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Original article

Neuro-protective and redox potential of troxerutin against cypermethrin-induced neurotoxicity and oxidative stress in mice

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Abstract

The present study was designed to evaluate the protective efficacy of troxerutin against cypermethrin-induced behavioral defects, motor function abnormalities, and oxidative stress in mice. Twenty-four adult female albino mice were randomly divided into four equal groups. The first group served as control, the second group was treated with cypermethrin (20 mg/kg b.w) intraperitoneally at day 21, and the remaining two groups were orally supplemented with TRX (150, 300 mg/kg b.w) for 20 days and with cypermethrin (20 mg/kg b.w) intraperitoneally at day 21. Behavior activities recorded after cypermethrin exposure showed significantly impaired motor function ($p \leq 0.05$) as evidenced by the beam balance and pole test. The cypermethrin was also found to cause significant memory dysfunction. Moreover, the oxidative stress in terms of increased tissue malondialdehyde level ($p \leq 0.05$) was recorded in the cypermethrin group. The antioxidant activities of catalase and glutathione peroxidase were decreased ($p \leq 0.05$) after cypermethrin exposure. Troxerutin supplementation significantly improved the cypermethrin-induced motor impairment and memory dysfunction. The supplementation of troxerutin significantly restored the redox status. Troxerutin attenuates the neurotoxic and behavioral deficits caused by cypermethrin. Furthermore, troxerutin also provides significant protection against cypermethrin-induced oxidative stress by improving the oxidative stress markers.

Keywords: antioxidant enzymes, behavioral tests, oxidant level, pesticides, rodents, water soluble flavonoids

Introduction

A pesticide is a combination of different noxious chemical constituents, used purposefully to prevent or kill insects, fungi, rodents, and other injurious pests (Mahmood et al. 2016). Cypermethrin (CYP) is a very potent class II pyrethroid pesticide which is comprehensively used across the globe for both household and agricultural pest control. According to reports, most human exposure to CYP occurs as a result of pyrethroid residues found in foods including bread, milk, vegetables, and fruits. CYP is absorbed mostly through the gastrointestinal system; however, it can also be absorbed through mist inhalation and skin (Tian et al. 2008). Due to its lipophilic nature, CYP can pass the blood brain barrier, which may lead to neurotoxicity and motor impairments (Ali et al. 2020). CYP produces neurotoxicity by prolonging the brief rise in sodium permeability of neuronal membrane channels following stimulation, the repetitive nerve impulses have the potential to cause oxidative stress, which raises the probability of increased reactive oxygen species formation in response to cypermethrin exposure (Sharma et al. 2014). Chronic CYP exposure in rats results in oxidative stress, apoptosis, DNA damage, neuro-inflammation and microglial activation (Yadav et al. 2021).

Troxerutin (TRX) is derived from the natural bioflavonoid rutin, exhibits antioxidant, antithrombotic, anti-inflammatory, fibrinolytic, antidiabetic, anticancer, hepatoprotective, edema-protective, radio-protective, neuro-protective, and rheological properties. Studies have showed that rats exposed to TRX have improved memory and learning by attenuating the anxiety and depression (Bayandor et al. 2019). Rats treated with TRX exhibit ample potential to upgrade antioxidant properties and reduce the generation of lipid peroxidation. (Raja et al. 2019). The beneficial effects of TRX in suppressing the motor dysfunction, anxiety, memory, and oxidative stress are known; however, studies concerning the effectiveness of TRX on reducing motor impairments, behavioral toxicity, and ROS induced by CYP are scarce. The present study was designed to evaluate the protective effects of TRX against CYP induced toxicity on the behavior and redox status of mice.

Materials and Methods

Chemicals used

α-Cypermethrin, purity 98.0% (Sigma-Aldrich, CAS No.: 67375-30-8) and troxerutin (Sigma-Aldrich Y000497). All chemicals used in this experiment were of analytical grade.

Animals and experimental design

All the procedures used in this study were approved by the Ethical Review Committee, UVAS, Lahore--Pakistan (DR/272/2021). Post acclimatization, twenty--four healthy adult female albino mice (25-35 g) were arbitrarily divided into 4 groups. Each group consisted of 6 mice. The first group was the control group and mice in this group were administered 0.9% normal saline orally for 20 days followed by a single dose of 0.9% normal saline intraperitoneally at day 21. The second group was the CYP group and mice in this group received 0.9 % normal saline orally for 20 days followed by a single dose of 20mg/kg of body weight CYP, dissolved in olive oil, intraperitoneally at day 21 (Mezni et al. 2020). The third group was the TRX-150 + CYP group and mice in this group were supplemented with 150 mg/kg body weight TRX dissolved in 0.9% normal saline orally for 20 days followed by a single dose of CYP (20 mg/kg of body weight dissolved in olive oil) intraperitoneally at day 21. The fourth group was the TRX-300+CYP group and mice in this group were supplemented with 300 mg/kg/body weight dissolved in 0.9% normal saline TRX orally for 20 days followed by a single dose of CYP (20 mg/kg of body weight dissolved in olive oil) intraperitoneally at day 21. The mice were maintained under a controlled temperature (24±2°C) and in a photoperiod of 12 hour light dark cycle; they were allowed unrestricted access to feed and water.

Evaluation of motor functions

On day 22 of the experiment, mice in each group were subjected to motor function assessment via beam balance test, pole test and foot print test.

Motor coordination was measured by the beam balance test described by Yaqub et al. (2020). In this test, the time was recorded to cross the beam to the mounted end. Each mouse traversed the beam twice; the time to cross the beam was noted.

For the pole test, the mice were positioned on a cylindrical pole as reported by Yaqub et al. (2020) and the time taken by the mice to descend to the base of the pole was recorded.

The footprint test was done using the method reported by Yaqub et al. (2020), to calculate and compare the distance between the hind limb and forelimb. Stride length was measured using a ruler. The average distance between two consecutive footprints was measured.

Assessment of anxiolytic effects

An open field test was performed using the method described by Bayandor et al. (2019). Parameters studied

in the open field test were time spent in the center, the number of times the mouse enters the center, number of crossed squares (ambulation), mobility duration, and total distance.

An elevated plus maze test was performed to assess anxiety in the mice. Entry of each animal in the open arm and closed arm was calculated manually. The standard of anxiety indices %OAT and %OAE was calculated as follows: %OAE: ratio of entries in open arms as compared to total entries into any arms ×100. %OAT: ratio of time spent in the open arms as compared to total time spent in any arms ×100. The index of locomotor activity is the total number of arm entries (Bayandor et al. 2019).

Memory testing

Using the Y-maze test, the spatial memory of mice exposed to cypermethrin was evaluated. Mice which had a good memory remembered the previously visited arm and entered the new arm (Hussein et al. 2018). The percentage of spontaneous alteration (%SAP) was calculated using this formula: %SAP= {number of spontaneous alterations/total number of arm entries-2}*100

Sample collection

After 24 hours of CYP administration, following the behavioral tests, the mice were anesthetized with light ether anesthesia. Blood was collected by cardiac puncture and added to the non-heparinized vacutainers in order to separate the serum and was kept at -20°C until biochemical testing. After blood collection, the mice were decapitated for the collection of brain, kidney, and muscle tissues. Collected serum and tissue samples were labeled and kept at -40°C until required.

Preparation of tissue homogenates

The collected tissues (brain, kidney, and muscle) were homogenized after being separately weighed and added to an ice cold 0.01 M of phosphate buffer saline (pH 7.4) using homogenizer analog. The centrifugation of homogenates was done at 5000 x g at 4°C for 20 minutes. The resultant supernatant was used for the evaluation of oxidant and antioxidants levels.

Biochemical assays

The amount of MDA was evaluated by measuring thiobarbituric acid reactive substances (TBARS) according to AMDCC (Animal Models of Diabetic Complications Consortium) protocols (2004). The activity of CAT in serum and tissues was assessed using the decomposition of hydrogen peroxide, as described by Hadwan and Abed (2016). The GPx catalyzed the reaction of reduced GSH into oxidized GSH. It also reduced H_2O_2 to water. The spontaneous reaction is terminated by the addition of acidic stop solution (Flohé and Günzler 1984). The maximum absorbance is measured at 450nm. A Bioassay Technology Laboratory-UK kit (CAT No. E1579Ra) was used for GPx estimation.

Statistical analysis

The data were analyzed using one-way ANOVA via SPSS software and presented as mean ± SEM. The means values were compared using the Tukey test to compare differences between the groups and a probability level of $p \leq 0.05$ was assumed as significant.

Results

Motor behavior of mice supplemented with troxerutin against cypermethrin

CYP caused significant motor impairments. The traverse time to cross the beam increased in CYP treated mice compared to the control group. On the other hand, mice supplemented with 150 mg TRX had a decreased ($p \leq 0.05$) traverse time as compared to the CYP treated group (Fig. 1a). CYP treated mice showed prolonged duration to descend from the pole in comparison with the control group, whereas TRX supplemented mice took less ($p \leq 0.05$) time to descend from the pole as compared to CYP treated mice (Fig. 1b). Stride length measurements of the hind limb showed no significant effect with TRX supplementation compared to the control groups (Fig. 1c).

Anxiogenic effects of mice supplemented with troxerutin against cypermethrin

The open field test demonstrated that the number of explored areas, total distance, mobility rate, and inside time were significantly decreased in mice supplemented with CYP as compared to the control group. Mice supplemented with 150 mg TRX showed increase ($p \leq 0.05$) in the number of explored areas, total distance, mobility rate, and inside time in comparison to CYP treated mice. No significant change was observed in outside time (Fig. 2a-e).

Elevated plus maze test results showed a significant decrease in open arm time when supplemented with CYP as compared to the control group. In contrast, supplementation of 150 mg TRX increased ($p \leq 0.05$) the time spent in open arms compared with CYP treated

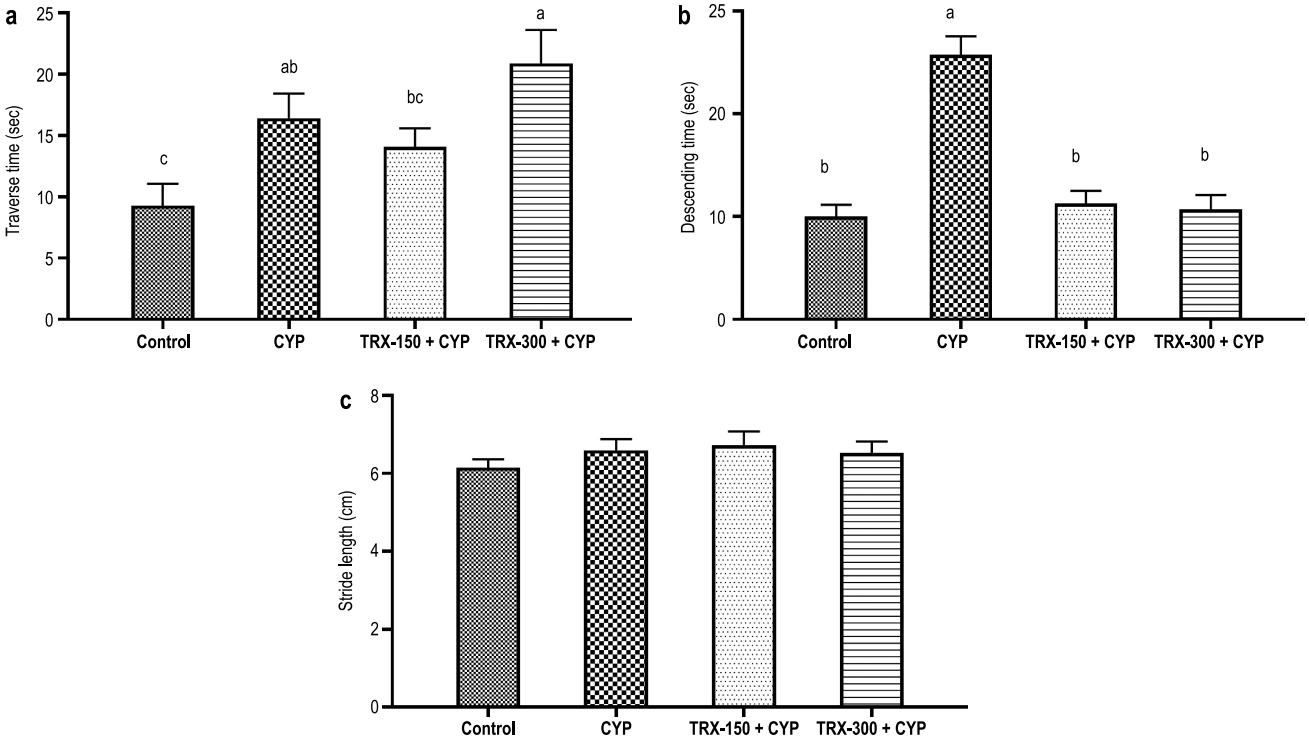

Fig. 1. Motor behavior of mice supplemented with troxerutin (TRX) against cypermethrin (CYP) **(a)** beam balance test **(b)** tail suspension test **(c)** foot print test. Data presented as Mean ± S.E.M. Different superscripts[a-c] on bars indicate significant difference between groups ($p<0.05$). **Control:** normal saline orally administrated followed by a single dose of normal saline; **CYP:** normal saline followed by a single dose of 20 mg/kg b.w. cypermethrin; **TRX-150 + CYP:** supplemented with 150 mg/kg b.w. troxerutin followed by a single dose of 20 mg/kg b.w. cypermethrin; **TRX-300 + CYP:** supplemented with 300 mg/kg b.w. troxerutin followed by a single dose of 20 mg/kg b.w. cypermethrin.

mice. However, the number of entries remained unaffected ($p\leq0.05$) with supplementation of TRX (Fig. 3a-b).

Memory deficit supplemented with troxerutin against cypermethrin

A significant decrease was observed in the number of spontaneous entries in the Y-maze test when treated with CYP as compared to the control group. Mice supplemented with 150 mg TRX had an increased ($p\leq0.05$) number of entries compared to the CYP treated group (Fig. 4).

Serum redox status in mice supplemented with troxerutin against cypermethrin

The present study showed that administration of CYP significantly increased ($p\leq0.05$) serum MDA level compared to the control group. In contrast, pre-supplementation with TRX at a dose of 150 mg/kg significantly decreased the MDA level compared to the CYP group. The CAT and GPx level in serum was significantly decreased in the CYP group compared with the control group while TRX supplementation did not affect the CAT and GPx activity in mice compared with the CYP group. The GPx level in serum remained unaffected with TRX supplementation (Fig. 5a-c).

Brain redox status in mice supplemented with troxerutin against cypermethrin

It was observed that the MDA level in brain tissue was significantly increased in mice treated with CYP as compared to the control group. The mice supplemented with 150 mg TRX has lower ($p\leq0.05$) MDA activity compared to the CYP group. The catalase level in brain tissue was significantly decreased in mice given CYP as compared to the control group. However, mice supplemented with 150 mg TRX increased ($p\leq0.05$) brain catalase level compared with the CYP treated group. The results of GPx level in brain tissue remained non-significant with TRX supplementation and CYP compared to the control group (Fig. 6a-c).

Kidney redox status in mice supplemented with troxerutin against cypermethrin

The kidney MDA level was found non-significant in the CYP treated group compared with the control group. Similarly, TRX supplementation did not alleviate the oxidative stress in mice compared with the CYP group. There were no significant ($p\leq0.05$) changes in CAT and GPx activity in kidney tissue with TRX supplementation in mice compared with the CYP group (Fig. 7a-c).

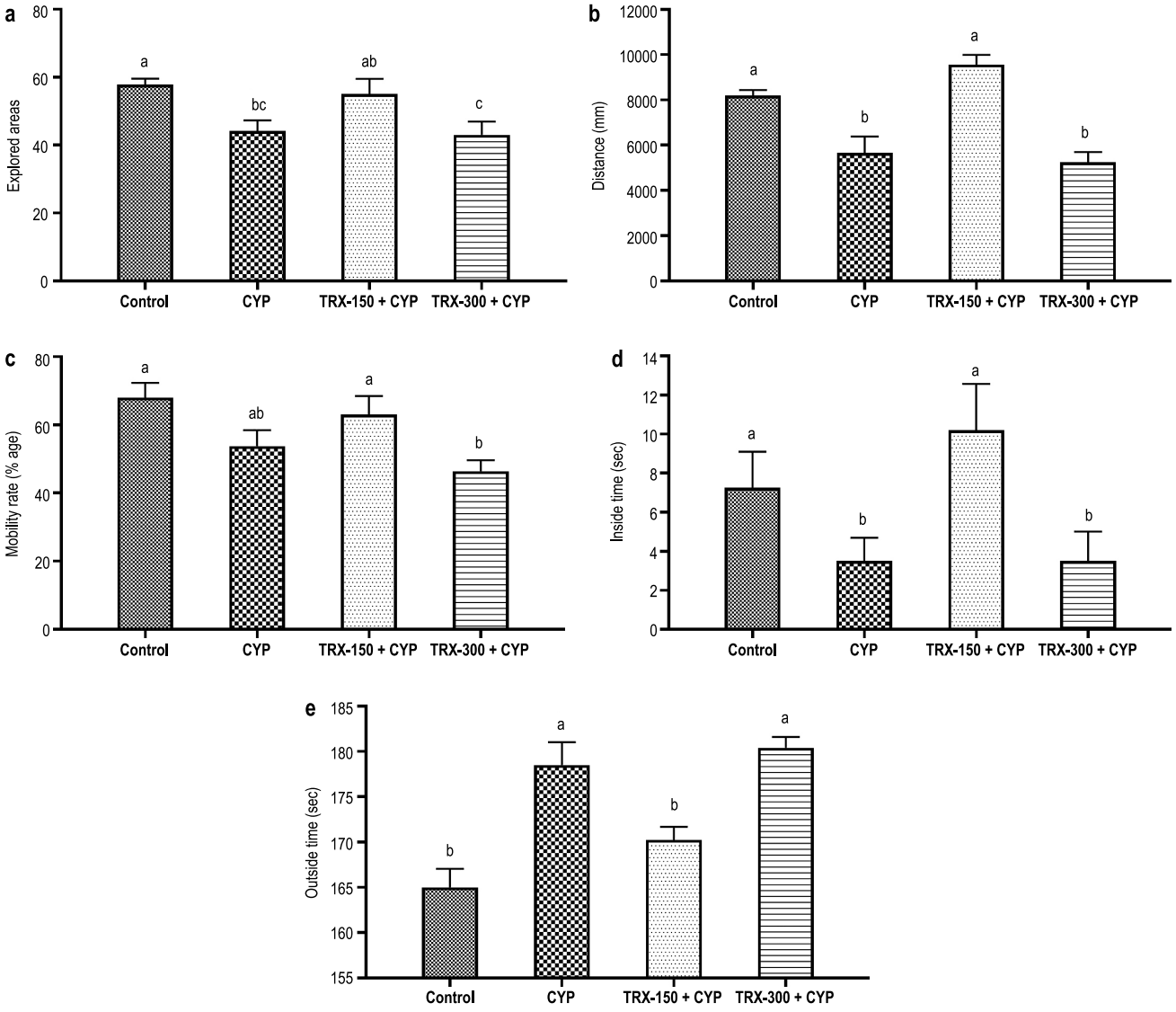

Fig. 2. Anxiogenic effects of mice supplemented with TRX against CYP. Open field test demonstrated **(a)** explored areas **(b)** total distance **(c)** mobility rate **(d)** inside time **(e)** outside time. Data presented as Mean ± S.E.M. Different superscripts[a-c] on bars indicate significant difference between groups ($p<0.05$).

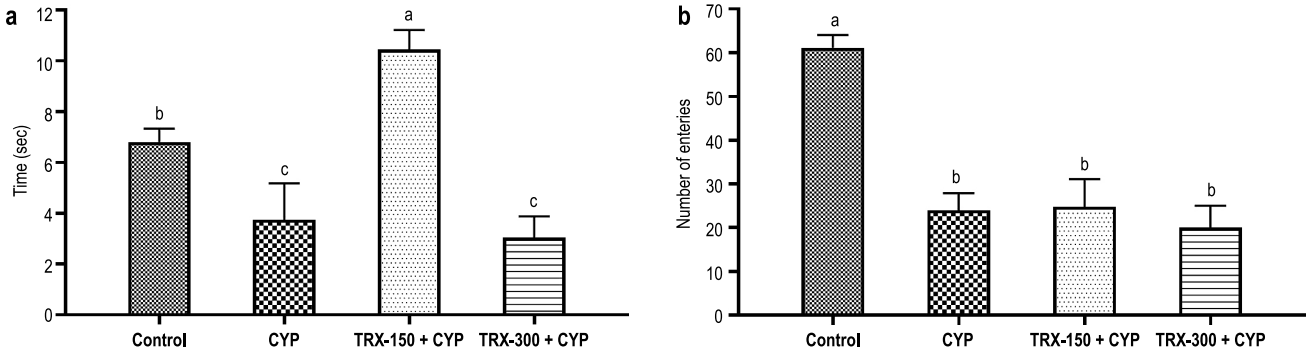

Fig. 3. Anxiogenic effects of mice supplemented with TRX against CYP. Elevated plus maze test showed **(a)** open arm time (OAT) **(b)** open arm entries (OAE). Data presented as Mean ± S.E.M. Different superscripts[a-c] on bars indicate significant difference between groups ($p<0.05$).

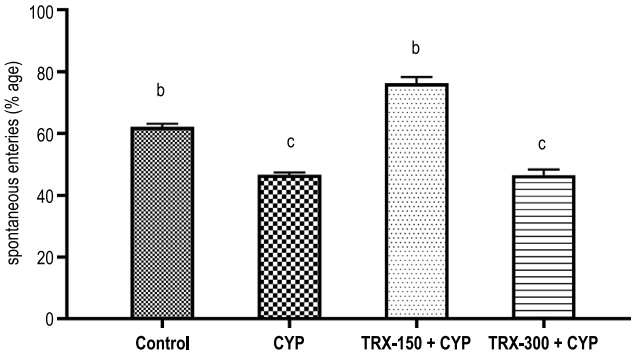

Fig. 4. Memory deficit of mice supplemented with TRX against CYP. Data presented as Mean ± S.E.M. Different superscripts[a-c] on bars indicate significant difference between groups ($p<0.05$).

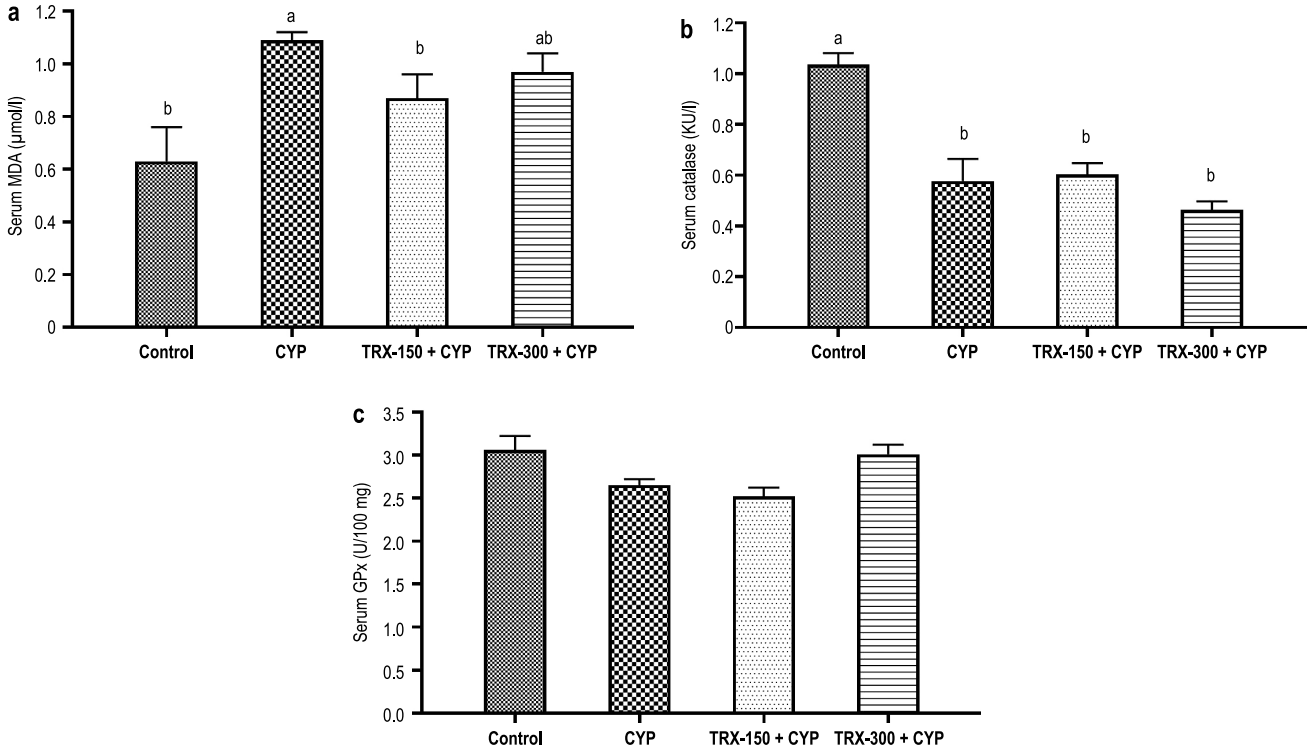

Fig. 5. Serum redox status of mice supplemented with TRX against CYP. **(a)** serum MDA **(b)** serum catalase **(c)** serum GPx. Data presented as Mean ± S.E.M. Different superscripts[a-b] on bars indicate significant difference between groups ($p<0.05$).

Muscle redox status in mice supplemented with troxerutin against cypermethrin

The muscle MDA level in the CYP group was increased ($p\leq0.05$) as compared to the control group. Groups of mice pre-supplemented with TRX had a significantly decreased the muscle MDA level compared to the CYP group. The CAT level was found significantly lower in the CYP group compared to the control group. However, the CAT muscle activity level was significantly restored ($p\leq0.05$) in the TRX supplemented group at a dose of 300 mg/kg. The muscle GPx level remained unaffected ($p\leq0.05$) with TRX supplementation compared with the CYP group (Fig. 8a-c).

Discussion

Cypermethrin was evaluated for its neurotoxicity and oxidative stress in mice. The pre-supplementation of TRX counters behavioral defects and alleviates oxidative stress in mice. The beam balance test is used for the assessment of any impairment in motor coordination and balance in rodents. According to Colle et al. (2020), co-administration of pesticides (paraquat and maneb), during the early postnatal and adulthood period in mice showed remarkable motor dysfunction as determined by the beam balance test. The results of the beam balance test in our study showed an increase in traversing time after exposure to CYP. Our results are similar to Nasuti et al. (2017), where rats treated with permethrin exhibited extended time in traversing the beam

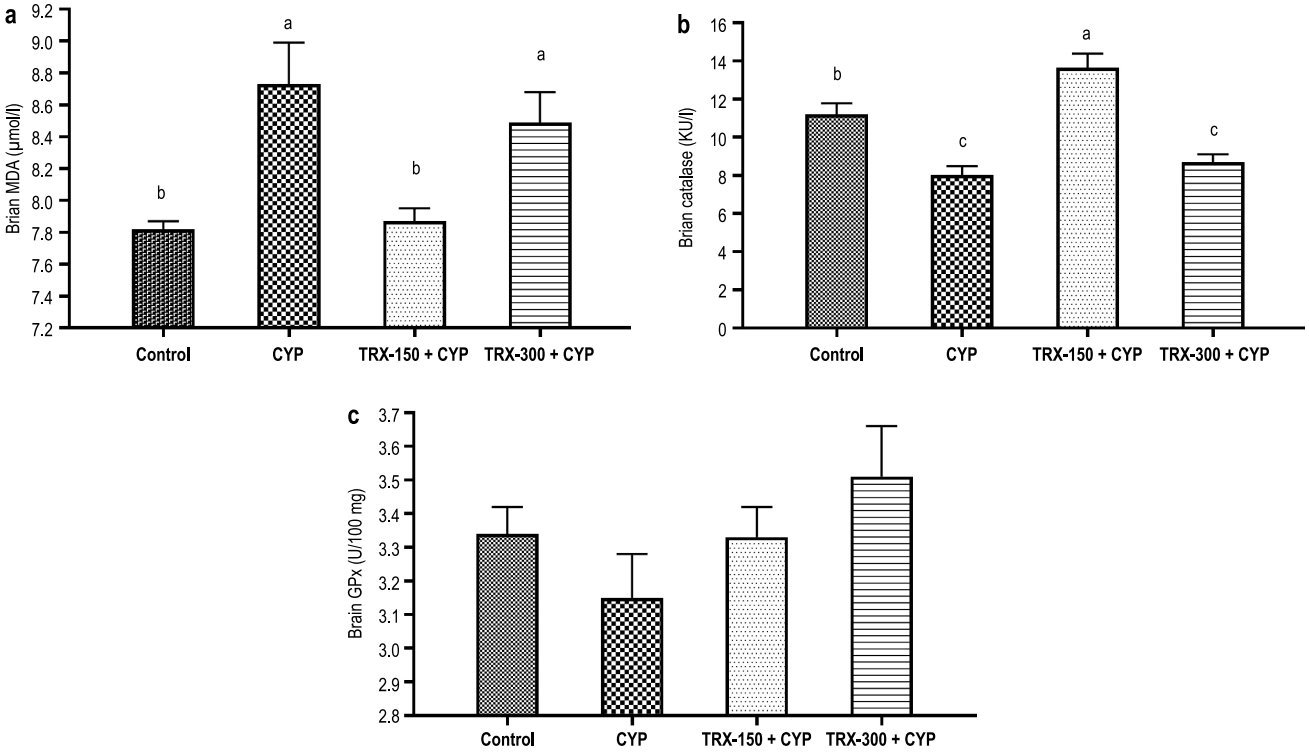

Fig. 6. Brain redox status of mice supplemented with TRX against CYP. **(a)** brain MDA **(b)** brain catalase **(c)** brain GPx. Data presented as Mean ± S.E.M. Different superscripts[a-c] on bars indicate significant difference between groups ($p<0.05$).

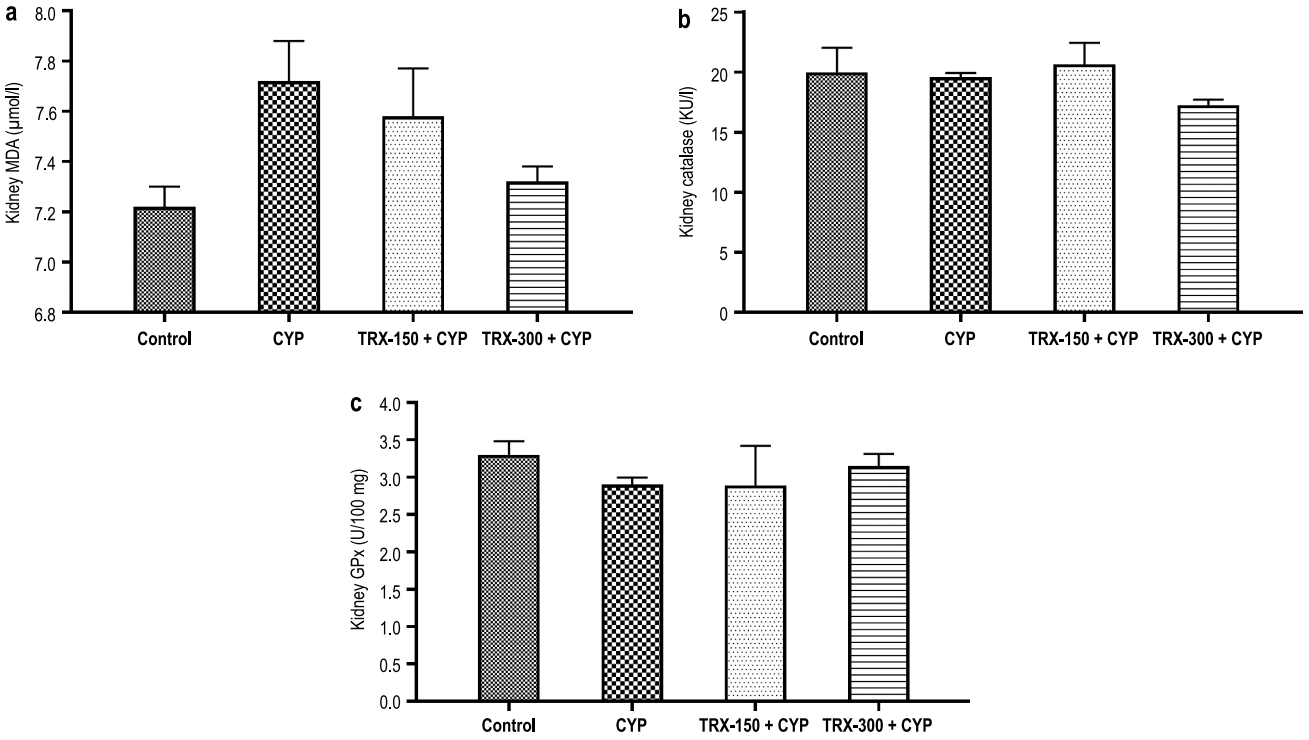

Fig. 7. Kidney redox status of mice supplemented with TRX against CYP. **(a)** kidney MDA **(b)** kidney catalase **(c)** kidney GPx. Data presented as Mean ± S.E.M. Different superscripts[a-b] on bars indicate significant difference between groups ($p<0.05$).

with asymmetric posture and dragged hind limbs. According to our findings, mice supplemented with flavonoid TRX 150 mg/kg clearly manifested decreased latency time as well as fewer errors while crossing the beam. Likewise, quercetin used against rotenone showed a decrease in latency of time to cross (Madiha et al. 2021). With TRX supplementation against CYP, the motor coordination outcome of the current investi-

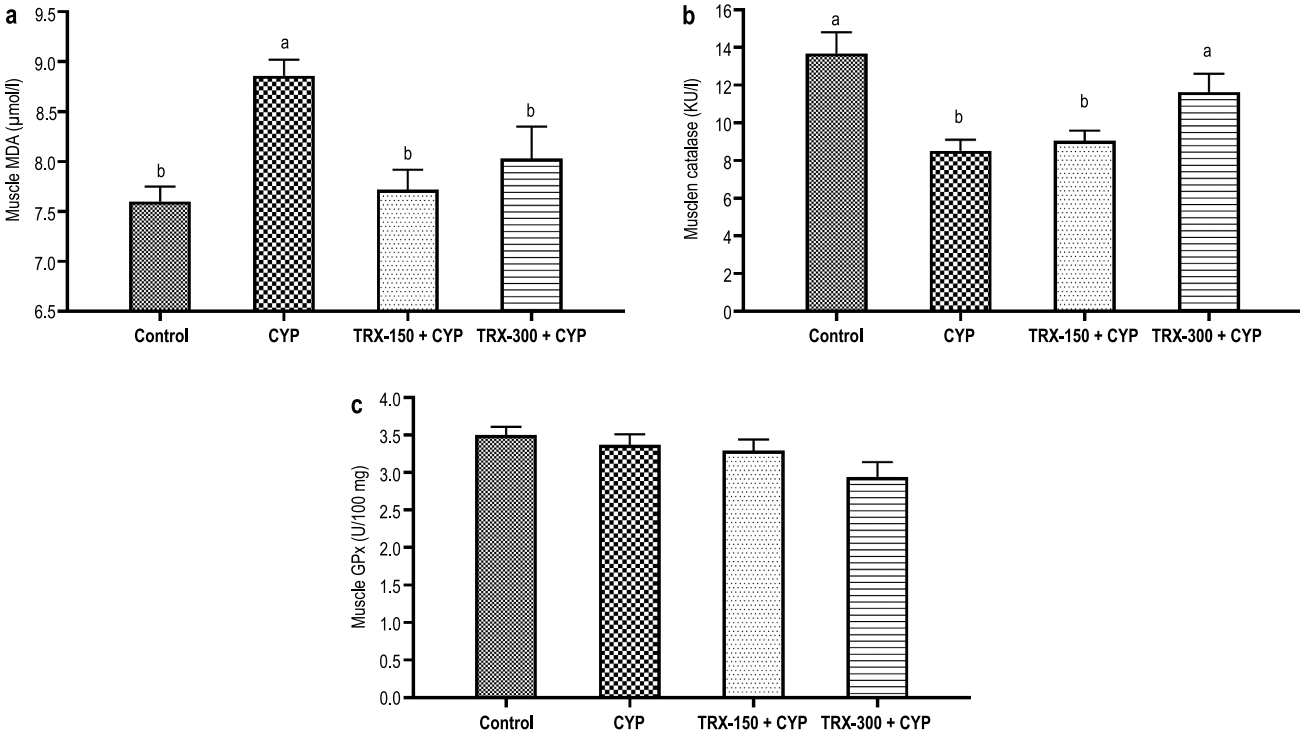

Fig. 8. Muscle redox status of mice supplemented with TRX against CYP. **(a)** muscle MDA **(b)** muscle catalase **(c)** muscle GPx. Data presented as Mean ± S.E.M. Different superscripts[a-b] on bars indicate significant difference between groups ($p<0.05$).

gation showed improved results. This may suggest that TRX has beneficial effects in preserving dopamine levels in the brain (Baluchnejadmojarad et al. 2017). The pole test is broadly used to evaluate the basal ganglia associated movement disorders and locomotive function of the rodents (Yaqub et al. 2020). The results of our research demonstrate that mice took more time to climb down the pole when treated with a single dose of CYP. However, the pole test time was decreased in mice treated with TRX. Our results are in line with Wu et al. (2021), who demonstrated that rats subjected to a single injection of fenpropathrin insecticide displayed an increased pole test time. However, rats treated with flavonoid quercetin against rotenone revealed a decrease in descent time (Madiha et al. 2021). Gait analysis was done using the foot print test. In our study, no significant change in stride length was observed in mice groups either exposed to CYP or TRX. Unlike the results reported by Garabadu and Agrawal (2020), cypermethrin and permethrin induced chronic toxicity in rats showed a significant decrease in stride length and, when treated with naringin, resulted in an increased stride length. Similarly, the rats given rotenone over a period of three weeks displayed a decrease in stride length and an improvement was seen when treated with quercitin (Madiha et al. 2021). This discrepancy in our results might be because motor neuron degeneration is a slow process and develops after chronic exposure to pesticides (Knippenberg et al. 2010). However, in our study a single dose of CYP was used at the end of the study to induce the acute toxicity.

The open field test is frequently used to assess the rodents' anxiety-like behaviors. Various studies reported that exposure to pyrethroid pesticides and insecticides bring about anxiety like behaviors in rodents. According to Rajawat et al. (2019), the mobility of swiss albino mice was impaired by the use of β-cyfluthrin in terms of lowered average number of squares crossed and increased immobility duration. Our results are parallel to a previous study of Righi and Palermo-Neto (2003), where pyrethroid cyhalothrin induced anxiety like behavioral changes as it significantly decreased the mobility duration, number of crossed squares, and the time spent in the inside and outside zone. Our findings in the open field test showed a significant reduction in mobility rate, explored areas, total distance, and time spent inside the zone in the group treated with CYP as compared to the control, while supplementation with TRX 150 mg/kg/b.w showed an improvement in the number of entries, total distance, mobility rate, and outside time and inside time. Our results are in line with the previous work of Bayandor et al. (2019), which reported an improvement in the central entries and time was seen in offspring of female wistar rats which were supplemented with 150 mg/kg TRX during the gestation period against a high-fat diet. Likewise, different doses of TRX (50, 150, 300 mg/kg) were administrated in male wistar rats, against anxiety and depressive like

behaviors induced by chronic mild stress. The results revealed an increased time spent in the center and more number of center entrances by rats with a dose of 50 and 150 mg/kg of TRX (Azarfarin et al. 2018). The elevated plus maze is another test used to assess anxiety in rodents. Our results showed that CYP administration significantly reduced the percentage of time spent in the open arm of the elevated plus maze test, but the percentage of entries remained non-significant. Similarly, cyhalothrin subjected rats spent a reduced percentage of time occupied in the open arms in contrast to the closed arms and the percentage of entries showed non-significant results (Righi and Palermo-Neto 2003). Flavonoids have been found to exhibit anxiolytic properties. In our study, mice treated with 150 mg/kg TRX showed a significant increase in the open arm test time. However, there was no significant difference in the results of open arm entries. Azarfarin et al. (2018), also described that rats treated with TRX (150 and 300 mg/kg) exhibited a rise in the number of entrances and time spent in the open arms against stress induced anxiety like behaviors. In addition, administration of 150 mg/kg TRX in high fat diet rats showed a further increase in the percentage of entries and percentage of time spent, respectively (Bayandor et al. 2019). These results indicate that TRX can neutralize CYP induced-anxiety in mice.

The Y- maze test, also known as the spontaneous alternation test, is used to determine short term memory. The outcomes of the Y- maze test indicated a decrease in the number of spontaneous alterations in behavior in the CYP administered group of mice. Bali et al. (2019) reported that mice treated with glyphosate showed a significant decrease in number of spontaneous entries. Furthermore, rats treated with chlorpyrifos revealed a significantly low number of spontaneous alterations in behavior (Hussein et al. 2018). Our findings also revealed that the group of mice pre-supplemented with TRX 150 mg/kg/b.w exhibited elevated spontaneous alterations during the Y-maze test. Our outcome is similar to the results of Jamali-Raeufy et al. (2019), in which rats supplemented with TRX (100 mg/kg/b.w) against lipopolysaccharides displayed an increase in spontaneous alteration behavior. Moreover, kaempferol improved the memory against chlorpyrifos-induced toxicity in rats as indicated by an increase in the spontaneous alteration percentage (Hussein et al. 2018).

Malonaldehyde is the major end product of lipid peroxidation. Lipid peroxidation is a significant indicator of oxidative damage due to reactive oxygen species. Several studies have revealed that administration of CYP results in lipid peroxidation. According to Gabbianelli et al. (2004), an oral dose of CYP (12.5 mg/kg/b.w) for 60 days caused an increased MDA concentration in rats. A high content of MDA is an established cause of CYP-induced lipid peroxidation, which results in tissue damage. Our work showed that administration of a single dose of CYP induced oxidative stress in the serum, brain, and muscle, indicated by elevated levels of MDA. The results of our study are similar to those of Sankar et al. (2012), who reported that administration of CYP causes an increase in levels of MDA in brain tissue, while it reduces the activities of antioxidant enzymes. Ateşşahin et al. (2005) also reported that CYP causes an elevation in the levels of MDA in serum. The high level of MDA brings about reduction in the activities of antioxidant enzymes (Mezni et al. 2020). Most of the studies for TRX are made in order to assess the role of TRX supplementation as a protective agent for certain disorders or to reduce their harmful effects. According to our findings, the MDA level appeared to be notably escalated in the serum, brain, and muscle of mice exposed to CYP as compared to mice administrated with TRX. The mice administrated with TRX 150 mg/kg/b.w showed lowest MDA level in brain and, muscle. However, mice supplemented with TRX 300 mg/kg/b.w showed a significantly lower MDA level in serum. Our results are similar to previous studies such as Gao et al. (2021), which also indicated that supplementation of mice with 10 and 20 mg/kg/ b.w TRX for seven days mitigates the cerebral ischemic reperfusion damage induced oxidative stress by decreasing the MDA level in the brain. Nitric oxide deficient hypertensive rats, when treated with 100 mg/kg/b.w TRX for one month, exhibited ample potential to upgrade antioxidant properties and reduce the generation of lipid peroxidation (Raja et al. 2019).

Catalase is an important antioxidant enzyme in organisms' cellular systems. It reduces oxidative stress by catalyzing the hydrogen peroxide into molecular oxygen and H_2O (Burton et al. 1983). Moreover, on exposure to CYP, lipid peroxidation may be a major factor in reducing CAT activity (Ateşşahin et al. 2005). Studies revealed that oral administration of 10 mg/kg b.w. CYP for 28 days in mice reduces the CAT activity in the liver, erythrocytes and kidney. The decrease in CAT activity might be due to the utilization of enzymes in overcoming the effect of oxidative stress (Ince et al. 2012). Our study aligns with the other studies, Ziada et al. (2020) showed that oral supplementation of 200 mg/kg/b.w. CYP for 28 days brings about oxidative stress in the serum and brain, as indicated by reduced CAT activity. In our research, we found that CAT activity significantly decreased in the serum, brain, and muscle of mice exposed to CYP. However, mice supplemented with 150 mg/kg/b.w. TRX for 20 days showed higher CAT activity in the serum and brain. The group of mice administrated with 300 mg/kg/b.w.

TRX showed higher CAT levels in muscle. No notable variances were noticed in the activity of CAT in kidney tissues in our research. Our results are supported by the study of Badalzadeh et al. (2017), who found an elevated activity of antioxidant enzyme CAT in serum after pre-treatment of type-1 diabetic rats with troxerutin (150 mg/kg/b.w.) for 4 weeks. This is because troxerutin plays a crucial role in restoring the activities of the antioxidant enzymes. Another study conducted by Gao et al. (2021) on rats also supports this data. According to this study, the ischemic/reperfusion induced brain injured rats, when treated with troxerutin (10 and 20 mg/kg/b.w.) for 7 days, showed increased CAT activity in the brain. This indicates the role of TRX in improving the enzyme defense system.

Glutathione is a major antioxidant molecule in many cells and along with a number of enzymes such as GPx, performs various functions to maintain the cellular homeostatic state by removing ROS (Meister 1974). GPx is not specific for hydrogen peroxide and its reduction results in the escalation of lipid peroxidation due to oxidative stress. GPx converts reduced glutathione to an oxidized form and H_2O_2 to H_2O (Li et al. 2012). According to Hussien et al. (2013), GPx activity in the brain of female mice is reduced following exposure to 12 mg/kg/b.w CYP for 30 days. According to Raja et al. (2019), 100 mg/kg/b.w. TRX improved the activity of GPx in the liver, heart and kidney of L-NAME induced hypertension in rats. In our research the GPx level remained unaffected by TRX and CYP. The results of our study corroborate the results of the study of Zamanian et al. (2017), where rats treated with TRX (75, 150, and 300 mg/kg/b.w.) for 30 days did not reveal any change of GPx activity. The endogenous GPx antioxidant enzyme not only decomposes harmful RO-OH and H_2O_2 but it also contributes substantially to reducing the level of lipid peroxidation (Zamanian et al. 2017). The unchanged level of GPx in our study might be due to lower production of lipid peroxidation or due to an increase in the activity of the compensatory antioxidant defense system (GSH and SOD) to mitigate the production of free radicals generated by CYP.

It is concluded that troxerutin attenuates the abnormalities in neurobehavioral (memory, anxiety) and locomotive functions in mice. Additionally, troxerutin neutralizes the oxidative stress induced by cypermethrin in term of the redox status of mice. Further studies are required to evaluate the molecular pathways regulating the neuromotor functions of troxerutin against cypermethrin induced neurotoxicity.

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