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Research Article
Subchronic Chlorpyrifos and Cypermethrin-induced Sensorimotor Changes in Male Wistar Rats: Ameliorative Effects of Melatonin

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Abstract

Background: Exposure to chlorpyrifos have been shown to induce short term sensorimotor changes in rats, which has been linked to oxidative stress, cypermethrin also induces oxidative stress. This study was therefore aimed at evaluating the ameliorative effects of melatonin on sensorimotor changes induced by co-administration of chlorpyrifos and cypermethrin in male Wistar rats. **Materials and Methods:** Fifty adult male Wistar rats were divided into five groups of ten rats each. Group I and II were given distilled water and soya oil (2 mL kg^{-1}), respectively. Group III was administered with melatonin at 0.5 mg kg^{-1} only. Group IV was administered with CPF (8 mg kg^{-1} to 1/10th LD_{50}) and CYP (30 mg kg^{-1} to 1/10th LD_{50}) group V was administered with CPF (8 mg kg^{-1} to 1/10th LD_{50}), CYP (30 mg kg^{-1} to 1/10th LD_{50}) and 30 min later melatonin (0.5 mg kg^{-1}). The regimens were administered orally by gavage once daily for 12 weeks. Sensorimotor performances were determined at intervals during the study thereafter the brain was evaluated for malonaldehyde and acetylcholinesterase concentrations. **Results:** There was reduced loco motor activity, decreased grip time, impaired efficiency of locomotion, reduced inclined plane performance, increased brain malondialdehyde concentration and decreased brain acetylcholinesterase concentration in the chlorpyrifos and cypermethrin treated group which were all ameliorated by melatonin pre-treatment. **Conclusion:** The study showed that treatment with melatonin ameliorated the sensorimotor deficits induced by sub-chronic co-administration of chlorpyrifos and cypermethrin.

Key words: Acetylcholineserase, sensorimotor, chlorpyrifos, cypermethrin, melatonin

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The use of pesticide mixtures is on the increase especially in the developing countries due to the resistance posed by pests to pesticides, though these new mixtures are useful but its use is not without side or long term effects[1]. Organophosphates (OP) were first developed in the 1930's by a group of German scientists, they were used as chemical warfare agent[2]. This group of chemicals has been used indiscriminately in large amounts leading to progressive global environmental pollution. Chlorpyrifos (CPF; 0,0-didsethyl0-3,5,6-trichloro-2-pyridylthiophosphate) is a broad spectrum chlorinated OP pesticide, synthesized by Dow chemical company in the USA is used against a wide variety of insect pest[3]. It is one of the most readily available and widely used organophosphate insecticides, despite restricted domestic use by United States Environmental Protection Agency[4]. Its acts mainly by inhibiting acetylcholinesterase[5]. Though oxidative stress has been suggested as one of the ways it exerts its action[6]. Cypermethrin (CYP) a type II pyrethroid, causes adverse effects on the nervous system[7], though oxidative stress has also been implicated in its mechanism of action. Cypermethrin is metabolized to cyanohydrins which decomposes further to cyanides and aldehyde, which can induce oxidative stress[8]. Interaction between OPs and pyrethroids leads to a non-reversible inhibition of esterases by the OPs which slow down of the activity of enzymes that break down the ester bond in the pyrethroid molecule[9]. Blocking this hydrolysis of pyrethroids by these enzymes significantly reduces the metabolism of these pesticides, resulting in a more potent and lasting insecticidal effect of the OP-pyrethroid mixture.

Antioxidants are substance that delays, prevents or removes oxidative damage to a target molecule[10]. Melatonin (N-acetyl-5-methoxytriptamine), secreted by the pineal gland is a free-radical scavenger and a strong antioxidant molecule, biosynthesized from tryptophan[11]. It acts directly as an antioxidant by scavenging free radicals and as an indirect antioxidant by inducing the expression of main antioxidant enzymes[12].

MATERIALS AND METHODS

Experimental animals: Fifty adult male Wistar rats, aged 14 weeks and weighing between 170-200 g obtained from the Laboratory Animal House of the Former, Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria served as subjects. They were housed in cages in the Department of Veterinary Physiology and Pharmacology Students Teaching Laboratory, Ahmadu Bello University, Zaria. The rats were given access to pellets of food prepared from growers mash, maize bran and groundnut cake at the ratio of 4:2:1 and water was provided *ad libitum*. The rats were pre-conditioned for 2 weeks prior to the commencement of the experiment.

Chemical acquisition and preparation: Commercial grade chlorpyrifos (CPF) 20% emulsifiable concentration (Sabero Organics Gujarat Limited, India) and cypermethrin (CYP) (Jiangsu Yangnog Chemical Co., Limited, China) were obtained from reputable Agrochemical Stores in Zaria. They were reconstituted in soya oil (Grand Cereal and Oil Mills Limited, Jos, Nigeria) to appropriate studies concentrations (200 mg mL^{-1} for CPF and 100 mg mL^{-1} for cypermethrin). 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^{-1} suspension daily before administration.

Sub-chronic toxicity study: They rats were divided at random into five groups of ten rats each. Rats in each group were weighed and marked on the tail with a permanent board marker for identification. Group I (DW) was administered distilled water at 2 mL kg^{-1} while group II (SO) was dosed with soya oil only at 2 mL kg^{-1}. Group III (MEL) was administered with melatonin (0.5 mg kg^{-1})[13], while 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^{-1} then dosed with CPF (1/10th LD_{50}) and cypermethrin (1/10th LD_{50}). The regimens were administered once daily by oral gavage for 12 weeks. The rats were monitored for neurobehavioral changes measuring motor activity, motor strength, efficiency of locomotion and neuromuscular coordination at various intervals during the study period using the appropriate neurobehavioral evaluation test apparatus.

Evaluation of motor activity: The effects of treatments on motor activity of each rat were measured in an open-field apparatus as described by Zhu *et al.*[14]. The open-field apparatus was constructed using a cardboard box (50×50×46 cm high) with clear plexiglas on the inner surface. The floor was divided into 25 equal squares. Each animal was placed in the central square and observed for 3 min to familiarize itself with the environment. Then the number of squares crossed with all the paws during the next 2 min was recorded as an indication of loco motor activity. Soapy water

followed by 90% alcohol solution was used to clean the inner surface of the box between trials to remove the interfering odours left by the previous animal. The open-field assessment was evaluated on 0, 2, 6, 8 and 12 weeks of the study.

Evaluation of motor strength: The fore-paw grip time was used to evaluate the motor strength in rats as described by Abou-Donia *et al.*[15]. This was conducted by having rats hung down from a 5 mm diameter wooden dowel gripped with both fore-paws. The time spent by each rat before releasing their grips was recorded in seconds. This parameter was evaluated on 0, 2, 6, 8 and 12 weeks of the study.

Assessment of efficiency of locomotion: The ladder walk apparatus was used to measure the efficiency of locomotion[16]. Each rat was encouraged to walk across a black wooden ladder (106×17 cm with 0.8 cm diameter rungs and with 2.5 cm spaces between them). The number of times each rat missed a rung was counted by one rater on each side. Performance on ladder walk was evaluated on 0, 6 and 12 weeks of the study.

Assessment of neuromuscular coordination: The effect of CPF and cypermethrin and the ameliorating effect of melatonin on neuromuscular coordination of each rat was evaluated based on the ability of the rat to walk through obstacles using inclined plane test as described by Ambali and Aliyu[17]. Briefly, each rat was evaluated by placing each rat on an apparatus made with an angled, rough wooden plank with a thick foam pad at its bottom end. The plank was first raised to an inclination of angle of 35° and thereafter, gradually increased in step-wise fashion by 5° until the rat was unable to stay and be situated horizontally on the plank for 3 sec, without sliding down. Angles were measured and marked on the apparatus beforehand and was obtained by propping the plank on a vertical bar with several notches. The test was conducted with the head of the rat first facing left and then right of the experimenter. The highest angle at which each rat stayed and stood horizontally while facing each direction was recorded. Two trials were performed for each testing period. The procedure was conducted on each rat on 0, 6 and 12 weeks of the study.

Determination of malondialdehyde concentration in whole brain: The principle of the method was based on spectrophotometric method of the colour, developed during the reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA). The level of thiobarbituric acid-reactive substance (TBARS) and MDA is an index of lipid peroxidation. For determination of lipid peroxidation in the whole brain, the method of Draper and Hadley[18] as modified by De Freitas *et al.*[19] was used. Whole brain samples from each animal in all the groups were weighed immediately after dissection and then homogenized in a known sample of ice-cold phosphate-buffer to obtain a 10% homogenate, which was centrifuged at 6000×g for 10 min using a centrifuge, IEC HN (Damon/IEC Division, UK). About 0.5 mL of the supernatant obtained following centrifuge was mixed with 1 mL of 10% trichloroacetic acid (TCA) solution and 1 mL of 0.67% TBA. The mixture was heated in boiling water bath for 15 min. Butan-2-ol (2:1 v/v) was added to the solution. After centrifugation (800×g for 5 min), the MDA concentration was determined from the absorbance at 532 nm using a UV visible spectrophotometer (T180+PG instrument Limited, United Kingdom). The MDA concentration in each sample was calculated from the absorbance coefficient of MDA-TBA complex (1.56×10^5 cm^{-1} M^{-1}) and expressed as nmol mg^{-1} of tissue protein.

The concentration of protein in the whole brain homogenate was determined using the method of Lowry *et al.*[20].

Evaluation of whole brain acetylcholinesterase activity: Acetylcholinesterase activity was determined by the method of Ellman *et al.*[21] using acetylcholine iodide as substrate. Briefly, the whole brain were weighed using the Mettler weighing balance (Mettler^R P161, Mettler instrument AG, CH806, Geifensee Zurich, Switzerland) and then homogenized in cold (0-4°C) 20 mM phosphate-buffered saline (PBS), incubated with 0.01 M 5,5-dithio-bis -(2-nitro benzoic acid) in 0.1 M PBS, pH 7.0. Incubation was allowed to proceed at room temperature (26°C) for 30 min. Then acetylcholine iodide (0.075 M in 0.1 M PBS, pH 8.0) was added to the tube and absorbance at 412 nm was measured with a Shimadzu UV spectrophotometer (Model UV 160, Kyoto, Japan). The changes in the absorbance were recorded for a period of 10 min at intervals of 2 min after the addition of acetylthiocholine (30 µL, final concentration = 0.5 mM) to the mixture. Thus, the change in the absorbance per minute was determined.

The enzyme activity was calculated by using the equation:

$$R = \frac{5.74\times10^{-4}\times A}{CO}$$

where, 5.74×10^{-4} is a dissociation coefficient, R is the rate in moles of substrate hydrolyzed per minute per gram tissue, A is the change in absorbance per minute, CO is the original concentration of the brain tissues.

Statistical analysis: Data obtained were expressed as Mean±SEM. Repeated measures analysis of variance, followed by Tukey's *post hoc* test was used to evaluate sensorimotor parameters measured repeatedly during the study. One way analysis of variance followed by Tukey's *post hoc* test was used to analyse brain malondialdehyde and acetylcholinesterase activity.

RESULTS

Locomotor activity: The effect of treatments on locomotor activity is shown in Fig. 1. On the dynamics of locomotor activity within the group, the number of squares crossed in the CC group increased significantly ($p<0.05$) at 0 week when compared to those recorded in 6 and 12 weeks, respectively. There was no significant change ($p>0.05$) in the number of squares crossed at 6 weeks when compared to that of 12 weeks in the CC group. There was no significant ($p>0.05$) difference in the number of squares crossed in the DW, SO, MEL and MCC groups at 0 week when compared to those recorded at 6-12 weeks. No significant decrease ($p>0.05$) in the number of squares crossed was also recorded in the MCC group at 6 compared to 12 weeks.

The dynamics of locomotor changes between the groups showed no significant ($p>0.05$) change in the number of squares crossed in between the groups at 0 week. At 6 weeks there was a significant decrease ($p<0.05$) in the number of squares crossed in the CC group compared to that of SO, DW and MEL groups respectively but no significant change ($p>0.05$) when compared to that of MCC group. There was also a significant decrease ($p<0.05$) in the number of squares crossed in the MCC group compared to that recorded in DW, SO and MEL groups, respectively.

Motor strength: The effect of treatments on motor strength is shown in Fig. 2. There was no significant change ($p>0.05$) in grip time of rats in the SO group at 0 week compared to that at 6 and 12 weeks. The grip time of MEL group at week 0 was not significantly ($p>0.05$) different compared with that obtained at week 6. However, there was a significant increase ($p<0.05$) in grip time in MEL group in 12 weeks when compared to that of 6 weeks. There was significant ($p<0.05$) decrease in grip time of rats in the CC group at 0 week compared to that obtained at 6 and 12 weeks, respectively. There was no significant ($p>0.05$) change in the grip time of rats in the MCC group at 0 week compared to that of 6 weeks but a significant ($p<0.05$) decrease was recorded at 12 weeks compared to that of 6 weeks. At 0 week, there was no

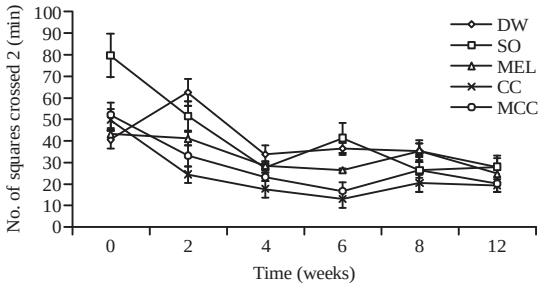

Fig. 1: Effect of distilled water, soya oil, melatonin and/or chlorpyrifos+cypermethrin on dynamics of locomotor activity in Wistar rats (n = 10)

significant change ($p>0.05$) in the grip time between the groups. At 6 weeks, there was a significant ($p<0.05$) decrease in the grip time in CC group when compared to those of the SO, MEL or MCC group but there was no significant change in grip time between the DW and CC groups. At 12 weeks there was a significant ($p<0.05$) decrease in grip time in the CC group when compared respectively to those of DW, SO, MEL and MCC groups.

Effect of treatments on efficiency of locomotion: The number of rungs missed in the CC group (Fig. 2b) increased insignificantly ($p>0.05$) at week 0 compared to those of 6-12 weeks. The number of rungs missed in CC group at 12 weeks increased insignificantly ($p>0.05$) compared to that of 6 weeks. There was no significant ($p>0.05$) change in the number of missed rungs recorded in the DW, SO, MEL groups at 0 week, compared to those of 6 and 12 weeks, respectively. No significant ($p>0.05$) increase was also recorded in the MCC group at 0 week compared to that of 6 and 12 weeks.

There was no significant ($p>0.05$) change in the number of missed rungs between the groups at 0 week. However, at 6 and 12 weeks, there was a significant ($p<0.05$) decrease in the number of rungs missed in the CC group compared respectively to those of the DW, SO, MEL or MCC groups. There was significant ($p<0.05$) increase in the number of missed rungs between the MCC group when compared respectively to those of the DW, SO or MEL group at 6 and 12 weeks.

Neuromuscular coordination: The effect of treatments on neuromuscular coordination assessed on the inclined plane performance is shown in Fig. 2c. There was no significant change ($p>0.05$) in the angle of slip in the CC group at 0 week compared to that of 6 weeks but, there was a significant decrease ($p<0.05$) at 12 weeks compared to that of 6 weeks.

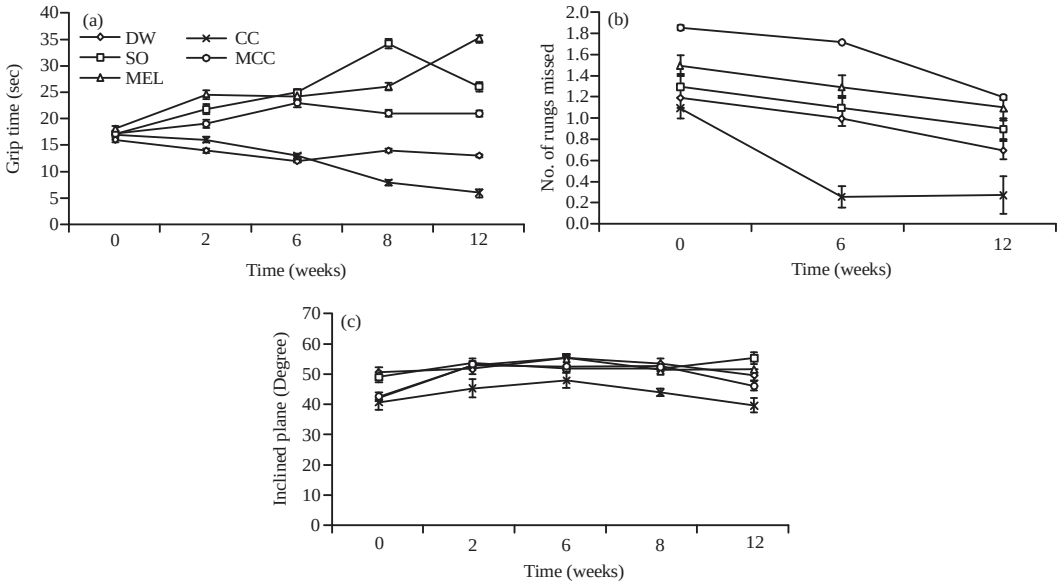

Fig. 2(a-c): Effect of distilled water (DW), soya oil (SO), melatonin (MEL), chlorpyrifos+cypermethrin (CC) and melatonin+chlorpyrifos+cypermethrin (MCC) on (a) Grip time, (b) Ladder walk performance and (c) Dynamic of inclined plane performance in Wistar rats (n = 10)

There was no significant difference ($p>0.05$) in the angle of slip recorded at 0, 6 and 12 weeks in the MCC group compared to those of the DW, SO or MEL group.

There was no significant ($p>0.05$) change in the angle of slip at 0 week in between the groups. At 6 and 12 weeks, there was a significant decrease ($p<0.05$) in the angle of slip in the CC group compared respectively to that in the DW, SO, MEL or MCC group. There was no significant change ($p>0.05$) in the MCC group compared to the DW, SO and MEL groups at 12 weeks.

Effect of treatments on malondialdehyde concentration: There was a significant increase ($p<0.05$) in the MDA concentration of the CC group when compared to that of the DW, SO, MEL and MCC groups. There was no significant change ($p>0.05$) in the MDA concentration of the MCC group compared to that of the DW, SO or MEL group (Fig. 3a).

Effect of treatments on acetylcholinesterase activity: The effects of treatments on AChE activity is shown in Fig. 3b. The AChE activity was significantly decreased ($p<0.05$) in CC group compared to that of the DW, SO, MEL or MCC group. The AChE activity in the MCC group was significantly ($p<0.05$) higher compared to that of the CC group but no significant change ($p>0.05$) relative to that of the DW, SO and MEL groups, respectively.

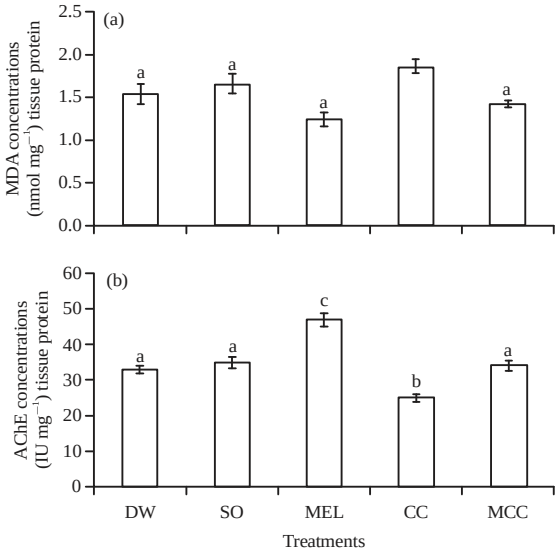

Fig. 3(a-b): Effects of distilled water, soya oil, melatonin and/or chlorpyrifos+cypermethrin on brain (a) Malondialdehyde concentration and (b) Acetylcholinesterase activity in Wistar rats (n = 10), ^{a,b,c}Values with different superscript letters are significantly ($p<0.05$) different

DISCUSSION

The lower Locomotor activity recorded in the CC group compared to the other groups contradicted the findings of

Latuszynska *et al.*[9] following 20 days dermal application of CPF and CYP. The contradiction may have arisen from apparently shorter duration of exposure and the dermal route of application, in that study, which may have resulted in low grade sustained AChE inhibition, cholinergism and hence increased locomotor activity. The lowered motor activity recorded in the CC group in the present study may be partly due to prolonged AChE activity leading to its exhaustion with consequent impairment of neuromuscular activity. Furthermore, Ambali and Ayo[16] recorded increased oxidative damage and lowered glycogen levels in the muscle following chronic CPF exposure and may have partly accounted for reduced motor activity in the CC group. This is also in agreement with findings from Wolansky *et al.*[22] that recorded a decreased locomotor activity following administration of type II pyrethroids. Nieradko-Iwanicka and Borzecki[23] also reported that brain ischemia following exposure to sub toxic doses of cypermethrin transiently impairs spontaneous movement activity in mice. The improvement in locomotor activity following melatonin pre-treatment may be partly attributed to its antioxidative and AChE restoration properties.

The decrease in grip time recorded in CC group agreed with the findings of Ambali *et al.*[6] and Wolansky *et al.*[22] were indicative of a deficit in motor strength. Both CPF[24] and CYP[25,26] have been shown to cause oxidative damage to neurones which could be responsible for the decrease in grip time. Furthermore, the decreased grip time recorded in the present study was in agreement with that of Ambali *et al.*[6] following CPF exposure. Pyrethroids, on the other hand have been shown to weaken the neuromuscular response in rats[22,26]. Exposure to CPF caused increased oxidative damage and lowered glycogen levels in the muscle following chronic CPF exposure[16] and may partly account for the reduced motor strength seen as decreased grip time in the CC group. Rats in melatonin pre-treated group showed an increase in grip time hence improved motor strength because melatonin apparently protected neurones from oxidative stress[27].

The decrease in number of rungs missed by the CC group showed that their legs were frequently being held stationary above the rungs for a relatively longer period, implying impaired locomotion efficiency. The finding is in agreement with studies carried out by Ambali and Aliyu[17] who recorded a decrease in number of rungs missed by rats, administered CPF, which may be attributed to either oxidative damage to the muscle as shown by Ambali and Ayo[16] who recorded increased oxidative damage and lowered glycogen levels in the muscles following chronic CPF exposure. Impairment of neuronal transmission as a result of paralysis of the neuromuscular junction, apparently, resulting from prolonged AChE inhibition may contributed to this locomotion deficiency[28]. The increase in the number of rungs missed by rats in melatonin pre-treated groups showed that melatonin ameliorated the impaired locomotion efficiency induced by co-administration of CPF and CYP, which may be due to its antioxidant, neuroprotective and AChE restoration properties.

The inclined plane is used to access neuromuscular coordination. The result of the present study revealed a deficit in inclined plane performance and consequently, neuromuscular coordination following co-administration of CPF and CYP. Previous studies have shown a deficit in neuromuscular coordination following CPF[6,17] and CYP[29,30] exposures. The impairment of neuromuscular coordination may be due to increase in brain lipoperoxidative changes induced by CPF[31] and cypermethrin[25] which alters the morphological and functional capacity of the brain region involved in neuromuscular coordination. The higher angles of slip exhibited by MCC group showed that melatonin may have ameliorated the adverse effects of the pesticide mixture on neuromuscular coordination. Furthermore, the neuroprotective effect of melatonin may have accentuated the improved neuromuscular coordination.

The higher MDA concentration recorded in the CC group agrees with the findings of Wielgomas and Krechniak[32] following exposure to in CPF and CYP. Similarly, Gultekin *et al.*[13] and Ambali *et al.*[6] showed that MDA concentration in the brain was elevated following CPF exposure. The MDA is a product of lipid peroxidation, which results from the reaction of oxygen radicals with polyunsaturated fatty acids residues in membrane phospholipids and this has been shown to damage proteins and DNA[33]. This compound is a reactive aldehyde and is one of the many reactive electrophile species that cause toxic stress in cells and form advanced glycation end-products[34]. The production of this aldehyde is used as a biomarker to measure the level of lipid peroxidation in an organism[34]. The brain is vulnerable to oxidative stress due to its biochemical and physiological properties[35]. Apart from harbouring large amount of oxygen in a relatively small mass, the brain contains a significant quantity of metals (Fe) and has fewer antioxidant mechanisms than other tissues[10]. The ROS can directly attack the polyunsaturated fatty acids of the cell membranes and induce lipid peroxidation. The increased MDA concentration in the present study therefore suggests the elevation of brain lipoperoxidation and that ROS and RNS are involved in the

mediation of these damages in the brain. Furthermore, Gultekin *et al.*[13] recorded that pre-incubation of cells with melatonin prevented an increase in thiobarbituric acid reactive substance (TBARS) in human hep G2 cell lines treated with chlorpyrifos.

The markedly decreased AChE activity in CC group agreed with the findings of Latuszynska *et al.*[9] and Wielgomas and Krechniak[32] who demonstrated that AChE activity in the brain markedly decreased in rats co-administered with CPF and CYP. The reduction in AChE activity may also be linked to induction of oxidative stress, since the hydroxyl radical has been shown to cause significant reduction in AChE activity in the rat brain[36]. Also, apart from the direct inhibition of AChE through occupation of the esteratic site of the enzyme, oxidative stress has been involved in many pathologies caused by CPF. It has also been indirectly linked with AChE inhibition[35]. The AChE is one of the membrane bound enzymes and lipoperoxidation of the membrane has been shown to alter its activity[34] which may be responsible for the decrease in AChE activity seen in the CC group. The increase in AChE activity recorded in melatonin treated group showed that melatonin exerts decreased lipid peroxidation and increased antioxidant status. The increased AChE activity in the MCC group showed that melatonin restored AChE activity following its inhibition by CPF and CYP. The AChE is one of the membrane bound enzymes and lipoperoxidation of the membrane has been shown to alter its activity and oxidative stress has been linked with most of the pathologies caused by CPF[34,36] and CYP[25,26,37].

CONCLUSION

In conclusion, melatonin ameliorated the sensorimotor changes induced by subchronic co-administration of chlorpyrifos and cypermethrin.

REFERENCES

1. Babu, H.S., P. Jayaraman and P. Aarthy, 2010. Screening of ach ease inhibition in blood and plasma and brain of Wistar rats by Neurella D (combination pesticide). Int. J. Pharma Bio Sci., 1: 574-578.
2. Minton, N.A. and V.S.G. Murray, 1998. A review of organophosphate poisoning. Med. Toxicol., 3: 350-370.
3. Eaton, D.L., R.B. Daroff, H. Autrup, J. Bridge and P. Buffler *et al.*, 2008. Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment. Crit. Rev. Toxicol., 38: 1-125.
4. US EPA., 2000. Chlorpyrifos: HED preliminary risk assessment for the reregistration eligibility decision (RED) document. Chemical No. 059101, Barcode: D260163, Case: 818975, Submission: S568580, United States Environmental Protection Agency, Washington, DC.
5. Vishwanathan, M. and K. Srinivasan, 1964. Treatment of organo-phosphorous compound poisoning. J. Indian Med. Assoc., 43: 494-495.
6. Ambali, S.F., S.B. Idris, C. Onukak, M.U. Shittu and J.O. Ayo, 2010. Ameliorative effects of vitamin C on short-term sensorimotor and cognitive changes induced by acute chlorpyrifos exposure in Wistar rats. Toxicol. Ind. Health, 26: 547-558.
7. Ray, D.E., 1991. Pesticides Derived from Plants and other Organisms. In: Handbook of Pesticide Toxicology. Hayes, Jr., (Ed.). Academic Press, Inc., New York, USA., pp: 2-3.
8. Leja-Szpak, A., J. Jaworek, R. Tomaszewska, K. Nawrot and J. Bonior *et al.*, 2004. Melatonin precursor, L-tryptophan protects the pancreas from development of acute pancreatitis through the central site of action. J. Physiol. Pharmacol., 55: 239-254.
9. Latuszynska, J., S. Luty, G. Raszewski, M. Tokarska-Rodak, D. Przebirowska, E. Przylepa and A. Haratym-Maj, 2001. Neurotoxic effect of dermally-applied chlorpyrifos and cypermethrin in Wistar rats. Ann. Agric. Environ. Med., 8: 163-170.
10. Halliwell, B. and J.M.C. Gutteridge, 2007. Free Radicals in Biology and Medicine. 4th Edn., Oxford University Press, Oxford, UK., ISBN-13: 978-0198568698, Pages: 704.
11. Reiter, R.J., D.X. Tan and A. Korkmaz, 2009. The circadian melatonin rhythm and its modulation: Possible impact on hypertension. J. Hypertens., 27: S17-S20.
12. Tomas-Zapico, C. and A. Coto-Montes, 2007. Melatonin as antioxidant under pathological processes. Recent Patents Endocr. Metab. Immune Drug Discov., 1: 63-82.
13. Gultekin, F., S. Patat, H. Akca, M. Akdogan and I. Altuntas, 2006. Melatonin can suppress the cytotoxic effects of chlorpyrifos on human hepG2 cell lines. Hum. Exp. Toxicol., 25: 47-55.
14. Zhu, H., R.W. Rockhold, R.C. Baker, R.E. Kramer and I.K. Ho, 2001. Effects of single or repeated dermal exposure to methyl parathion on behavior and blood cholinesterase activity in rats. J. Biomed. Sci., 8: 467-474.
15. Abou-Donia, M.B., L.B. Goldstein, K.H. Jones, A.A. Abdel-Rahman and T.V. Damodaran *et al.*, 2001. Locomotor and sensorimotor performance deficit in rats following exposure to pyridostigmine bromide, DEET and permethrin, alone and in combination. Toxicol. Sci., 60: 305-314.
16. Ambali, S.F. and J.O. Ayo, 2011. Sensorimotor performance deficits induced by chronic chlorpyrifos exposure in Wistar rats: Mitigative effect of vitamin C. Toxicol. Environ. Chem., 93: 1212-1226.

17. Ambali, S.F. and M.B. Aliyu, 2012. Short-term sensorimotor and cognitive changes induced by acute chlorpyrifos exposure in wistar rats: Ameliorative effect of Vitamin E. Pharmacologia, 3: 31-38.
18. Draper, H.H. and M. Hadley, 1990. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol., 186: 421-431.
19. De Freitas, R.M., S.M. Vasconcelos, F.C. Souza, G.S. Viana and M.M. Fonteles, 2005. Oxidative stress in the hippocampus after pilocarpine-induced status epilepticus in wistar rats. FEBS J., 272: 1307-1312.
20. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265-275.
21. Ellman, G.L., K.D. Courtney, V. Andres Jr. and R.M. Featherstone, 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol., 7: 88-95.
22. Wolansky, M.J., C. Gennings and K.M. Crofton, 2006. Relative potencies for acute effects of pyrethroids on motor function in rats. Toxicol. Sci., 89: 271-277.
23. Nieradko-Iwanicka, B. and A. Borzecki, 2008. Effect of cypermethrin on memory, movement activity and co-ordination in mice after transient incomplete cerebral ischemia. Pharm. Rep., 60: 699-705.
24. Zama, D., Z. Meraihi, S. Tebibel, W. Benayssa, F. Benayache, S. Benayache and A.J. Vlietinck, 2007. Chlorpyrifos-induced oxidative stress and tissue damage in the liver, kidney, brain and fetus in pregnant rats: The protective role of the butanolic extract of *Paronychia argentea* L. Indian J. Pharm., 39: 145-150.
25. Gabbianelli, R., G. Falcioni, C. Nasuti and F. Cantalamessa, 2002. Cypermethrin-induced plasma membrane perturbation on erythrocytes from rats: Reduction of fluidity in the hydrophobic core and in glutathione peroxidase activity. Toxicology, 175: 91-101.
26. Singh, A.K., M.N. Tiwari, O. Prakash and M.P. Singh, 2012. A current review of cypermethrin-induced neurotoxicity and nigrostriatal dopaminergic neurodegeneration. Curr. Neuropharmacol., 10: 64-71.
27. Gonenc, S., N. Uysal, O. Acjkgoz, B.M. Kayatekin and A. Sonmez *et al*., 2005. Effects of melatonin on oxidative stress and spatial memory impairment induced by acute ethanol treatment in rats. Physiol. Res., 54: 341-348.
28. Alavanja, M.C.R., J.A. Hoppin and F. Kamel, 2004. Health effects of chronic pesticide exposure: Cancer and neurotoxicity. Ann. Rev. Public Health, 25: 155-197.
29. Ray, D.E., 2001. Pyrethroid Insecticides: Mechanisms of Toxicity, Systemic Poisoning Syndromes, Paresthesia and Therapy. In: Handbook of Pesticide Toxicology, Krieger, R.I. (Eds.). Academic Press, USA., pp: 1289-1303.
30. Ray, D.E., 1982. The contrasting actions of two pyrethroids (deltamethrin and cismethrin) in the rat. Neurobehavioral Toxicol. Teratol., 4: 801-804.
31. Verma, R.S. and N. Srivastava, 2001. Chlorpyrifos induced alterations in levels of thiobarbituric acid reactive substances and glutathione in rat brain. Indian. J. Exp. Biol., 39: 174-177.
32. Wielgomas, B. and J. Krechniak, 2007. Effect of alpha-cypermethrin and chlorpyrifos in a 28-day study on free radical parameters and cholinesterase activity in Wistar rats. Polish J. Environ. Stud., 16: 91-95.
33. Krishnamoorthy, G., P. Venkataraman, A. Arunkumar, R.C. Vignesh, M.M. Aruldhas and J. Arunakaran, 2007. Ameliorative effect of vitamins (α-tocopherol and ascorbic acid) on PCB (Aroclor 1254) induced oxidative stress in rat epididymal sperm. Reprod. Toxicol., 23: 239-245.
34. Ambali, S.F., A.O. Makinde, M. Shittu, S.A. Adeniyi and F.O. Mowuogwu, 2012. Alleviating effect of *Phyllanthus niruri* on sensorimotor and cognitive changes induced by subacute chlorpyrifos exposure in Wistar rats. Am. J. Med. Med. Sci., 2: 50-58.
35. Drewa, G., M. Jakbczyk and A. Araszkiewicz, 1998. Role of free redicals in schizophrenia. Med. Sci. Monitor, 4: 1111-1115.
36. Tsakiris, S., P. Angelogianni, K.H. Schulpis and J.C. Stavridis, 2000. Protective effect of L-phenylalanine on rat brain acetylcholinesterase inhibition induced by free radicals. Clin. Biochem., 33: 103-106.
37. Hussien, H.M., H.M. Abdou and M.I. Yousef, 2013. Cypermethrin induced damage in genomic DNA and histopathological changes in brain and haematotoxicity in rats: The protective effect of sesame oil. Brain Res. Bull., 92: 76-83.