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ANTIDEPRESSANT-*LIKE* ACTION OF PHYTOL, POSSIBLY *VIA* REDUCING OXIDATIVE STRESS IN THE MICE BRAIN

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Abstract

The diterpenoid phytol (PYT) is a chlorophyll-derived essential oil with promising biological activities. This study evaluated the antidepressant effect of PYT along with its antioxidant capacity in mice. For this, adult male *Swiss* mice were treated intraperitoneally (i.p.) with vehicle (NC: 0.05% tween 80 dissolved in 0.9% NaCl solution, 10 mL/kg), PYT (25, 50 and 75 mg/kg), imipramine (50 mg/kg), paroxetine (20 mg/kg) and reserpine (0.25 mg/kg). The animals were subjected to forced swimming test, and following to determine the levels of thiobarbituric acid reactive substances (TBARS), nitrite content (NO_2^-), and the activity of catalase (CAT) and superoxide dismutase (SOD) in their hippocampus, striatum, frontal cortex and cerebellum. The results suggest that PYT dose-dependently reduce the immobility of the test animals. PYT at all doses significantly ($p<0.05$) reduced the levels of TBARS and the NO_2^- contents, while increasing in CAT and SOD activities in the test animals. Moreover, PYT at 75 mg/kg with imipramine, paroxetine and reserpine significantly ($p<0.05$) reversed the behavioral as well as oxidant and antioxidant biomarekrs in the animals. In conclusion, PYT produced an antidepressant effect, possibly *via* modulation of serotonergic and adrenergic neurotransmission, and antioxidant-mediated neuroprotection pathway.

Keywords: *antidepressant, antioxidant, Mus musculus, oxidative stress, phytol*

Introduction

Depression is a mental disorder with a high degree of psychological distress. This condition involves numerous clinical, etiopathogenic and therapeutic aspects that, if not treated early and/or properly, can lead to the incapacitation or even death to the patients (Holzmann et al. 2015). Notably, the side effects and cost are the two consequences in current antidepressant treatments, despite of their levels of security and effectiveness (Neis et al. 2015).

Although, the exact pathophysiological mechanisms of depression are yet to be elucidated, but some study suggesting that oxidative stress may contribute directly to its etiology. Oxidative stress may be due to an increase in reactive oxygen and nitrogen species (ROS/RNS) and/or reduction of the body enzymatic or non-enzymatic defense systems, which cause damage to the cellular macromolecules and affect essential cellular functions (Chang et al. 2015). The above circumstances encourage to seek new, safer and economic antidepressants (Siwek et al. 2013).

The alcoholic diterpenoid, phytol (3,7,11,15-tetrametilhexadec-2-en-1-ol) (PYT) is already evident to have potent antioxidant capacity in a number of non- and pre-clinical studies (Islam et al. 2016a). In a recent study, Islam et al. (2016b) have been reported a number of diterpenes having antiodiant and antidepressant potentials, that can be used in the treatment of neurological disorders.

From the above viewpoint, our current study evaluated a possible antidepressant effect of PYT in *Swiss* albino mice through the forced swim test (FST). Additionally, we investigated the levels of TBARS, nitrite (NO_2^-), CAT and SOD in brain areas (hippocampus, striatum, frontal cortex and cerebellum) of the animal after FST.

Methods

Reagents and chemicals

PYT and all the other required chemicals and reagents were purchased from Sigma-Aldrich (Chem Ex. Co. St. Louis, Missouri, USA).

Animals

Swiss mice (*Mus musculus*) of approximately 2 months old, weighing between 25 and 30 g, were provided by the Central Animal Laboratory of the Federal University of Piaui (Teresina, Brazil). The animals were kept in a controlled environment (temperature: 25 ± 2°C; humidity 50 ± 5%; 12 h dark/light cycle; food Purina® pellets and filtered water: *ad libitum*). The animals were then acclimated for seven days prior to the tests commenced. Experimental protocol was approved by the Ethics Committee in Animal Experimentation of UFPI (No. 013/2011).

Experimental protocol

PYT was emulsified with 0.05% Tween 80 dissolved in 0.9% normal saline (vehicle). Test animals were divided into ten groups (seven animals in each). Group-I was treated with vehicle (10 mL/kg), while group-II, III and IVwith imipramine (50 mg/kg; IMP 50), paroxetine (20 mg/kg; PAR 20) and reserpine (0.25 mg/kg; RES 0.25), respectively. Group-V to VII were treated with PYT (25, 50 and 75 mg/kg; PYT 25, PYT 50 and PYT 75, respectively). All the treatments were administered *via* intraperitoneal (i.p.) injection. Finally, three groups of animals were combined with PYT 75 and IMP 50, PAR 20 and RES 0.25 groups. After 30 minutes of the above treatments, the bellow mentioned parameters were analyzed.

Evaluation of antidepressant activity by forced swim test (FST)

The FST was conducted according to the earlier described method by Detke et al. (1995). Briefly, mice was placed in a transparent water tank with 22 cm in diameter and 40 cm in height, containing fresh water (half portion of it) at 26 ± 1°C. The mobility and immobility parameters were recorded in seconds (s) for five minutes.

Dissection of brain of the animals and preparation of homogenates

After 48 h of FST, the animals were decapitated with a guillotine (insight) and then the brains were rapidly removed, sectioning the above mentioned portions. The homogenates were prepared as 10% (w/v) in 50 mM sodium phosphate buffer (pH 7.4) and were subjected to the analysis of TBARS and NO_2^- contents, and SOD and CAT activities.

Determination of thiobarbituric acid reactive substances (TBARS)

The extent of lipid peroxidation was measured by quantifying the TBARS level according to the method described by Draper and Hadley (1990). Briefly, to the 0.1 mL homogenates (10%, w/v), 1 mL of 10% trichloroacetic acid and 1 mL of 0.67% thiobarbituric acid was added and then the mixtures was stirred. Subsequently, the mixtures were kept in a boiling water bath for 15 minutes, cooled with running tap water and followed by an addition of 2 mL of n-butanol, stirring for 1 min and centrifugation at 8,00 *g* for 5 min. The absorbance was read out by using a UV spectrophotometer at 535 nm. The results were expressed as nM of malondialdehyde (MDA)/g tissue (nmol of MDA/g tissue).

Determination of nitrite (NO_2^-) content

In this occasion, 0.5 mL of tissue homogenate was added to an equal volume of Griess reagent (1% sulfanilamide in phosphoric acid and 0.1% N-1-naphthylethylenediamine dihydrochloride). The mixture was kept at room temperature for 10 min and absorbance was measured at 560 nm. The results were determined based on the Griess reaction and expressed in nM (Greenet al., 1981).

Determination of catalase (CAT) activity

The reaction medium was prepared with hydrogen peroxide (H_2O_2; 18 ml) in 1 M Tris HCl, 5 mM EDTA pH 8.0 (1.0 mL) and H_2O (0.8 mL). Then, 0.980 mL of reaction medium and 0.02 mL of tissue homogenate was placed in a quartz cuvette and the absorbance was measured for 6 min by using a UV spectrophotometer at 230 nm. A blank was constituted with 1 mL of the reaction medium. The results were expressed in mM/min/mg protein (Chance and Maehly, 1955).

Determination of superoxide dismutase (SOD) activity

The tissue homogenates were centrifuged at 8,00 g for 20 minutes, and the supernatants were used in this assay. SOD activity was tested by the rate of reduction of cytochrome-*c* by superoxide radicals ($O_2^{\bullet-}$). The xanthine oxidase system was used as a source of superoxide anion (O_2-) (Arthur and Boyne, 1985). The results were expressed as U/mg protein. One unit (U) of SOD activity corresponds to 50% of inhibition in the reaction of O_2 with cytochrome-*c*.

Ststistical analysis

The results were expressed as mean ± standard error of the mean (S.E.M.). Statistical analysis was performed using one-way ANOVA for multiple comparisons, followed by *t*-Student–Newman–Keuls as a *post hoc* test by using GraphPad Prism (version: 6.0) Software (GraphPad San Diego, California, USA. Copyright © 1994-1999) considering $p<0.05$ at 95% confidence level.

Results

FST

PYT at all doses decreased the immobility time significantly ($p<0.05$) as compared to the vehicle group. The IMP 50 and PAR 20 also reduced the immobility time by 63 and 56%, respectively in comparison to the vehicle group. However, PYT 75 showed a lower immobility time when compared to the RES (88%), IMP (34%), and PAR (29%) (Figure 1).

On the other hand, groups pre-treated (15 min prior) with PYT 75 and combined with RES, IMP and PAR, exhibited a reverse phenomena in the immobility time. In comparison to the individual group IMP 50, PAR 20 and RES 0.25, PYT 75 reduced the immobility time by 69.80, 35 and 29%, respectively (Figure 1).

TBARS content in the brain areas

Figure 2 represents the results of the lipid peroxidation test in the mouse brain areas. PYT in all cases, dose-dependently decreased the levels of TBARS in the hippocampus, striatum, frontal cortex and cerebellum of mice as compared to the vehicle and IMP 50, PAR 20 and RES 0.25 groups. There was an augment in TBARS level in the IMP 50, PAR 20 and RES 0.25 treated groups. On the other hand, PYT 75 combined with them was found to reverse the situation. However, more reduction in TBARS levels was observed in the PYT 75 and IMP 50 + PYT 75 groups in the frontal cortex area of the experimental animals.

NO_2^- content in the brain areas

The results presented in Figure 3 depicts that PYT exhibited a dose-dependent reduction in NO_2^- content in the brain areas of the experimental

animals. However, more reduction in NO_2^- content was observed in the striatum and frontal cortex regions. The positive controls used in this study, exhibited almost similar results that of the vehicle in all cases. PYT 75 when combined with them, significantly ($p<0.05$) reduced the NO_2^- content in the brain areas of the animals. However, more reduction in NO_2^- content was observed in striatum.

CAT activity in the brain areas

The activity of CAT was found to increase dose-dependently with PYT treatment in comparison to the vehicle group. IMP 50, PAR 20 and RES 0.25 also decreased CAT levels in all areas in the brain of the test animals, whichever, in turn reversed by the co-treatment with PYT 75. On the other hand, the group treated with PYT 75 + RES 0.25 was found to augment the CAT activity better than the RES 0.25 alone and other combination groups (PYT 75 + IMP 50 and PYT 75 + PAR 20) (Figure 4).

SOD activity in the brain areas

In this case, PYT also dose-dependently (with an exception in the striatum) augmented the SOD activity in the mouse brain areas. All the standards were found to reduce SOD levels in the brain areas of the test animals, where a sharp reduction was found in the striatum area. However, PYT 75 augmented the SOD level again when treated with them. Only in the hippocampus area, PYT 75 combindly increased the SOD levels with PAR 20 and RES 0.25, while in other areas PYT 75 + IMP 50 group augmented SOD level better than the other co-treatment groups (Figure 5).

Discussion

In a recent study, Costa et al. (2014) showed that the PYT at 75 mg/kg in mice showed better anxiolytic activity without muscle relaxant and sedative effect. In this study, PYT also produced an antidepressant-*like* effect in a dose-dependent manner, where significant ($p<0.05$) activity was observed with the same dose treated. The activity should be considered strong as compared to the standards used in this study. Moreover, PYT 75 pre-treated with IMP, PAR and RES reversed the situation occurred by the individually treated groups, suggesting a possible synergistic effect of PYT with them.

RES produces depressant and sedative effects *via* promoting depletion of noradrenalin, dopamine and 5-HT neurons (central and peripheral), and generate oxidative stress by increasing the production of by-products of lipid peroxidation as well as a decrease in enzyme activity antioxidants (Wang et al. 2015). Therefore, the effects of PYT can be connected to the serotonergic and noradrenergic neurotransmission, as it reversed the effects of RES in all cases.

In some pre-clinical and clinical studies, it has been suggested that the depression is associated with an altered level of oxidative stress markers such as lipid peroxidation, nitric oxide (NO), CAT, SOD and glutathione peroxidase (GPx) (Siwek et al. 2013; Vargas et al. 2013). A research conducted on human emphasized that the depressed patients showed an increase in lipid peroxidation as compared to the healthy control groups (Kotan et al. 2011). Almeida et al. (2014) suggested that an increased level of NO during depression, and reducing NO_2^- levels can be used to treat depression and other neurological diseases. On the other hand, the CAT and SOD prevent and/or control the formation of free radicals and non-radical species involved with the initiation of chain reactions that culminate in oxidative damage (Barbosa et al. 2010). In our study, we found that PYT dose-dependently decreased TBARS and NO_2^- contents, while increased in CAT and SOD levels in the brain of mice. Thus, the reduction of oxidative stress mediators and an augmentation of the physiological antioxidant system in the test animals may attribute neuroprotection and antidepressant effects. The diterpenes are promising antioxidants and therefore cytoprotective in nature (Islam et al. 2016c). Although the antioxidant capacity of PYT, in this study was seen in a region-dependent manner, but a dose-dependent and sinergistic antioxidant as well as the antidepressant effect of it was observed throughout.

In summary, phytol exerted significant antidepressant-*like* effect *via* reducing the immobility time in forced swimming test, as well as reducing the lipid peroxidation and nitrite contents, with the increasing in catalase and superoxidase activity in mouse brain areas. The antidepressant activity of PYT may be plugged in its antioxidant and

serotonergic and adrenergic neurotransmission pathways.

Acknowledgments

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rat striatum. Pharmacol Biochem Behav 2015;131:71-76.

Figure 1. Effect of treatments onimmobility time of mice subjected to the forced swimming test. [Values are mean ± S.E. M. (n = 7);[a]p <0.05 compared to the vehicle; [b]p <0.05 compared to the IMP 50; [c]p <0.05 compared to the PAR 20; [d]p <0.05 compared to the RES 0.25; [e]p <0.05 compared to the PYT 75; PYT: phytol; IMP: imipramine; RES: reserpine; PAR: paroxetine; one-way ANOVA for multiple comparisons followed by *t*-Student–Newman–Keuls.]

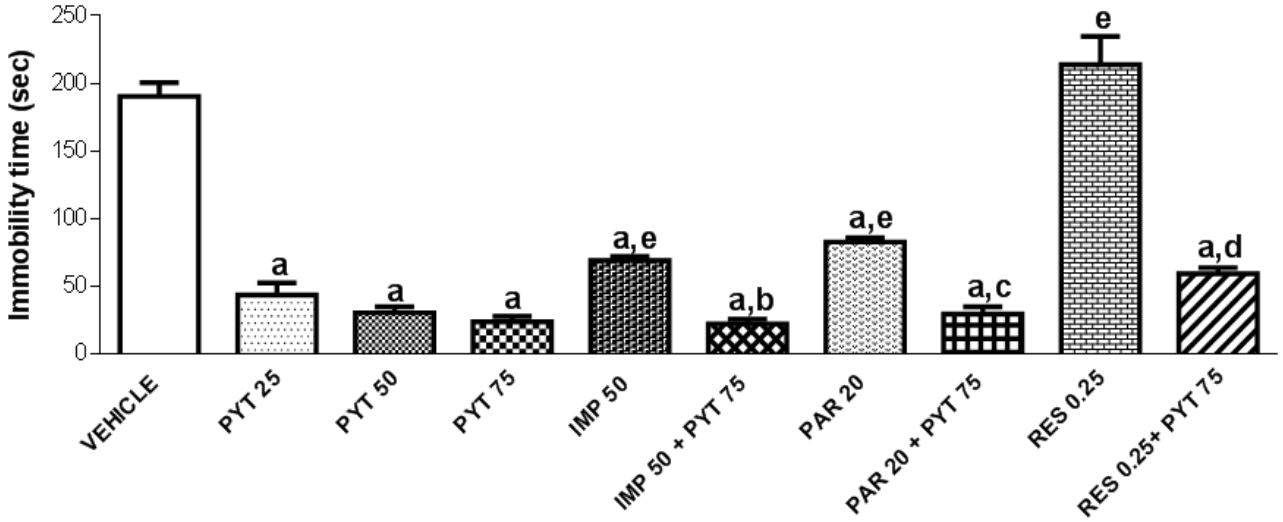

Figure 2. Extent oflipid peroxidation in the hippocampus, striatum, frontal cortex and cerebellum of mice subjected to the forced swimming test. [Values are mean ± S.E.M. (n = 7); [a]p <0.05 compared to the vehicle; [b]p <0.05 compared to the IMP 50; [c]p <0.05 compared to the PAR 20; [d]p <0.05 compared to the RES 0.25; [e]p <0.05 compared to the PYT 75; PYT: phytol; IMP: imipramine; RES: reserpine; PAR: paroxetine; one-way ANOVA for multiple comparisons followed by *t*-Student–Newman–Keuls.]

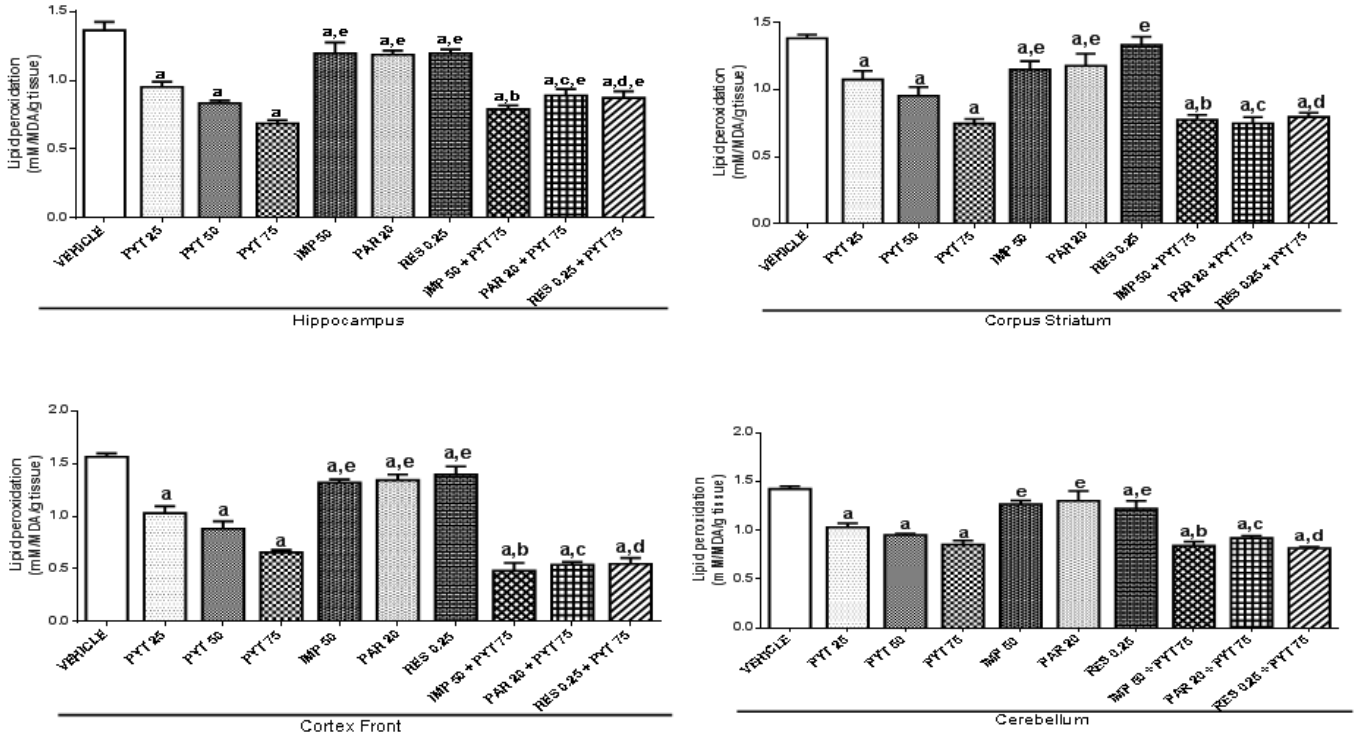

Figure 3. Nitrite content in the hippocampus, striatum, frontal cortex and cerebellum of mice subjected to the forced swimming test. [Values are mean ± S.E.M. (n = 7); [a]p <0.05 compared to the vehicle; [b]p <0.05 compared to the IMP 50; [c]p <0.05 compared to the PAR 20; [d]p <0.05 compared to the RES 0.25; [e]p <0.05 compared to the PYT 75; PYT: phytol; IMP: imipramine; RES: reserpine; PAR: paroxetine; one-way ANOVA for multiple comparisons followed by *t*-Student–Newman–Keuls.]

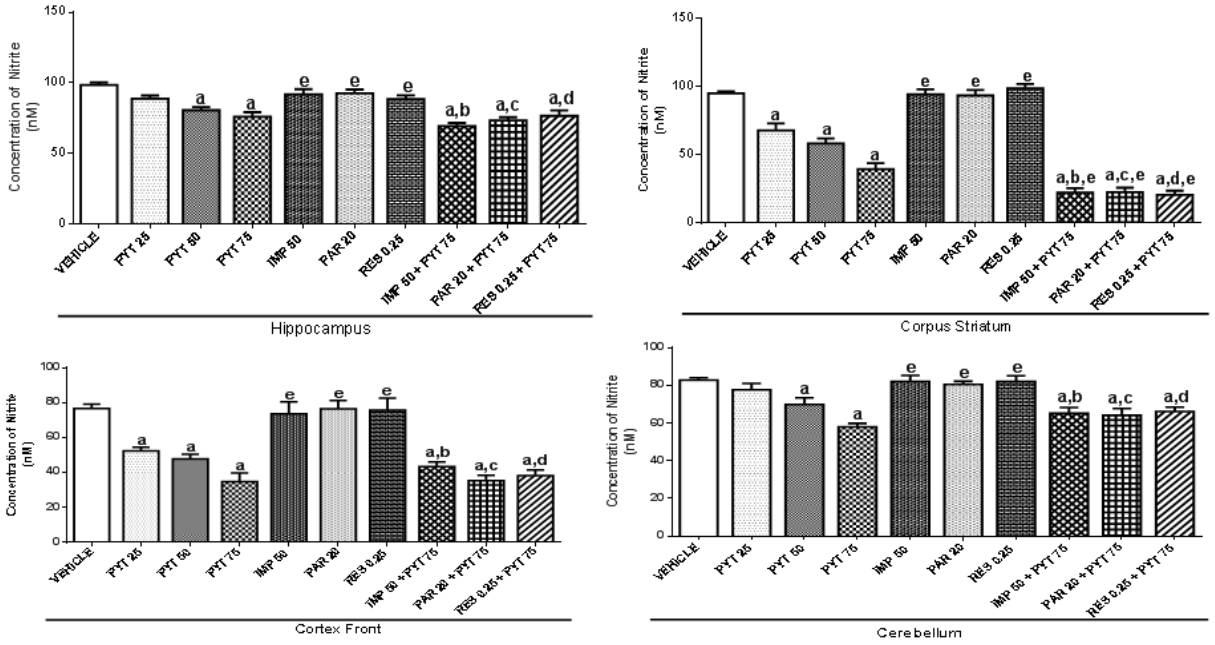

Figure 4. Catalase activity in the hippocampus, striatum, frontal cortex and cerebellum of mice subjected to the forced swimming test. [Values are mean ± S.E.M. (n = 7); [a]p <0.05 compared to the vehicle; [b]p <0.05 compared to the IMP 50; [c]p <0.05 compared to the PAR 20; [d]p <0.05 compared to the RES 0.25; [e]p <0.05 compared to the PYT 75; PYT: phytol; IMP: imipramine; RES: reserpine; PAR: paroxetine; one-way ANOVA for multiple comparisons followed by *t*-Student–Newman–Keuls.]

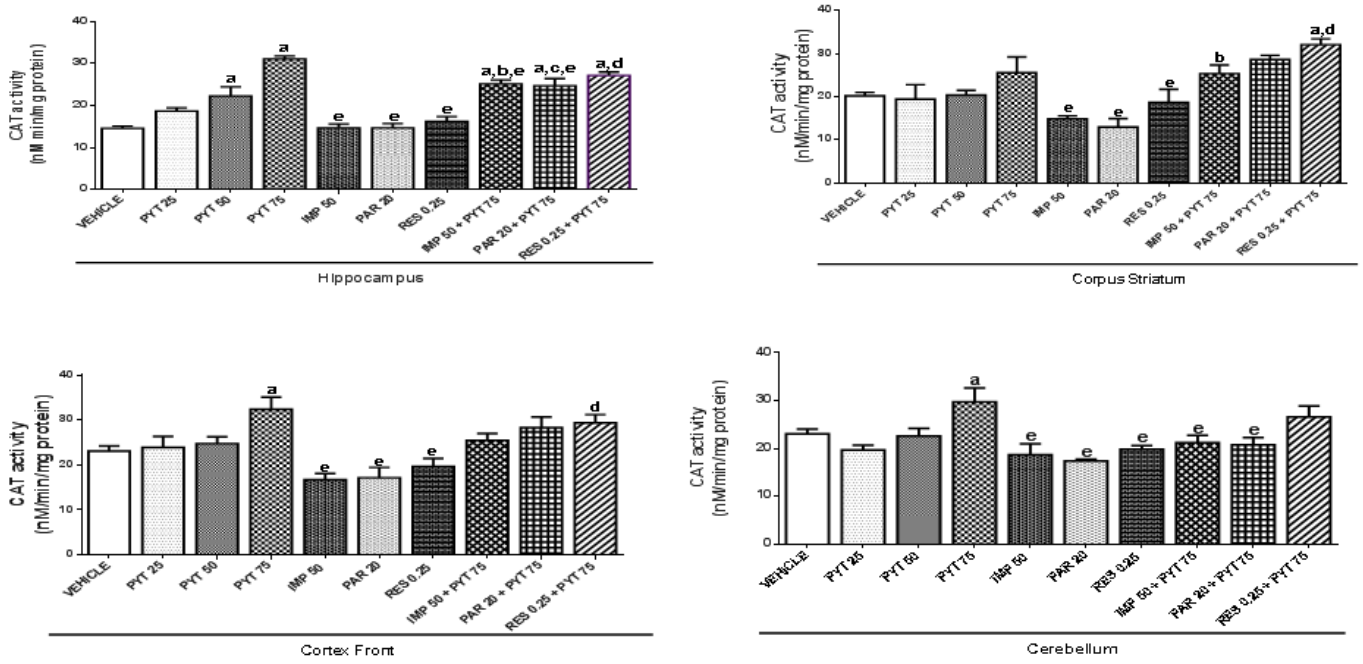

Figure 5. Superoxide dismutase activity in the hippocampus, striatum, frontal cortex and cerebellum of mice subjected to the forced swimming test. [Values are mean ± S.E.M. (n = 7); [a]p <0.05 compared to the vehicle; [b]p <0.05 compared to the IMP 50; [c]p <0.05 compared to the PAR 20; [d]p <0.05 compared to the RES 0.25; [e]p <0.05 compared to the PYT 75; PYT: phytol; IMP: imipramine; RES: reserpine; PAR: paroxetine; one-way ANOVA for multiple comparisons followed by *t*-Student–Newman–Keuls.]