Hyperglycemia induced by subchronic co-administration of chlorpyrifos and lead in Wistar rats: Role of pancreatic lipoperoxidation and alleviating effect of vitamin C

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Abstract
Studies were conducted to evaluate the role of pancreatic lipoperoxidation on hyperglycemia induced by subchronic co-administration of chlorpyrifos (CPF) and lead (Pb) in Wistar rats and the ameliorative effect of vitamin C. Forty male Wistar rats divided into 4 groups of 5 animals in each group were used for this study. Rats in group were dosed with corn oil (2 ml/kg) while those in group II were dosed with vitamin C (100 mg/kg). Group III were co-administered CPF (4.25 mg/kg ~ 1/20th LD50) and Pb (250 mg/kg~1/20th LD50) while those in group IV were pretreated with vitamin C (100 mg/kg) and then co-administered with CPF (4.25 mg/kg) and Pb (250 mg/kg) 30 min later. The regimen were administered once daily by gavage for a period of 9 weeks. The rats were sacrificed and serum obtained from the blood samples were analyzed for glucose concentration. The liver and pancreas samples were analyzed for glycogen and malonaldehyde (MDA) concentrations, respectively. The study showed that co-administration of CPF and lead caused increased glucose and MDA concentrations, and a reduced glycogen concentration. Pretreatment with vitamin C restored the concentrations of glucose, glycogen and MDA to apparently normal level. In conclusion, pretreatment with vitamin C restored the hyperglycemia and reduced glycogen concentration induced by co-administration of CPF and Pb partly due to its antioxidant properties.

Keywords: Chlorpyrifos; lead; hyperglycemia; lipoperoxidation; amelioration; vitamin C.

Introduction
Man and animals are exposed to a mixture of chemical contaminants in the environment, which directly or indirectly affect their health and well-being. The environment is pervasive with multiple chemicals that directly or indirectly interact with each other and the ecosystem. Few studies have evaluated the effect of multiple chemical contaminants on human and animal health as efforts have centered on evaluating the effect of a single contaminant. Pesticides and heavy metals constitute the most widespread environmental contaminants due to their ubiquitous use in all aspects of human endeavor.

Organophosphate (OP) insecticides are one of the most widely used insecticides accounting for 50% of the global insectical use (Casida and Quistad, 2004). The compelling needs to improve human and animal nutrition and promote public health has led to an increase in OP usage in recent time as they are used extensively to control agricultural, household and structural pests (Pope, 1999). Although, neurotoxicity is the hallmark of OP insecticide poisoning, other systemic toxicity have been observed following acute or repeated exposure.

Hyperglycemia is one of the side effects of OP poisoning in humans with blood glucose rising by about five folds (Namba et al., 1971; Hayes et al., 1978; Meller et al., 1981). Similarly, an epidemiological study has found a direct relationship between consistent exposure to OP insecticides (including CPF) and incidence of diabetes among the pesticide applicators (Montgomery et al., 2008). Hyperglycemia has been observed following OP exposure in animal models (Seifert, 2001; Ambali, 2009).

Chlorpyrifos (CPF) is one of the most widely used OP insecticides in agriculture and public health. Due to their wide availability, poisoning by CPF is common (Garcia et al., 2003) as residual amounts have been detected in the soil, water bodies, vegetables, grains and other food products (Poet et al., 2004). Similarly, lead is one of the most pervasive heavy metal contaminants in the environment (Krishna and Ramachandran, 2009). Exposure to lead has been known to adversely affect human and animal health in urbanized communities (Wang et al., 2006). Hyperglycemia is one of the signs associated with lead poisoning (Stevenson et al., 1976; Shaffi, 1979).


Although many mechanisms are involved in both CPF and Pb poisoning, the induction of oxidative stress is central to the two contaminants (Ercal et al., 1996; Gultekin et al., 2001; Olaleye et al., 2007; Ambali et al., 2010a-d). The animal body has evolved an effective antioxidant system to combat the menace posed by oxidative stress. Vitamin C is one of the most important free radical scavengers in extracellular fluid, trapping radicals in the aqueous phase, and protecting biomembranes from peroxidative damage (Yavuz et al., 2004). Vitamin C has shown promise in alleviating toxicity induced by CPF (Ambali et al., 2007; 2010c,d) and lead (Houston and Johnson, 2000; Oladipo, 2010). It is therefore conceivable that pretreatment with vitamin C will reduce lipoperoxidative changes induced by co-administration of CPF and Pb. The aim of the present study is therefore to evaluate the alleviating effect of vitamin C on hyperglycemia and hepatic glycogen depletion induced by subchronic co-administration of CPF and Pb in Wistar rats.

Materials and Methods

Experimental animals
Forty 6-week old adult male Wistar rats were obtained from the Animal House of the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria, Nigeria. The rats were fed on standard rat pellets and water provided ad libitum. The experimental procedures were conducted in accordance with the guideline on the use of laboratory animals (NRC, 1996).

Chemicals
Commercial grade CPF (Termicot® 20% EC, Sabero Organics, Gujarat, India) was dissolved in soya oil to make 10% stock solution, which was subsequently used for the experiment. Analytical grade lead acetate (Kiran Light Laboratories, Mumbai, India) used for the study was made into a 20% stock solution in distilled water. Commercial grade vitamin C tablets (Emzor Pharmaceutical Ltd, Nigeria, BN: 618N) was prepared in distilled water to make 10% stock solution.

Animal treatments
Forty weaned male Wistar rats were divided into four groups of 10 animals per group. The rats in group I were administered corn oil (2ml/kg), while those in group II were administered Vitamin C (100mg/kg). Rats in group III were co-administered CPF [4.25mg/kg,~1/20th LD50 (Ambali, 2009)] and lead acetate [225mg/kg,~1/20th LD50 (Oladipo, 2010)], respectively. Rats in group IV were pretreated with vitamin C, and then co-administered with CPF (4.25mg/kg) and Pb (225mg/kg), 30 min later. These regimens were administered orally by gavage once a day for a period of 9 weeks. At the end of the study period, the rats were sacrificed by jugular venesection after light chloroform anesthesia. Serum obtained from each blood sample was used to evaluate the concentration of glucose while the liver was assayed for glycogen concentration. The pancreas was assayed for the concentration of malonaldehyde (MDA) as an index of lipoperoxidation.

Evaluation of serum glucose concentration
The serum glucose concentration was determined using the glucose oxidase method. The principle of the method is based on the ability of glucose oxidase to catalyse the oxidation of β-D-glucose to D-glucono-δ-lactone with the concurrent release of hydrogen peroxide (H2O2). In the presence of peroxidase (POD) this H2O2 enters into a second reaction involving p-hydroxybenzoic acid and 4-aminoantipyrine with the quantitative formation of a quinoneimine dye complex which is measured at 510 nm.

Evaluation of hepatic glycogen concentration
The hepatic glycogen concentration was evaluated using the gravitational method of Good et al. (1933). Briefly, 0.5g of liver was extracted with 3mls of 30% KOH, incubated for 30 min. at 100°C, and then brought to acidic pH by addition of 20% trichloroacetic acid. Precipitated protein was removed by centrifugation for 10 min at 3000xg. Glycogen was precipitated by ethanol and weighed. The results were expressed in g of glycogen/100g of liver sample.

Evaluation of pancreatic lipoperoxidation
The MDA concentration of the pancreas was assayed using the double heating method of Draper and Hadley (1990). 0.3g of pancreas was homogenized in 30 ml cold phosphate buffered saline to obtain and centrifuged at 3000xg for 10 min. The supernatant from each homogenate was divided into two parts, for MDA and protein concentrations, respectively. The protein concentration was determined using the
method described by Lowry et al. (1951). For the determination of MDA concentrations, 0.25ml of supernatant was mixed with 0.5ml of 10% trichloroacetic acid, and then heated in a boiling water bath for 15 min. After cooling under running tap water for 5 min, the mixture was centrifuged at 1600g for 10 min, 1ml of the supernatant was then added to 0.5ml of 6.7g/L TBA solution in a test tube and placed in a boiling water bath for 15 min. The solution was then cooled under running tap water and the absorbance was then measured at 532nm using a UV spectrophotometer (T80*UV/VIS Spectrometer® PG Instruments Ltd, UK). The MDA concentration was calculated by the absorbance co-efficient, MDA-TBA complex \(1.56 \times 10^5/\text{cm}\) and expressed in nmol/mg of protein.

Statistical analysis
Values were expressed as mean±SEM and subjected to one-way analysis of variance followed by Tukey's test. Value of \(P<0.05\) was considered significant.

Results

Effect of treatments on serum glucose concentration
The effect of treatments on serum glucose is shown in Figure 1. There was no significant difference (\(P>0.05\)) between the groups. However, a comparative increase in the concentration of serum glucose was recorded in CPF+ Pb group.

![Figure 1: The effect of corn oil (C/oil), vitamin C (VC) and/or chlorpyrifos (CPF) and lead on serum glucose concentration.](image)

Effect of treatments on hepatic glycogen concentration
The effect of treatments on hepatic glycogen concentration is shown in Figure 2. A significant (\(P<0.05\)) decrease in the hepatic glycogen concentration was recorded in the CPF + Pb group compared to the other group.
Effect of treatments on pancreatic malondialdehyde concentration

The effect of treatments on pancreatic malondialdehyde concentration is shown in Figure 3. There was a significant increase (P<0.01) in the concentration of pancreatic MDA in the CPF+Pb group compared to the other groups. Similarly, there was a significant increase (P<0.05) in the MDA concentration in the VC+CPF+Pb group compared to the VC group.
Discussion
A high glucose concentration was recorded in the group co-administered with CPF and Pb. Hyperglycemia has been recorded following CPF (Ambali, 2009) and lead poisoning (Krishna and Ramachandran, 2009). The hyperglycemic response recorded in the group co-administered with CPF and Pb in the present study may be due to increased pancreatic lipoperoxidation, which ultimately affect the elaboration of insulin that is critical in the regulation of glucose concentration in the blood. Similarly, OP compounds have been shown to increase glycogenolysis by inhibiting insulin activity and stimulating glucagon activity (Rahimi and Abdollahi, 2007). In addition, the elevated serum glucose concentration increases the peroxide levels in the islets, which normally have poor antioxidant enzymes mRNA contents and activity levels (Vousough-Ghanbari et al., 2007). This glucotoxic condition causes a decrease in the levels of two critical regulatory proteins, PDX-1 and MafA (a family of Maf family of transcription factors) that normally bind to the insulin promoter and stimulate insulin gene transcription (Vousough-Ghanbari et al., 2007). Decrease in the levels of these two proteins causes decrease in insulin promoter activity, insulin gene expression and insulin secretion (Harmon et al., 1999; Robertson et al., 2003). This may have contributed to the apparent increase in glucose concentration in CPF+Pb group.

Similarly, the induction of toxic stress may have played some role in the hyperglycemia recorded in the group co-administered CPF and Pb. Stress generally activates the hypothalamo-pituitary-adrenal (HPA) axis and the sympathetic nervous system resulting in hyperglycemia (Bateman et al., 1989; Blalock, 2002; Mechanick, 2006). The activation of HPA axis results in increased elaboration of glucocorticoid from the adrenal cortex, which eventually results in increased glucose concentration through increased gluconeogenesis (Rahimi and Abdollahi, 2007) and impairment of glucose uptake in skeletal muscle (Oda et al., 1995). Similarly, the stimulation of sympathetic nervous system during stress leads to enhanced release of catecholamines, glucagon, and growth hormone which result in promotion of gluconeogenesis, glycogenolysis, insulin resistance, and constitution of hyperglycemia (Gustavson et al., 2003; Gearhart et al., 2006). It has been shown that OP compounds induces insulin resistance by inhibiting glucose transport in skeletal muscle via impinging on the component of insulin signaling pathway (Chiasson et al., 1981; Hunt and Ivy, 2002). The oxidative stress induced by both CPF and Pb in the body may have been exacerbated by the hyperglycemia through the formation of of advanced glycation end products (Rahimi et al., 2005; Gillery, 2006). Therefore, hyperglycemia is considered as a mechanism for development of oxidative stress in OP poisoning (Rahimi and Abdollahi, 2007).

Furthermore, increased muscular activity resulting from increased cholinergic activity (Shi and Screming, 1992; Garg et al., 2004) and pancreatitis due cholinergic stimulations (Harputluoglu et al., 2003; Markrides et al., 2005) have been shown to escalate OP-induced hyperglycemia. Pancreatic β-cells contain muscarinic ACh receptors, which are involved in the glucose-dependent production of insulin (Duttaroy et al., 2004). The cholinergic system plays an essential role in insulin release (Balkan and Dunning, 1995; Ahren et al., 1999; D’Alessio et al., 2001). CPF and lead are known inhibitor of AChE activity (Ambali et al., 2010; Ademuyiwa et al., 2007), the enzyme responsible for the degradation of acetylcholine (ACh). Thus, exposure to the CPF and Pb may have resulted in increased accumulation of ACh, potentially leading to overstimulation and eventual downregulation of its receptors (van Koppen and Kaiser, 2003) and consequent reduction of insulin production (Montgomery et al., 2008). Furthermore, prolonged stimulation by ACh may reduce β-cell sensitivity to glucose (Gilon and Henquin, 2001). AChE inhibition has been shown to be partly responsible for OP-induced hyperglycemia (Pourkahliili et al., 2009; Joshi and Rashini, 2010). Lead has equally been shown to increase glucose synthesis as well as suppressed pancreatic function (Stevenson et al., 1976). All these factors either individually or collectively may have contributed to increased glucose concentration in group co-administered CPF and Pb.

Pretreatment with vitamin C restored the serum glucose concentration to apparently normal level, indicating the role of oxidative stress in the hyperglycemic response observed in rats exposed to a combination of CPF and Pb. This may be due to the ability of the antioxidant vitamin to prevent oxidative damage to the pancreatic islets. A previous study has shown that α-tocopherol, a lipid soluble antioxidant vitamin restored diazinon-induced insulin
secretion (Pourkalili et al., 2009). Apart from lipoperoxidation, vitamin C has been shown to restore AChE activity inhibited by OPs (Yavuz et al., 2004; Ambali et al., 2010d). This may have apparently restored the level of ACh, a potent secretagogue of both insulin and glucagon (Duttaroy et al., 2004). This may have aided pancreatic β cell activity, increasing the elaboration of insulin and therefore suppressing the hyperglycemia. Some other non-antioxidant activity of vitamin C may have complemented the restoration of normal serum glucose level following alteration by CPF and Pb.

The present study has also revealed a decrease in hepatic glycogen concentration in the group co-administered CPF and Pb. This may be due to pancreatic damage as a result of lipoperoxidative changes, which causes an increase in blood glucose concentration and a decrease in intracellular glucose that can be used to synthesize glycogen. Furthermore, studies have shown that CPF causes impairment in hepatic function due to oxidative changes (Goel et al., 2006; Ambali et al., 2007; Ambali, 2009). This reduces the ability of the liver to synthesize glycogen. OP compounds also cause inhibition of AChE activity at the neuroeffector sites in the adrenal medulla, resulting in increased adrenal secretion (Gupta, 1974) and consequent elevation in glycogenolytic processes in the liver and skeletal muscle (Gustavson et al., 2003). Similarly, OP compounds have been shown to increase lipolysis, resulting in the elevation of free fatty acids that is known to have an inhibitory effect on the insulin signaling and inhibit glycogen synthesis (Itani et al., 2002). In addition, oxidative stress has been shown to induce an impairment of insulin action in glucose transport and glycogen synthesis. The decrease in insulin-stimulated glycogen synthesis during oxidative stress has been linked to the impairment of insulin to stimulate the activity of glycogen synthase (Dokken et al., 2008). The consequences of these are hyperglycemia and reduced glycogen synthesis.

Pretreatment with vitamin C restored the hepatic glycogen level to apparently normal level, indicating the role of oxidative stress in the depleted hepatic glycogen reserves observed in rats exposed to combination of CPF and Pb. The cytoprotection offered by vitamin C pretreatment on oxidative stress-induced hepatic and pancreatic damage may have played a very significant role in the apparent restoration of hepatic glycogen reserves in the vitamin C pretreated group. In addition, the ability of vitamin C to restore AChE activity (Yavuz et al., 2004; Ambali et al., 2010d) may have reduced the activity of the adrenal medulla to elaborate adrenaline and consequently reduced hepatic glycogenolysis.

The increase in pancreatic MDA concentration in the group co-administered CPF and Pb was an indication of lipoperoxidation. Oxidative stress is being increasingly implicated in the pathogenesis of pancreatic inflammation (Leung and Chan, 2009). The lipoperoxidative damage to the pancreas may have compromised its structural and functional integrity. Diazinon, an OP insecticidal compound, had equally been shown to reduce glucose-stimulated insulin secretion through the induction of oxidative and toxic stress in the islet of Langerhans (Pourkalili et al., 2009). In addition to being a potential inhibitor of insulin gene transcription and suppressor of the promoter, reactive oxygen species (ROS) also causes a decrease in mRNA and protein, and impairs the homeodomain transcription factor pancreatic/duodenal homeobox-1 (PDX-1) activity (Matsuoka et al., 1997), which is an important insulin transcription factor. The result is a decreased insulin secretion and consequent hyperglycemia.

Pretreatment with vitamin C restored the pancreatic MDA concentration to apparently normal level, affirming the role of oxidative stress in the pancreatic lipoperoxidative response observed in rats exposed to combinations of CPF and Pb. Vitamin C prevented oxidative damage to the pancreatic islets, thereby aiding in the maintenance of its structural and functional status. Vitamin C, like many other nutritional antioxidants act as free radical scavengers by directly neutralizing them, reduce peroxide concentration and repair oxide membranes and by quenching iron to decrease ROS production. Furthermore, they are known to regulate a number of genes and signal regulatory pathways thereby preventing the incidence of cell death (Young and Woodside, 2001).

**Conclusion**

The present study has shown that vitamin C pretreatment restored the hyperglycemia and decreased liver glycogen reserve induced by subchronic co-administration of CPF and Pb. This may be partly due to antioxidant property of the vitamin, which prevented oxidative damage to the pancreas. This study therefore
underscores the role of oxidative stress in the etiopathogenesis of hyperglycemia following exposure to combination of CPF and Pb.

References


Ameliorative effect of antioxidant vitamins C and E on neurotoxicological, haematological and biochemical changes induced by chronic chlorpyrifos exposure in Wistar rats. PhD Dissertation, Ahmadu Bello University, Zaria, Nigeria.


Ambali SF, Ayo JO, Ojo SA, Esievo KAN, 2010c. Ameliorative effect of vitamin C on chlorpyrifos-induced increased erythrocyte fragility in Wistar rats. Human and Experimental Toxicology. Epub ahead of print


Ercal N, Treratphan B, Hammond TC, Mathews RH, Grannemann NH, Spitz DR. 1996. In vivo indices of oxidative stress in lead exposed C57BL/6 mice are reduced by treatment with mesa-2, 3-dimercaptosuccinic acid or N-acetyl cysteine. Free Radical Biology and Medicine, 21: 157-161.


Acute and chronic manifestations. American Journal of Medicine, 50(4): 475-492.


Oladele OO, 2010. Ameliorative effects of ascorbic acid on neurobehavioural, haematological and biochemical changes induced by subchronic lead exposure in Wistar rats. MSc thesis, Ahmadu Bello University, Zaria, Nigeria.


