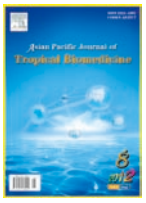
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb



Document heading doi:10.1016/S2221-1691(12)60113-0

Alleviating effects of melatonin on oxidative changes in the testes and pituitary glands evoked by subacute chlorpyrifos administration in Wistar rats

Angela J Umosen[1], Suleiman F Ambali[1,2*], Joseph O Ayo[2], Bisala Mohammed[3] , Chidiebere Uchendu[1]

[1]*Department of Veterinary Pharmacology, Ahmadu Bello University, Zaria, Nigeria*
[2]*Department of Veterinary Physiology, Ahmadu Bello University, Zaria, Nigeria*
[3]*Department of Veterinary Pathology, Ahmadu Bello University, Zaria, Nigeria*
[4]*Department of Veterinary Physiology and Pharmacology, University of Ilorin, Ilorin, Nigeria*

ARTICLE INFO

Article history:
Received 12 January 2012
Received in revised form 20 January 2012
Accepted 25 February 2012
Available online 28 August 2012

Keywords:
Chlorpyrifos
Oxidative stress
Testes
Pituitary glands
Alleviation
Melatonin

ABSTRACT

Objective: To evaluate the alleviating effects of melatonin on oxidative changes in the testes and pituitary gland induced by subacute chlopyrifos (CPF) exposure in rats. **Methods:** Forty adult male Wistar rats divided into 4 groups of 10 animals were used for the study. Group I received soya oil (2 mL/kg) while group II was administered with melatonin (0.5 mg/kg). Group III was administered CPF only (8.5 mg/kg ~ 1/10th of the LD_{50}) while group IV was pretreated with melatonin (0.5 mg/kg) and then exposed to CPF (8.5 mg/kg), 10 min later. The regimens were administered by gavage once daily for a period of 28 d. At the end of the exposure period, the rats were sacrificed and the testicular tissues and pituitary glands were evaluated for the malonaldehyde (MDA) concentration and activities of superoxide dismutase (SOD) and catalase (CAT). **Results:** CPF increased MDA concentrations and reduced the activities of SOD and CAT in the testes and pituitary gland. Melatonin pretreatment reduced the testicular and pituitary MDA concentrations and improves the SOD and CAT activities. **Conclusions:** the study showed that subacute CPF-induced oxidative stress in the testes and pituitary glands were alleviated by melatonin due to its antioxidant property.

1. Introduction

The global increase in infertility, especially in Sub-Saharan Africa with the highest prevalence is becoming a source of concern[1]. In conjunction with this are reports that indicate a significant decrease in the quality of human semen[2-4]. It is estimated that male-related disorders are probably present in up to 40% to 50% of childless couples, alone or in combination with female factors[5,6]. Environmental chemical contaminants, including pesticides such as organophosphates (OP) insecticides have been largely implicated in this reproductive deficit[7]. There is increasing evidence to suggest an association between environmental exposure to OPs and adverse reproductive outcomes[8].

Chlorpyrifos (CPF; 0,0 - diethyl 0-3,5,6-trichloro-2-pyridyl phosphothionate) is a broad spectrum OP insecticide used in the control of a wide variety of insects, including fleas, wasps, bees, cockroaches and agricultural pests[9]. Association between low sperm concentration and serum concentrations of 3,5,6-trichloropyridinol, a metabolite of CPF has been reported in humans[10]. Similarly, studies using animal models have demonstrated reduced sperm concentrations and alterations in sex hormone following repeated CPF exposure[11,12]. There are several possible mechanisms for the anti-gonadal actions of OPs: they may exert a direct inhibitory action on the testis; they may affect the pituitary, causing changes in gonadotrophin concentrations and thus subsequent spermatogenic impairment; or they may change the concentration of neurotransmitters[13]. Like other OP insecticides, the principal mechanism of CPF toxicity is due to inhibition of acetylcholinesterase (AChE) activity, resulting in the accumulation of the neurotransmitter, acetylcholine (ACh), at the cholinergic receptors in the peripheral and central

*Corresponding author: Department of Veterinary Physiology and Pharmacology University of Ilorin, Ilorin, Nigeria.
Tel: +2348037015411
E-mail: fambali2001@yahoo.com; atunluse@gmail.com
Foudation Project: Partly supported by Ahmadu Bello University Board of Research (Grant No. ABU/UBR/125/09).

nervous systems[9]. However, toxicity has been reported at doses that do not inhibit AChE[14,15]. Therefore, other mechanisms including the induction of oxidative stress have been implicated in CPF-induced toxicity[16-23]. During oxidative stress, the body's antioxidant mechanisms are overwhelmed by the oxidants, resulting in cellular damage. In this type of situation, therefore, supplementation with antioxidant molecules have been shown to mitigate oxidative stress and the consequent cellular damage[17-21].

Melatonin (N-acetyl-5-methoxytryptamine), the principal secretory product of the pineal gland, produced during the dark phase of the circadian cycle, is a highly conserved antioxidant molecule[24,25]. Melatonin along with its metabolites has been shown to scavenge reactive oxygen species (ROS) or reactive nitrogen species (RNS)[26]. This cascade reaction makes melatonin a highly effective antioxidant, even at low concentrations, in protecting cells from oxidative stress[27]. Melatonin diminishes the destruction of DNA, proteins and lipids that occur as a result of their reactions with ROS and RNS[28] as it easily crosses the cell membranes and the blood-brain barrier[29]. Since antigonadal action of OPs may involve the pituitary gland and testes couple with the established role of oxidative stress in the etiopathogenesis of OP-evoked toxicity, the present study was therefore aimed at evaluating the alleviating effect of melatonin on subacute CPF-induced oxidative stress in both organs in Wistar rats.

2. Materials and methods

2.1. Chemicals and preparation

Commercial grade CPF (20% EC; Termicot® Sabero Organics, Gujarat, India) was reconstituted to 1% solution in soya oil (Grand oil® Grand Cereal, Jos, Nigeria). Melatonin tablet (5 mg, NATROL, Inc. Chatsworth, USA) was prepared by dissolving in 10 ml of distilled water to make 0.5 mg/mL.

2.2. Animals and treatments

Forty young adult male Wistar rats weighing (126±3) g were obtained from the Animal House of the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria, Nigeria. They were housed in cages and fed on standard rat pellets and water was provided ad libitum. The rats were preconditioned for two weeks in the laboratory prior to the commencement of the study. They were weighed and divided at random into four groups, consisting of 10 rats per group. Group I (S/oil) was administered soya oil (2 mL/kg) while group II (Mel) was dosed with melatonin (0.5 mg/kg; Gultekin *et al*[30]). Group III (CPF) was administered CPF only (8.5 mg/kg ~ 1/10th of the LD_{50} ; Ambali[31]), while group IV (Mel+CPF) was pretreated with melatonin (0.5 mg/kg), and then administered CPF (8.5 mg/kg) 10 min later. The regimens were administered by gavage once daily for a period of 28 days.

At the end of the treatment period, the rats were killed by jugular venesection after light chloroform anesthesia. The testes and pituitary glands were evaluated for malonaldehyde (MDA) and protein concentrations, and activities of superoxide dismutase (SOD) and catalase (CAT). The study was conducted in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals[32].

2.3. Pituitary gland and testicular tissue preparations

The pituitary glands and the testicular tissue from each animal were rapidly removed and known weight of each of the organs was homogenized in a known volume of ice cold 20 mM phosphate buffer to obtain a 10% homogenate. This was then centrifuged at 3 000×*g* for 10 min to obtain the supernatant. The supernatant was then used to assess the concentration of proteins and MDA, and activities of superoxide dismutase and catalase in both tissues.

2.4. Evaluation of the effect of treatments on pituitary gland and testicular protein concentrations

The protein concentration from supernatants prepared from the pituitary gland and testicular tissues were evaluated using the method of Lowry[33].

2.5. Evaluation of the effect of treatments on testicular tissue and pituitary gland lipoperoxidation

The level of thiobarbituric acid reactive substance, malonaldehyde (MDA) as an index of lipid peroxidation was evaluated in the testicular tissue and pituitary gland samples using the double heating method of Draper and Hadley[34]. The MDA concentrations in the testes and pituitary glands were calculated by the absorbance coefficient of MDA-TBA complex 1.56×10^5/cm and expressed in nmol/mg of protein.

2.6. Evaluation of antioxidant enzymes activities in testicular and pituitary glands

Catalase and superoxide dismutase activities in the pituitary glands and testicular tissues were evaluated using commercial kits (Northwest Diagnostics of Virginia, U.S.A.) and then expressed as IU/mg of protein.

2.7. Statistical analysis

Data obtained were expressed as mean ± SEM and subjected to statistical analysis using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc multiple comparison test using Graphpad prism 4.0, San Diego, California, USA (www.graphpad.com). Values of $P<0.05$ were considered significant.

3. Results

3.1. Effect of treatments on testicular and pituitary gland malonaldehyde concentrations

There was a significant increase ($P<0.01$) in the concentration of testicular MDA in the CPF group when compared to S/oil, Mel or Mel+CPF group. There was no significant change ($P>0.05$) in the MDA concentration in the Mel+CPF group when compared to either S/oil or Mel group (Figure 1).

The pituitary MDA concentration in the CPF group was significantly ($P<0.01$) higher in the CPF group when compared to S/oil, Mel or Mel+CPF group. There was no significant change ($P>0.05$) in the MDA concentration in the Mel+CPF group when compared to either the S/oil or Mel group (Figure 2).

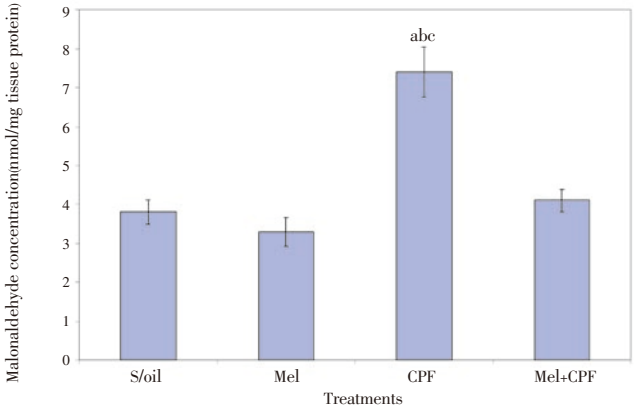


Figure 1. Effect of subacute soya oil (S/oil), chlorpyrifos (CPF) and/or melatonin (Mel) exposure on testicular malonaldehyde concentration in Wistar rats ($n=10$). $^{abc}P<0.01$ *vs* S/oil, Mel and Mel+CPF groups, respectively.

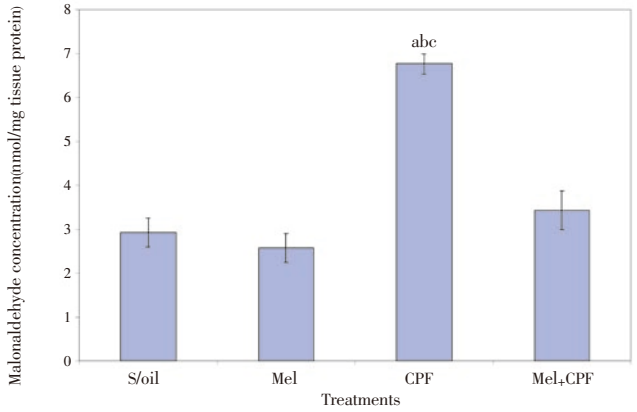


Figure 2. Effect of soya oil (S/oil), chlorpyrifos (CPF) and/or melatonin (Mel) on pituitary gland's malonaldehyde concentration in Wistar rats ($n=10$). $^{abc}P<0.01$ *vs* S/oil, Mel and Mel+CPF groups, respectively.

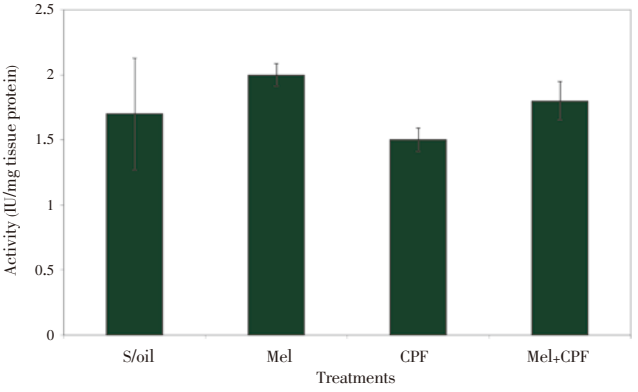


Figure 3. Effect of subacute soya oil (S/oil), chlorpyrifos (CPF) and/or melatonin (Mel) exposure on testicular superoxide dismutase activity in Wistar rats ($n=10$).

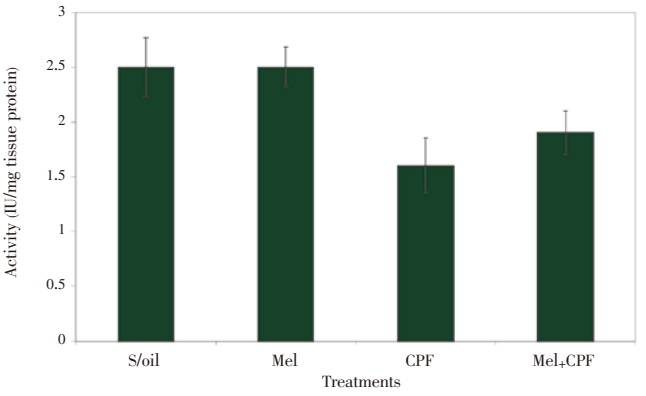


Figure 4. Effect of soya oil (S/oil), chlorpyrifos (CPF) and/or melatonin (Mel) on pituitary gland's superoxide dismutase activity in Wistar rats ($n=10$).

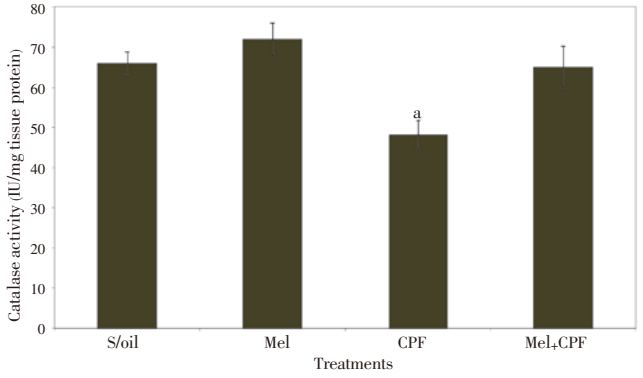


Figure 5. Effect of soya oil (S/oil), chlorpyrifos (CPF) and/or melatonin (Mel) on testicular catalase activity in Wistar rats ($n=10$). $^{a}P<0.05$ *vs* Mel group.

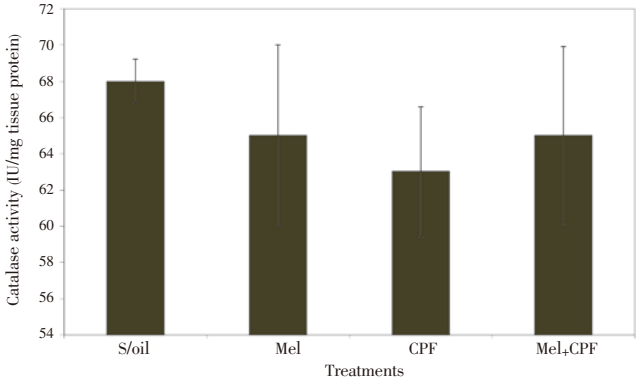


Figure 6. Effect of soya oil (S/oil), chlorpyrifos (CPF) and/or melatonin (Mel) on pituitary gland's catalase activity in Wistar rats ($n=10$).

3.2. Effect of treatments on testicular and pituitary gland superoxide dismutase activities

The effect of subacute exposure to soya oil, CPF and/or melatonin on testicular SOD level is shown in Figure 3. There was no significant change ($P>0.05$) in activity level of SOD between the groups. However, CPF group had the lowest mean testicular SOD activity decreasing by 11% compared to that recorded in the S/oil group. The Mel+CPF group showed a comparatively higher (6%) testicular SOD activity compared to that of CPF or S/oil group but was comparatively lower (18%) than that of the Mel group.

There was no significant change ($P>0.05$) in pituitary gland activity level of superoxide dismutase between groups. However, CPF group had the lowest SOD activity decreasing by 36% compared to the S/oil group. The Mel+CPF group showed a comparatively higher (24%) mean pituitary gland SOD activity compared to the CPF group but was comparatively lower than those recorded in S/oil (32%) or Mel (32%) group (Figure 4).

3.3. Effect of treatments on testicular and pituitary gland catalase activities

The effect of treatments on testicular CAT activity is shown in Figure 5. There was a significant decrease ($P<0.05$) in testicular activity of CAT in the CPF group when compared to the Mel group, but no significant change ($P>0.05$) when compared to that of S/oil or Mel+CPF group. The CPF group had the lowest mean CAT activity decreasing by 27% compared to the S/oil group. The Mel+CPF group showed a comparatively higher testicular CAT activity (26%) compared to the CPF group but it was comparatively lower than those in S/oil (2%) or Mel (11%) group.

There was no significant change ($P>0.05$) in pituitary gland's CAT activity between the groups. However, the CPF group had the lowest mean pituitary gland's CAT activity decreasing by 7% compared to that of the S/oil group. The Mel+CPF group showed a comparatively higher pituitary gland's CAT activity (4%) compared to the CPF group but it was comparatively lower than that of the S/oil group (24%) and the same with that of the Mel (4%) group (Figure 6).

4. Discussion

The study had also shown the ability of CPF to increase the concentration of MDA in both the pituitary gland and the testes. This confirms the increase in MDA concentration following CPF exposure in previous studies[18-23,35]. MDA is a lipoperoxidation by-product resulting from interaction of oxygen radicals with polyunsaturated fatty acids residues in membrane phospholipids and has been shown to damage proteins and DNA[36]. The high MDA concentrations of the pituitary and the testicular tissues in the CPF group are indications of the level of lipoperoxidative changes, reflecting alteration in structural and perhaps functional status of these organs. Lipid peroxidation involves the formation and propagation of lipid radicals, the uptake of oxygen and rearrangement of the double and unsaturated lipids, resulting in a variety of degraded products that eventually cause destruction of membrane lipids[37]. However, pretreatment with melatonin has been shown by the present study to decrease the testicular and pituitary MDA concentrations, indicating its anti-lipoperoxidative effect.

Decrease in the pituitary and testicular SOD activities were recorded in the CPF group. SOD, which catalyzes the dismutation of $O_2^{\cdot-}$ to H_2O_2 is the first line antioxidant defense in the body. The decreased activity in the CPF group may be due to decreased synthesis, increased degradation or even outright inactivation of the enzyme. SOD is considered the most important antioxidant enzyme in the spermatozoa where it has a key physiological role in sperm motility[38]. Therefore, the low SOD activity in the testes recorded in the CPF group may also indirectly reflect its activity status in the spermatozoa. The resulting impairment of sperm motility may have contributed to the decrease in its fertilizing capacity reported in CPF exposure. The increase in both pituitary and testicular SOD activities in the group pretreated with melatonin is a demonstration of the ability of the antioxidant agent to protect the SOD from the ravaging effect of CPF-induced ROS through boosting of the antioxidant reserves in both the testes and the pituitary glands.

Similarly, the study also showed a comparative decrease in CAT activities in both the pituitary glands and the testes in the CPF group. CAT is known to neutralize H_2O_2 to H_2O and O_2. The decline in the CAT activity observed in CPF group may be due to the reduced conversion of $O_2^{\cdot-}$ to H_2O_2 by SOD, which then leads to the accumulation of $O_2^{\cdot-}$. This accumulation of $O_2^{\cdot-}$ has been shown to inhibit the activity of CAT[39] as $O_2^{\cdot-}$ converts ferroxy state of CAT to ferryl state, which is an inactive form of the enzyme[40]. Several studies have shown that CPF reduced the activities of both SOD and CAT[41]. The ability of melatonin to improve CAT activity is a demonstration of the ability of this antioxidant molecule to boost the endogenous antioxidant reserve and protect CAT from the ROS induced by CPF.

These lipoperoxidative changes couple with low antioxidant status represented by low level of activity of SOD and CAT in the CPF group is an indication of oxidative stress. Oxidative stress may therefore be partly responsible for the alteration in the synthesis of some sex hormones and other substances essential for sustaining reproductive activity evoked by CPF and other OPs[11,12], culminating in low sperm count. Therefore, oxidative stress may have been partly involved in the male reproductive toxicity reported in CPF poisoning[11,12].

Melatonin has been found to protect cells, tissues and organs against oxidative damage induced by a variety of free radical generating agents and processes[42]. Melatonin

acts as a direct free radical scavenger[43] and as an indirect antioxidant via its stimulatory actions on antioxidative enzymes[44]. The radical-scavenging potency of melatonin is much greater than that of the most important endogenous radical scavenger, glutathione, and the classical hydroxyl radical scavenger, mannitol[45]. Melatonin has also been reported to alter the activities of enzymes that improve the total antioxidative defense capacity of the organism [46]. Its hydrophilic and lipophilic nature also aids in its free radical scavenging and antioxidant actions. Its lipophilic nature enhances the easiness at which it crosses the morphophysiological barriers, such as the blood-brain barrier, and then effectively enters the cells and subcellular compartments[47,48]. It distributes in all cell compartments, being especially high in the nucleus and mitochondria[49,50]. Furthermore, several metabolites such as 6-hydroxymelatonin and N-acetyl-N-formyl-5-methoxykynurenamine that are formed when melatonin neutralizes damaging reactants are themselves scavengers suggesting that there is a cascade of reactions that greatly increase the efficacy of melatonin in stymieing oxidative mutilation. Other processes suggested to contribute to melatonin's ability to reduce oxidative stress include stimulation of glutathione synthesis (an important antioxidant which is at high concentrations within cells), reducing electron leakage from the mitochondrial electron transport chain (which would reduce free radical generation), limiting cytokine production inflammatory processes (actions that would also lower toxic reactant generation), and synergistic effects with other classical antioxidants such as vitamins C and E[52]. All these may have aided the improvement in the antioxidant status recorded in the group pretreated with melatonin.

In conclusion, the present study has shown that the induction of oxidative stress in the testes and pituitary glands evoked by CPF may have been partly responsible for the male reproductive toxicity reported in previous studies. Melatonin was able to mitigate the oxidative changes evoked by subacute CPF exposure partly due to its antioxidant property and therefore may be useful in protecting the pituitary gland and testicular tissue from toxicity induced by the OP insecticide.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

We thank the Ahmadu Bello University Board of Research for partly funding this research through the grant number ABU/UBR/125/09. We also appreciate the technical support of Chief E. Nwosu and Mr. A. Olu.

References

[1] Pennings G, de Wert G, Shenfield F, Cohen J, Tarlatzis B, Devroey P. Providing infertility treatment in resource-poor countries. *Hum Reprod* 2009; **24**(5): 1008-11.

[2] Nelson CMK, Bunge RG. Semen analysis evidence for changing parameters of male fertility potential. *Fert Ster* 1974; **25**: 503-7.

[3] Murature DA, Tang SY, Steinhardt G, Dougherty PC. Phthalate esters and semen quality parameters. *Biomed Environ Mass Spect* 1987; **14**: 4731-7.

[4] Bendvold E. Semen quality in Norwegian men over a 20-year period. *Int J Fert* 1989; **34**: 401-4.

[5] Bungum M. Sperm DNA integrity assessment: A new tool in diagnosis and treatment of fertility. *Obstet Gynecol Int* 2012 (2012), Article ID: 531042, 6 pages doi:10.1155/2012/531042.

[6] Tournaye H. Male factor infertility and ART. *Asian J Androl* 2012; **14**: 103-108;

[7] Volk M, Jaklič H, Zorn B, Peterlin B. Association between male infertility and genetic variability at the PON1/2 and GSTM1/T1 gene loci. *Reprod Bio Med Online* 2011; **23**(1): 105-10.

[8] Peiris-John RJ, Wickremasinghe R. Impact of low-level exposure to organophosphate on human reproduction and survival. *Trans Royal Soc Trop Med Hyg* 2008; **102**(3): 239-45.

[9] Eaton DL, Daroff RB, Autrup H, Buffler P, Costa LG, Coyle J, et al. Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment. *Crit Rev Toxicol* 2008; **S2**: 1-125.

[10] Meeker JD, Ryan L, Barr D, Hauser R. Exposure to non-persistent insecticides and male reproductive hormones. *Epidemiol* 2006; **17**(1): 61-68.

[11] Zidan NA. Evaluation of the reproductive toxicity of chlorpyrifos methyl, diazinon and profenofos pesticides in male rats. *Int J Pharmacol* 2009; **5**(1): 51-57.

[12] Fattahi E, Parivar K, Jorsaraei SGA, Moghadamnia AA. The effects of diazinon on testosterone, FSH and LH levels and testicular tissue in mice. *Iranian J Rep Med* 2009; **7**(2): 59-64.

[13] Sarkar R, Mohanakumar KP, Chowdhury M. Effects of an organophosphate pesticide, quinalphos, on the hypothalamo-pituitary-gonadal axis in adult male rats. *J Rep Fert* 2000; **118**: 29-38.

[14] Slotkin TA, MacKillop EA, Ryde IT, Seidler FJ. Ameliorating the developmental neurotoxicity of chlorpyrifos: a mechanisms-based approach in PC12 cells. *Environ Health Perspect* 2007; **115**: 1306-13.

[15] Chakraborti TK, Farrar JD, Pope CN. Comparative neurochemical, and neurobehavioral effects of repeated chlorpyrifos exposures in young rats. *Pharmacol Biochem Behav* 1993; **46**: 219-24.

[16] Mansour SA, Mossa AH. Adverse effects of lactational exposure to chlorpyrifos in suckling rats. *Hum Exp Toxicol* 2010; **29**(2): 77-92.

[17] Uchendu C, Ambali SF, Ayo JO, Esievo KAN, Laisisi IO, Surakat LS. Protective effect of acetyl-L-carnitine on subacute chlorpyrifos-induced biochemical changes in Wistar rats. *Int J Biol Chem* 2011; **5**: 300-13

[18] Ambali SF, Abubakar AT, Shittu M, Yaqub LS, Anafi SB, Abdullahi A. Chlorpyrifos-induced alteration of hematological

parameters in Wistar rats: Ameliorative effect of zinc. *Res J Env Toxicol* 2010; **4**(2): 55-66.

[19] Ambali SF, Abubakar AT, Shittu M, Yaqub LS, Kobo PI, Giwa A. Zinc ameliorates chlorpyrifos-induced erythrocyte fragility and lipoperoxidative changes in Wistar rats. *New York Sci J* 2010; **3**: 117-22.

[20] Ambali SF, Idris SB, Onukak C, Shittu M, Ayo JO. Ameliorative effects of vitamin C on short-term sensorimotor and cognitive changes induced by acute chlorpyrifos exposure in Wistar rats. *Toxicol Ind Health* 2010; **26**(9): 547-58.

[21] Ambali SF, Ayo JO, Ojo SA, Esievo KAN. Ameliorative effect of vitamin C on chlorpyrifos-induced increased erythrocyte fragility in Wistar rats. *Hum Exp Toxicol* 2011; **30**(1): 19-24.

[22] Shittu M, Ayo JO, Ambali SF, Kawu MU, Salami SO. Vitamin E mitigates chronic chlorpyrifos-induced oxidative changes in the testes and pituitary glands in Wistar rats. *Am J Appl Sci* 2012; **9**(1): 75-82.

[23] Shittu M, Ayo JO, Ambali SF, Fatihu MY, Onyeanusi BI, Kawu MU. Chronic chlorpyrifos-induced oxidative changes in the testes and pituitary glands of Wistar rats: Ameliorative effect of vitamin C. *Pest Biochem Physiol* 2012; **102**: 79-85.

[24] Kudo T, Horikawa K, Shibata S. Circadian rhythms in CNS and peripheral clock disorders.The circadian clock and hyperlipidemia. *J Pharmacol Sci* 2007; **103**: 139-43.

[25] Reiter RJ, Tan D, Fuentes-Broto L. Melatonin: a multitasking molecule. *Prog Brain Res* 2010; **181**(08): 127-51

[26] Hardeland R, Tan DX, Reiter RJ. Kynuramines, metabolites of melatonin and other indoles: the resurrection of an almost forgotten class of biogenic amines. *J Pineal Res* 2009; **47**: 109-26.

[27] Tan DX, Manchester LC, Terron MP, Flores LJ, Reiter RJ. One molecule, many derivatives; a never-ending interaction of melatonin with reactive oxygen and nitrogen species. *J Pineal Res* 2007; **42** (1): 28-42.

[28] Chojnacki J, Wiśniewska-Jarosińska M, Śliwiński T, Błasiak J, Chojnacki C. Melatonin modulates DNA damage and repair in colonocytes of subjects with ulcerative colitis. *Gastroenterol Pol* 2011; **18** (2): 67-71.

[29] Hardeland R. Melatonin: signaling mechanisms of a pleiotropic agent. *Biofactors* 2009; **35**: 183-192.

[30] Gultekin F, Patat S, Akca H, Akdogan M, Altuntas I. Melatonin can suppress the cytotoxic effects of chlorpyrifos of human HepG2 cell lines. *Hum Exp Toxicol* 2006; **25**(2): 47-55.

[31] Ambali SF. *Ameliorative effect of antioxidant vitamins C and E on neurotoxicological, haematological and biochemical changes induced by chronic chlorpyrifos exposure in Wistar rats*. Zaria: Ahmadu Bello University; 2009, p. 356.

[32] Guide for the care and use of laboratory animals, DHEW Publication No. (NIH) 85-23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20892.

[33] Lowry OH, Rosebrough NJ, Fair AL, Randall RJ. Protein measurement with folin-phenol reagent. *J Biol Chem* 1951; **193**: 265-75.

[34] Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. *Methods Enzymol* 1990; **186**: 421-31.

[35] Gultekin F, Ozturkn M, Akdogan M. The effect of organophosphate insecticide chlorpyrifos-ethyl on lipid peroxidation and antioxidant enzymes (*in-vitro*). *Arch Toxicol* 2000; **74**: 533-8.

[36] Krishnamoorthy G, Ventaraman P, Arunkumar A, Vignesh RC, Aruldhas MM, Arunakaran J. Ameliorative effect of vitamins (α - tocopherol and ascorbic acid) on PCB (Aroclor 1254)-induced oxidative stress in rat epididymal sperm. *Rep Toxicol* 2007; **23**: 239-45.

[37] Dattani JJ, Moid N, Highland HN, George LB, Desai RR. Ameliorative effect of curcumin on hepatotoxicity induced by chloroquine phosphate. *Env Toxicol Pharmacol* 2010; **30**(2): 103-9.

[38] Marzec-Wróblewska U, Kamiński P, Łakota P, Szymański M, Wasilow K, Ludwikowski G, et al. Zinc and iron concentration and SOD activity in human semen and seminal plasma. *Biol Trace Elem Res* 2011; **143**(1): 167-77.

[39] Kono Y, Fridovich I. Superoxide radical inhibits catalase. *J Biol Chem* 1982; **257**: 5751-4

[40] Freeman BA, Crapo JD. Biology of disease: free radicals and tissue injury. *Lab Invest* 1982; **47**: 412-26.

[41] Astiz M, María JT, De Alaniz, Marra CA. The impact of simultaneous intoxication with agrochemicals on the antioxidant defense system in rat. *Pest Biochem Physiol* 2009; **94**: 93-9.

[42] Rao MV, Purohit AR. Neuroprotection by melatonin on mercury induced toxicity in the rat brain. *Pharmacol Pharm* 2011; **2**: 375-385.

[43] Korkmaz A, Reiter RJ, Topal T, Manchester LC, Oter S, Tan DX. Melatonin: an established antioxidant worthy of use in clinical trials. *Mol Med* 2009; **15**: 43-50.

[44] Hibaoui Y, Roulet E, Ruegg UT. Melatonin prevents oxidative stress-mediated mitochondrial permeability transition and death in skeletal muscle cells. *J Pineal Res* 2009; **47**: 238-252.

[45] Poeggeler B, Saarela S, Reiter RJ, Tan DX, Chen LD, Manchester LC, et al. Melatonin - a highly potent endogenous scavenger and electron donor: New aspects of the oxidation chemistry of this indole assessed *in vitro*. *Ann NY Acad Sci* 1994; **738**: 419-20.

[46] Bharti VK, Srivastava RS, Subramaian P, Spence DW, Pandi-Perumal SR, Brown GM. Cerebral epiphyseal proteins and melatonin modulate the hepatic and renal antioxidant defense of rats. *Int J Nephrol* 2011; 2011, Article ID 142896, 5 pages doi:10.4061/2011/142896.

[47] Johns J. Estimation of melatonin blood brain barrier permeability. *J Bioanal Biomed* 2011; **3**: 064-069. doi:10.4172/1948-593X.1000045.

[48] Erukainure OL, Ajiboye JA, Adejobi RO, Okafor OY, Adenekan SO. Protective effect of pineapple (*Ananas cosmosus*) peel extract on alcohol-induced oxidative stress in brain tissues of male albino rats. *Asian Pac J Trop Dis* 2011; **1**(1): 5-9.

[49] Venegas C, García JA, Escames G, Ortiz F, López A, Doerrier C, et al. Extrapineal melatonin: analysis of its subcellular distribution and daily fluctuations. *J Pineal Res* 2011; DOI: 10.1111/j.1600-079X.2011.00931.x.

[50] Srinivasan V, Spence DW, Pandi-Perumal SR, Brown GM, Cardinali DP. Melatonin in mitochondrial dysfunction and related disorders. *Int J Alzheimer's Dis* 2011; 2011, Article ID 326320, 16 pages, doi:10.4061/2011/326320.

[51] Sharma AK, Mehtab AK, Rathor N, Hanumantappa MKC, Khanna N, Bhattacharya SK. Melatonin attenuates cognitive dysfunction and reduces neural oxidative stress induced by phosphamidon. *Fund Clin Pharm* 2011 doi: 10.1111/j.1472-8206.2011.00977.

[52] Reiter RJ, Tan DX, Gitto E. Pharmacological utility of melatonin in reducing oxidative cellular and molecular damage. *Pol J Pharmacol* 2004; **56**(2): 159-70.