RED WINE POLYPHENOLS REVERSE DEPRESSIVE-LIKE BEHAVIORS IN MICE INDUCED BY REPEATED CORTICOSTERONE TREATMENT

POLIFENÓIS DO VINHO TINTO REVERTEM COMPORTAMENTOS DEPRESSIVOS EM RATOS INDUZIDOS PELO TRATAMENTO REPETIDO COM CORTICOSTERONA

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INTRODUCTION

Major depression disorder (MDD) is one of the most common debilitating mood disorders worldwide and becoming the second leading disease contributing to the years lived with disability by 2013 (Dean and Keshavan, 2017). The prominent and persistent low mood, mental retardation, cognitive impairment, volitional decline and somatic symptoms companied the patients’ lifelong and impaired their social

SUMMARY

The aim of this study was to investigate the antidepressant-like effect of red wine phenolic extracts in mouse model exposed to exogenous corticosterone. The results showed that 3-week corticosterone injections caused depression-like behavior in mice, as indicated by the significant decrease in sucrose consumption and increase immobility time in the forced swimming test (FST). Red wine phenolic extracts treatment significantly reduced serum corticosterone levels. Moreover, it was found that red wine phenolic extract increased the brain-derived neurotrophic factor protein (BDNF) and tropomyosin-related kinase B (TrkB) phosphorylation and cAMP-responsive element binding protein (CREB) phosphorylation levels in the hippocampus and prefrontal cortex. However, K252a, an inhibitor of TrkB, completely abolished those antidepressant-like effects. These results suggested that the red wine phenolic extracts produce an antidepressant-like effect in corticosterone-treated mice, at least in part, which is possibly mediated by modulating hypothalamic-pituitary-adrenal (HPA) axis, BDNF, TrkB and CREB phosphorylation levels in the brain region of mice.

RESUMO

O objetivo deste trabalho foi estudar o efeito antidepressivo de extratos fenólicos do vinho tinto em rato-modelo exposto à corticosterona exógena. Os resultados mostraram que injeções de corticosterona de 3 semanas causaram comportamento semelhante à depressão em ratos, como indicado pela diminuição significativa no consumo de sacarose e aumento do tempo de imobilidade em teste de natação forçada (FST). O tratamento com extratos fenólicos de vinho tinto reduziu significativamente os níveis séricos de corticosterona. Além disso, verificou-se que o extrato fenolico do vinho tinto aumentou a proteína do fator neurotrófico derivado do cérebro (BDNF) e fosforilação do TrkB e os níveis de fosforilação da proteína de ligação ao elemento cAMP-responsivo (CREB) no hipocampo e no córtex pré-frontal. No entanto, K252a, um inibidor de TrkB, aboliu completamente os efeitos do tipo antidepressivo. Estes resultados sugerem que os extratos fenólicos do vinho tinto produzem um efeito antidepressivo em ratos tratados com corticosterona, pelo menos em parte, o que é possivelmente mediado pela modulação do eixo hipotálamo-hipófise-adrenal (HPA), BDNF, TrkB e níveis de fosforilação de CREB na região do cérebro dos ratos.

Key words: depressive behavior, polyphenols, red wine.
Palavras-chave: comportamento depressivo, polifenóis, vinho tinto.
functions. The lifetime and 12-month prevalence estimates for MDD were 5.8% and 2.2% in an Asian multi-racial population, respectively (Chong et al., 2012). On the other hand, diabetes, cardiometabolic disease, obesity and other comorbidity associated with MDD (Badescu et al., 2016; Jani et al., 2017), emerge as a serious health concern.

Despite its higher prevalence, the mechanisms associated with the pathogenesis of MDD have yet to be completely understood and current treatments remain ineffective in a large subset of patients (Lima-Ojeda et al., 2018). A growing literature has shown that the hypothalamic–pituitary–adrenal (HPA) axis plays a major role in the regulation of a variety of physiological disorders, such as depression (Menke, 2019). In this classic neuroendocrine circuit, limbic and hypothalamic brain structures coordinate emotional, cognitive, neuroendocrine and autonomic inputs, which together determine the magnitude and specificity of an individual’s behavioral, neural and hormonal responses to stress. This response is mediated by glucocorticoid hormones (corticosterone in rodents and cortisol in humans) (Gerritsen et al., 2017). Increased level of corticosterone has mostly been ascribed to impaired feedback regulation of the HPA axis, possibly caused by altered function of the glucocorticoid receptor and induced depressive disorder (Leistner and Menke, 2018).

Moreover, brain-derived neurotrophic factor (BDNF) and its receptor, tropomyosin receptor kinase B (TrkB) downstream signaling are integral to a range of neural functions, including synaptic plasticity and exhibits activity-dependent regulation of expression. The neurotrophic model of depression hypothesizes that the level of BDNF is decreased during the depression, which has been certified by the concentration of BDNF detected in the serum and hippocampus of postmortem in several publications (Buttenschon et al., 2015; Reinhart et al., 2015). Additionally, cAMP-response element binding protein (CREB) signaling also can increase the transcription of BDNF in the soma or transportation to dendrites (Wang et al., 2017), contribute to the actions of antidepressant treatments.

A depression animal model by repeated corticosterone treatment has been performed widely in mice, which resulted in depression-like behavior marked by significant changes in behavioral traits, neurochemistry and brain (Ding et al., 2018). Corticosterone-induced depression model has advantages over the stress models (such as restraint stress exposure) that avoid the possibility of potential habituation effects and variation in HPA axis response to stress stimuli (Obasi et al., 2019). Previous reports have shown that exogenous corticosterone administration develops depression-like behavior in mice during forced swim test, sucrose consumption test and tail suspension test (Fenton et al., 2015; Zhang et al., 2015). Therefore, these findings suggest that a chronic corticosterone treatment appears to model depression-like state in mice is suitable for evaluating the efficacy of potential antidepressant candidates and to explore the mechanism of action of antidepressants.

Recent studies have shown that plant polyphenols possess a number of beneficial properties, such as reducing the risks of cancer and heart diseases (Mattera et al., 2017; Amor et al., 2018); green tea and grape powder have shown effect on alleviating cognitive impairments and leading to a lower prevalence of depressive symptoms (Mulero et al., 2015; Patki et al., 2015; Solanki et al., 2015). It has been recognized that red wines are one of the richest sources of polyphenols and thus possess beneficial effects on human health when drunk in moderation (Nash et al., 2018). However, it is unknown whether red wine has a potential effect on alleviating depressive disorder, and the impact of polyphenols content of antidepressant-like effect. Therefore, the objective of this study was to verify the antidepressant-like effects of red wine phenolic extracts in a mouse model of depression induced by repeated injections of corticosterone, and to further investigate the relationship between BDNF signaling and the antidepressant-like effect of red wine phenolic extracts.

MATERIAL AND METHODS

Red wine phenolic extracts

The two different red wine phenolic extracts tested, one from red wine at 2 days of maceration (TPx-MT2) and another from red wine at 7 days of maceration (TPx-MT7), were obtained as described in the previous work (Sun et al., 2011). Briefly, red wine was made by classic vinification method with Vitis vinifera varieties (Castelão:Tinta Miuda; 3:2; w/w) harvested at maturity. After 7 days of maceration when alcoholic fermentation was finished, the mash was pressed. About 80 mL of the wine samples was evaporated under vacuum at less than 30 °C to remove ethanol and then loaded onto an open column (200 mm × 25 mm i.d.) packed with LiChroprep RP-18 (25-40 μm particle size) already preconditioned with distilled water. The column was washed with 200 mL of distilled water, followed by 150 mL of methanol to recover TPs. The methanol fraction was added with an equal volume of distilled
water, evaporated under vacuum at less than 30 °C to remove methanol, and then lyophilized to obtain TPs extract (TPx). The obtained polyphenol extracts from the wine at 2 and 7 days of maceration named TPx-MT2 and TPx-MT7, respectively. Both red wine polyphenolic extracts present high purity in polyphenols (> 91%; w/w) but TPx-MT2 contains more anthocyanins than TPx-MT7 (14.00% w/w versus 8.43% w/w) while TPx-MT7 contains more proanthocyanidins than TPx-MT2 (6.17% w/w versus 4.61% w/w) (Sun et al., 2011).

Animals

Adult male Kunming mice (weighing 20 ± 2 g) were purchased from the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China). All of them were maintained under standard laboratory conditions of constant temperature (23 ± 1 °C), relative humidity (50 ± 10%) and a 12 h light/dark cycle (light from 7:00 a.m. to 7:00 p.m.) with food and water available ad libitum and were allowed to habituate to the novel environment for one week prior to use in experiment. The experiment was carried out in compliance with the National Institutes of Health and institutional guidelines for the humane care of animals and was approved by the Animal Care Committee of Shenyang Pharmaceutical University. Every effort was made to minimize the number of animals used and any pain and discomfort experienced by the subjects.

Drug administration and experimental groups

The mice were randomly assigned eleven groups (n=8/group): control group, vehicle group, corticosterone groups: corticosterone only, TPx-MT2 (10 mg/kg), TPx-MT2 (10 mg/kg) + K252a, TPx-MT2 (20 mg/kg), TPx-MT2 (20 mg/kg) + K252a, TPx-MT7 (10 mg/kg), TPx-MT7 (10 mg/kg) + K252a, TPx-MT7 (20 mg/kg), TPx-MT7 (20 mg/kg) + K252a. Corticosterone (TCI, Japan) was dissolved in normal saline and injected i.p. in a volume of 10 mL/kg before 30 min of gavage administration (Luo et al., 2015). The dose of TPx-MT was chosen based on the results of preliminary experiment. The behavioral tests were carried out 24 h after the last injection. One animal from each group was tested in sequence.

Sucrose preference test

Sucrose preference test was carried out 24 h after the last injection as described previously (Chiba et al., 2012). Briefly, prior to testing, mice were trained to adapt to the sucrose solution (1%, w/v): two bottles of sucrose solution were placed in each cage for 24 h, and then one bottle of sucrose solution was replaced with water for 24 h. After adaptation, mice were deprived of water and food for 24 h. Sucrose preference test was conducted with mice housed in the individual cage and free access to the two bottles, one containing 100 mL of sucrose solution (1% w/v) and the other 100 mL of water. After 1 h, the volumes of consumed sucrose solution and water were recorded and the sucrose preference was calculated as the sucrose preference (%) = sucrose consumption/(sucrose consumption + water consumption) ×100%.

Forced swimming test

The forced swimming test (FST) was carried out on mice, according to the method of Kruk-Slomka et al. (2015). Briefly, the individual mouse was subjected to swimming stress session for 15 min (pre-test), in a vertical glass cylinder (25 cm high, 14 cm in diameter) containing 10 cm of water, maintained at 25 ± 2 °C. After 24 h, FST was carried out and the total duration of immobility (seconds) was recorded during the last 4 min of a single 6 min test session. A mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only small movements necessary to keep its head above water. The water in the container was changed after each trial.

Serum corticosterone measurement

After the behavioral test, mice were euthanized by decapitation and blood was collected (Yu et al., 2015). Serum corticosterone level was measured using a commercially customized ELISA kit (Liyu Bioengineering Ltd., Shanghai, China) according to the manufacturer’s protocol. Briefly, 50 μL of sample and standard solutions were added to the already precoated antibody plate provided with the kit and incubated for 30 min at 37 °C. The reaction was ended and followed by washing, 50 μL of the TMB color reagent was added and incubated for 20 min without shaking. The reaction was stopped by adding 50 μL of stop solution and absorbance was read at
450 nm using a microplate reader (Varioskan flash, Thermoscientific, USA).

**Tissue collection and biochemical analysis**

Whole brains were rapidly removed from the mice and chilled in an ice-cold saline solution after blood collection. Brain regions of the hippocampus and prefrontal cortex were dissected on a cold plate and immediately frozen in liquid nitrogen. The tissue samples were stored at −80 °C until assay.

The level of BDNF, pCREB, CREB, pTrkB and TrkB were measured using commercially available enzyme-linked immunosorbent assay ELISA kits (Liyu Bioengineering Ltd., Shanghai, China) according to the manufacturer’s instructions. Absorbance was measured at 450 nm using a microplate reader (Varioskan flash, Thermoscientific, USA).

**Statistical analysis**

All data were analyzed using a one-way analysis of variance (ANOVA) with repeated measures, followed by Tukey HSD post-hoc test when significant main effects were indicated. All analyses were two-tailed and *p<0.05 was considered significant a priori.

**RESULTS AND DISCUSSION**

**Sucrose consumption**

As shown in Figure 1, a 3-week corticosterone exposure significantly reduced the percentage of sucrose consumption in the stressed mice in comparison with the control animals [F(10, 77) = 9.616, p<0.01]. However, post-hoc analysis revealed that long-term treatment of corticosterone mice with TPx-MT7 (10, 20 mg/kg) increased sucrose preference, as compared to corticosterone-exposed mice (respectively, p<0.05; p<0.01). But with TPx-MT2 treatment, there were no similar results obtained. Chronic treatment with TPx-MT2 or TPx-MT7 showed no effects on sucrose preference of K252a-injected animals. The sucrose preference test is an indicator of anhedonia-like behavioral change. Anhedonia a core symptom of major depression among humans is modeled by inducing a decrease in responsiveness to rewards, as reflected by the reduced consumption of and/or preference for sweetened solutions (Rzepa and McCabe, 2019). In the present study, the data are in line with previous findings showing that a significant decrease in the percentage of sucrose consumption of mice (Cerniauskas et al., 2019). Red wine extract significantly reversed this behavioral change, which suggested the antidepressant-like function.

![Figure 1. Effect of TPx-MT2 and TPx-MT7 on sucrose consumption. All the values are given as mean ± standard error of mean (SEM) (n=8), *p<0.05, **p<0.01 vs CORT; #p<0.05 vs control.](image1)

**Immobility time in the FST**

The effects of treatment with TPx-MT2 and TPx-MT7 in the immobility time were present in Figure 2. TPx-MT2 (20 mg/kg) and TPx-MT7 (10, 20 mg/kg) treatment significantly increased the immobility time of stressed animals compare to the corticosterone-treated only group [F(10, 77) = 20.077, p<0.01].

![Figure 2. Effect of TPx-MT2 and TPx-MT7 on the immobility time in forced swimming test. All the values are given as mean ± SEM (n=8), **p<0.01 vs CORT; #p<0.01 vs control.](image2)

On the contrary, there were no significant differences of the immobility time between K252a-treated groups
and corticosterone-treated group also including TPx-MT2 (10 mg/kg) group. FST is a behavioral despair test useful for probing the pathological mechanism of depression and for the evaluation of antidepressant drugs (Zhang et al., 2019a). This neurobehavioral alteration was also ameliorated by red wine extract, thereby underlining the effectiveness of red wine as an antidepressant candidate.

**Serum corticosterone levels**

As shown in Figure 3, there was a significant effect of corticosterone exposure on serum corticosterone concentrations \( F(10,77) = 10.092, p<0.05 \). The corticosterone-induced increases in serum corticosterone levels were significantly reduced in mice treated with TPx-MT2 (20 mg/kg) \( p<0.05 \) and TPx-MT7 (10, 20 mg/kg) \( p<0.05 \). However, these reductions were robust by K252a injection. Chronic treatment with TPx-MT2 (10 mg/kg) showed no effect on the serum corticosterone level of corticosterone-treated animals. This means that exogenous corticosterone results in an absolute increase in circulating serum corticosterone levels, an indicator of stress and depression in mice. Thus, the present study revealed that the behavioral consequences of repeated corticosterone administration were accompanied by dysregulation of the HPA axis.

**Figure 3.** Effect of TPx-MT2 and TPx-MT7 on serum corticosterone concentration. All the values are given as mean ± SEM (n=8), *p<0.05 vs CORT; †p<0.05 vs control.

**Efeito do TPx-MT2 e TPx-MT7 na concentração sérica de corticosterona. Todos os valores são dados como média ± EPM (n = 8), *p<0.05 vs CORT; †p<0.05 vs controlo.**

**BDNF levels**

As shown in Figure 4, exposure to corticosterone significantly decreased both hippocampal \( F(10,77)=11.382, p<0.01 \) (Figure 4A) and prefrontal cortex BDNF levels \( F(10,77)=8.092, p<0.05 \) (Figure 4B) as compared to the control animals. Treatment with a daily dose of TPx-MT2 (20 mg/kg) and TPx-MT7 (20 mg/kg) significantly attenuated the decrease in BDNF protein level \( p<0.05 \) and \( p<0.05 \), respectively) in the hippocampus as compared to the only corticosterone-treated mice. And this attenuated effect in the hippocampus was blocked by K252a. But the BDNF levels in the prefrontal cortex of both TPx-MT2 and TPx-MT7 treatment groups have no alterations compared to the corticosterone-treated group.

**Figure 4.** Effect of TPx-MT2 and TPx-MT7 on BDNF level of hippocampus (A) and prefrontal cortex (B). All the values are given as mean ± SEM (n=8), *p<0.05 vs CORT; †p<0.05, ‡p<0.01 vs control.

**Efeito de TPx-MT2 e TPx-MT7 no nível de BDNF do hipocampo (A) e do córtex pré-frontal (B). Todos os valores são dados como média ± SEM (n = 8), *p<0.05 vs CORT; †p<0.05, ‡p<0.01 vs controlo.**

The role of BDNF in the pathogenesis of depression and in the mechanism of action of antidepressants has been well appreciated. In humans, brain BDNF levels have been found to be reduced in postmortem samples from depressed patients, and antidepressant therapies restored brain BDNF level to the normal range (Al-Hatamleh et al., 2019). As well as clinical studies, it has also been shown that BDNF expression
was decreased in the hippocampus and prefrontal cortex of depressive animals, which could be reversed by long term antidepressant treatment (Mendez-David et al., 2015; Sahin et al., 2015). In line with the previous reports, long term red wine extract treatment reversing the reduction of BDNF which suggested that the behavioral improvement in the depressed mice might be related with the regulation of BDNF.

**TrkB phosphorylation / TrkB**

As shown in Figure 5, the ratio of pCREB/CREB in the hippocampus \[F(10,77) = 10.291, p<0.05\] (Figure 5A) and prefrontal cortex \[F(10,77) = 9.357, p<0.05\] (Figure 5B) of the corticosterone-treated mice were significantly decreased as compared to the control mice.

**CREB phosphorylation/ CREB**

As shown in Figure 6, the ratio of pCREB/CREB in the hippocampus \[F(10,77) = 13.527, p<0.01\] of corticosterone-treated mice was significantly decreased as compared to the control group. TPx-MT2 or TPx-MT7 up-regulated the ratio of pCREB/CREB, and K252a has not abolished it. Similar results were obtained in the prefrontal cortex; all treated groups were not different compared to the control group. CREB is up-regulated by chronic antidepressant treatment, and increasing CREB levels in rodent model results in antidepressant-like behaviors. Furthermore, postmortem studies indicate that CREB levels are increased in subjects taking antidepressants at the time of death (Tian et al., 2019). In agreement with that, the red wine extract treatment significantly increased the ratio of pCREB/CREB in the hippocampus and prefrontal cortex. And K252a did not show any inhibitory effect.
Thus, the CREB signaling pathway might be involved in the antidepressant-like effect as well.

![Figure 6](image)

**Figure 6.** Effect of TPx-MT2 and TPx-MT7 on the ratio of pCREB/CREB of hippocampus (A) and prefrontal cortex (B). All the values are given as mean ± SEM (n=8), *p*<0.05 vs control.

Efeito de TPx-MT2 e TPx-MT7 na relação de pCREB / CREB do hipocampo (A) e córtex pré-frontal (B). Todos os valores são dados como média ± SEM (n = 8), *p* <0.05 vs controlo.

Based on the different preparation procedures, both red wine phenolic extracts present high purity in polyphenols (> 91%; w/w), TPx-MT2 contains more anthocyanins than TPx-MT7 while TPx-MT7 contains more proanthocyanidins than TPx-MT2 (Sun *et al.*, 2011). Comparing the red wine extract treatment groups, it was found that BDNF level and pTrkB/TrkB ratio in hippocampus of TPx-MT7 is higher than TPx-MT2, especially in TPx-MT7 (20 mg/kg) group. All the above results illustrated that proanthocyanidins are exerting greater antidepressant-like effect than anthocyanins, which is consistent with the data of *in vitro* antioxidant activities previously published (Sun *et al.*, 2009). However, it was also found that the ratio of pCREB/CREB among TPx-MT2 and TPx-MT7 treatment groups both in the hippocampus and prefrontal cortex is similar; this may be caused by the dose or other involved mechanisms and should be investigated in our further studies.

**CONCLUSIONS**

Although both red wine phenolic extracts showed their potent reversing the anhedonia effects in the depressed mouse model, TPx-MT7 containing more proanthocyanidins appeared more effective on the antidepressant-like activity than TPx-MT2 containing more anthocyanins, indicating that proanthocyanidins would have a greater antidepressant-like effect than anthocyanins. The antidepressant-like effects may be speculated to be mediated by its modulatory action on the HPA axis function and its ability to prevent the alterations of BDNF, pTrkB/TrkB and pCREB/CREB levels in the hippocampus and prefrontal cortex of depressed mice.

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