The effects of prolonged oral administration of the disinfectant calcium hypochlorite in Nigerian commercial cockerels

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This study was designed to investigate the effects of prolonged oral administration of calcium hypochlorite in the drinking water of commercial cockerels. It was carried out in order to ascertain probable toxicity associated with prolonged exposure to calcium hypochlorite. Thirty-two healthy birds were used; they were grouped into four groups of eight. Group 1, which served as the control, received 10 mL/kg body weight of physiological saline. Groups 2, 3 and 4 received 0.0375 g, 0.375 g and 0.75 g of calcium hypochlorite per 10 litres of drinking water for six weeks respectively. Six weeks after the administration of calcium hypochlorite, blood was collected from the jugular vein to assess liver function, lipid profiles and for markers of oxidative stress. The results revealed a significant ($p < 0.05$) increase in alanine aminotransferase activity in a dose-dependent manner when compared with the control. Also, there was a significant ($p < 0.05$) increase in aspartate aminotransferase and alkaline phosphatase activity. Similarly, there was a significant ($p < 0.05$) increase in total cholesterol, triglycerides, high-density lipoprotein and low-density lipoprotein levels compared with the control. There was a significant increase in malondialdehyde and hydrogen peroxide generation with a concomitant significant ($p < 0.05$) decrease in serum glutathione level in a dose-dependent manner when compared with the control. In this study, calcium hypochlorite-induced hepatic damage via oxidative stress and decrease in antioxidant defense system was found. Therefore, prolonged exposure of chickens to calcium hypochlorite is potentially harmful.

Introduction

This study is aimed at investigating tissue damage induced by prolonged exposure to calcium hypochlorite in the drinking water of commercial chickens and its mechanism of action via increased generation of hydrogen peroxide and malondialdehyde (MDA).

In an attempt to provide potable and germ-free water for domestic animals and household use in developing countries such as Nigeria, the use of calcium hypochlorite ($Ca[ClO]_2$) as water disinfectant is on the increase. This is administered indiscriminately without any recourse as to the probable direct toxicological effects on the livestock and humans, or on the indirect effects on the final consumers of animal products. Meanwhile, $Ca(ClO)_2$ has been reported to cause oxidative stress despite its desirable role in water disinfection (Semenza *et al.* 1998).

Calcium hypochlorite is a solid, white powder, tablet, briquette or crystalline with a distinct chlorine odour. Hypochlorite contains a high concentration of available chlorine, which is a proven germicide capable of controlling pathogenic microorganisms such as the bacteria, fungi and algae commonly found in water sources. It destroys a variety of organisms including bacteria, yeast, fungus, spores, and viruses (Nassar *et al.* 1997; Lang, Ingham & Ingham 2000; Lombardi *et al.* 2008).

The mechanism of hypochlorite-induced lipid peroxidation has been proposed through myeloperoxidase catalysis initiation of lipid peroxidation (LPO) in phospholipid membranes and human blood lipoproteins. This reaction is accompanied by the production of free radicals (but not singlet oxygen), probably alkoxyl radicals, which may play a role in the initiation of hypochlorite-induced LPO (Panasenko 1997; Spickett *et al.* 2000).

Activated phagocytes generate the potent oxidant hypochlorite (OCl) via the release of the enzyme myeloperoxidase and hydrogen peroxide (H_20_2). Hypochlorite has been reported to react with and damage a number of biological targets including proteins, DNA, lipids and cholesterol. Proteins are likely to be major targets for reaction with hypochlorite due to their abundance and high reactivity with it within a cell (Harrison 1947; Pattison, Hawkins & Davies 2007).

A 100% increase in alanine aminotransferase (ALT) activity in broilers fed with aflatoxin B1 has been reported (Davi & Ademoyero 1984). Similarly, a sharp rise in the activities of serum transaminases (aspartate aminotransferase (AST) and ALT), albumin, lactate dehydrogenase (LDH) and bilirubin in the birds that have been given a single dose of aflatoxin is indicative of an acute liver injury and kidney damage (Friedwald, Levy & Fredickson 1997). Studies have suggested an association between chlorination disinfection by-products and congenital anomalies, induction of oxidative stress, apoptosis and mutagenicity (Nieuwenhuijsen *et al.* 2009; Schenck *et al.* 2009). However, other studies have failed to establish an association between cancers or miscarriage and exposure to chlorinated drinking water (Sharma & Goel 2007; Goel 2008).

Materials and methods

Chemicals

Thiobarbituric acid and 1-chloro-2, 4-dinitrobenzene (CDNB) and Xylenol orange were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of analytical grade and were obtained from the British Drug Houses (Poole, Dorset, UK).

Animal treatment

Thirty-two cockerels were used in this study. The chickens were purchased at eight weeks of age and acclimatised to their surroundings for two weeks before the start on the experiment. The animals were kept in wire mesh cages under a controlled light cycle (12 h of light or 12 h of dark) and fed a commercial grower feed *ad libitum.* They were grouped into four groups of eight birds. Birds in Group 1 served as a control and were administered 10 mL/kg body weight of normal saline. The birds in Group 2 received the recommended dose of 0.0375 g of calcium hypochlorite/25 litres of drinking water, whilst Group 3 and Group 4 were treated with 0.375 g and 0.75 g calcium hypochlorite per 25 litres of drinking water respectively. The experiment lasted for six weeks. All of the animals received humane care according to the criteria outline in the *Guide for the Care and the Use of Laboratory Animals* prepared by the National Academy of Science and published by the National Institute of Health. The ethics regulations were followed in accordance with national and institutional guidelines for the protection of the animals' welfare during experiments (Public Health Service [PHS] 1996).

Biochemical assays

Protein concentrations were determined by the method of Lowry *et al.* (1951). Activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to standard method (Reitmann & Frankel 1957). Alkaline phosphatase (ALP) activity was measured according to Rec GSCC (DGKC) (1972). Serum was analysed for total cholesterol (TC), triglycerides (TAG) and high-density lipoprotein cholesterol (HDL-C) determined by an enzymatic method coupled with spectrophotometry using an assay kit (Randox Lab. Ltd., Co. Antrim, UK). Low-density lipoprotein cholesterol was extrapolated from Friedewald's formula (Friedewald *et al.* 1972). The creatinine and blood urea nitrogen concentration were determined according to Harrison (1947). Serum sodium potassium and phosptate ions were determined by flame photometry. The concentration of potassium was calculated using the standard calibration method (Kolthoff & Elving 1976). Bicarbonate and chloride anions were measured as described previously (Schales & Schales 1971; Van Slyke & Aulle 1977).

Markers of oxidative stress and antioxidant defence system

Reduced serum glutathione (GSH) was determined at 412 nm using the method as described (Jollow *et al.* 1974). Hydrogen peroxide generation was determined as described (Woff 1994). The MDA level was calculated (Farombi *et al.* 2000). Lipid peroxidation in units/mg protein or gram of tissue was computed with a molar extinction coefficient of $1.56 \times 10^5\ M^{-1}Cm^{-1}$.

Statistical analysis

All values are expressed as mean ± standard deviation. The test of significance between two groups was estimated by the Student's t test according to Bailey (1992). One-way ANOVA with Dunnett's post test was also performed using GraphPad Prism version 4.00 (GraphPad Software 2003).

Results

The results show that there is a significant ($p < 0.05$) increase in the serum total protein of birds exposed to oral calcium hypochlorite when compared with the control group (Table 1). The results also show a significant ($p < 0.05$) increase in malondialdehyde (MDA) levels in birds treated with calcium hypochlorite when compared with the control birds. The levels of hydrogen peroxide (H_2O_2) generation increased significantly with the increasing dose of calcium hypochlorite in the exposed birds when compared with the control birds. The serum levels of the antioxidant-defence system (GSH) also show a significant ($p < 0.05$) decrease in exposed animals when compared with the non-exposed

TABLE 1: Effect of calcium hypochlorite on markers of oxidative stress and serum glutathione.

Groups (n = 8)	Total protein(mg/dL)	MDA (µmoL/mg protein)	H_2o_2 generation (µmoL/min/mg protein)	GSH (µg/mL)
Control	5.73 ± 0.42	56.05 ± 8.85	35.67 ± 2.67	56.67 ± 6.67
Group 2	6.05 ± 0.70	75.08 ± 3.33*	39.50 ± 0.50*	38.50 ± 1.92*
Group 3	12.10 ± 2.01*	81.80 ± 3.20*	47.50 ± 4.50*	39.33 ± 1.16*
Group 4	13.30 ± 2.97*	82.75 ± 10.55*	50.00 ± 6.00*	49.33 ± 1.33*

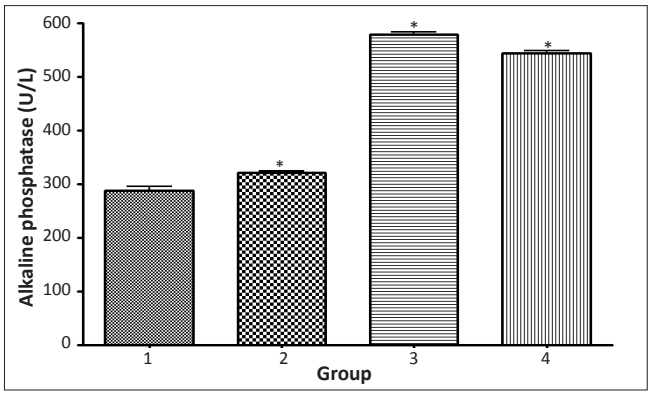
MDA, malondialdehyde; H_2o_2, Hydrogen peroxide; GSH, glutathione.
*, $p < 0.05$, compared with control.
Note: Values expressed as mean ± standard deviation where (n = 8).

birds (Table 1). The results of lipid profiles show a significant ($p < 0.05$) increase in serum total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol in birds treated with calcium hypochlorite compared with the control birds (Table 2). Serum blood urea nitrogen (BUN) shows a significant ($p < 0.05$) increase in a dose-dependent manner in birds that were administered with calcium hypochlorite (Table 3). The levels of serum bicarbonate (HCO3) also increased significantly ($p < 0.05$) in the group that received the highest dose of the disinfectant compared with the control group (Table 3). Serum bicarbonate (HCO3) levels also increased in Group 3 birds, but not significantly.

There was also a significant ($p < 0.05$) increase in alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity in the chickens exposed to calcium hypochlorite compared with the control group (Figure 1, Figure 2 and Figure 3).

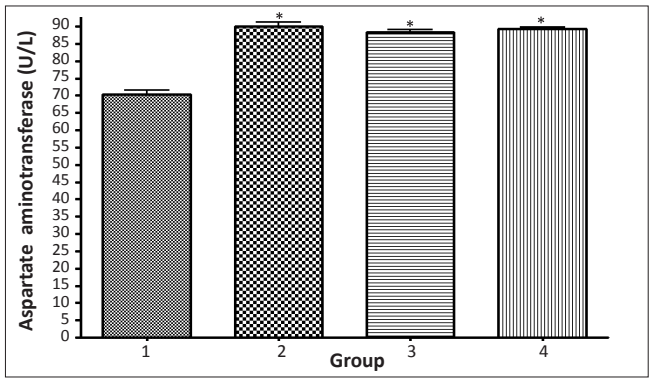
Discussion

The data showed that birds that were exposed to prolonged oral administration of calcium hypochlorite had a significant increase in serum alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase activity. Increases in serum AST and ALT levels are attributed to hepatic structural damage because these enzymes are normally localised within the cytoplasm and released into circulation after cellular damage has occurred (Kumar *et al.* 2004). It has been reported that elevated activity of AST and ALT in plasma are indicative of cellular leakage and loss of functional integrity of the cell membrane in the liver and that their estimations are useful quantitative markers of the extent of hepatocellular damage (Lombardi *et al.* 2008; Recknagel *et al.* 1989). The data therefore confirm hepatic damage associated with prolonged exposure of experimental animals to calcium hypochlorite. The increased serum total protein was found to be associated with chronic renal failure (CRF) accompanied by abnormal muscle protein degradation and synthesis, metabolic acidosis and uraemia (Johansen 2009; Muscaritoli *et al.* 2009; Tessari *et al.* 2010; Workeneh & Mitch 2010). This, as well as a significant increase in (BUN), was supported by this work. The increase in chloride and bicarbonate ions could indicate metabolic alkalosis. This could precipitate hyperventilation in order to remove the excess bicarbonate ions via CO_2 expiration. The prolonged



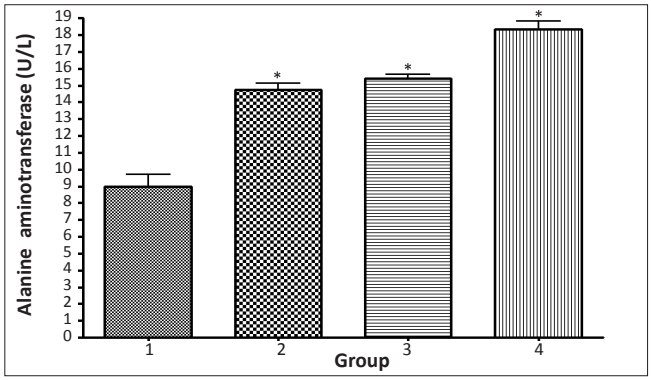
* $p < 0.05$, compared with control.
Note: Values expressed as mean ± standard deviation where ($n = 8$).

FIGURE 1: Effect of calcium hypochlorite on alkaline phosphatase activity.



* $p < 0.05$, compared with control.
Note: Values expressed as mean ± standard deviation where ($n = 8$).

FIGURE 2: Effect of calcium hypochlorite on aspartate aminotransferase activity.



* $p < 0.05$, compared with control.
Note: Values expressed as mean ± standard deviation where ($n = 8$).

FIGURE 3: Effect of calcium hypochlorite on alanine aminotransferase activity.

TABLE 2: Effect of calcium hypochlorite on serum lipid profiles.

Groups ($n = 8$)	TC (mg/dL)	TAG (mg/dL)	HDL-cholesterol (mg/dL)	LDL-cholesterol (mg/dL)
Group 1	76.00 ± 4.51	180.00 ± 19.70	15.67 ± 1.20	36.50 ± 7.32
Group 2	109.30 ± 5.54*	146.60 ± 9.45*	19.40 ± 1.50*	55.83 ± 2.92*
Group 3	113.30 ± 4.27*	218.80 ± 13.12*	20.17 ± 1.09*	58.83 ± 1.58*
Group 4	120.60 ± 6.70*	225.00 ± 13.69*	22.60 ± 2.06*	66.20 ± 4.85*

*, $p < 0.05$, compared with control.
Note: Values expressed as mean ± standard deviation where ($n = 8$).

TABLE 3: Effect of calcium hypochlorite on serum electrolytes and metabolites.

Groups ($n = 8$)	Na+ (mmol/L)	K+ (mmol/L)	CL- (mmol/L)	HCO3 (mmol/L)	Creatinine (mmol/L)	Urea (mmol/L)
Group 1	145.80 ± 1.49	4.35 ± 0.47	114.50 ± 2.33	18.75 ± 0.95	0.17 ± 0.02	5.67 ± 0.88
Group 2	141.70 ± 3.16	3.83 ± 0.21	108.70 ± 5.06*	19.33 ± 0.67	0.18 ± 0.03	9.25 ± 1.11*
Group 3	143.40 ± 2.05	5.24 ± 0.34	116.30 ± 2.06	19.25 ± 0.75	0.18 ± 0.03	10.00 ± 0.55*
Group 4	146.10 ± 1.50	5.63 ± 0.62	112.80 ± 0.75	19.75 ± 0.25*	0.18 ± 0.02	10.80 ± 1.02*

*, $p < 0.05$, compared with control.
Note: Values expressed as mean ± standard deviation where ($n = 8$).

use in animals with impaired renal function may further compound the metabolic alkalosis because of the inability of the kidney to eliminate chloride ions.

One of the by-products of lipid peroxidation is malondialdehyde (MDA), which has been used in various biochemical assays to monitor the extent of peroxidative damage. The increase in MDA levels in all the treated groups compared with the control group indicates the oxidative damage in the liver following exposure to the treatment and may be related to the elevated level of H_2O_2. The increased levels of H_2O_2 have been reported to mediate the toxic effect through the formation of hydroxyl radical, a potent activator of lipid peroxidation (Teo, Pohl & Hapert 1986; Chance, Sies & Boveris 1979). H_2O_2 and MDA levels were elevated in all treatment groups.

The biochemical basis of CHC toxicity might be associated with oxidative stress through the generation of free radicals and reactive oxygen species (ROS). Antioxidants play a major role by continuously inactivating ROS to a level necessary to maintain normal cell function (Al-Dabbas *et al.* 2006)**.** Oxidative damage occurs when the production of ROS overwhelms the antioxidant defense mechanisms (Spickett *et al.* 2000). The level of reduced glutathione (GSH) has been shown to be a measure of the cellular redox status (Al-Dabbas *et al.* 2006). Therefore, changes in glutathione concentration may affect the overall redox status of the cell (Kumar *et al.* 2004). In this study, the level of glutathione (GSH) was significantly reduced in the chickens exposed to calcium hypochlorite. The increase in the level of lipid peroxidation product (MDA) and H_2O_2 with a concomitant decrease in reduced glutathione suggests that calcium hypochlorite increases oxidative stress and depletes the antioxidant defense system, which probably contributed to the observed liver damage.

Previous studies have reported the association between total cholesterol, triglycerides, LDLs and the plasma concentration of HDLs to be inversely related to the risk of cardiovascular disease (Muscaritoli *et al.* 2009). In this study, serum triglycerides, total cholesterol, LDL-cholesterol and HDL-cholesterol exhibited a significantly high concentration. Hypercholesterolemia, as observed in this study, has been reported to be due to the presence of abnormally high levels of particles that are rich in free cholesterol and apolipoprotein B (apoB) associated with obstructive jaundice (Vaziri 2006). The impaired HDL metabolism has been reported to contribute to the disturbance of triglyceride-rich lipoprotein metabolism, the risk of arteriosclerotic cardiovascular disease and may adversely affect progression of renal disease and energy metabolism in chronic renal failure (Quaschning *et al.* 2001; Vaziri 2006). The mechanism underlying this aberration in lipid metabolism (CRF-induced hypertriglyceridaemia) has been proposed to be due to down-regulation of lipoprotein lipase, hepatic lipase and the very low-density lipoprotein receptor, as well as the up-regulation of hepatic acyl-CoA cholesterol acyltransferase (Vaziri 2009). Recently, it was reported that HDL deficiencies and/or dysfunction and the associated atherosclerosis and cardiovascular disease have been found to be related to end-stage renal disease (Vaziri 2009).

Conclusion

In conclusion, exposure of poultry to calcium hypochlorite disinfectant could induce liver and kidney damage as well as atherosclerosis via free radical generation. The prolonged administration of calcium hypochlorite as water disinfectant in the poultry industry could potentially reduce productivity and increase morbidity and economic loss. It is therefore recommended that in order to avoid toxicity that calcium hypochlorite should not be used for more than one week as a water disinfectant in the poultry industry.

Acknowledgement

Competing interests

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Authors' contributions

T.O.I. (Federal College of Animal Health and Production Technology, Moor Plantation); A.A.O. (University of Ibadan) and O.I.A. (University of Ibadan) contributed equally to the design and conduct of the study, as well as writing and proofreading of the manuscript.

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