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Effects of melatonin on changes in cognitive performances and brain malondialdehyde concentration induced by sub-chronic co-administration of chlorpyrifos and cypermethrin in male Wister rats

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PEER REVIEW

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Comments

This is a good study in which the authors evaluated the toxic effects of CPF and CYP and the protective role of melatonin as an antioxidant.
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ABSTRACT

Objective: To evaluate the ameliorative effect of melatonin on sub-chronic chlorpyrifos (CPF) and cypermethrin (CYP)-evoked cognitive changes in male Wistar rats.

Methods: Fifty adult male Wistar rats, divided into five groups of ten rats each, were used for the study. Groups I and II were given distilled water and soya oil (2 mL/kg) respectively. Group III was administered with melatonin at 0.5 mg/kg only. Group IV was administered with CPF [7.96 mg/kg (1/10th LD_{50})] and CYP [29.6 mg/kg (1/10th LD_{50})] , and Group V was administered with CPF [7.96 mg/kg (1/10th LD_{50})] and CYP [29.6 mg/kg (1/10th LD_{50})] 30 min after melatonin (0.5 mg/kg). The regimens were administered by gavage once daily for 12 weeks. Thereafter, cognitive performances were determined and the brain was evaluated for malonaldehyde concentration.

Results: CPF and CYP induced cognitive deficits and increased brain malonaldehyde concentration, which were all ameliorated by melatonin.

Conclusion: Cognitive deficits elicited by CPF and CYP was mitigated by melatonin due to its antioxidant property.

KEYWORDS

Chlorpyrifos, Cypermethrin, Cognition, Brain malondialdehyde, Melatonin

1. Introduction

The application of pesticides, which plays a pivotal role in the control of vector-borne crop diseases, has resulted in tremendous increase in crop production and, consequently, meeting the food demand of the escalating global human and animal population. Unfortunately, most of the applied pesticides are dispersed in the environment[1], and they adversely affect the health of both humans and animals[2,3]. The application of combined pesticides with different modes of action is fast gaining popularity in pest control programmes because such applications result in manifestation of broad spectrum of activity, with better efficacy in pest control[4]. Thus, the

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combination of pesticides exhibits a different toxicological profile, when compared with the toxicity of the individual pesticides in the combination[4]. Concurrent exposure to chlopyrifos (CPF) and cypermethrin (CYP) leads to the inhibition of esterases, responsible for hydrolysis of the latter and, consequently, slows down its metabolism[5]. Such interaction enables the use of smaller doses of both pesticides[6]. Although beneficial in combating pest resistance, the formulations of combined pesticides pose a new challenge to human and animal health since the outcomes of such interactions are unknown. CPF [O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl) phosphorothionate] is a broad-spectrum organophosphate insecticide utilized extensively in agriculture and for residential pest control throughout the world[7].

Chlorpyrifos is one of the most widely used organophosphate insecticides in agriculture and public health, despite restrictions placed on some of its domestic uses by United States Environmental Protection Agency in 2000. It is an irreversible inhibitor of acetylcholinesterase in the central and peripheral nervous systems that causes accumulation of acetylcholine, which in turn results in neurotoxicity in animals and humans[8]. The nervous system is the primary target because acetylcholinesterase catabolizes acetylcholine, thereby terminating its synaptic function[8]. Oxidative stress is one of the mechanisms implicated in CPF-evoked neurotoxicity[9,10].

CYP is a pyrethroid insecticide, which acts as a stomach and contact insecticide, and it is widely used in the production of cotton, cereals, vegetables and fruits, for food storage, in public health and animal husbandry. Its structure is based on pyrethrum, a natural insecticide which is contained in chrysanthemum; but it has a higher biological activity, and is more stable than its natural model[11]. The main mechanism of CYP is through its interferance with sodium channels in nerve cells, by delaying its closure, which results in repetitive firing and eventually impairment of neuronal transmission[12]. Furthermore, CYP induces oxidative damage by increasing lipid packing and decreasing membrane fluidity in cells. It decreases the activity of gluthathione peroxidase[13].

Melatonin (N-acetyl-5-methoxytriptamine) is a potent antioxidant hormone secreted by pineal gland. The function of this indole amine, as a free-radical scavenger, is facilitated by the ease, with which it crosses morphophysiological barriers like blood-brain barrier, intracellular and subcellular barriers[14]. Umosen *et al*. showed that melatonin ameliorated the subacute CPF-induced oxidative changes in the testes and pituitary glands[15]. Melatonin is also effective in protecting nuclear DNA, membrane lipids, and possibly, cytosolic proteins from oxidative damage. Melatonin, associated with the cellular antioxidant defence[16], acts at two levels: firstly, as a direct antioxidant, due to its ability to act as a free-radical scavenger; and secondly as an indirect antioxidant, since it is able to induce the expression and/or the activity of the main antioxidant enzymes[16].

The aim of the present study was to investigate the effect of exposure to a combination of CPF and CYP for 12 weeks on cognitive performance in male Wistar rats.

2. Materials and methods

2.1. Experimental animals

Fifty adult male Wistar rats obtained from the laboratory animal house of the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria, Nigeria served as subjects. They were housed in cages in the Department of Veterinary Physiology and Pharmacology laboratory, Ahmadu Bello University, Zaria, Nigeria. The rats were given access to pellets, prepared from growers' mash, maize bran and groundnut cake at the ratio of 4:2:1, and water *ad libitum*. They were pre-conditioned for two weeks prior to the commencement of the experiment.

2.2. Chemical acquisition and preparation

Commercial grade CPF (Sabero Organics Gujurat Limited, India) and CYP (Jiangsu Yangnog Chemical Co. Limited, China) were obtained. They were reconstituted in soya oil (Grand Cereal and Oil mills Limited, Jos, Nigeria) to appropriate working concentrations. Melatonin tablet (3 mg, Nature Made Nutritional Products, Mission Hills, USA) was dissolved in 6 mL of distilled water to make 0.5 mg/mL suspension daily before administration.

2.3. Subchronic toxicity study

Fifty adult male Wistar rats aged 4 weeks were used for this phase of the study. They were divided by simple random selection into four groups of ten animals each. Rats in each group were weighed and marked on the tail with a board marker for identification. Group I (DW) was given distilled water while Group II (SO) were dosed with soya oil only at 2 mL/kg. Rats in Group III (MEL) was administered with melatonin (0.5 mg/kg)[9]. Rats in Group IV (CC) was co-administered with CPF (1/10th LD_{50}) and cypermethrin (1/10th

LD_{50}). Group V (MCC) was pre-treated with melatonin 0.5 mg/kg then dosed with CPF (1/10th LD_{50}) and CYP (1/10th LD_{50}). The regimens were administered once daily by oral gavage for 12 weeks. During this period, the rats were evaluated for clinical signs and body weight using a digital weighing balance (Citizen Scales PVT. LTD.) on weekly basis.

2.4. Effect of treatments on cognition

The effect of treatment on learning was evaluated 48 h to the termination of the experiment, using the step-down inhibitory avoidance learning task as described by Zhu *et al*[17]. Briefly, an acrylic chamber, 40×25×25 cm, consisting of a floor made of parallel 2 mm calibre stainless steel bars spaced 1 cm apart was used for this test. An electric shock was delivered through the floor bars. A 2.5 cm high, 8×25 cm wooden platform was placed on the left extreme of the chamber. Upon stepping down, the rat immediately received a single 80 V foot-shock. If the animal did not return to the platform, the foot-shock was repeated every 5 seconds. A rat was considered to have learned the avoidance task, if it remained on the platform for more than 2 min. The number of foot-shocks was recorded as an index of learning acquisition.

Step-down avoidance inhibitory task was used to assess short-term memory in individual rat from each group, using the method of Zhu *et al*[17], 24 h after the assessment of learning. Briefly, the apparatus used for the memory test was the same as that used earlier for learning. In this test, the rat again was placed gently on the platform 24 h after performing the learning task. The time an animal remained on the platform was recorded as an index of memory retention. Staying on the platform was recorded for 2 min, and was counted as the maximum memory retention (ceiling response).

2.5. Determination of brain malondialdehyde (MDA) concentration

The principle of the method was based on spectrophotometric method of the colour developed during the reaction of thiobarbituric acid (TBA) with MDA, while the level of TBA-reactive substance and MDA is an index of lipid peroxidation. For determination of lipid peroxidation in the brain, the method of Draper and Hadley[18] as modified by Freitas *et al*[19] was used. Briefly, brain samples from animals in each group were obtained immediately after sacrificing the rats following light chloroform anaesthesia. They were weighed and then homogenized in a known sample of ice-cold phosphate buffer to obtain a 10% homogenate, which was centrifuged at 7329 r/min for 10 min using a centrifuge (IEC HN, Damon/IEC Division, UK). About 0.5 mL of the supernatant obtained from each animal was mixed with 1 mL of 10% trichloroacetic acid solution and 1 mL of 0.67% TBA. The mixture was heated in boiling water bath for 15 min. Buta-2-ol (2:1 v/v) was added to the solution. After centrifugation at 2676 r/min for 5 min, the MDA concentration was determined from the absorbance at 532 mm using a UV visible spectrophotometer (T180+UV/VIS Spectrometer® PG instrument Limited, Liicestershire, LE 175BE, United Kingdom). The MDA concentration in each brain sample was calculated from the absorbance coefficient of MDA-TBA complex [1.56×10^5/(cm · mol/L)] and expressed as nmol/mg of tissue protein. The concentration of protein in the brain homogenate was determined using the method of Lowry *et al*[20]. The study was conducted in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals[21].

2.6. Statistical analysis

Obtained values expressed as mean±SEM were subjected to One-way analysis of variance (ANOVA), followed by Tukey's *post hoc* test, using GraphPad Prism version 4.0 for windows from GraphPad Software, San Diego, California, USA. Values of $P<0.05$ were considered significant.

3. Results

3.1. Effect of treatments on learning acquisition

The effect of treatments on learning acquisition is shown in Figure 1. There was a significant increase ($P<0.05$) in learning in CPF+CYP treated rats compared to those in the SO, DW or MEL groups. The number of foot-shocks in rats in the CPF+CYP group was higher ($P<0.05$) than that in the MCC group. Rats in MEL group had the lowest number of foot-shocks.

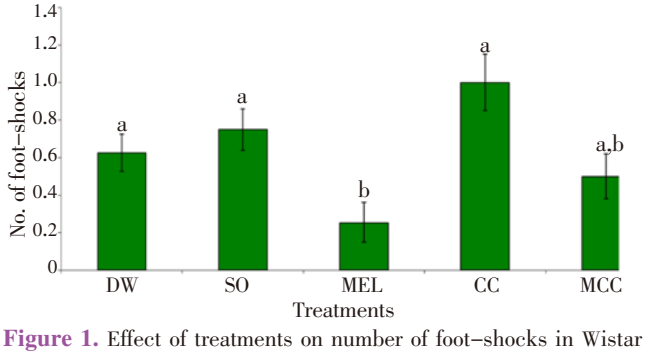

Figure 1. Effect of treatments on number of foot-shocks in Wistar rats (n=10).
DW: Distilled water, SO: Soya oil, MEL: Melatonin, CC: CPF+CYP, MCC: Melatonin+CPF+CYP. a,b: Values with different superscript letters are significantly ($P<0.05$) different.

3.2. Effect of treatments on short-term memory

The effect of treatment on short-term memory is shown in Figure 2. The latency on platform was significantly (P<0.001) lower in the CPF+CYP group compared to that of the DW, SO or MEL group. The latency on platform was significantly (P<0.001) higher in the MCC group than that recorded in the CPF+CYP group. There was no significant (P>0.05) change in the latency on platform in the MCC group compared to the DW, SO, or MEL group.

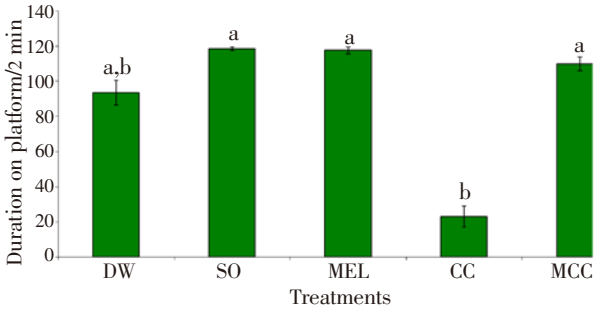

Figure 2. Effect of treatments on duration on platform in Wistar rats (n=10).
DW: Distilled water, SO: Soya oil, MEL: Melatonin, CC: CPF+CYP, MCC: Melatonin+CPF+CYP. a,b: Values with different superscript letters are significantly (P<0.05) different.

3.3. Effect of treatments on brain MDA activity

There was a significant increase (P<0.05) in the MDA concentration of the CC group when compared to the DW, SO, MEL and MCC groups (Figure 3). There was an increase (P>0.05) in the MDA concentration in the CC group when compared to the SO and DW group. There was no significant change (P>0.05) in the MDA activity of the MCC group compared to that of the DW, SO and MEL group.

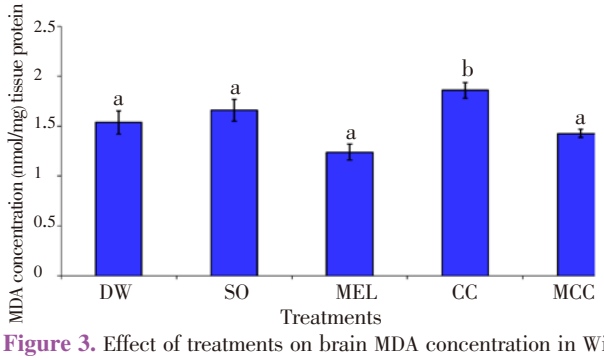

Figure 3. Effect of treatments on brain MDA concentration in Wistar rats (n=10).
DW: Distilled water, SO: Soya oil, MEL: Melatonin, CC: CPF+CYP, MCC: Melatonin+CPF+CYP. a,b: Values with different superscript letters are significantly (P<0.05) different.

4. Discussion

4.1. Learning acquisition

The deficit in learning acquisition obtained following co-administration of CPF and CYP in the present study is consistent with the findings from previous studies, which observed learning deficits following CPF exposure[9,22]. This deficit may be caused by neuronal injury, partly due to induction of oxidative stress in the cerebral cortex[23], which is the centre for learning. Furthermore, the deficit recorded in the present study may be due to neuronal damage partly caused by increased oxidative stress in the brain structures responsible for learning acquisition, especially in the cerebral cortex. Pyrethroids have been reported to alter the levels of consciousness and cause confusion in rats[24]. The reduced number of foot-shocks in the pre-treated group showed that melatonin may prevent cognitive deficits and this result is in agreement with the finding of Sharma and Gupta[25], who observed an improved learning ability following melatonin administration in rats induced with oxidative stress.

4.2. Short-term memory

The shorter duration of staying on the platform by rats in the CC group indicating a deficit in memory caused by the administration of CPF and CYP, CPF poisoning in rats agree with the findings of impairment of memory[9,10]. The cognitive decline may be partly due to oxidative damage to the hippocampus, which plays an important role in learning and memory processes[26]. The result of the present study is in agreement with finding that a single application of CYP produces apoptotic cell death in the central nervous systems of exposed animals[27], and that CYP causes pyknosis in the cytoplasm of neurones in the brain tissues of rats[28]. The improvement in memory following pre-treatment with melatonin underlies the significance of oxidative stress in the pathogenesis of CPF and CYP-induced cognitive decline. The finding is in agreement with the result obtained by Sharma and Gupta[25], who showed that melatonin improved cognitive decline caused by free-radical generation in the brain of rats[29]. Melatonin exerts a positive effect on the Morris water maze performances, which is a model used to evaluate the cognitive functions of rats[9].

4.3. Effect on brain malondialdehyde

The higher MDA activity recorded in the CC group agrees with the findings of Weilgomas and Krechniack[9,10,30] who showed that MDA concentration in the brain was elevated after CYP and CPF exposure. MDA is a product of lipid peroxidation, which results from the reaction of oxygen radicals with polyunsaturated fatty acid residues in memberane phospholipids, and it has been shown to damage proteins and DNA[31]. The increase recorded in MDA concentration in the present study, therefore, suggests the elevation of brain lipoperoxidation and that reactive oxygen and nitrogen species are involved in the mediation of these damages in the brain. MDA concentration was reduced in the melatonin-pretreated group. This result agrees with the findings of Gonenc *et al*[29], who showed that melatonin decreased ethanol-induced lipid peroxidation using TBA-reactive substance as an indicator of lipid peroxidation. This suggests that melatonin protected the cells against oxidative stress, it reduced MDA levels in the brain of CC administered rats.

In conclusion, cognitive deficits induced by subchronic co-administration of CPF and CPF in rats were ameliorated by melatonin.

Conflict of interest statement

We declare that we have no conflict of interest.

Comments

Background

Pesticide mixtures pose a worldwide public health problem, especially in developing countries in the bid to control pests. Although the use of combined pesticides is widespread, its toxicological profile is unknown. Antioxidants have assumed an important role in combating oxidative stress.

Research frontiers

The authors evaluated the toxic effects of CPF and CYP on cognition and MDA concentration. The mixture caused a decline in learning and memory and increased MDA concentrations, suggesting involvement of oxidative stress. Other authors have evaluated the effects of both pesticides singly on learning and have found that they adversely affect learning. Melatonin has been used by others as an antioxidant to improve cognition in rats.

Related reports

The data from this work agrees with the findings of Wielgomas and Kriechnac (2007) that increased MDA concentration occur following CPF and CYP exposure, and Sharma and Gupta (2001) that melatonin, as an antioxidant, improved cognitive decline in rats.

Innovations and breakthroughs

This paper reveals that prolonged exposure to combination of both pesticides has attendant side-effects, and melatonin ameliorated the effects.

Applications

The result of the present study suggests the use of melatonin for protection before application or exposure to pesticides.

Peer review

This is a good study in which the authors evaluated the toxic effects of CPF and CYP and the protective role of melatonin as an antioxidant.

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