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Effects of troxerutin and selenium supplementation in miti-gating cypermethrin-induced behavioral impairments and oxidative stress in mice

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Abstract: The current research aimed to investigate the potential effectiveness of single and combined supplementation with troxerutin (Trx) and selenium (Se) against cypermethrin (Cyp)-induced neurotoxicity and oxidative stress in mice. Thirty mice were randomly divided into five groups. The control group received 0.9% normal saline orally; the Cyp group received Cyp (5 mg/kg) orally; the Cyp+Trx group received Trx (150 mg/kg) in combination with Cyp; the Cyp+Se group received Se (25 μg/kg) with Cyp; and the Cyp+Trx+Se group received Trx (150 mg/kg) and Se (25 μg/kg) together with Cyp, all administered orally for 28 days. The individual and combined supplementation of Trx and Se significantly improved motor impairment, memory function, hepatic and renal health markers, and reduced anxiety levels (inside time/time in centar zone) in Cyp-exposed mice. Moreover, all supplementations reduced the Cyp-induced oxidative stress, but the SOD activity was increased in the serum and brain by the supplementation of Trx and Se, respectively. Liver and kidney SOD activities were improved with all the supplementations. Combined supplementation of Trx and Se enhanced brain acetylcholinesterase activity compared to the Cyp group. These findings suggest that both single and combined supplementation with Trx and Se confer neuroprotection against pesticide-induced oxidative and behavioral alterations; however, brain acetylcholinesterase activity and renal health markers were improved only by the combined Trx and Se supplementation.

Keywords: behavior, cypermethrin, neurotoxicity, oxidative stress, selenium, troxerutin

INTRODUCTION

Cypermethrin (Cyp) belongs to the type-II pyrethroid class and functions as a key pest control agent worldwide for veterinary, agricultural, and domestic applications. The widespread domestic and commercial use of Cyp increases exposure to non-target species. Recurrent exposure to pesticides leads to multiple disorders and toxicities [1]. Primarily, Cyp alters nervous and muscular functions and can also induce a wide array of neuronal toxicity, genotoxicity, and immunotoxicity [2]. Due to its lipophilic nature, it crosses the blood-brain barrier and subsequently alters the cellular antioxidant enzyme system, leading to neurotoxicity and motor impairments [3]. The neurotoxicity of Cyp is attributed to its modification of sodium channels, leading to prolonged sodium permeability in neuronal membranes and disrupted nerve impulse conduction. Prolonged opening of sodium channels also results in increased generation of reactive oxygen species, ultimately leading to oxidative stress [4]. Additionally, Cyp intoxication disrupts acetylcholine levels by inhibiting acetylcholinesterase, leading to locomotor deficits and paralysis [5].

The toxic effects of Cyp can be mitigated by antioxidants. Troxerutin (Trx) is a trihydroxyethylated, naturally occurring derivative of the bioflavonoid rutin. It is also recognized as vitamin P4 and exhibits anti-oxidative, neuroprotective, antidiabetic, anti-inflammatory, and anti-cancer activities [6]. Earlier studies have shown that Trx supplementation alleviates anxiety and fear by improving the learning and memory functions in rodents [7-8]. The administration

of Trx reduces MDA levels and enhances the activities of superoxide dismutase, catalase, and glutathione peroxidase in the rodents [9]. Our research group has also reported a reduction in MDA levels and improvements in catalase and glutathione activities when Cyp-exposed mice were pre-supplemented with Trx (150 and 300 mg/kg). In addition, it also improved behavioral performance, including motor coordination, anxiety, and memory-related parameters in Cyp-exposed mice [10]. As both doses showed comparable efficacy, the 150 mg/kg dose was used in the present study.

Selenium (Se) is an essential dietary mineral required for numerous physiological functions, including metabolic pathways, redox balance, thyroid hormone metabolism, and immune-related activities [11]. It is also important for normal central nervous system function, and its deficiency induces anxiety- and depression-like behavior, which can be mitigated by Se supplementation [12]. Likewise, nano-selenium administration in Cyp-treated rats displayed protective effects by improving the redox status and reducing inflammation, resulting in improved locomotor performance [3].

In biological systems, pesticide exposure produces oxygen-derived free radicals (ROS) that lead to oxidative stress. Because the brain contains high levels of polyunsaturated fatty acids, has a high oxygen demand, and possesses a relatively low antioxidant defense capacity, it is particularly vulnerable to ROS-induced neuronal disruption and oxidative stress [22]. In order to overcome the pesticide-induced damage, the current study aims to investigate the single and combined effects of Trx and Se supplementation against Cyp-induced behavioral deficits and oxidative stress. Hence, behavioral attributes like motor, anxiety, and memory functions, brain acetylcholinesterase activity, and redox status were analyzed in multiple mouse tissues to assess whether single and combined supplementation of Trx and Se counteract Cyp-induced toxicity. To the best of our knowledge, no studies have reported the effects of combined Trx and Se supplementation on Cyp-induced behavioral alterations and neurotoxicity. Although Trx and Se individually improved several Cyp-induced behavioral and biochemical alterations, their combined supplementation produced a more consistent recovery in selected endpoints, including brain acetylcholinesterase activity and renal function markers, supporting an additive protective effect in this model.

MATERIALS AND METHODS

Ethics statement

All procedures were conducted in compliance with the Institutional Guidelines of the Ethical Review Committee of the University of Veterinary and Animal Sciences Lahore, Pakistan (Directive Approval No. DR/119, 26th April 2024).

Chemicals used

Cypermethrin (Cyp, 10% EC) was purchased from Symans Pharmaceuticals (Lahore, Pakistan). Selenium (Se as Na_2SeO_3, 98% purity) and troxerutin (Trx) were purchased from Sigma-Aldrich, USA.

Experimental animals, management, and grouping

Thirty adult male albino mice (6-8 weeks old, weighing 25-35 g) were used in the study. They had free access to standard mouse chow and freshwater and were maintained under controlled temperature conditions (24±2°C) with a 12 h light-dark cycle. After one week of acclimatization, the mice were randomly divided into five groups of six mice each. The experimental groups were designated as follows: Control: mice received 0.9% normal saline orally; Cyp: mice were orally administered 5 mg/kg body weight Cyp only; Cyp+Trx: mice were orally supplemented with 150 mg/kg body weight Trx, along with 5 mg/kg body weight Cyp; Cyp+Se: mice were orally supplemented with 25 µg/kg body weight Se, along with 5 mg/kg body weight Cyp; Cyp+Trx+Se**:** mice were orally supplemented with 150 mg/kg body weight Trx and 25 µg/kg body weight Se, along with 5 mg/kg body weight Cyp. The experiment was conducted for 28 days. To assess motor coordination, anxiety, and memory functions, various tests were performed.

Motor coordination tests

Beam balance test: Motor coordination was assessed by the beam balance test, in which the time taken by mice to traverse the beam was recorded [10].

Pole test: The pole test was performed as described by Shehzad et al. [10]. Briefly, mice were placed on the top of a round pole and allowed to descend along its length to the base. The time required to reach the base was recorded, and the mean descent time was calculated.

Footprint test: The footprint test was conducted to determine the motor activity of the hind limb [10]. Three consecutive steps were analyzed to calculate the average values for each measurement, including stride length, step length, and step width.

Anxiety level tests

Open field test: Anxiety, locomotor, and exploration activity were assessed using the open field test. The test was conducted according to Bayandor et al. [7]. A camcorder was used to record the time spent in the central area of the arena. "Inside time" was defined as the cumulative time spent in the central zone during the 5-min trial. The number of center entries, the number of crossed squares (ambulation), mobility duration, and total distance covered during the 5-min period were also recorded.

Elevated plus maze test: An elevated plus maze test was carried out using the method described by Bayandor et al. [7]. The entry of each mouse into the open and closed arms was recorded. The parameters of anxiety, such as percent open arm time (OAT) and percent open arm entry (OAE), were calculated.

Memory test – Y-maze test

The Y-maze test assesses spatial memory in rodents by measuring the percentage of spontaneous alternations, defined as consecutive entries into all three arms of the maze without repetition [10].

Sample collection

After conducting the behavior tests, the mice were anesthetized. Blood samples were taken by cardiac puncture, placed in vacutainer tubes, and centrifuged at 1,500 x g for 10 min to separate the serum. The mice were then decapitated to remove the brain, liver, and kidney. Finally, the collected serum and tissue samples were labeled and kept at -80°C until further analysis.

Serum biochemical analysis

Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, and urea were estimated using commercial kits (DiaSys Diagnostic Systems GmbH, Germany). Brain, liver, and kidney tissues were individually weighed, homogenized in ice-cold 0.01 M phosphate-buffered saline (pH 7.4), and centrifuged at 5,000 × g for 20 min at 4 °C.

Redox status analysis

The amount of malondialdehyde (MDA) in serum and tissue homogenates of brain, kidney, liver, and muscle was estimated by measuring thiobarbituric acid reactive substances (TBARS) as per the method described by Feldman [13]. The MDA values were presented as μM. The serum and tissue homogenates' superoxide dismutase (SOD) activity was measured using the method described by Li [14]. Initially, 5 μL of the sample solution was added to Tris-HCl buffer (pH 8.2) containing ethylenediaminetetraacetic acid disodium salt (Na_2EDTA). Subsequently, 5 μL of pyrogallol (12 mM) was added, and the mixture was incubated at room temperature for 1 min. Absorbance was measured at 325 nm, and superoxide dismutase (SOD) free radical scavenging activity was expressed as percentage inhibition. Catalase (CAT) activity in serum and tissue homogenates was assessed using the method described by Hadwan and Abed [15]. CAT activity was expressed as U/mL for serum and tissue samples.

Brain acetylcholinesterase activity

Acetylcholinesterase activity in brain supernatants was determined according to the method of Ellman et al. [16] and expressed as mmol/min/g tissue.

Statistical analysis

Data normality was checked with the Shapiro-Wilk test, and Levene's test was used to evaluate variance homogeneity. The collected data were analyzed using one-way ANOVA through SPSS software (Version 22) and presented as mean±SE. The mean values were compared using Tukey's post hoc test to compare differences between the groups. A probability level of less than 0.05 was assumed significant.

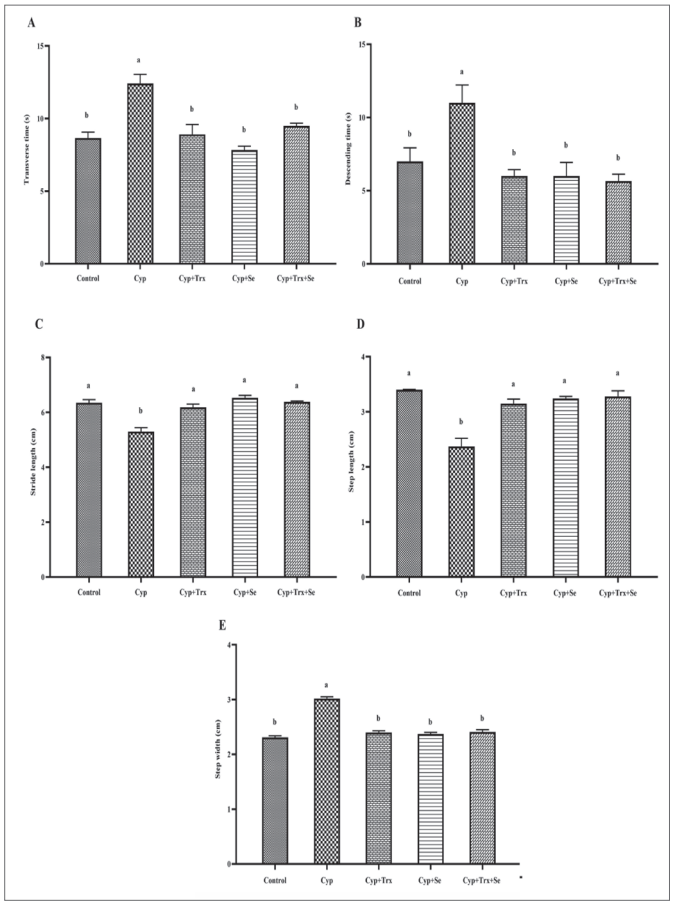

Fig. 1. Motor coordination in mice supplemented with Trx and Se during 28-day exposure to Cyp. **A** – Beam balance test; **B** – pole test; **C** – footprint test (stride length); **D** – footprint test (step length); **E** – footprint test (step width). Data are presented as the mean±SE (n=6/Group). Different superscripts[a-b] on bars indicate significant differences between groups (P<0.05). Control – saline; Cyp – 5 mg/kg body weight cypermethrin; Cyp+Trx – 150 mg/kg body weight troxerutin and 5 mg/kg body weight cypermethrin; Cyp+Se – 25 μg/kg body weight Se and 5 mg/kg body weight cypermethrin; Cyp+Trx+Se – 150 mg/kg body weight troxerutin+25 μg/kg body weight Se, and 5 mg/kg body weight cypermethrin.

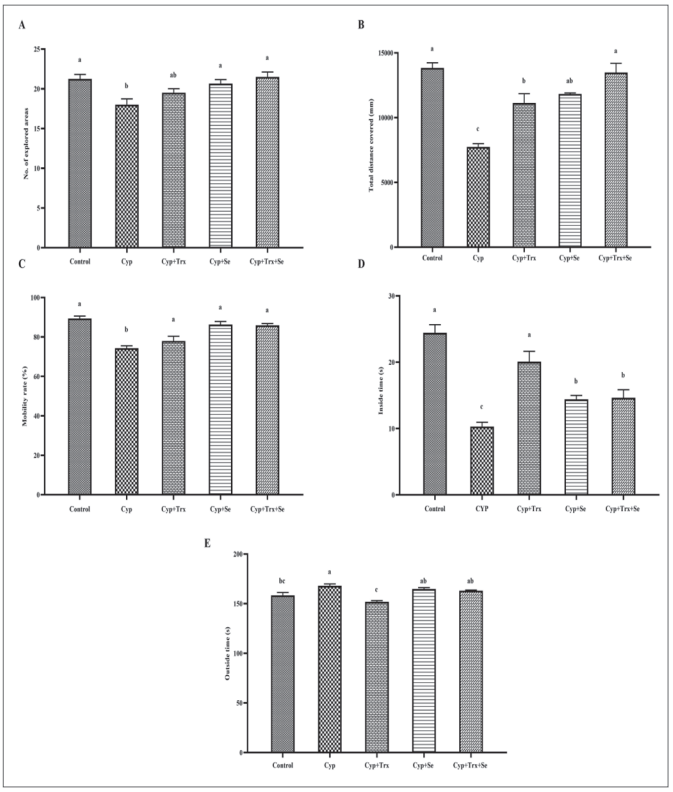

Fig. 2. Anxiolytic effects (open field test) in mice supplemented with Trx and Se during 28-day exposure to Cyp. **A** – explored areas; **B** – total distance; **C** – mobility rate; **D** – inside time; **E** – outside time. Data are presented as the mean±SE (n=6/Group). Different superscripts[a-c] on bars indicate significant differences between groups (P<0.05).

RESULTS

This study evaluated the neuroprotective efficacy of Trx and Se individually and in combination, against cypermethrin-induced oxidative stress and neurotoxicity in mice through behavioral, biochemical, and redox analyses.

Motor coordination

The results indicate that Cyp significantly compromised the performance of the mice in the beam balance, pole, and footprint tests compared to the control group. However, single or combined supplementation of Trx and Se significantly improved performance compared to the Cyp group (Fig. 1A-E).

Anxiety levels

In the open field test, Cyp exposure induced an anxiety-like phenotype, evidenced by a reduced time spent in the center zone (inside time) and reduced locomotor performance compared with the control group. In contrast, supplementation with Trx, Se, or Trx+Se significantly increased inside time and improved locomotor activity parameters (total distance, mobility rate, explored areas) compared with the Cyp group, indicating attenuation of Cyp-induced anxiety-like behavior along with restoration of exploratory activity (Fig. 2A-E).

In the elevated plus maze test, open-arm time, a standard index of anxiety, indicated that Se and Trx+Se supplementation significantly reduced anxiety-like

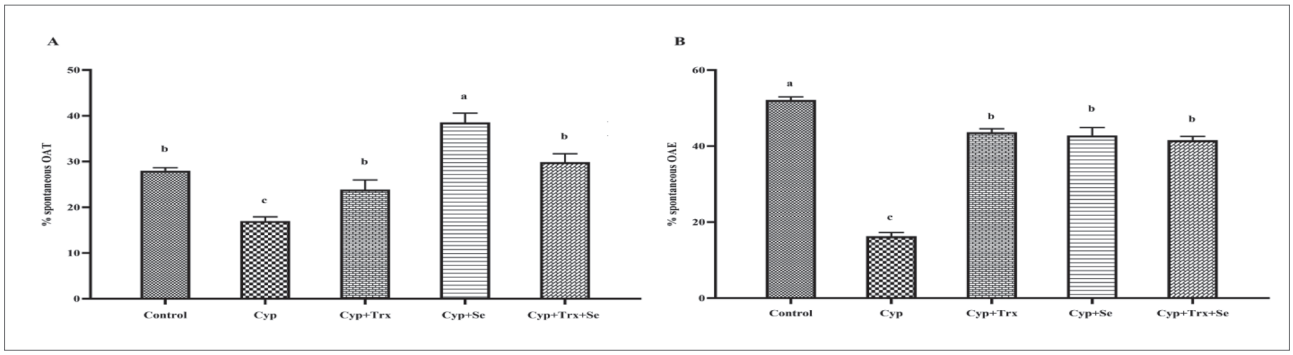

Fig. 3. Anxiolytic effects (elevated plus maze test) in mice supplemented with Trx and Se during 28-day exposure to Cyp. **A** – open arm time (OAT); **B** – open arm entries (OAE). Data are presented as the mean±SE (n=6/Group). Different superscripts[a-c] on bars indicate significant differences between groups (P<0.05).

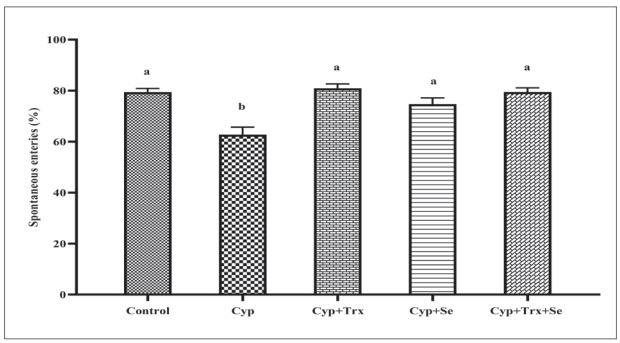

Fig. 4. Memory performance (Y-maze test) of mice supplemented with Trx and Se during 28-day exposure to Cyp. Data presented as mean±SE (n=6/Group). Different superscripts[a-b] on bars indicate significant differences between groups (P<0.05).

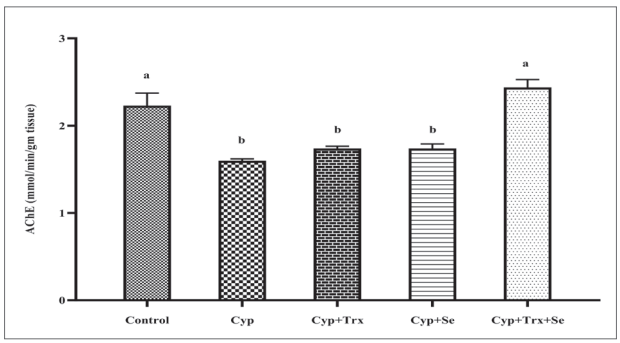

Fig. 5. Acetylcholinesterase activity in the brain of mice supplemented with Trx and Se against 28-day exposure to Cyp. Data presented as mean±SE (n=6/Group). Different superscripts[a-b] on bars indicate significant differences between groups (P<0.05).

behavior compared with the Cyp group (Fig. 3A). In addition, both single and combined supplementation with Trx and Se increased exploratory behavior, as reflected by a higher number of open-arm entries relative to the Cyp group (Fig. 3B).

Memory functions

Results from the Y-maze test showed that the Cyp group exhibited significantly lower spontaneous alternation compared with the control group. In contrast, spontaneous alternation was significantly increased (P<0.05) in the Trx, Se, and Trx+Se groups compared to the Cyp group (Fig. 4).

Serum biochemistry

Serum ALT, AST, creatinine, and urea levels were significantly higher in the Cyp group than in the control group, whereas all supplementations significantly reduced these parameters (P<0.05) compared with the Cyp group. Notably, a more pronounced reduction in creatinine levels was observed in the Trx+Se group (Table 1).

Serum and tissue redox status

Serum MDA levels were significantly increased in the Cyp group compared with the control group, whereas significant reductions (P<0.05) were observed in the Trx and Trx+Se groups relative to the Cyp group. Serum SOD activity was significantly decreased in the Cyp group but increased (P<0.05) in the Se-supplemented group compared with the Cyp group. Serum CAT activity remained unchanged across all groups (Table 2).

Brain MDA levels were significantly higher in the Cyp group than in the control group, whereas MDA levels were significantly reduced (P<0.001) in the Trx and Trx+Se groups compared with the Cyp group.

Table 1. Serum biomarkers of mice supplemented with Trx and Se during a 28-day exposure to Cyp

Biomarkers	Control	Cyp	Cyp+Trx	Cyp+Se	Cyp+Trx+Se	P value
ALT (U/L)	8.32±0.49^{b}	24.57±1.00^{a}	9.90±0.53^{b}	10.52±0.34^{b}	8.98±0.33^{b}	<0.001
AST (U/L)	19.34±0.23^{c}	38.29±1.35^{a}	25.73±0.40^{b}	24.68±1.02^{b}	22.94±0.61^{b}	<0.001
Creatinine (mg/dL)	1.62±0.02^{d}	5.56±0.26^{a}	3.74±0.16^{b}	3.38±0.09^{bc}	2.78±0.10^{c}	<0.001
Urea (mg/dL)	20.17±0.54^{c}	34.27±0.59^{a}	25.51±0.38^{b}	24.63±0.62^{b}	20.89±1.08^{c}	<0.001

Data are presented as the mean±SE (n=6/Group). Different superscripts^{a-d} on bars indicate significant differences between groups (P<0.05).

Table 2. Serum redox status of mice supplemented with Trx and Se during 28-day exposure to Cyp

Biomarkers	Control	Cyp	Cyp+Trx	Cyp+Se	Cyp+Trx+Se	P value
MDA (μM)	4.77±0.27^{c}	11.89±0.45^{a}	4.90± 0.266^{c}	11.02±0.207^{a}	7.88±0.27^{b}	<0.001
SOD (% inhibition)	18.71±0.27^{a}	16.91± 0.29^{b}	18.49±0.52^{a}	17.66±0.34^{ab}	17.92±0.12^{ab}	0.008
CAT (U/mL)	8.63±0.36	8.47±0.53	8.95±0.68	8.72±0.47	8.48±0.34	0.958

Data are presented as the mean±SE (n=6/Group). Different superscripts^{a-c} on bars indicates significant differences between groups (P<0.05); MDA – malondialdehyde; SOD – superoxide dismutase; CAT – catalase.

Table 3. Brain redox status of mice supplemented with Trx and Se during 28-day exposure to Cyp

Biomarkers	Control	Cyp	Cyp+Trx	Cyp+Se	Cyp+Trx+Se	P value
MDA (μM)	6.12±0.40^{c}	10.72±0.42^{a}	8.21±0.18 ^{b}	9.44±0.19^{ab}	8.47±0.63^{b}	<0.001
SOD (% inhibition)	18.31±0.13^{a}	16.94± 0.09^{b}	17.87±0.30^{ab}	18.19±0.29^{a}	17.95±0.30^{ab}	0.005
CAT (U/mL)	24.47±1.06	27.21±1.13	24.90±1.14	25.20±0.50	27.92±0.52	0.092

Data are presented as the mean±SE (n=6/Group). Different superscripts^{a-c} on bars indicates significant differences between groups (P<0.05); MDA – malondialdehyde; SOD – superoxide dismutase; CAT – catalase.

Table 4. Liver redox status of mice supplemented with Trx and Se during 28-day exposure to Cyp

Biomarkers	Control	Cyp	Cyp+Trx	Cyp+Se	Cyp+Trx+Se	P value
MDA (μM)	5.24±0.13^{b}	7.68±0.33^{a}	5.71±0.24^{b}	5.12±0.27^{b}	3.94±0.20^{c}	<0.001
SOD (% inhibition)	16.92±0.13^{a}	15.79± 0.35^{b}	17.10±0.08^{a}	17.81±0.35^{a}	17.72±0.30^{a}	<0.001
CAT (U/mL)	36.46±1.21	32.57±1.67	33.16±1.77	35.04±1.53	31.52±0.82	0.289

Data are presented as the mean±SE (n=6/Group). Different superscripts^{a-b} on bars indicates significant differences between groups (P<0.05); MDA – malondialdehyde; SOD – superoxide dismutase; CAT – catalase.

Table 5. Kidney redox status of mice supplemented with Trx and Se during 28-day exposure to Cyp

Biomarkers	Control	Cyp	Cyp+Trx	Cyp+Se	Cyp+Trx+Se	P value
MDA (μM)	4.55±0.27^{b}	6.42±0.17^{a}	5.10±0.21^{b}	4.96±0.05^{b}	4.71±0.20^{b}	<0.001
SOD (% inhibition)	17.25±0.21^{a}	16.02± 0.11^{b}	17.88±0.20^{a}	17.39±0.50^{a}	18.43±0.22^{a}	<0.001
CAT (U/mL)	52.47±2.05	55.28±0.86	57.74±1.88	54.43±1.85	51.58±1.47	0.114

Data are presented as the mean±SE (n=6/Group). Different superscripts^{a-b} on bars indicates significant differences between groups (P<0.05); MDA – malondialdehyde; SOD – superoxide dismutase; CAT – catalase.

Brain SOD activity was significantly decreased in the Cyp group relative to the control group; however, SOD activity was significantly improved (P<0.05) in the Se-supplemented group compared with the Cyp group. No significant differences in brain CAT activity were observed among the groups (Table 3).

A significant increase was observed in liver MDA levels in the Cyp group compared with the control group, whereas a decrease (P<0.05) was observed in Trx, Se, and Trx+Se groups (P<0.05) as compared to the CYP group. Liver SOD activity was decreased in the Cyp group compared with the control group; however, SOD activity was significantly increased (P<0.05) in the Trx, Se, and Trx+Se groups relative to the Cyp group. Liver CAT activity remained unchanged across all groups (P>0.05) (Table 4).

Kidney MDA levels were significantly increased in the Cyp group compared with the control group, whereas they were significantly reduced (P<0.05) in the Trx, Se, and Trx+Se groups relative to the Cyp group.

Kidney SOD activity was significantly decreased in the Cyp group compared with the control group; however, SOD activity was significantly increased ($P<0.05$) in the Trx, Se, and Trx+Se groups compared with the Cyp group. Kidney CAT activity was not significantly affected among the treatment groups (Table 5).

Brain acetylcholinesterase activity

Brain acetylcholinesterase activity was significantly decreased in the Cyp group compared with the control group. A significant increase in acetylcholinesterase activity ($P<0.05$) was observed only in the Trx+Se group compared to the Cyp group (Fig. 5).

DISCUSSION

Cyp is a well-known neurotoxic pesticide that rapidly crosses the blood-brain barrier and disrupts neuronal excitability through modulation of the voltage-gated sodium channels [3]. It has been well established that behavior serves as a biological biomarker of neurotoxicity induced by chemical agents [20]. We investigated the adverse effects of Cyp exposure and their mitigation by Trx and Se, supplemented either individually or in combination, on motor coordination, anxiety-like behavior, and memory functions, along with redox status in multiple organs of mice. Based on the existing literature, no earlier work has been reported on the combined efficacy of Trx and Se against Cyp-induced toxicity in mice. Our study manifested the possible neuroprotective and redox potential of single and combined supplementation of Trx (150 mg/kg) and Se (25 μg/kg), which was indicated by the behavioral outcomes in terms of improved locomotor activity, enhanced spatial memory, and anxiolytic behavior in supplemented mice. Trx and Se supplementation reduced oxidative stress by lowering lipid peroxidation and enhancing the primary antioxidant SOD activity, ameliorated renal and hepatic health markers, and subsequently improved the acetylcholinesterase activity in the brain. The findings of the current study support the efficacy of Trx and Se as neuroprotective and antioxidant agents for Cyp-induced toxicity.

Motor, anxiety, and memory functions are among the most important neurobehavioral endpoints for evaluating pyrethroid poisoning, as these attributes are affected by all pyrethroids, regardless of species or mode of application [20]. In the current study, the mice receiving Cyp exhibited motor dysfunction, indicated by a marked reduction in transverse time, increased time to climb down the pole, and gait impairment as evidenced by a decrease in stride length and step length, as well as a wider step width. Our findings are consistent with earlier reports, which found that exposure of Cyp in rodents displayed significant motor deficits as Cyp toxicity resulted in potentiated skeletal muscle contraction, increased extensor tone in the hind limbs, as well as brought about rolling gait and incoordination in movements, which later led to coarse tremors in rodents [18,21]. The results of the present study showed that mice administered with single and combined supplementation of Trx and Se displayed improved motor coordination. Our findings agree with previous studies, which reported that flavonoids and Se improve motor functions by increasing the antioxidant enzyme activity and preventing the loss of dopaminergic neurons due to redox imbalance [21,22]. It has also been reported that Cyp exposure inhibits the activity of acetylcholinesterase (AChE) in the neural tissues [23]. This inhibition of AChE activity has been associated with impaired neuromuscular signaling and loss of coordinated movement in rodents. Furthermore, as Cyp reduced brain AChE activity in the current study, the subsequent increase in motor coordination by Trx and Se supplementation is probably due to their antioxidant and neuroprotective properties, which were further enhanced by the combination, as indicated in the recovery of cholinergic brain AChE activity.

Hypolocomotion and inside-time duration are key indicators used in the assessment of anxiogenic behavior. The current results show that Cyp administration produced anxiety-like behavior and decreased locomotor activity in the open field and elevated plus maze tests. In the open field test, Cyp-exposed mice displayed anxiety-like behavior characterized by reduced center exploration, accompanied by impaired locomotor performance. Increased avoidance of the center zone is widely used as an index of elevated anxiety-like behavior, although locomotor impairment may act as a confounder when neurotoxicants reduce mobility. Cyp exposure has been associated with anxiety-like behavior in rodents, which is indicated by the poor performance in the elevated plus maze and open field tests [24,25]. This anxiogenic effect has been

linked to augmented oxidative stress and inflammatory alterations in the hippocampus, as well as decreased brain-derived neurotrophic factor (BDNF), which is involved in emotional regulation. A combination of these neurotoxic changes might have resulted in the Cyp-induced anxiety-like behaviors. Importantly, Trx, Se, and Trx+Se supplementation improved center-zone exploration and locomotor indices, suggesting that antioxidant supplementation alleviated Cyp-induced behavioral deficits rather than merely increasing general activity. It was reported [26,27] that both Trx and Se mitigate hypoactive exploratory and anxiety-like behaviors in rodents. Trx has anxiolytic effects as it reduces neuroinflammatory and oxidative stress radicals, whereas Se enhances antioxidant capacity, maintains synaptic function, and decreases neuronal oxidative stress. Hence, Trx and Se reversed the effects of Cyp-induced behavioral anxiety.

Memory function was assessed by using the Y-maze test, where Cyp-exposed mice displayed a marked decrease in the percentage of spontaneous alterations. Narwanto et al. [28] also reported that exposure to Cyp impaired short-term memory in rats, as evidenced by reduced spontaneous alternation in the Y-maze, which was associated with hippocampal neuronal loss and increased oxidative stress, reflected by elevated brain MDA levels. Our results indicate that the individual and combined supplementation of Trx and Se boosts spatial memory behavior in Cyp-exposed mice. Memory assessments in previous studies [26,29] have shown that the supplementation of Trx and Se alone results in improved working memory impairments in rodents by enhancing antioxidant capacity and reducing neuroinflammation and synaptic apoptosis. The role of specific brain regions in these behavioral functions is well established, with the hippocampus playing a central role in anxiety-like behavior and memory functions, and the cerebellum and cerebral cortex being critical for motor coordination. All endpoints of our study used to assess anxiety and memory function are closely linked to motor functions, as they connect the measurement of anxiety and memory to the animals' ambulatory capacity for surrounding exploration. The decline in locomotor performance, memory, and exploratory behavior following Cyp exposure appeared to be closely linked with the observed reduction in brain AChE activity. As Trx and Se supplementation resulted in significant behavioral improvements, this can probably be attributed to the antioxidant and neuroprotective actions that are independent of cholinergic effects.

Beyond the central nervous system, Cyp also induces systemic toxicity, as indicated by elevated liver and kidney biomarkers in mice. According to our findings, serum ALT, AST, creatinine, and urea levels were elevated in the Cyp-exposed group. These results are consistent with earlier reports [30,31], which demonstrated that cellular and membrane damage caused by Cyp-induced oxidative stress leads to increased serum levels of ALT, AST, creatinine, and urea. Individual and combined Trx and Se supplementation in our study showed a reduction in serum ALT, AST, creatinine, and urea levels. Our findings are in line with previous investigations that reported protective effects of Se and Trx on liver and kidney functions [32,33]. Trx may enhance renal and hepatic cellular structure and function by increasing antioxidant enzyme levels, thereby enhancing reactive oxygen species scavenging and alleviating oxidative stress. Similarly, Se maintains cell viability as a key constituent of selenocysteine, thereby protecting mammalian cells against oxidative injury. The observed improvement in hepatic and renal biomarkers with Trx and Se supplementation may be attributed to their attenuation of oxidative stress, as evidenced by reduced MDA levels and enhanced SOD activity in the liver and kidney.

Excessive production of reactive free radicals and oxidative stress is considered one of the major mechanisms by which pyrethroid pesticides disrupt cellular function. MDA serves as an important indicator of oxidative stress as it is the primary product of lipid peroxidation [13]. Redox status analyses of serum, brain, liver, and kidney tissues showed increased MDA levels and decreased SOD activity in Cyp-treated mice. Our results are in accordance with previous studies where Cyp exposure displayed escalated MDA levels and reduction in antioxidant enzyme activities [3,34]. As Cyp is lipophilic in nature and can easily cross the lipid bilayer, these changes exhibited its ability to disrupt redox balance and damage cell integrity. In the present study, Trx and Se supplementation suppresses the generation of lipid peroxidation in serum and tissues by lowering the MDA level and substantially increasing SOD activity due to their ROS scavenging properties. Consistent with our results, several studies have demonstrated the antioxidant roles of Trx and

Se, reporting increased SOD activity in rodents and protection against oxidative damage in blood and brain tissues [27,35]. Although CAT is found in almost all tissues that require oxygen and plays an important role in neutralizing hydrogen peroxide, it is not the first enzyme to respond during oxidative stress [36]. In the current study, the results of CAT activity remained non-significant. As in most cases, SOD converts the superoxide radicals to hydrogen peroxide at higher rates. This reflects the decomposition of hydrogen peroxide in cellular compartments such as mitochondria, where CAT is not the primary antioxidant enzyme, and glutathione peroxidase (GPx) mainly performs this function in coordination with SOD. Even though the current research demonstrated the neuroprotective properties of Se supplementation, the activity of GPx was not measured. Although considerable alterations in MDA and SOD indicate an antioxidant effect, the role of Se-dependent GPx pathways is inferential, especially in the context of insignificant CAT activity, which is the limitation of this study and therefore requires further investigation.

AChE is synthesized by cholinergic neurons and hydrolyzes acetylcholine in the synaptic cleft. The high lipid content of synaptic membranes increases their susceptibility to oxidative stress [37]. AChE also serves as a neurotoxicity indicator that is associated with behavioral changes and motor dysfunctions [5]. As in previous studies [5,38], our study also showed decreased AChE activity in the brain of Cyp-exposed mice. However, this activity was improved only by the combined supplementation of Trx and Se. Bayandor et al. [7] reported that the administration of Trx alone reduced AChE activity in the hippocampus of rats. Even though Trx lowers AChE activity, it stimulates the expression of α7-nAChR and improves the cholinergic function. Similarly, many studies claimed that Se treatment increased AChE activity in the rodent brain [11,39]. This result indicates that co-administration of Trx and Se may exert additive neuroprotective effects, enhancing AChE activity more effectively than either compound alone. This may be attributed to their complementary mechanisms of action, with Se contributing to redox regulation and selenoprotein-mediated neuroprotection, and Trx providing potent antioxidant and anti-inflammatory effects. Together, these mechanisms may mitigate oxidative stress and improve AChE activity in the brain.

As discussed, Trx and Se exhibit well-established protective roles through multiple mechanisms, including the enhancement of endogenous antioxidant defenses and reduction of lipid peroxidation. Together, these effects preserve neuronal integrity, improve cognitive and behavioral functions, and attenuate systemic organ toxicity in models of chemically induced neurotoxicity. In the present study, supplementation with Trx and Se in Cyp-exposed mice improved behavioral performance and supported hepatic and renal health, likely by alleviating oxidative stress, as evidenced by increased SOD activity and reduced MDA levels. While Trx and Se supplementation alone did not improve brain AChE activity, their combined supplementation effectively improved it, suggesting an additive interaction between Trx and Se.

CONCLUSIONS

In conclusion, single and combined supplementation with Trx and Se ameliorated Cyp-induced neurotoxicity and behavioral impairments in mice through their antioxidant and neuroprotective properties. In addition, Trx and Se supplementation, administered individually or in combination, improved hepatic and renal function markers. Moreover, co-supplementation alleviated the inhibitory effects of Cyp on brain AChE activity, thereby reducing neuronal hyperexcitability. Future studies are warranted to elucidate the precise molecular mechanisms underlying the neuroprotective actions of Trx and Se against Cyp-induced neuronal dysfunction.

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Data availability: The data supporting this article are available in the online dataset: https://www.serbiosoc.org.rs/NewUploads/Uploads/Shehzad%20et%20al_Dataset.xlsx

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