Reducing Power Assay

As shown in Figure 4, reducing powers of ODV and VEN were all increased with the elevation of sample concentration, the absorbance (A) reached the peak values at a concentration of 1.0 mg/mL (0.84 *vs.* 0.21), and the peak A value of vitamin C was 1.14 at a concentration of 0.2 mg/mL. The results indicated that both ODV and VEN were electron donors, which combined with radicals to terminate the reaction. Owing to the existence of phenolic hydroxyl group, the reducing power of ODV was stronger than that of VEN, but inferior to that of vitamin C.

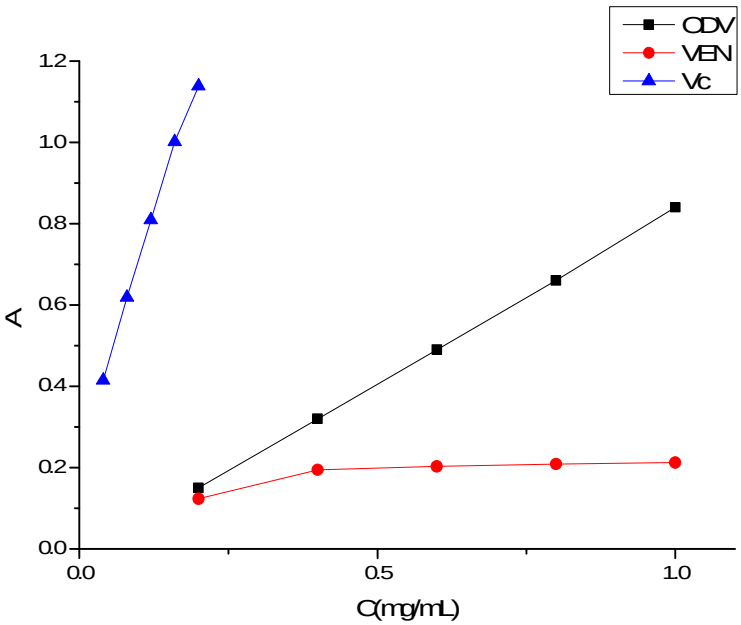


**Figure 4. Reducing powers of ODV and VEN**

### 4. Ferrous Ion-Chelating Assay

As shown in Figure 4, at a concentration of 4 mg/mL, the CR values of ODV, VEN, and vitamin C were 3.80 %, 5.41 %, and 104.01% respectively, the corresponding $IC_{50}$ were 13.34 mg/mL, 13.29 mg/mL, and 9.49 mg/mL. The results indicated that ferrous ion-chelating capacities ofODV was a little inferior to that of VEN, both of them were greatly inferior to that of EDTA. From the slope of C *vs.* CR, we can observe that CR values were not well consistent with the concentration increased.

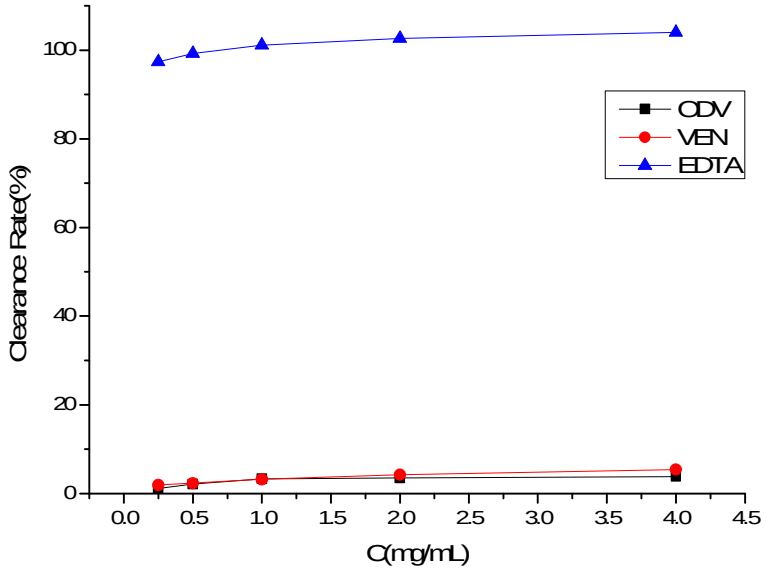


**Figure 5. Ferrous ion-chelating capacities of ODV and VEN**

**Acknowledgments**
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## CONCLUSION

In present investigation, the in *vitro* antioxidant activities of venlafaxine (VEN) and *O*-desmethylvenlafaxine (ODV) were studied in four different antioxidant models. The results indicated that the phenolic hydroxyl group of ODV contributed to the antioxidant capacities in DPPH and reducing power models, where the in *vitro* antioxidant activities of ODV was better than that of VEN. Nevertheless, in hydroxyl radical and ferrous ion-chelating models, the antioxidant capacities of ODV was equal or somewhat inferior to that of VEN, which indicated that the phenolic hydroxyl contributed a little in these in *vitro* antioxidant models. Our present research may provide some proof for the further investigation of the antidepressant mechanism of ODV and VEN.

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