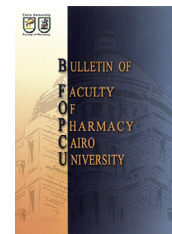HOSTED BY

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ORIGINAL ARTICLE

Combined hepatoprotective and antidepressant effects of resveratrol in an acute model of depression

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Abstract There are numerous herbal medicines that have been introduced into psychiatric practice because of greater compliance and milder side effects. *Polygonum cuspidatum* is a native Asian plant; known for its medicinal properties and traditionally used in the treatment of neuropsychiatric disorders, such as psychosocial stress, dementia and Parkinson's disease. Resveratrol is the active ingredient of *P. cuspidatum*. Researchers have suggested that the trans-isomer of resveratrol demonstrates a variety of pharmacological activities including antioxidant, anti-inflammatory, hepatic and neuroprotective properties. In this study we examined the hepatoprotective and antidepressant effects of trans-resveratrol against fluoxetine in an acute reserpine model of depression in rats. Main methods: depression-like behaviors were induced by single reserpine intraperitoneal injection (6 mg/kg, i.p.). Trans-resveratrol (15, 30 and 60 mg/kg bwt) and fluoxetine (24 mg/kg bwt) were administered orally for the following 3 days. Behavioral effects namely open field test (OFT) and forced swimming test (FST) and biochemical parameters namely neurotransmitters levels and antioxidant contents were assessed. Liver histopathological examination was performed. Key findings: Results revealed that resveratrol (60 mg/kg bwt) showed a potential hepatoprotective and an antidepressant-like effects compared to those of fluoxetine.

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1. Introduction

Depression is a common and invalidating mental illness affecting approximately 2.5% of the general population. It has neg-

ative social consequences in terms of reduced employment and psychosocial impairment. Based on the World Health Organization surveys; it has been suggested to become the second leading cause of disability by 2020.[1] Despite a steady increase in the number of antidepressants over the years, the prevalence of the disorder remains stable which may be due to unclear pathophysiology or the inconsistent efficacy of currently available antidepressants with undesirable side effects. However, there is a direct correlation between the catecholaminergic neuronal systems and depression.[2]

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Fluoxetine is a widely prescribed selective serotonin reuptake inhibitor with antidepressant properties.[3] However, it has recently been postulated to induce liver damage and mediates free radical reactions due to its fluorine content.[4]

A variety of consumable plant-derived phytochemicals exhibit nutraceutical properties because they produce physiological benefits and combat disease processes. Emerging evidence suggests that widely accessible and safe organic polyphenolic phytochemicals, in particular, treat depression at much lower concentrations than clinical doses of classical drugs.[5]

Resveratrol (3,5,4′-trihydroxystilbene) is a polyphenolic compound that has been detected in fruits and some flowering plants. It was first isolated from the roots of white hellebore (*Veratrum grandiflorum* O. Loes) in 1940 and later, in 1963 from the roots of *Polygonum cuspidatum*, a plant used in traditional Chinese and Japanese medicine. Other major dietary sources containing resveratrol include grapes, wine, peanuts, and peanut products. The first real interest in this compound came when in 1992 resveratrol was postulated to explain some of the cardio-protective effects of red wine and was suggested to be an important factor in the French Paradox, a term coined to describe the observation that the French population has a very low incidence of cardiovascular disease, despite a diet high in saturated fat. Five years later, in 1997, resveratrol was reported to work as a chemo-preventive agent, by the ability to inhibit carcinogenesis at multiple stages. Meanwhile, also anti-inflammatory and antioxidant properties were identified for resveratrol.[6–9] Additionally, the protective role of resveratrol against a number of hepatic injuries (e.g. cholestasis) due to oxidative damage of primary rat hepatocytes was reported by several authors.[10–12] Moreover, intraperitoneal administration of resveratrol in rats with ligated bile ducts maintained antioxidant defenses and reduced liver oxidative damage and ductular proliferation.[13] Neuro-pharmacological activities such as amelioration of learning and memory impairment and neuroprotective properties have also been reported.[14–17]

The present study aimed to investigate the combined hepatoprotective and antidepressant effects of trans-resveratrol against fluoxetine in a reserpine model of depression in rats.

2. Materials and methods

2.1. Animals

Adult male Wister rats, weighing 130–150 g each, were purchased from the animal house at the National Research Centre (NRC, Giza, Egypt). All animals received care in compliance with the guidelines of the animal care and use committee of the NRC. Upon arrival, the animals were kept in a quiet place, housed eight per cage and acclimatized to a colony room with controlled ambient temperature (22 ± 1 °C), humidity (50 ± 10%) and a 12 h natural light/dark cycle. They were fed a standard diet, water was provided *ad libitum* and they were acclimated for 7 days before entry into the subsequent study. They were allowed free access to water and food throughout the period of investigation. All the procedures described below were carried out in accordance with the guidelines of the EU Directive 2010/63/EU for animal experiments. The experiments were performed with 8 rats per treatment group according to a randomized schedule. In behavioral tests, animals in every group were intermixed during the observation and the observers were unaware of the treatment conditions.

2.2. Drugs and drug administration

Fluoxetine hydrochloride (Prozac 20 mg dispersible tablets, Lilly, Spain), the tablets were freshly suspended in distilled water prior to oral administration. Trans-resveratrol was provided as a generous gift from Jing Tea LLC, it was provided as Harmoni-T micronized trans-resveratrol capsules for ingestion. The powder in the capsules was freshly dissolved in distilled water just before oral administration. Reserpine was a generous gift from Novartis co. Egypt, it was provided as pure powder for injection and it was freshly dissolved in a DMSO/saline mixture (0.1: 10 ml) before intraperitoneal injection.

2.3. Experimental design

Rats were divided into six different groups (8 rats each) and treated as follows; Group (1): normal control (DMSO/saline group). Group (2): depressed group (reserpine group). Group (3): receiving fluoxetine (24 mg/kg, p.o.).[4] Groups (4, 5 and 6): receiving trans-resveratrol (15, 30 and 60 mg/kg, p.o.).

2.4. Experimental procedure

Groups 2, 3, 4, 5 and 6 were administered reserpine (6 mg/kg, i.p.) once on day one of the experiment. This dose results in depression symptoms that persist for 72 h. after injection.[18] Group (1) was administered DMSO/Saline i.p. injections on the same day. Group (1) was kept in separate cages and had free access to food and water till the end of the experiment. All other groups received the corresponding drugs orally for the following 3 days. On day number 2 behavioral tests, namely open field test (OFT) and forced swimming test (FST) were performed. Twenty-four hours later, the rats were killed by decapitation 30 min after the last drug ingestion. Brain and liver tissues were isolated and each brain or liver was washed with cold sterile physiological saline, blotted between two damp filter papers and stored at −80 °C for further biochemical analysis. Parts of the liver tissues were isolated in formalin and used for the histopathology.

2.5. Behavioral tests

2.5.1. Open field test
The open field test was carried out in a square wooden arena (80 cm × 80 cm × 40 cm high) with red walls and white smooth polished floor divided by black lines into 16 equal squares. The test was performed under white light in a quiet room. Each rat was placed at the same corner square and observed during 5 min. The floor and walls were cleaned after testing each rat. The following parameters were recorded during the 5 min observation period; latency: time taken by each animal till it starts moving in the arena, ambulation frequency: number of squares crossed by the animal, rearing frequency: number of times the animal stood stretched on its hind limbs with or without forelimb support.[19–21]
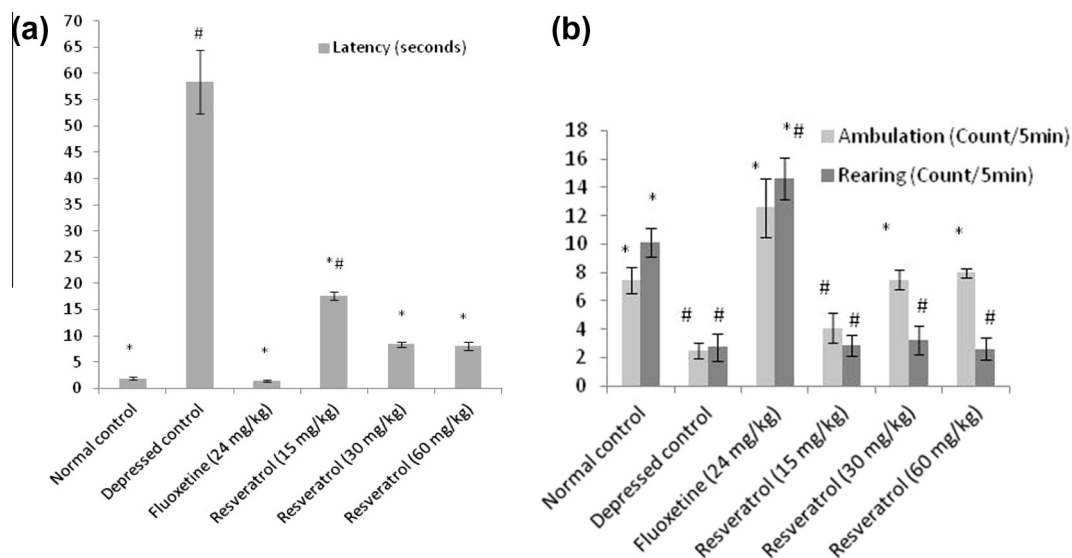
Figure 1 Depression was induced by acute reserpine injection. Statistical analysis for the latency time was carried out by one way ANOVA followed by Tukey HSD post hoc multiple comparison test. Statistical analysis for the ambulation and rearing frequencies was carried out by non parametric K independent samples Kruskal–Wallis *H* test followed by Two-independent-samples Mann–Whitney *U* test. Data were expressed as mean ± SEM, *n* = 8 rats/group. *, significant from depressed control at *P* < 0.05; #, significant from normal control at *P* < 0.05.

2.5.2. Forced swimming test

The forced swimming test was performed according to the method described by Porsolt et al.[22] Each rat was placed for 5 min in a cylindrical water tank (70 cm high, 40 cm diameter) where, water level was about 40 cm and water temperature was maintained at 23–25 °C. The total duration of immobility of each animal was recorded. The tank was emptied and washed with fresh water flush between each rat to remove any traces of urine or feces.

2.6. Biochemical analysis

2.6.1. Determination of brain monoamine contents

Each brain tissue was weighed and homogenized in 75% aqueous HPLC grade methanol (10% w/v). The homogenate was spun at 4000 r.p.m. for 10 min. and the supernatant was isolated. Brain monoamines were detected by HPLC according to the method described by Pagel et al.[23]

2.6.2. Determination of brain and liver MDA and GSH levels

Each brain or liver tissue was homogenized in ice-cold saline (20% w/v).[24] The homogenate was divided into 2 portions for the determination of malondialdehyde (MDA) and reduced glutathione (GSH) levels. The level of MDA was determined according to the method of Ruiz-Larea et al.[25] The level of GSH was determined according to the method of Ellman modified by Bulaj et al.[26,27]

2.7. Statistical analysis

Statistical analyses for the ambulation and rearing frequencies were carried out by non parametric K independent samples Kruskal–Wallis *H* test followed by two-independent-samples

Mann–Whitney *U* test. Statistical analyses for the latency time in the open field test and the immobility duration in the forced swimming test were carried out by one way analysis of variance (ANOVA) followed by Tukey HSD post hoc multiple comparisons test. For all the other parameters, statistical analyses were performed by one way ANOVA followed by the Least Significant Difference post hoc multiple comparisons test (LSD test).

3. Results

3.1. Effect of trans-resveratrol on the open field parameters

The results are represented in Fig. 1a and b. Depression-like behavior induced by reserpine could be clearly demonstrated by the significant decrease in the activity of all rats compared to vehicle control values. Trans-resveratrol (15, 30 and 60 mg/kg, p.o.) dose dependently increased the rat activity significantly compared to the depressed control where, the latency time was significantly decreased at the three dose levels and the ambulation frequency was normalized at the higher two doses. Fluoxetine (24 mg/kg, p.o.) treatment normalized the open field parameters.

3.2. Effect of trans-resveratrol on the immobility duration in the forced swimming test (FST)

Fig. 2 shows the effects of trans-resveratrol (15, 30 and 60 mg/kg, p.o.) and fluoxetine (24 mg/kg, p.o.) on the duration of immobility in the forced swimming test. Post hoc analysis revealed that trans-resveratrol, at doses of 15 and 30 mg/kg, led to a dose-dependent reduction in the immobility period as compared to the depressed control group. Resveratrol
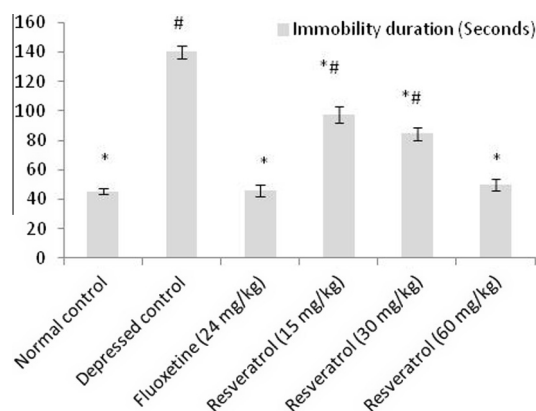
Figure 2 Depression was induced by acute reserpine injection. Statistical analysis was carried out by one way ANOVA followed by Tukey HSD post hoc multiple comparison test. Data were expressed as mean ± SEM, $n = 8$ rats/group. ∗, Significant from depressed control at $P < 0.05$; #, significant from normal control at $P < 0.05$.

60 mg/kg and the classical antidepressant fluoxetine (24 mg/kg, p.o.) normalized the immobility time.

3.3. Effect of trans-resveratrol on the neurotransmitter levels

The levels of monoamines detected are summarized in Table 1. Statistics revealed that both serotonin and dopamine levels were normalized after the administration of fluoxetine (24 mg/kg) and the three dose levels of trans-resveratrol. Nor-epinephrine level was only normalized in case of trans-resveratrol (60 mg/kg, p.o.) and fluoxetine. The two lower doses of trans-resveratrol had no effect on the nor-epinephrine level compared to the depressed group.

3.4. Effect of trans-resveratrol on the brain GSH and MDA levels

Results are represented in Table 2. Acute reserpine injection significantly increased the brain level of MDA compared to the vehicle control group but had no effect on the GSH content. Both fluoxetine (24 mg/kg, p.o.) and trans-resveratrol (60 mg/kg, p.o.) normalized the MDA level.

Table 2 Effect of trans-resveratrol on the brain GSH and MDA levels in depressed rats using the acute reserpine injection model of depression.

Antioxidant parameter	GSH (μmol/g tissue)	MDA (nmol/g tissue)
Groups		
Normal control	2.38 ± 0.046	126.62 ± 2.33*
Depressed control	2.38 ± 0.071	249.14 ± 12.012#
Fluoxetine (24 mg/kg)	2.47 ± 0.077	129.61 ± 4.143*
Resveratrol (15 mg/kg)	2.46 ± 0.125	248.59 ± 11.72#
Resveratrol (30 mg/kg)	2.48 ± 0.141	172.98 ± 5.656*,#
Resveratrol (60 mg/kg)	2.53 ± 0.290	128.16 ± 4.847*

Depression was induced by acute reserpine injection. Statistical analysis was carried out by one way ANOVA followed by LSD post hoc multiple comparison test. Data were expressed as mean ± SEM, $n = 8$ rats/group.
* Significant from depressed control at $P < 0.05$.
# Significant from normal control at $P < 0.05$.

3.5. Effect of trans-resveratrol on the liver GSH and MDA levels

Results are illustrated in Table 3. Acute reserpine injection significantly decreased the GSH content and increased the MDA level compared to the vehicle control. Fluoxetine (24 mg/kg, p.o) showed a significant increase in the GSH content and decrease in the MDA level. Trans-resveratrol at the higher two doses showed better results where the dose of trans-resveratrol (60 mg/kg, p.o.) normalized both the GSH and MDA levels.

3.6. Histopathological examination

Results are represented in Fig. 3a–f. Histopathological examination of the liver sections revealed that induction of depression by reserpine resulted in slight hydropic degeneration of the hepatocytes. Neither fluoxetine nor any of the three resveratrol doses used showed any histopathological changes in the hepatocytes.

4. Discussion

This study was designed to investigate the antidepressant and hepatoprotective effects of resveratrol against fluoxetine in an

Table 1 Effect of trans-resveratrol on the neurotransmitter levels in depressed rats using the acute reserpine injection model of depression.

Neurotransmitter parameter	Serotonin (μg/g tissue)	Norepinephrine (μg/g tissue)	Dopamine (μg/g tissue)
Groups			
Normal control	0.220 ± 0.012*	0.116 ± 0.012*	0.427 ± 0.03*
Depressed control	0.182 ± 0.011#	0.085 ± 0.001#	0.359 ± 0.02#
Fluoxetine (24 mg/kg)	0.253 ± 0.008*,#	0.121 ± 0.005*	0.480 ± 0.02*
Resveratrol (15 mg/kg)	0.229 ± 0.008*	0.093 ± 0.001#	0.439 ± 0.02*
Resveratrol (30 mg/kg)	0.233 ± 0.006*	0.096 ± 0.001#	0.473 ± 0.01*
Resveratrol (60 mg/kg)	0.243 ± 0.007*	0.108 ± 0.001*	0.476 ± 0.01*

Depression was induced by acute reserpine injection. Statistical analysis was carried out by one way ANOVA followed by LSD post hoc multiple comparisons test. Data were expressed as mean ± SEM, $n = 8$ rats/group.
* Significant from depressed control at $P < 0.05$.
# Significant from normal control at $P < 0.05$.

Table 3 Effect of trans-resveratrol on the liver GSH and MDA levels in depressed rats using the acute reserpine injection model of depression.

Antioxidant parameter	GSH (μmol/g tissue)	MDA (nmol/g tissue)
Groups		
Normal control	13.24 ± 0.53[*]	29.4 ± 1.4[*]
Depressed control	8.81 ± 0.18[#]	41.62 ± 2.12[#]
Fluoxetine (24 mg/kg)	10.40 ± 0.85[*,#]	35.82 ± 1.17[*,#]
Resveratrol (15 mg/kg)	9.01 ± 0.7[#]	37.3 ± 2.67[#]
Resveratrol (30 mg/kg)	11.09 ± 0.38[*,#]	34.74 ± 1.73[*]
Resveratrol (60 mg/kg)	11.99 ± 0.35[*]	30.57 ± 2.48[*]

Depression was induced by acute reserpine injection. Statistical analysis was carried out by one way ANOVA followed by LSD multiple comparison test. Data were expressed as mean ± SEM, $n = 8$ rats/group.

[*] Significant from depressed control at $P < 0.05$.

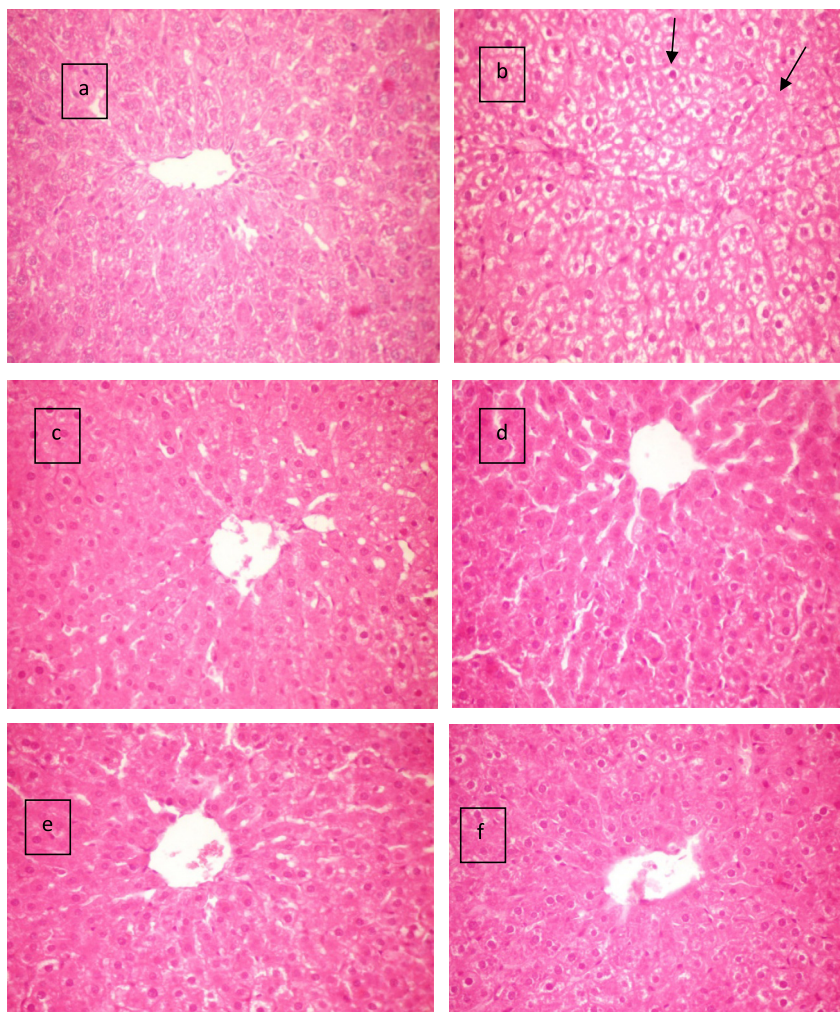[#] Significant from normal control at $P < 0.05$.

Figure 3 (a) Liver of rat from the normal control group showing no histopathological changes (H& E x 400). (b) Liver of rat from the depressed group showing slight hydropic degeneration of hepatocytes (H& E x 400). (c) Liver of rat from the Fluoxetine group showing no histopathological changes (H& E x 400). (d) Liver of rat from the Resveratrol 15 group showing no histopathological changes (H& E x 400). (e) Liver of rat from the Resveratrol 30 group showing no histopathological changes (H& E x 400). (f) Liver of rat from the Resveratrol 60 group showing no histopathological changes (H& E x 400).

acute reserpine model of depression in rats. Soon after the introduction of reserpine, it became apparent that the drug could induce depression in patients being treated for hypertension as well as in normal subjects. Reserpine model is based on the capability of antidepressants to reverse the depressive-like effects of reserpine in rodents.[28–30] Reserpine induces in rats a behavioral and biochemical syndrome that has been extensively studied as a potential animal model of depression.[31–33] Qingjun et al. reported that i.p. injection of reserpine increased floating time in the Porsolt swim test in a dose- and time-dependent manner in rats.[34] Moreover, reserpine was found to induce a syndrome of locomotor hypomotility in the open field test and an increase in the immobility period in the tail suspension test (TST).[35–37] Subsequent research in rats showed that reserpine-induced behavioral depression was correlated to the depletion of brain monoamines. However, although the concentrations of norepinephrine (NE), dopamine (DA), and serotonin (5-HT) in brain remained at low levels for more than a week after acute reserpine treatment (5 mg/kg), the behavioral depression usually ended in 72 h.[18] Furthermore, biochemical analysis revealed that chronic reserpine treatment significantly induced lipid peroxidation, decreased GSH level as well as the levels of antioxidant defense enzymes; superoxide dismutase (SOD) and catalase (CAT) in rat brain tissues.[38–40]

In the present investigation, reserpine resulted in a significant hypo-locomotor activity in the open field test and also a pronounced increase in the immobility duration in the forced swimming test as compared to vehicle control. Moreover, a significant decrease in the brain neurotransmitters was recorded as well as a significant increase in the brain and liver MDA level. On the other hand, acute injection of reserpine only caused a significant decrease in the liver GSH without affecting brain GSH content.

Trans-resveratrol (60 mg/kg, p.o.) reversed reserpine-induced hypo-motility in the open field test and forced swimming test in a manner comparable to that of fluoxetine. These findings are in rhythm with former investigations.[17,41] In addition trans-resveratrol reversed the reserpine-induced reduction in the brain neurotransmitter content. Previous studies showed that resveratrol is an inhibitor of norepinephrine and 5-HT uptake activity in rats.[42] Recent researches also indicated that trans-resveratrol inhibits monoamine oxidase (MAO) activity.[17]

Many studies have focused on the adverse effects of fluoxetine exposure on the liver in both patients and animal models. Elevated levels of aminotransferases, hepatocellular changes and acute hepatitis have been observed among animals as well as patients treated with fluoxetine in clinical trials.[4,43] Thompson et al. (2000) have shown that one of fluoxetine's metabolites, 4-trifluoromethylphenol decreases intracellular glutathione concentration in liver slices.[44] However, Zafir and Banu observed no changes in the antioxidant defense components and oxidative stress markers in the liver of non-stressed rats exposed to 20 mg fluoxetine/kg for 21 days. Moreover; they ascertained that treatment with fluoxetine ameliorates stress-induced oxidative damage.[45] On the other hand, Adachi et al. reported that; hepatic dysfunction was confirmed by a significant increase in liver enzyme activities and uric acid concentration in liver of fluoxetine treated rats providing an evidence for the pathogenic role of fluoxetine in inducing oxidative liver injury.[46] In the present study, fluoxetine ingestion for 3 days following induction of depression by reserpine, normalized the brain MDA level and resulted in a significant decrease in liver MDA level and significant increase in the GSH level compared to the depressed group.

On the other hand, trans-resveratrol; previously reported as hepatoprotective,[47–49] normalized the brain and liver MDA level and liver GSH content and it is worth mentioning that results obtained from the 60 mg/kg dose level were better than those obtained from fluoxetine.

5. Conclusion

Results revealed that acute ingestion of resveratrol (60 mg/kg bwt) for 3 days after induction of depression by reserpine, showed a hepatoprotective and an antidepressant-like effects comparable to those of fluoxetine. Further investigations are required to evaluate long term efficacy and safety of resveratrol as antidepressant.

6. Conflict of interest

None declared.

Acknowledgments

References

1. Serafini G. Neuroplasticity and major depression, the role of modern antidepressant drugs. *World J Psychiatry* 2012;**2**:49–57.
2. Mahesh R, Bhatt S, Devadoss T, Jindal A, Gautam B, Pandey D. Antidepressant potential of 5-HT3 receptor antagonist, N-n-propyl-3-ethoxyquinoxaline-2-carboxamide (6n). *J Young Pharm* 2012;**4**:235–44.
3. Mahaffey PJ, Vu MH, Ritter EJ. Fluoxetine acts as an antagonist at 5-HT$_{2C}$ receptors in the Crayfish neuromuscular junction. *Pioneering Neurosci* 2010;**11**:27–30.
4. Inkielewicz-Stępniak I. Impact of fluoxetine on liver damage in rats. *Pharmacol Rep* 2011;**63**:441–7.
5. Ogle WO, Speisman RB, Ormerod BK. Potential of treating age-related depression and cognitive decline with nutraceutical approaches: a mini-review. *Gerontology* 2013;**59**:23–31.
6. Baur JA, Sinclair DA. Therapeutic potential of resveratrol: the in vivo evidence. *Nat Rev Drug Discov* 2006;**5**:493–506.
7. Chen S, Li Z, Li W, Shan Z, Zhu W. Resveratrol inhibits cell differentiation in 3T3-L1 adipocytes via activation of AMPK. *Can J Physiol Pharmacol* 2011;**89**:793–9.
8. Timmers S, Auwerx J, Schrauwen P. The journey of resveratrol from yeast to human. *Aging* 2012;**4**:146–58.
9. Vang O, Ahmad N, Baile CA, Baur JA, Brown K, Csiszar A, et al. What is new for an old molecule? Systematic review and recommendations on the use of resveratrol. *PLoS One* 2011;**6**:e19881.
10. Ara C, Kirmlioglu H, Karabulut AB, Coban S, Ay S, Harputluoglu M, et al. Protective effect of resveratrol against oxidative stress in cholestasis. *J Surg Res* 2005;**127**:112–7.
11. Cerný D, Kutinová Canová N, Martínek J, Horínek A, Kmonícková E, Zídek Z, et al. Effects of resveratrol pretreatment on tert-butylhydroperoxide induced hepatocyte toxicity in immobilized perifused hepatocytes: involvement of inducible nitric oxide synthase and hemoxygenase-1. *Nitric Oxide* 2009;**20**:1–8.

12. Farghali H, Cerný D, Kameníková L, Martínek J, Horínek A, Kmonícková E, et al. Resveratrol attenuates lipopolysaccharide-induced hepatitis in D-galactosamine sensitized rats: role of nitric oxide synthase 2 and heme oxygenase-1. *Nitric Oxide* 2009;**21**:216–25.

13. Farghali H, Kutinová Canová N, Lekić N. Resveratrol and related compounds as antioxidants with an allosteric mechanism of action in epigenetic drug targets. *Physiol Res* 2013;**62**:1–13.

14. Chen LW, Wang YQ, Wei LC, Shi M, Chan YS. Chinese herbs and herbal extracts for neuroprotection of dopaminergic neurons and potential therapeutic treatment of Parkinson's disease. *CNS Neurol Disord Drug Targets* 2007;**6**:273–81.

15. Kumar A, Naidu PS, Seghal N, Padi SS. Neuroprotective effects of resveratrol against intracerebroventricular colchicine induced cognitive impairment and oxidative stress in rats. *Pharmacology* 2007;**79**:17–26.

16. Ranney A, Petro MS. Resveratrol protects spatial learning in middle-aged C57BL/6 mice from effects of ethanol. *Behav Pharmacol* 2009;**20**:330–6.

17. Xu Y, Wang Z, You W, Zhang X, Li S, Barish PA, et al. Antidepressant-like effect of trans-resveratrol: Involvement of serotonin and noradrenaline system. *Eur Neuropsychopharmacol* 2010;**20**:405–13.

18. Huang QJ, Jiang H, Hao XL, Minor TR. Brain IL-1 beta was involved in reserpine-induced behavioral depression in rats. *Acta Pharmacol Sin* 2004;**25**:293–6.

19. Bellés M, Albina ML, Linares V, Gmez M, Sánchez DJ, Domingo JL. Combined action of uranium and stress in the rat: I. Behavioral effects. *Toxicol Lett* 2005;**158**:176–85.

20. Kim SH, Han J, Seog DH, Chung JY, Kim N, Park YH, et al. Antidepressant effect of Chaihu-Shugan-San extract and its constituents in rat models of depression. *Life Sci* 2005;**76**:1297–306.

21. Pruus K, Vaarmann A, Rudissaar R, Allikmets L, Matto V. Role of 5-HT1A receptors in the mediation of acute citalopram effects. A 8-OH-DPAT challenge study. *Prog Neuropsychopharmacol Biol Psychiat* 2002;**26**:227–32.

22. Porsolt RD, Le Pichon M, Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. *Nature* 1977;**266**:730–2.

23. Pagel P, Blome J, Wolf HU. High-performance liquid chromatographic separation and measurement of various biogenic compounds possibly involved in the pathomechanism of Parkinson's disease. *J Chromatogr B* 2000;**746**:297–304.

24. Mansour MA, Nagi MN, El-Khatib AS, Al-Bekairi AM. Effects of thymoquinone on antioxidant enzyme activities, lipid peroxidation and DT-diaphorase in different tissues of mice: a possible mechanism of action. *Cell Biochem Funct* 2002;**19**:1–10.

25. Ruiz-Larea MB, Leal AM, Liza M, Lacort M, De Groot H. Antioxidant effects of estradiol and 2-hydroxyestradiol on iron-induced lipid peroxidation of rat liver microsomes. *Steroids* 1994;**59**:383–8.

26. Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959;**82**:70–7.

27. Bulaj G, Kortemme T, Goldenberg D. Ionization-reactivity relationships for cysteine thiols in polypeptides. *Biochemistry* 1998;**37**:8965–72.

28. Kumar V, Singh PN, Jaiswal AK, Bhattacharya SK. Antidepressant activity of Indian hypericum perforatum Linn in rodents. *Indian J Exp Biol* 1999;**37**:1171–6.

29. Kim KW, Kim HD, Jung JS, Woo RS, Kim HS, Suh HW, et al. Characterization of antidepressant-like effects of p-synephrine stereoisomers. *Naunyn Schmiedebergs Arch Pharmacol* 2001;**364**:21–6.

30. O'Donnell KC, Gould TD. The behavioral actions of lithium in rodent models: leads to develop novel therapeutics. *Neurosci Biobehav Rev* 2007;**31**:932–62.

31. Leith NJ, Barrett RJ. Effects of chronic amphetamine or reserpine on self stimulation responding: animal model of depression? *Psychopharmacol* 1980;**72**:9–15.

32. Minor TR, Huang Q, Foley EA. Cytokine–purine interactions in behavioral depression in rats. *Integr Physiol Behav Sci* 2003;**38**:189–202.

33. Rojas-Corrales MO, Berrocoso E, Gibert-Rahola J, Micó JA. Antidepressant-like effect of tramadol and its enantiomers in reserpinized mice: comparative study with desipramine, fluvoxamine, venlafaxine and opiates. *J Psychopharmacol* 2004;**18**:404–11.

34. Qingjun H, Xinling H, Minor TR. Adenosine A2a receptor mediates reserpine-induced depression in rats. *Acta Psychol Sin* 2003;**35**:106–11.

35. Dhingra D, Valecha R. Evaluation of the antidepressant-like activity of *Convolvulus pluricaulis* choisy in the mouse forced swim and tail suspension tests. *Med Sci Monit* 2007;**13**:155–61.

36. Tadaiesky MT, Andreatini R, Vital MABF. Different effects of 7-nitroindazole in reserpine-induced hypolocomotion in two strains of mice. *Eur J Pharmacol* 2006;**535**:199–207.

37. Woode E, Amidu N, Owiredu WKBA, Boakye-Gyasi E, Ansah C, Duwiejua M. Antidepressant-like effects of an ethanolic extract of *Sphenocentrum jollyanum* pierre roots in Mice. *Int J Pharmacol* 2009;**5**:22–9.

38. Abílio VC, Vera Jr JAR, Ferreira LSM, Duarte CRM, Carvalho C, Grassl C, et al. Effects of melatonin on orofacial movements in rats. *Psychopharmacol* 2002;**161**:340–7.

39. Naidu PS, Singh A, Kulkarni SK. Reversal of reserpine-induced orofacial dyskinesia and cognitive dysfunction by quercetin. *Pharmacology* 2004;**70**:59–67.

40. Naidu PS, Singh A, Kulkarni SK. Effect of *Withania somnifera* root extract on reserpine-induced orofacial dyskinesia and cognitive dysfunction. *Phytother Res* 2006;**20**:140–6.

41. Wang Z, Gu J, Wang X, Xie K, Luan Q, Wan N, et al. Antidepressant-like activity of resveratrol treatment in the forced swim test and tail suspension test in mice: the HPA axis, BDNF expression and phosphorylation of ERK. *Pharmacol Biochem Behav* 2013;**112**:104–10.

42. Yáñez M, Fraiz N, Cano E, Orallo F. Inhibitory effects of cis- and trans-resveratrol on noradrenaline and 5-hydroxytryptamine uptake and on monoamine oxidase activity. *Biochem Biophys Res Commun* 2006;**344**:688–95.

43. Beasley Jr CM, Nilsson ME, Koke SC, Gonzales JS. Efficacy, adverse events, and treatment discontinuations in fluoxetine clinical studies of major depression: a meta-analysis of the 20 mg/day dose. *J Clin Psychiatry* 2000;**61**:722–8.

44. Thompson DC, Perera K, London R. Spontaneous hydrolysis of 4-trifluoromethylphenol to a quinone methide and subsequent protein alkylation. *Chem-Biol Interact* 2000;**126**:1–14.

45. Zafir A, Banu N. Antioxidant potential of fluoxetine in comparison to *Curcuma longa* in restraint-stressed. *Eur J Pharmacol* 2007;**572**:23–31.

46. Adachi Y, Horii K, Suwa M, Tanihata M, Ohba Y, Yamamoto T. Serum glutathione S-transferase in experimental liver damage in rats. *J Gastroenterol* 2007;**16**:129–33.

47. Bishayee A, Darvesh AS, Politis T, McGory R. Resveratrol and liver disease: from bench to bedside and community. *Liver Int* 2010;**30**:1103–14.

48. Hamadi N, Mansour A, Hassan MH, Khalifi-Touhami F, Badary O. Ameliorative effects of resveratrol on liver injury in streptozotocin-induced diabetic rats. *J Biochem Mol Toxicol* 2012;**26**:384–92.

49. Lee ES, Shin MO, Yoon S, Moon JO. Resveratrol inhibits dimethylnitrosamine-induced hepatic fibrosis in rats. *Arch Pharm Res* 2010;**33**:925–32.