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Original Article

Subchronic Chlorpyrifos-Induced Clinical, Hematological and Biochemical Changes in Swiss Albino Mice: Protective Effect of Vitamin E

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ABSTRACT

Forty adult Swiss albino mice of either sex divided into 4 groups of 10 mice in each group were used to evaluate the ameliorating effect vitamin E on hematological and serum biochemical changes induced by subchronic chlorpyrifos (CPF) exposure. Group I (control~C/oil) and group II (VE) were administered corn oil (2 ml/kg) and vitamin E (75 mg/kg), respectively. Group III was administered CPF (21.3 mg/kg~ 1/5th LD50) only while group IV (VE + CPF) was pretreated with vitamin E (100 mg/kg) followed by CPF administration, 30 min later. The regimens were administered orally by gavage, every other week days for a period of ten weeks. The mice were evaluated for signs of toxicity and weekly body weight changes. At the end of the dosing period, blood samples collected were analyzed for packed cell volume, total red blood cell, white blood cell and total protein. The sera obtained from the blood samples were analyzed for the levels of Na+, K+, Cl-, total protein, urea, creatinine, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and malonaldehyde. The results showed that pretreatment with vitamin E ameliorated deficits in clinical, body weight, hematological and biochemical changes induced by repeated CPF administration in mice, partly due to its antioxidant properties.

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1. Introduction

Organophosphate (OP) insecticides which have largely replaced the organochlorine compounds are one of the most widely used estimated to account for about 50% of all insecticides used globally [1]. They are used in agriculture, horticulture and public health. Their widespread use is however accompanied by attendant adverse effect on animal and human health. Toxic effects of OP compounds are predominantly produced through inhibition of acetylcholinesterase (AChE), causing accumulation of acetylcholine at peripheral and central cholinergic receptors, resulting in overstimulation of the cholinergic system [2, 3] and subsequent paralysis. However, doses below the threshold for the AChE inhibition have been shown to induce toxicity [4]. Therefore, other associated mechanisms have been implicated in the molecular mechanisms of OP-induced toxicosis. Among these mechanisms, the induction of oxidative stress by the OP compounds has continued to receive tremendous attention [3, 5-10].

Chlorpyrifos (CPF) is one of the most widely used OP insecticides in USA, with an annual usage of 8-10 million pounds in agricultural sector in 1999 [11]. Approximately 800 registered pesticide products in the market contain CPF [12]. It is also the most widely studied OP compound [13]. Despite the restrictions placed on some of its domestic uses in USA in 2000 [14], the use CPF is still on the increase, with its attendant consequence on the health and well being of man, animals and the environment. Since the use of CPF is on the increase, especially in agriculture, the need to identify agents that would mitigate the adverse health consequence posed by long term exposure to this chemical pesticide becomes pertinent. Oxidative stress has been implicated in the molecular mechanism of CPT-induced toxicity [7-10]. We

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have earlier demonstrated the mitigating effect of vitamin C on some hematological and serum biochemical changes induced by CPF [6]. The aim of this study was to evaluate the ameliorative of vitamin E on hematologic and biochemical changes induced by subchronic CPF exposure.

2. Materials and Method

2.1 Chemicals

Commercial grade CPF (20% EC, TermicotTM Sabero Organics, Gujarat Ltd., India) and vitamin E (100 mg DL-α-tocopherol) (Patterson Zoochonis Ltd, Nigeria) were reconstituted in corn oil to 1% solution and 20 mg/ml, respectively.

2.2 Animals and Treatments

Forty healthy Swiss albino mice of either sex, weighing between 17-21 g were obtained from the Animal house of the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria, Nigeria. They were housed in a steel cage, fed on pellets made from growers mash (Rebson Feeds, Zaria, Nigeria), maize bran and groundnut cake in the ratio of 4:2:1 and water was provided ad libitum. The experiment was performed in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals [15].

The mice were randomly divided into four groups of 10 animals per group and dosed as follows: group I (C/oil), which was a control was given only corn oil (2 ml/kg); group II (VE group) was administered vitamin E only (75 mg/kg); group III (CPF group) was dosed with CPF only [21.3 mg/kg ~1/5th LD50 Ambali et al [6]]; group IV (VE+CPF group) was pretreated with vitamin E (75 mg/kg) and then dosed with CPF (21.3 mg/kg), 30 min later. These regimens were administered three times per week at every other week days (Mondays, Wednesdays and Fridays) for a period of 10 weeks. The animals were examined for clinical signs, weekly body weight changes and death. At the end of the dosing period, the mice were sacrificed by severing the jugular vein, after light ether anesthesia, and 0.2 ml of blood samples collected into heparinized test tubes were analyzed for hematological parameters. Another 2 ml blood sample was collected from each animal into another test tube and was incubated for 30 minutes before being centrifuged at 1000g for 10 minutes. Thereafter, the serum sample collected from the test tube into a clean sample bottle was used for the analysis of serum biochemical parameters.

2.3 Evaluation of Hematological Parameters

The hematological parameters evaluated include packed cell volume (PCV), hemoglobin (Hb) concentration, total erythrocyte (RBC), and total and absolute differential leukocyte (WBC) counts. The PCV and concentrations of RBC and WBC were evaluated as described by Rodak [16], while Hb concentration was measured using the method of Van Kampen and Zilstra [17].

2.4 Evaluation of Biochemical Parameters

The serum biochemical parameters evaluated included urea, creatinine, electrolytes (Na+, K+ and Cl-), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and malonaldehyde (MDA). The activities of AST and ALT were evaluated using the method of Reitman and Frankel [18] while that of ALP was determined as described by King and Armstrong [19]. Serum creatinine concentration was measured using the method of Miller and Miller [20] (1951), while urea concentration was determined according to the modified method of Natelson et al [21] by using diacetyl-monoxime-thiosemicarbazide procedure. In addition, the concentration of Na+ and K+ were measured by flame photometry, while Cl- was analyzed using the method described by Schales and Schales [22]. Total serum protein (TP) was determined using Lowry method, while serum MDA level was also analyzed using the method of Draper and Hadley [23] as modified by Kanter et al [24].

2.5 Statistical analysis

Values obtained were expressed as Mean ± SEM and then subjected to one-way analysis of variance, followed by Tukey's test. The mean body weight of the mice in each group at the commencement of the study (week 1) was compared with those recorded at the termination of the study (week 10) using the Student's t-test. Values of $P < 0.05$ were considered significant.

3. Results

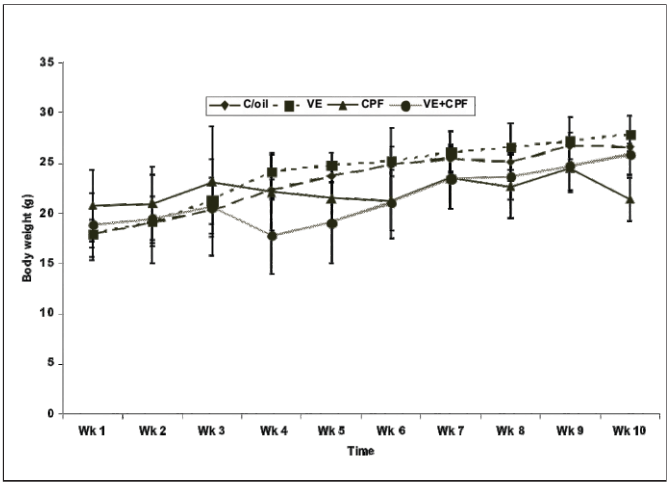
3.1 Effect of treatments on clinical signs

Both C/oil and VE groups did not show any apparent sign of toxicity. However, CPF group exhibited huddling, depression, conjunctivitis, tremor, piloerection, diarrhea and dyspnea. Also the mortality rate for the CPF was 20% by the end of the 10th week of treatment. On the other hand, mice pretreated with vitamin E and CPF showed milder toxic signs, which included mild diarrhea, tremor and huddling and a mortality rate of 10% by the end of 10 weeks of treatment.

3.2 Effect of treatments on body weight changes

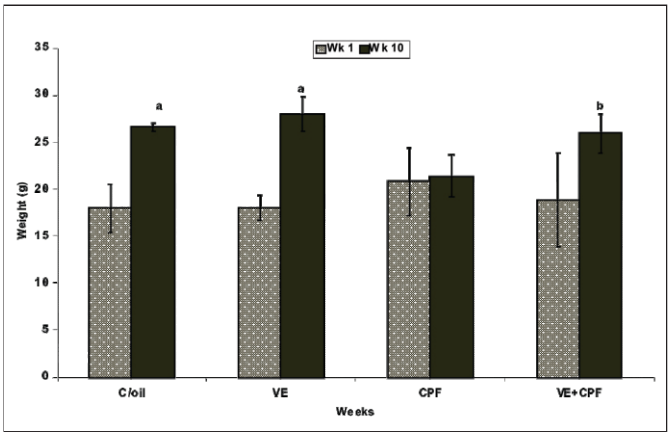
The effect of treatments on body weight is shown in Figures 1 and 2. The C/oil and the VE groups showed a consistent progressive increase in body weight gain over the ten-week period. The mean body weight of C/oil and VE groups at termination were significantly higher ($P < 0.05$) compared to the values obtained at commencement as they experienced 32% and 36% weight increase, respectively, throughout the study period . Mice in CPF group showed a comparatively less progressive elevation in body

Figure 1: The effect of corn oil (C/oil), chlorpyrifos (CPF) and/or vitamin E (VE) on dynamic of body weight changes in mice.



weight gain as they experienced a 3% increase over the ten-week period, and the mean body weight at termination was not significantly different to those obtained at the commencement of the study. VE+CPF group showed a comparative progressive increase in their body weight dynamics with a 27% increase over the ten week period and the values obtained at termination of the study was significantly higher ($P < 0.05$) compared to those obtained initially at the commencement of the study.

Figure 2: The effect of corn oil (C/oil), chlorpyrifos (CPF) and /or vitamin E (VE) on total mean body weight changes in mice. aP < 0.01 versus week 1; bP < 0.05 versus week 1



3.3 Effect of treatments on hematological parameters

There was a significant increase in PCV ($P < 0.01$) of the CPF group compared to either C/oil or VE group. There was no significant change ($P>0.05$) in the PCV of VC+CPF group relative to either C/oil or VE group (Figure 3). The RBC count of mice in the CPF group was significantly higher compared to C/oil ($P<0.01$), VE ($P<0.01$) or VE+CPF ($P < 0.05$) group. There was no significant change ($P < 0.05$) in RBC concentration in the VE+CPF group compared to either C/oil or VE group (Figure 4). The Hb concentration in the CPF group was significantly higher compared to C/oil ($P<0.01$), VE ($P<0.01$) or VE+CPF ($P < 0.05$) group. The Hb concentration in the VE+CPF group was not significantly different ($P>0.05$) compared to the C/oil or VE group (Figure 5).

Figure 3: The effect of corn oil, chlorpyrifos and/or vitamin E on packed cell volume in mice. abP < 0.05 versus C/oil and VE groups, respectively.

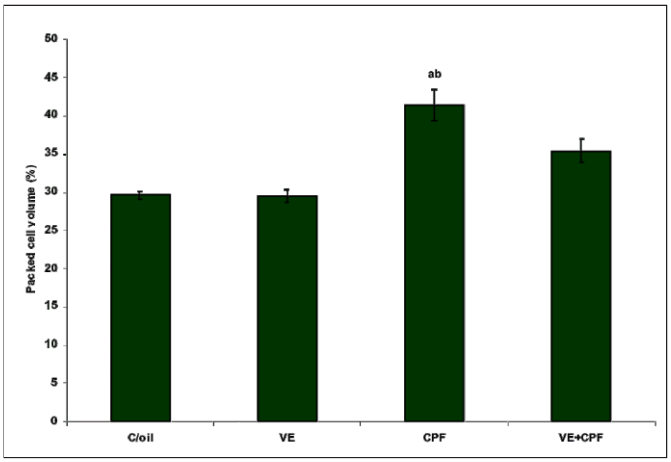


Figure 4: The effect of corn oil (C/oil), chlorpyrifos (CPF) and/or vitamin E (VE) on hemoglobin concentration in mice. abcP < 0.01 versus C/oil, VE and VE+CPF group.

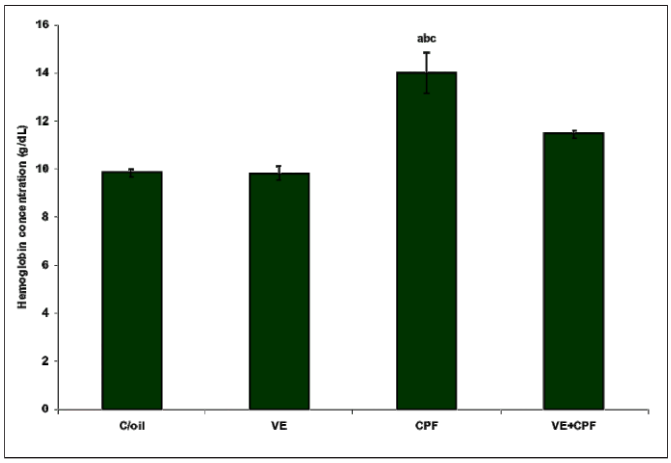
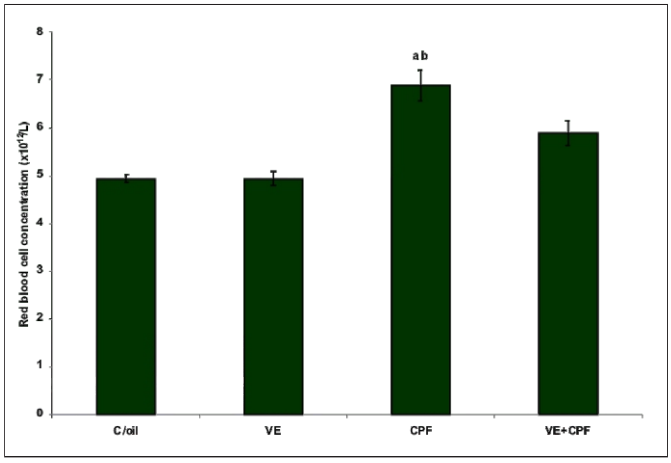


Figure 5: The effect of corn oil (C/oil), chlorpyrifos (CPF) and/or vitamin E (VE) on red blood cell count in mice. $^{ab}P < 0.05$ versus



The WBC count in the CPF group was significantly lower ($P<0.01$) compared to C/oil, VE or VE+CPF group. The WBC count of VE+CPF group also decreased significantly ($P < 0.01$) when compared to either C/oil or VE group (Figure 6). The absolute differential leukocyte count revealed that the neutrophil count was significantly lower ($P < 0.01$) in the CPF group compared to C/oil, VE, or VE+CPF group. The neutrophil count in the VE+CPF group was significantly lower ($P < 0.01$) compared to C/oil or VE group. The lymphocyte count in the CPF group was significantly lower ($P < 0.01$) compared to the C/oil, VE, or VE+CPF group. The lymphocyte count in the VE+CPF group was significantly lower ($P<0.01$) compared to the VE group but not significantly different when compared to the C/oil group (Figure 7).

3.4 Effect of treatments on serum biochemical parameters

The TP concentration in the CPF group was significantly higher ($P<0.05$) when compared to either C/oil or VE group. There was no significant change ($P>0.05$) in the TP concentration of mice in the VE+CPF group compared to C/oil, VE or CPF group (Figure 8)

Figure 6: The effect of corn oil (C/oil), chlorpyrifos (CPF) and/or vitamin E (VE) on white blood cell count in mice. abcP < 0.01 versus C/oil, VE and VE+CPF groups, respectively; deP<0.01 versus C/oil and VE groups, respectively.

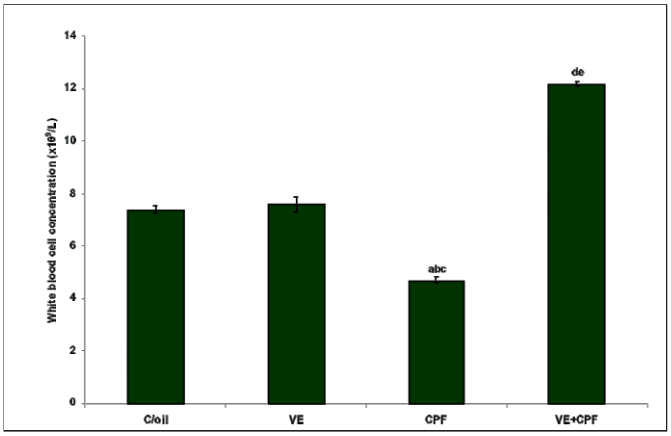


Figure 7: The effect of corn oil (C/oil), chlorpyrifos (CPF) and/or vitamin E (VE) on differential leukocyte count in mice. abcP < 0.01 versus C/oil, VE and VE+CPF groups, respectively; deP<0.01 versus C/oil and VE groups, respectively; fgP<0.01 versus VE group

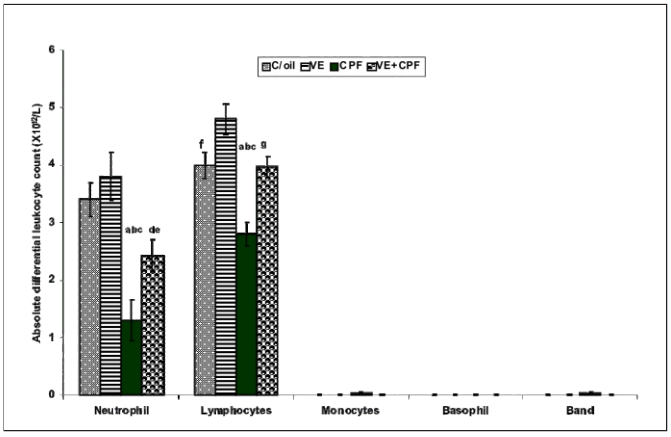
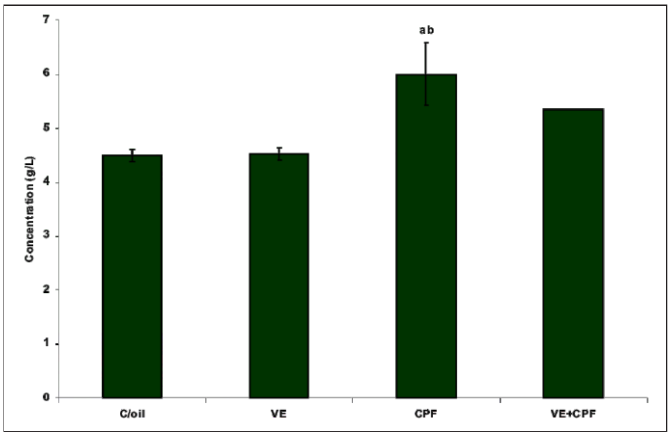
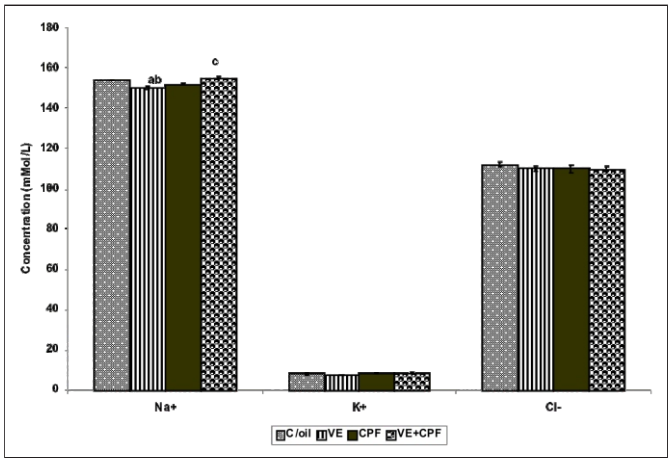


Figure 8: The effect of corn oil (C/oil), chlorpyrifos (CPF) and/or vitamin E (VE) on total proteins in mice. abP < 0.01 versus C/oil and VE, respectively.



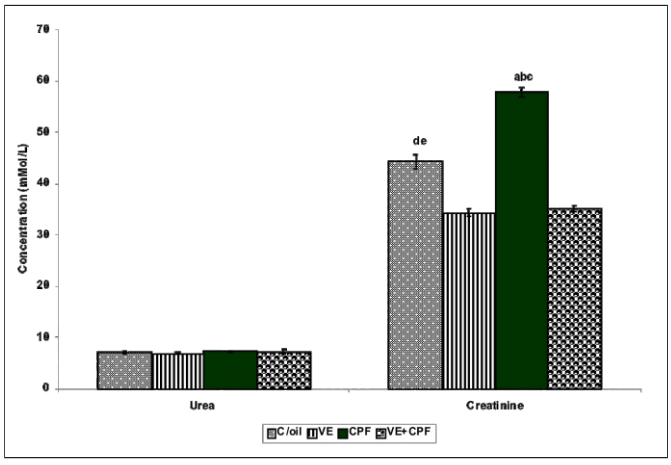
The Na+ concentration in the VE+CPF group was significantly higher when compared to the C/oil (P<0.05), VE (P<0.01) or CPF (P<0.05) group. There were no significant changes (P>0.05) in the concentration of K+ and Cl- between the groups (Figure 9).

Figure 9: The effect of corn oil (C/oil), chlorpyrifos (CPF) and/or vitamin E (VE) on serum electrolyte concentrations in mice. aP < 0.05 versus C/oil; bP<0.01 versus VE+CPF; cP<0.05 versus CPF



A non-significant (P>0.05) change in the urea concentration was observed when the values were compared to each other (Figure 10). The creatinine concentration was significantly higher (P < 0.01) in the CPF group when compared to the C/oil, VE or VE+CPF group. The creatinine concentration in the C/oil group was significantly higher (P<0.01) compared to VE and VE+CPF groups. There was no significant change (P>0.05) in the creatinine concentration in the VE+CPF group compared to VE group (Figure 10)

Figure 10: The effect of corn oil, chlorpyrifos and/or vitamin E on serum urea and creatinine concentrations in mice. abcP < 0.01 vs C/oil, VE, and VE+CPF, respectively; deP < 0.01 vsVE and VC+VE groups, respectively.

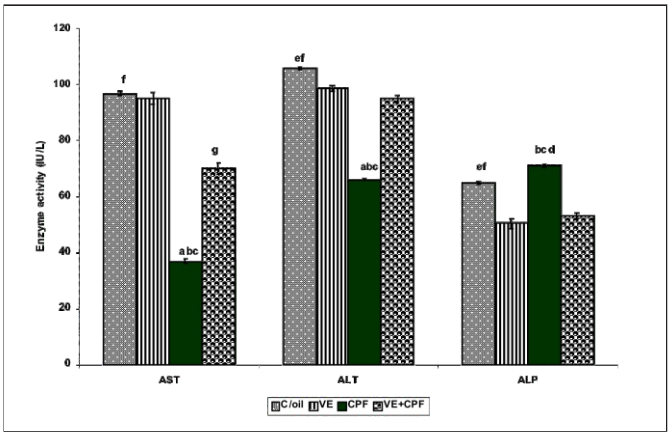


The activity of AST in the CPF group was significantly lower (P<0.01) relative to C/oil, VE or VE+CPF group. The AST activity in the VE+CPF group was significantly lower (P<0.01) when compared to C/oil and VE groups. There was no significant change (P>0.05) in the AST activity in the C/oil group compared VE group (Figure 11)

The ALT activity was significantly lower (P<0.01) in the CPF group compared to C/oil, VE or VE+CPF group. The ALT activity in the VE+CPF group was significantly higher (P<0.01) when compared to the C/oil group but there was no significant change (P>0.05) relative to the VE group (Figure 11).

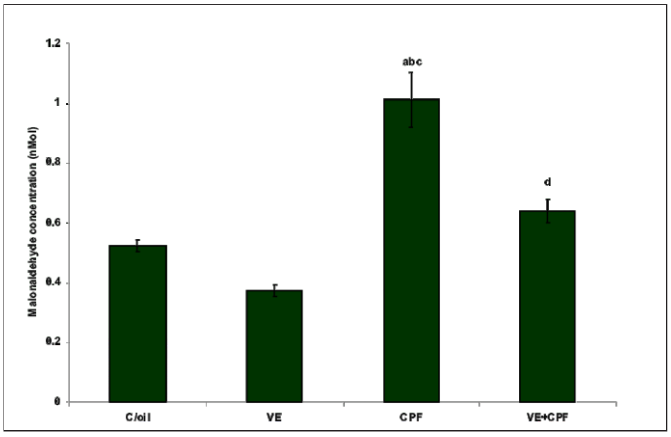
The ALP activity was significantly higher in the CPF group relative to the C/oil (P<0.05), VE (P<0.01) or VE+CPF (P<0.01) group. The C/oil group showed significantly higher (P<0.01) ALP activity when compared to the VE and VE+CPF group. There was no significant change (P>0.05) in the ALP activity in the VE+CPF group compared to VE group (Figure 11).

Figure 11: The effect of corn oil (C/oil), chlorpyrifos (CPF) and/or vitamin E (VE) on liver enzymes in mice. abcP < 0.01 versus the C/oil, VE and VE+CPF groups, respectively; dP < 0.05 vs C/oil group;efP<0.01 vs VE and VE+CPF, respectively; gP<0.01 vs VE group.



The serum MDA concentration in the CPF group was significantly higher (P < 0.01) than those obtained in the C/oil, VE or VE+CPF group. The serum MDA concentration in the VE+CPF group was significantly higher (P<0.01) compared to VE group, but there was no significant change (P>0.05) when compared to C/oil group (Figure 12).

Figure 12: The effect of corn oil (C/oil), chlorpyrifos (CPF) and/or vitamin E (VE) on serum malonaldehyde concentration in mice. abcP < 0.01 versus the C/oil, VE and VE+CPF groups, respectively; dP<0.01 versus VE group.



4. Discussion

The toxic signs observed in mice exposed to CPF only were due to stimulation of cholinergic receptors by the OP, apparently due to AChE inhibition [25] The toxic signs expressed by mice in the VE+CPF group were less severe compared to the CPF group, although there were mortalities in the two groups. The relatively mild toxic signs shown by the VE+CPF group demonstrated that oxidative stress may be playing some roles in toxic manifestations observed in OP poisoning. This finding agreed with previous works, which showed that some of the toxic manifestations by OPs may be associated with production of reactive oxygen species (5-10, 26, 27]. Besides, it has been shown that AChE inhibiting action of OPs is better compensated by vitamin E [28]. In addition, antioxidant vitamin E has been shown to enhance the restoration of cholinesterase activity [29]. All these properties may have contributed to the ability of vitamin E in mitigating CPF-evoked toxicity.

The present study also revealed that prolonged CPF administration caused a less appreciable increase in weight gain. This finding agreed with what had been reported in earlier studies [6, 8, 30, 31]. In fact, some other studies have shown that repeated CPF administration result in outright weight loss [32, 33]. Pretreatment with vitamin E has been shown by the present study to cause a marked (27%) increase in weight gain of mice compared to the mere 3% increase recorded in the CPF group. This shows that oxidative stress may be involved in the CPF-induced body weight suppression. Besides, chlorpyrifos-oxon, the toxic metabolite of CPF has been shown to inhibit cholesteryl ester hydrolase essential in promoting normal reaction to stress [34]. Earlier studies have shown that repeated CPF exposure causes vacuolization of the zona fasciculata [30, 35], the region of the adrenal cortex responsible for the elaboration of cortisol and corticosterone, essential for both immediate and long-term responses to stress. Therefore, it can be inferred that the adverse effect of CPF on body weight gain may be due to a combination of oxidative, toxic and cholinergic stress.

The increase in PCV, and concentrations of RBC and Hb in the CPF group may be due to diarrhea resulting in hemoconcentration. The fact that these hematological parameters in the VE+CPF group were not significantly different from those obtained in both the C/oil and VE groups shows that pretreatment with the antioxidant ameliorated the hematological changes induced by CPF Repeated exposure of mice to CPF resulted in a significant reduction in the WBC, attributable to neutropenia. This agreed with what was obtained in a previous study [6]. However, other studies attribute leucopenia following CPF exposure to lymphopenia [8, 31, 36]. Activated neutrophils have been demonstrated to play an essential role in free-radical mediated injury by inducing extracellular release of superoxide and other free radicals [37],which are toxic to the host cells including neutrophils itself, thereby resulting in their decrease [6]. However, pretreatment with vitamin E caused a significant elevation in WBC count compared to any of the other groups. The leukocytosis recorded in the vitamin E pretreatment group was due to lymphocytosis. The reason for the leukocytosis is not known and deserves further investigation. However, we speculate that vitamin

E, being the most important lipophilic antioxidant in cells, plays a vital role in the maintenance of cellular membrane integrity [38], especially in the face of oxidative assault by CPF [8-10]. This may have contributed to the improvement in leukocyte count by stabilizing its membrane from the effect of free radical mediated cytotoxicity. Besides, antioxidants have been shown to inhibit free-radical induced apoptosis [38, 39].

The increase in TP concentration in the CPF group may be due to the diarrhea, hence hemoconcentration. Similar result was obtained in our previous study [6]. This however contradicted the hypoproteinemia that had been reported by many workers following low dose CPF exposure [31, 40]. The difference may have arisen due to relatively higher dose in the present study, which causes diarrhea. Pretreatment with vitamin E, however, apparently normalized the TP concentration.

The significant decrease in Na+ concentration in the CPF group relative to the VE+CPF group may also be associated with diarrhea and consequent metabolic acidosis. The fact that vitamin E pretreatment apparently improved the Na+ showed the ability of the vitamin to restore the relative hyponatremia induced by CPF. The result also showed that repeated CPF exposure did not significantly alter the serum concentrations of K+ and Cl- and that generally, vitamin E pretreatment did not significantly affect the concentration of these electrolytes. Similarly, prolonged CPF exposure, and even pretreatment with vitamin E did not significantly alter the urea level. The marked elevation in the level of creatinine observed in the CPF group had been previously reported [6, 31], and probably result from pathological changes in the renal tissue (31,41]. Glomerular and renal tubular degenerative changes have been observed following CPF exposure [6, 31, 42]. However, vitamin E pretreatment markedly reduced the renal damage as evidenced by the lowered creatinine level. The normalization of creatinine level underscores the role of oxidative stress as a molecular mechanism of CPF-induced toxicity.

The significant decrease in the activities of ALT and AFST in the CPF group was consistent with findings from previous studies [6, 33]. This observation, however, contravened the increase in ALT and AST activities reported from other studies [31, 43]. The reason for the lowered AST and ALT activities recorded in CPF group in the present study is not known. Besides, the toxicological significance of lowered ALT and AST is not known. Although, there was still a significant decrease in the activities of AST and ALT in the group pretreated with vitamin E relative to the C/oil control. There was a relative increase in the activities of these two enzymes in the vitamin E pretreatment group. This showed that pretreatment vitamin E apparently normalized the ALT and AST activities. Furthermore, the significant elevation in the ALP level in the CPF group reflects the degree of damage to organs, such as the liver, muscle and intestine producing this enzyme. Either or all the organs producing ALP may have experienced various degrees of pathological changes as a result of CPF exposure. Similar observations have been reported in previous study following repeated CPF exposure [6, 31, 43]. CPF-induced hepatotoxicity has been reported by many workers [31,43] However, pretreatment with vitamin E mitigated the CPF-induced hepatotoxicity as demonstrated by reduction in the ALP level. Vitamin E, as an intracellular antioxidant may have protected hepatic membranes against CPF-induced free radical cytotoxicity. The hepatoprotective effect of vitamin E had been demonstrated in carbon tetrachloride-induced hepatotoxicity [44].

The high serum MDA concentration in the CPF group demonstrated the role of lipid peroxidation in the CPF-induced alteration in clinical, hematological and biochemical parameters obtained in the present study. This finding agreed with those obtained in previous studies[7-10, 45, 46]. MDA has been recognized as reactive products of membrane lipid peroxidation that plays a vital role in the pathogenesis of several diseases and inflammatory processes [47]. The low serum MDA in vitamin E pretreated mice reinforced the fact that the tissue protective effect of this antioxidant vitamin is due to suppression of lipoperoxidative damage. However, other non antioxidant activity of this vitamin may have contributed to the mitigation of CPF-induced cytotoxicity in the present study. For instance, vitamin E increases the activity of paraoxonase (PON) [48], a high-density lipoprotein-bound A esterase responsible for the metabolism of OP compounds. PON has been shown to have a very high activity towards chlorpyrifos-oxon [49], a more active metabolite of CPF. Similarly, the ability of this vitamin to enhance cholinesterase reactivation [9, 28, 29] may have contributed to the mitigation of CPF-induced hematologic and biochemical changes observed in the present study.

4. Conclusions

The present study has demonstrated the mitigating effect of vitamin E on clinical, hematological and serum biochemical parameters, altered by repeated CPF administration in mice. This amelioration may be partly due to the antioxidant effect of vitamin E, in addition to its other non-antioxidant effects. Therefore, the data obtained from the study further proved the usefulness of antioxidant vitamin E as part of the drug arsenals that may be recommended for the protection of farmers and other high risk pesticide users against toxicity that may likely develop from repeated CPF exposure.

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