Evaluation of the Impacts of Taurine on Oxidative Stress Indices in Sera and Brain of Rats Exposed to Cypermethrin

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Abstract: Cypermethrin is a pyrethroid insecticide applied for pest control on animals and the environment. Taurine is a putative antioxidant and bioprotective amino acid. The purpose of the research was to evaluate the impacts of taurine on oxidative stress indices in the sera and brains of rats exposed to cypermethrin. Forty rats were assigned to five groups of eight rats each. Distilled water was given to the first group, while the second group received soya oil (2 ml/kg). Cypermethrin (20 mg/kg) was administered to the third group. The Taurine50+Cypermethrin group received taurine (50 mg/kg) and cypermethrin, while the Taurine100+Cypermethrin group was administered with taurine (100 mg/kg) and Cypermethrin. The treatments were given once daily by oral gavage for 35 days. Sera were obtained from the blood samples of the rats after the completion of the study for the determination of the oxidative stress indices (malondialdehyde concentration and the activities of antioxidant enzymes). Oxidative stress indices were analysed in the brains. Taurine significantly (P < 0.05) augmented the superoxide dismutase activity in the sera. However, other oxidative stress indices were not ameliorated by taurine in the sera and the brains. Cypermethrin (20 mg/kg) did not overtly evoke oxidative stress in the sera and the brains of the rats in this study, probably because it is a moderately toxic insecticide. This is the first study that has investigated the effects of taurine on cypermethrin toxicity. Further research is warranted to expound the mechanisms of action of taurine and cypermethrin in biological systems.

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Pesticides are substances or mixtures of substances that are used in the environment for the control of pests, weeds and disease vectors (Nicolopoulos-Stamati et al., 2016). Cypermethrin (CYP) is a type II pyrethroid pesticide and it is utilized for agricultural, community and animal health schemes (Sharma et al., 2014). Additionally, CYP is a broad-spectrum insecticide and it kills beneficial insects as well as the targeted insects in the environment (Pascual and Peris, 1992). Consequently, a disruption of the ecosystem might occur. Cypermethrin elicits toxicity by interrupting sodium channels in neurons and this culminates in the destruction of neuronal transmission (Singh et al., 2012). Moreover, CYP induces oxidative stress (Mossa et al., 2015; Das et al., 2016). Oxidative stress refers to the alteration in the equilibrium between oxidants and antioxidants in preference of oxidants (Puttachary et al., 2015). However, antioxidants in cells delay, thwart or eliminate oxidative damage (Halliwell, 2007). Antioxidant enzymes are the body’s major defence against oxidative injury (Yildirim and Kilic, 2011).

Accordingly, an alteration in the activity of antioxidant enzymes might indicate oxidative stress (Mossa et al., 2013). Taurine (2-aminoethanesulfonic acid, TA) is an organic compound and an antioxidant that is commonly found in animal tissues (Schuller-Levis et al., 2003). It protects cells by its antioxidant property (Ito et al., 2008), and by scavenging reactive oxygen and nitrogen species (Oliveira et al., 2010). There is scarceness of reports on the influence of TA on oxidative injury evoked by pyrethroids in rodents. Cypermethrin is commonly utilized for the control of pests in Nigeria. Therefore, it is expedient to investigate the mitigation of the plausible harmful impacts of CYP in living systems. The purpose of the current research was to assess the effects of taurine on oxidative stress indices in the sera and brains of rats exposed to cypermethrin.

MATERIALS AND METHODS

Experimental Animals: The male Wistar rats used in this study were obtained from the Department of Physiology, Faculty of Veterinary Medicine, Ahmadu
Bello University, Zaria, Kaduna State, Nigeria. They were acclimatized for a period of two weeks before the study started, and they were housed in cages under standard environmental conditions (23–25°C, 12hrs/12hrs light/dark cycle). The experimental animals were provided with standard rat chow and tap water ad libitum. The research was approved by the University of Abuja Research Ethics Committee. The animals were handled in accordance with the stipulations of the National Institute of Health Guide for Care and Use of Laboratory animals (Garber et al., 2011). All the institutional and national guidelines for the care and utilization of laboratory animals for scientific research were adhered to.

**Chemicals:** The commercial grade of CYP (marketed as Cypro-10®) was purchased from an agrochemical company in Abuja, Nigeria, Federal Capital Territory, West Africa. A 5% solution of cypermethrin (emulsifiable concentrate) was reconstituted from Cypro-10® which was a 100% solution containing 100 mg/ml of CYP. It was prepared by reconstitution in soya oil (SO, Grand Cereals and Oil Mills Limited, Jos, Nigeria). An analytical grade of taurine (TA) was obtained from Sigma Aldrich® (Steinheim, Germany). 100 mg of TA was reconstituted in distilled water to obtain a 100 mg/ml suspension before the rats were administered with it on a daily basis.

**Subchronic Toxicological Study:** The rats were weighed and allocated to five groups with eight rats in each group. Distilled water was given to the DW group, while the animals in the SO group received soya oil at 2 ml/kg. An oral median lethal dose (LD₅₀) value of 200 mg/kg was selected for CYP based on the range of oral LD₅₀ of rats reported as 150 – 500 mg/kg by USEPA (1989). Cypermethrin was administered to the CYP group at 20 mg/kg (10% of the LD₅₀ of CYP that was selected as 200 mg/kg). The TA50+CYP group received TA at 50 mg/kg (Cetiner et al., 2005; Akande et al., 2014 a,b) and CYP at 20 mg/kg, successively, while the TA100+CYP group was administered with TA at 100 mg/kg (Abdel-Reheim, 2016; Heidari et al., 2018) and CYP at 20 mg/kg, consecutively. The treatments were administered once daily by oral gavage for 35 days. The animals were observed for clinical signs of toxicity during the investigation. The rodents were sacrificed and 3 ml of blood samples were collected into centrifuge tubes after the study was terminated. The blood samples were incubated at room temperature for 30 minutes and then centrifuged at 1000 x g for 5 minutes in order to obtain sera samples.

**Preparation of Tissue Homogenates:** The brain tissues were rinsed with physiological saline, patted with filter paper and weighed after excision from the rats. Sections of the brain tissues were combined with phosphate buffered saline (pH 7.4) in a 1:10 (w/v) ratio and homogenized with pestles and mortars. The ensuing mixtures were kept in ice baths and centrifuged at 3000 x g for 10 minutes. The supernatants obtained were used for the assessment of brain malondialdehyde (MDA) concentration and the activities of brain antioxidant enzymes.

**Determination of Serum and Brain Malondialdehyde Concentration:** The MDA concentration in the serum and brain samples were evaluated in accordance with the method of Draper and Hadley (1990). The solutions were cooled under tap water, and the absorbance was assessed with an ultraviolet (UV) spectrophotometer (T80 UV/Visible Spectrometer®; PG Instruments Ltd., Leicestershire, United Kingdom) at 532 nm. The MDA concentration in the serum and brain samples were computed with the use of the absorbance coefficient, MDA-TBA complex 1.56 x 10³/cm/M.

**Assays of the Activities of Serum and Brain Antioxidant Enzymes:** The activities of the antioxidant enzymes in the serum and brain samples were determined as follows: The activity of superoxide dismutase (SOD) was assessed with the Northwest Life Science Specialties SOD activity assay kit, as described by Martin et al., (1987). In addition, catalase (CAT) activity was evaluated by the Northwest Life Science Specialties CAT activity assay kit in line with the method of Beers and Sizer (1952). Glutathione peroxidase (GPx) activity was analysed with the Northwest Life Science Specialties GPx activity assay kit and the assay was based on the oxidation of reduced glutathione (GSH) to form oxidized GSH (Paglia and Valentine, 1967). The assay kits were procured from Northwest Life Science Specialties, LLC, Vancouver, Washington, District of Columbia, United States of America.

**Statistical Analysis:** Data are expressed as mean ± standard error. The biochemical parameters were analyzed using one-way ANOVA and Tukey’s multiple comparison post hoc test. Graphpad Prism version 7.03 for Windows (Graphpad software, San Diego, California, USA) was utilized as the statistical package. Values of P < 0.05 were considered as statistically significant.

**RESULTS AND DISCUSSION**

The Wistar rats did not exhibit any clinical sign of toxicity and there was no mortality during the research. This may be attributed to the fact that is CYP is a moderately toxic (toxicity class II) pyrethroid insecticide (USEPA, 1989).

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In the current research, there was no considerable difference in the serum malondialdehyde (MDA) concentration among the treatment groups (Table 1). There was no remarkable increase in the MDA level of the CYP group, although it was higher compared to those of the TA-treated groups. It is known that CYP induces oxidative stress in biological systems and this may be evidenced by an elevation in MDA level (Soliman et al., 2015; Abdul-Hamid et al., 2017).

Conversely, there was a diminution in the serum MDA level in the TA50+CYP and the TA100+CYP groups, although not statistically significant in this research. Taurine reduced serum MDA concentration in experimental animals exposed to various toxicants (Akande and Ahmed, 2017; Liu et al., 2017). Furthermore, there was a significant increase (P < 0.05) in the serum superoxide dismutase (SOD) activity of the TA50+CYP group relative to that of the CYP group (Fig. 1).

However, the reason why TA did not augment the activities of the serum CAT and GPx in this study is unknown. In the present research, the impacts of CYP and TA on MDA concentration as well as the activities of antioxidant enzymes in the brain samples of the male Wistar rats were evaluated. There was no significant difference in the brain MDA concentration, and the activities of brain SOD, CAT, and GPx in the experimental groups (Table 2). The brain MDA concentration of the CYP group was not overtly increased in accordance with the reports of other researchers (Sankar et al., 2012; Özok et al., 2019). This may be due to the fact that CYP elicits variable responses depending upon the doses, time, routes of exposure, strain, age, gender, and species of animals across multiple studies (Singh et al., 2012). Additionally, CYP has low mammalian toxicity (USEPA, 1989).

Therefore, these afore-mentioned assertions may provide an explanation for the apparent lack of induction of considerable oxidative stress in the serum and brains of the rats by CYP in the current research. Furthermore, insignificant increases were recorded in the activities of the brain antioxidant enzymes in the TA50+CYP and the TA100+CYP groups compared to the CYP group. Based on existing scientific literature, TA improves the activities of brain antioxidant enzymes in various experimental models (Patel and Lam-Cam, 2017; Niu et al., 2018). These corroborated the results in this research.

![Fig 1. Effects of the treatments on serum superoxide dismutase activity in male Wistar rats](image)

*p < 0.05 TA50+CYP group versus CYP group; DW: Distilled Water; SO: Soya Oil; CYP: Cypermethrin (20 mg/kg); TA50+CYP: Taurine (50 mg/kg) + Cypermethrin (20 mg/kg); TA100+CYP: Taurine (100 mg/kg) + Cypermethrin (20 mg/kg); MDA: Malondialdehyde; SOD: Superoxide dismutase; CAT: Catalase; GPx: Glutathione peroxidase

Superoxide dismutase enhances the breakdown of superoxide entities to hydrogen peroxide (Ikpeme et al., 2015). Cypermethrin has been reported to decrease SOD activity in rats (Ghazouani et al., 2019), and this agrees with the finding in our research. There was no distinction in the serum catalase (CAT) and serum glutathione peroxidase (GPx) activities of the experimental groups (Table 1). The activities of the serum CAT and GPx in the CYP group were not different from those in the other groups. Other investigators have stated that CYP elicits a decrease in the activities of serum antioxidant enzymes (Sharma et al., 2014; Eraslan et al., 2016). It has been shown that CAT and GPx reduce hydrogen peroxide into water and oxygen to avert oxidative injury and ensure homeostasis (Ikpeme et al., 2015). Moreover, TA improves the activities of serum antioxidant enzymes in rats (Akande and Ahmed, 2017) and fish (Zhang et al., 2018).

**Table 1. Effects of the treatments on serum oxidative stress parameters**

| Parameters   | DW (nmol/mL) | SO (nmol/mL) | CYP (nmol/mL) | TA50+CYP (nmol/mL) | TA100+CYP (nmol/mL) |
|--------------|--------------|--------------|---------------|--------------------|---------------------|
| Serum MDA    | 13.56 ± 0.28 | 14.04 ± 0.89 | 13.98 ± 1.47  | 13.16 ± 0.68       | 13.64 ± 0.41        |
| CYP          | 81.26 ± 0.94 | 85.76 ± 1.57 | 82.48 ± 2.27  | 82.36 ± 0.98       | 81.88 ± 0.76        |
| GPx          | 19.90 ± 0.97 | 18.60 ± 0.37 | 18.36 ± 0.24  | 18.20 ± 0.24       | 18.46 ± 0.44        |

**DW**: Distilled Water; **SO**: Soya Oil; **CYP**: Cypermethrin (20 mg/kg); **TA50+CYP**: Taurine (50 mg/kg) + Cypermethrin (20 mg/kg); **TA100+CYP**: Taurine (100 mg/kg) + Cypermethrin (20 mg/kg); **MDA**: Malondialdehyde; **SOD**: Superoxide dismutase; **CAT**: Catalase; **GPx**: Glutathione peroxidase

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This is the first study that investigated the impacts of TA on CYP-induced oxidative stress in the brains and sera of male Wistar rats. It may be deduced from the results that CYP at 20 mg/kg did not elicit oxidative stress to a noticeable extent, and this may have hampered the full expression of the antioxidant potential of TA in this research.

**Conclusion:** The results indicated that the subchronic low dose exposure of the rats to CYP did not induce significant oxidative stress, except with regard to the serum SOD activity. Probably, this was why TA did not elicit its antioxidant and bioprotective properties profoundly in the current study. Therefore, it is posited that the beneficial effects of TA in biological systems may be dependent on the level of induction of oxidative stress by cypermethrin and other pesticides.

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**Table 2. Effects of the treatments on brain oxidative stress parameters**

| Parameters | DW | SO | CYP | TA50+CYP | TA100+CYP |
|------------|----|----|-----|---------|-----------|
| Brain MDA (nmol/mg protein) | 24.48 ± 23.66 | 21.38 ± 21.06 ± 0.47 | 22.76 ± 1.01 |
| Brain SOD (IU/L) | 0.50 | 1.24 | 1.02 |
| Brain CAT (IU/L) | 0.14 | 0.20 | 0.23 |
| Brain GPx (IU/L) | 94.06 ± 90.82 | 89.14 ± 94.76 ± 1.28 | 90.56 ± 3.05 |
| | 3.72 ± 2.52 | 3.26 |
| | 37.30 ± 33.86 | 31.86 ± 33.60 ± 1.97 | 32.58 ± 3.41 |
| | 1.00 | 1.30 | 2.27 |

*DW: Distilled Water; SO: Soya Oil; CYP: Cypermethrin (20 mg/kg); TA50+CYP: Taurine (50 mg/kg) + Cypermethrin (20 mg/kg); TA100+CYP: Taurine (100 mg/kg) + Cypermethrin (20 mg/kg); MDA: Malondialdehyde; SOD: Superoxide dismutase; CAT: Catalase; GPx: Glutathione peroxidase*
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