eISSN: 09748369, www.biolmedonline.com

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

[1]Ambali SF*, [1]Shuaib K, [1]Edeh R, [1]Orieji BC, [1]Shittu M, [2]Akande M
[1]Toxicology Unit, Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria, Nigeria.
[2]Department of Veterinary Pharmacology and Toxicology, University of Abuja, Nigeria.

***Corresponding Author:** fambali2001@yahoo.com, atunluse@gmail.com

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 LD_{50}) and Pb (250 mg/kg~1/20th LD_{50}) 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 insecticidal 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 LD_{50} (Ambali, 2009)] and lead acetate [225mg/kg,~1/20th LD_{50} (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 venisection 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-σ-lactone with the concurrent release of hydrogen peroxide (H_2O_2). In the presence of peroxidase (POD) this H_2O_2 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^{o}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 usingthe

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 1600xg 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 1.56x105/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.

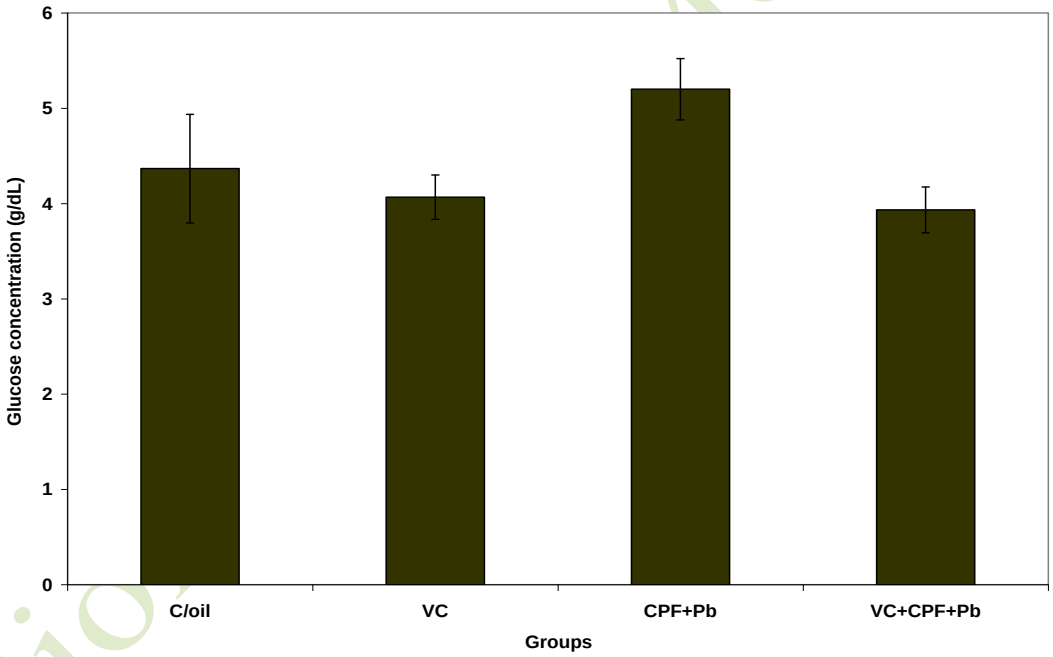

Figure 1: The effect of corn oil (C/oil), vitamin C (VC) and/or chlorpyrifos (CPF) and lead on serum glucose concentration.

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.

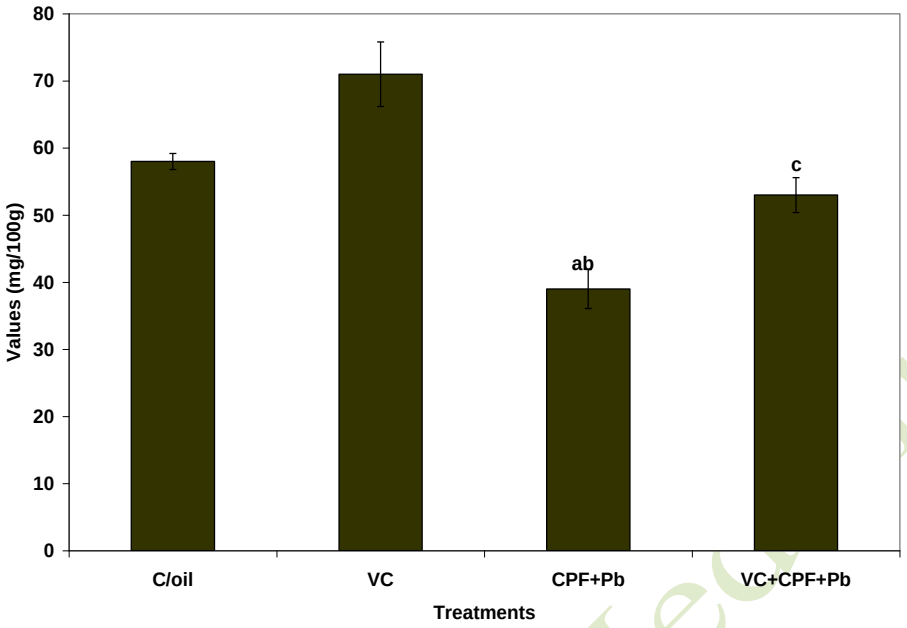

Figure 2: Effect of corn oil (C/oil), vitamin C (VC) and/or chlorpyrifos (CPF) + lead (Pb) on hepatic glycogen concentration. [a]P<0.01 vs VC group; [b]P<0.05 vs corn oil; [c]P<0.05 vs VC group.

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.

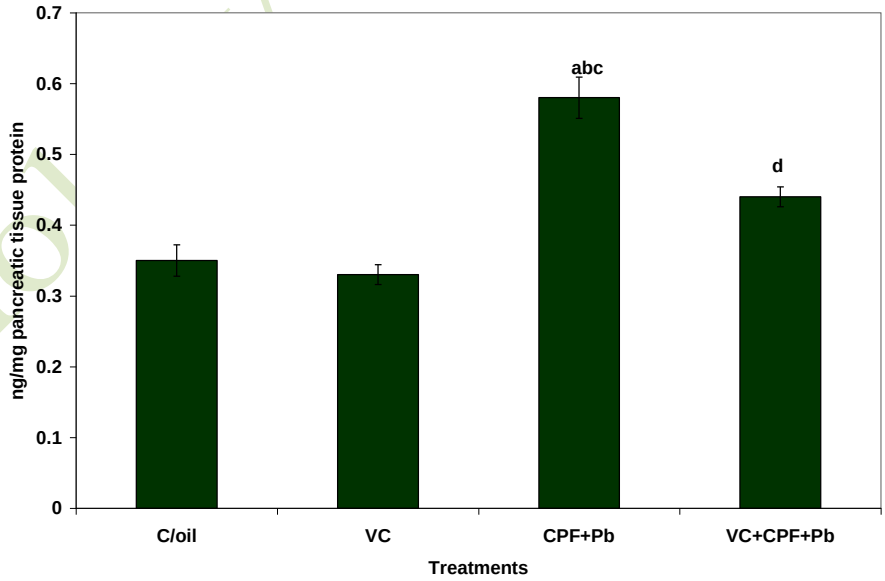

Figure 3: Effect of corn oil (C/oil), vitamin C (VC) and/or chlorpyrifos (CPF) + lead (Pb) on pancreatic malonaldehyde concentration. [ab]P<0.01 vs corn oil and VC groups, respectively; [c]P<0.05 vs VC+CPF+Pb group; [d]P<0.05 vs VC group.

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 (Pourkahlili *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 causes inhibition of AChE activity at the neuroeffector sites in the adrenal medulla, resulting in increased adrenalin 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 adrenalin 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 (Pourkhalili *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.

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