

Haematology and Erythrocyte Osmotic Fragility Indices in Domestic Chicken Following Exposure to a Polyvalent Iodophorous Disinfectant

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Abstract

The effect of prolonged use of Iodosteryl, a polyvalent iodophorous compound, as water disinfectant, on the hematology and erythrocyte osmotic fragility of the domestic chicken was investigated. Twenty eight adult male domestic chickens of the Nera black strain were divided into four groups of seven birds per group. Birds in groups B-D were given potable water containing 1 ml, 2 ml and 4 ml/l Iodosteryl respectively for six weeks. Group A served as the control. Blood samples were collected from each bird after six weeks and analyzed immediately. No significant changes were observed in the packed cell volume (PCV), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet total and differential leucocytes values. However, red blood cells (RBC) were slightly lower while erythrocyte osmotic fragility and erythrocyte sedimentation rate (ESR) was higher in those birds exposed to Iodosteryl compared with control. This study confirms that prolonged use of Iodosteryl is stressful and may lead to intravascular haemolysis as indicated by the higher erythrocyte fragility and ESR values, respectively. The damage observed may be due to peroxidation of erythrocyte membrane lipids, proteins or a generation of free radicals induced by iodine.

Keywords : Chicken, hematology, iodophorous compound, iodosteryl, osmotic fragility.

1. Introduction

Water, an essential component of all organic tissues without which organism cannot survive, is the major component of the earth. It constitutes about 75% of the Earth's surface in the form of ocean, lakes, rivers and streams. Despite the abundance of these water bodies however, only 1% is available as fresh water for human and animal consumption. This is principally because; the water is either salty or unwholesome for consumption. This therefore calls for water treatment before it is made available for use. In fact, more than one billion people lack access to safe drinking water, while more than 200,000 children die of one form of waterborne disease or the other on a daily basis worldwide (World Health Organization, 2010).

The need for water disinfection for the production of wholesome and potable water has been recognized since the medieval period. In recent time however, several more sophisticated procedures are being used in water purification, these include physical methods such as irradiation, ultrasound, ultra filtration, reverse osmosis, heating, freezing, and the use of ionizing radiation (Mako *et al.*, 2007; Lukhele *et al.*, 2010; Fittipaldi *et al.*, 2010).

Other methods include coagulation-flocculation or precipitation prior to sedimentation or filtration, adsorption with activated charcoal, clay etc., and ion exchange processes (World Health Organization, 2010). The chemical methods are so called because of the abilities of the compounds to destroy or inhibit water borne pathogenic organisms that may be present in the impure or contaminated water (Thomas *et al.*, 2009). This however, is not with its attendant consequences especially with prolonged or excessive use of these compounds (Morgans and Trotter, 1953).

One of the commonly used chemical agents in water sanitation and disinfection is Iodine and its compounds have been found to be an effective water disinfectant for short term use. According to WHO report on Water Sanitation and Health (World Health Organization, 2010), Iodine, either dissolved in water or in the form of an iodinated exchange resin, has been used for short-term water treatment by outdoor recreationists (campers, hikers, etc), field military personnel, and persons displaced by natural disasters and human conflicts in wars and other societal disruptions (Rogers and Vitaliano, 1977). The use of Iodine in short term water disinfection for military personnel with proven efficacy against viruses, bacteria and protozoan cysts has also been well documented (Clarke and Bettin, 2006). However, several iodine and

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iodine resin or iodophores are in circulation for use in water disinfection in forms of tablets, resins and solutions (Backer, 2002; Backer and Hollowell, 2000; Butkus *et al.*, 2005; Clark *et al.*, 2003; Crittenden *et al.*, 2005).

The most widely used iodine based water disinfectant in Nigeria, especially; among livestock producers is Iodosteryl, a polyvalent iodophorous compound used in disease prevention in poultry (Mako *et al.*, 2007). According to the manufacturer's claim and field reports from farmers, the compound has been reported to possess bacteriocidal and virucidal properties. It is also known to inhibit protozoan cysts especially coccidial oocysts. However, most of these claims are yet to be documented. This notwithstanding, the use of Iodosteryl is on the increase in disease prevention in poultry production in Nigeria (Mako *et al.*, 2007).

The present study was designed to investigate the effects of prolonged use of Iodosteryl in water on the haematological parameters and erythrocyte osmotic fragility of the domestic chicken.

2. Materials and Methods

2.1. Animals

Adult male domestic chickens of the Nera black strain were used for the study. Twenty-eight, adult (16 weeks old), male Nera black chickens were purchased from a local farm in Ibadan. The birds were stabilized for two weeks, during which they were dewormed with Piperazine hydrochloride (Alfasan, Holland), while antibiotics, Doxygen[®], containing gentamycin and doxycycline (Adamore, Nigeria Limited) was administered via their drinking water for 5 days.

2.2. Experimental design

The birds were randomly divided into 4 groups (A-D) consisting of seven birds per group. Group A, which served as the control received clean tap water while groups B-D received tap water with 1 part, 2 parts and 4 parts per million of Iodosteryl, which correspond to low, medium and high doses, respectively, incorporated into their water for a period of six weeks. All the birds were given grower mash and water *ad libitum* throughout the period of the experiment. 1 ml per litre of Iodosteryl is the manufacturer's recommendation for water disinfectant.

Iodosteryl was purchased from Crosley Sinbad & Co. Limited (Nigeria). Blood samples (5 ml) were collected from each bird (at the end of six weeks of exposure to Iodosteryl) into heparinized bottles for determination of haematological parameters and erythrocytes osmotic fragility. Packed cell volume (PCV) was determined by the micro haematocrit method, haemoglobin (Hb) by cyanmethaemoglobin method while red blood cell (RBC), white blood cell and platelet counts were determined using haemocytometer with the improved Neubauer slide (Douglas and Harold, 2004). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were then calculated according to Jain (1986). Differential leucocyte count was also determined from blood smears stained with Giemsa. Erythrocyte sedimentation rate was

determined from blood diluted with 4 parts citrate solution (3.3% sodium citrate) allowed to stand in Westergren tube.

Erythrocyte osmotic fragility was determined according to the method described by Oyewale (1992). Briefly, 0.02 ml of blood was added to tubes containing increasing concentration of phosphate-buffered sodium chloride (NaCl) solution at pH 7.4 (0, 0.1, 0.3, 0.5, 0.7, and 0.9% NaCl concentration).

The tubes were gently mixed and incubated at room temperature (29°C) for 30 minutes. The content of each tube was then centrifuged at 3500rev/min for 10 minutes and the supernatant removed for measurement. Optical density of the supernatant was determined spectrophotometrically at 540 nm using SM22PC Spectrophotometer (Surgienfield Instruments, England). Haemolysis in each tube was expressed as a percentage, taking haemolysis in distilled water (0% NaCl) as 100%.

2.3. Animal ethics

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 Science and published by the National Institute of Health. The ethic regulations have been followed in accordance with national and institutional guidelines for the protection of animals' welfare during experiments (Public Health Service, 1996). The experiment was carried out at Biochemistry Laboratory, Department of Veterinary Physiology, Biochemistry and Pharmacology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria.

2.4. Statistical analysis

All values are expressed as mean \pm SD. The test of significance between two groups was estimated by Student's t-test. One-way ANOVA with Dunnett's post-test was also performed using GraphPad Prism version 4.00.

3. Results

The effect of prolonged administration of Iodosteryl as water disinfectant on the haematological parameters is shown in Table 1.

Table 1. Haematological parameters of adult, male Nera black chicken after exposure to water treated with various concentrations of Iodosteryl

Parameters	Group A (Control, n = 7)	Group B (1 ppm, n = 7)	Group C (2 ppm, n = 7)	Group D (4 ppm, n = 7)
PCV (%)	26.00 \pm 4.80	26.00 \pm 2.00	25.70 \pm 2.50	24.67 \pm 0.87
Hb (g/dl)	8.15 \pm 1.73	8.64 \pm 0.07	8.53 \pm 0.86	8.32 \pm 0.87
RBC ($\times 10^6/\mu\text{l}$)	3.78 \pm 0.44	3.49 \pm 0.38	2.63 \pm 0.44	2.97 \pm 0.10
MCV (fl)	60.25 \pm 19.16	74.00 \pm 8.41	99.14 \pm 13.01	83.17 \pm 7.91
MCH (pg)	19.75 \pm 5.56	24.71 \pm 3.25	32.71 \pm 4.54	27.50 \pm 2.66
MCHC (g/dl)	33.17 \pm 0.62	33.24 \pm 0.28	33.22 \pm 0.42	33.02 \pm 0.45
Platelet ($\times 10^3/\mu\text{l}$)	105.00 \pm 19.10	104.30 \pm 29.40	107.10 \pm 27.50	131.70 \pm 18.00
WBC ($\times 10^3/\mu\text{l}$)	4.65 \pm 1.59	3.63 \pm 0.84	3.91 \pm 0.94	4.78 \pm 0.52
Lymph ($\times 10^3/\mu\text{l}$)	2.94 \pm 0.99	2.00 \pm 0.46	2.82 \pm 0.68	3.07 \pm 0.33
Heter ($\times 10^3/\mu\text{l}$)	1.67 \pm 0.05	1.62 \pm 0.38	1.06 \pm 0.26	1.66 \pm 0.18
ESR	4.00 \pm 0.82	7.43 \pm 2.94	8.86 \pm 4.71*	8.71 \pm 2.04*

Data are presented as means \pm SD, n = (numbers of birds) and values within the same row with asterisks are significant

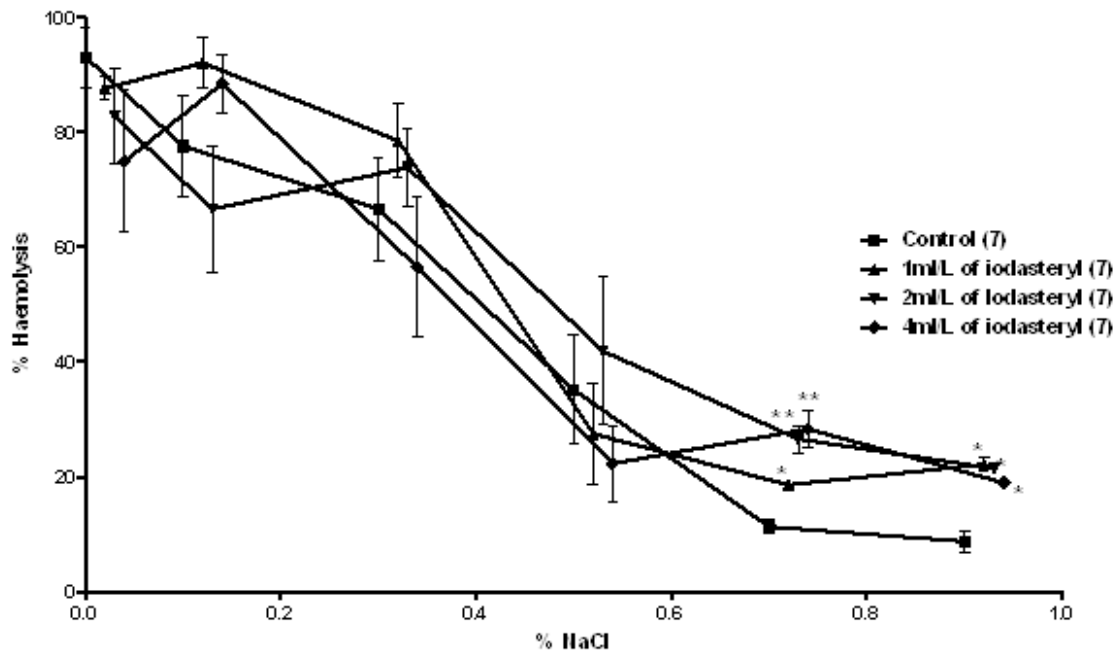
compared with the control group at $p \leq 0.05$ using analysis of variance (ANOVA) test

The PCV, Hb concentration, WBC and platelet counts as well as the erythrocyte indices of the test groups (groups B to D) that received various concentrations of Iodosteryl were not significantly different from those of the control. Similarly, the differential leucocytes count obtained in the birds treated with Iodosteryl in water were not significantly different from the control. However, the erythrocyte sedimentation rate of the birds exposed to 2ppm and 4ppm of Iodosteryl per litre of drinking water

were significantly higher ($p < 0.05$) than those of the control. The RBC counts in those birds that received 2 and 4ppm of iodosteryl was lower, though non-significantly than that of the control

As shown in Figure 1, the erythrocyte osmotic fragility of the domestic chicken given 1ppm Iodosteryl (Group B) was significantly higher than that of the control at 0.7% NaCl ($p < 0.05$) and 0.9% NaCl concentrations. Similarly, erythrocyte fragility of the chicken in Groups C and D was significantly higher than that of the control at 0.7% NaCl ($p < 0.01$) and 0.9% NaCl ($p < 0.05$).

Fig 1.0: Erythrocyte osmotic fragility of 24 weeks old Nera black cockerel after administration of Iodosteryl® as water sanitizer. Values are means \pm SD. Number of birds in parenthesis.



Asterisks indicate significant difference from the control. *= $P < 0.05$, **= $P < 0.01$

4. Discussion

Iodine and several iodine products have been in use for ages in disinfection of wounds because of the biocidal properties of the halogen and its compounds. This has been due to the oxidant property of halogens, precipitated by the ability of halogens to react with biological molecules, thereby leading to oxidative damage to the molecules. Iodine also forms hypiodous acid a potent oxidant when it reacts with water (Hollowell, 2000; Clarke and Bettin, 2006), a property that has been employed in the use of iodine products in water disinfection especially in the rural areas and third world countries or even during disaster and other emergencies (Hollowell, 2000).

The potency of iodine as water disinfectant notwithstanding, the problem of residue of halogens in drinking water and the effects on consumers have been an object of serious concern to health practitioners worldwide, especially in prolonged and excessive use of these water disinfectants. The evaluation of the effects of prolonged use of these water sanitizers or disinfectants is

especially important because, the effectiveness of iodine as a disinfectant is a function of the contact time with microorganism, water pH and the temperature (Clarke and Bettin, 2006). This tends to promote prolonged and continuous use of the disinfectant and the attendant complications on the consumers.

From the result obtained in this study, prolonged use of Iodosteryl appeared quite safe because the PCV, Hb and RBC values were similar to those in the control especially at low dose of 1 part per million. At higher doses of 2 and 4 part per million however, there were marginal decreases in the RBC counts while the ESR was significantly higher than the control. This is an indication that the use of Iodosteryl at these concentrations must have resulted in intravascular hemolysis especially with the concurrent increase in the erythrocyte osmotic fragility at 0.7 and 0.9% NaCl concentrations. The MCV and MCH values in these groups of birds also appeared higher, (though non-significantly) than those of the control because the RBC counts in these two groups were slightly lower than that of the control (Table 1).

The total and differential leucocyte values were also not affected despite the prolonged use of the compound in drinking water. However, the erythrocyte osmotic fragility increased significantly irrespective of the dosage of Iodosteryl used as water disinfectant. The effect of Iodosteryl on erythrocyte fragility is not farfetched; this is because erythrocyte fragility has been reported to increase in conditions associated with oxidative stress and free radical release into the blood circulation. These conditions include exercise (Kurkcu *et al.*, 2010), aging (Droge, 2002), diabetes mellitus (Baynes, 1991), neurodegenerative diseases and even HIV infection (Droge, 2002).

Increased erythrocyte fragility usually results when reactive oxygen or nitrogen radicals or other forms of oxidants react with integral and other proteins on the erythrocyte membrane leading to destruction of the membrane structure. They may also attack the membrane lipids, resulting in lipid peroxidation, membrane fluidity and ultimate destruction of the bilayer integrity of erythrocyte membrane (Girotti, 1985). Summarily, we can infer that stress, from whatever source increases erythrocyte osmotic fragility by lipid peroxidation and destruction of membrane proteins in erythrocytes. This shows that prolonged administration of Iodosteryl in this study is a form of stress associated with decreased osmotic resistance of the erythrocytes. This may also result in intravascular hemolysis as in the groups that received higher doses as evidenced by their lower RBC counts and higher ESR values. Of course, erythrocyte sedimentation rate is a measure of the rate of rouleaux formation by erythrocytes, a direct indicator of higher levels of acute phase protein especially fibrinogen and globulin during inflammatory conditions. Fibrinogen levels in the plasma increases progressively during inflammation and stress until the condition is resolved and can be measured directly or estimated by erythrocyte sedimentation (Barham *et al.*, 1979). But the leucocytes parameters in this study did not indicate presence of any inflammation; therefore, the increased ESR may be associated with stress induced by iodine and the associated intravascular hemolysis. Increase in ESR may also occur concomitantly with increased erythrocyte fragility as previously reported by Barham *et al.* (1979).

Iodine and its product have been used successfully as a water disinfectant among soldiers in field conditions with minimal complications (Hollowell, 2000). It has several advantages over chlorine for field use, including: greater chemical stability of the product and less reactivity with organic nitrogenous wastes and contaminants in water, leaving higher free residual concentration in water and more acceptable taste in equipotent doses. This use of iodine based water disinfectants has also been associated with few isolated cases of goitre (Henjum *et al.*, 2010; Kettel-Khan *et al.*, 1998; TWAS, 2002; Zhao *et al.*, 2000).

The present study showed that, prolonged use of Iodosteryl as water disinfectant is not entirely safe, especially when used indiscriminately. It could induce intravascular hemolysis at high doses as a result of decreased erythrocyte osmotic resistance associated with peroxidation of the lipid bilayer and damage to erythrocyte membrane proteins and lipids by iodine. We however,

recommend further evaluation of the prolonged use of Iodosteryl as water disinfectant on endogenous antioxidants such as MDA, SOD, Catalase and glutathione as well as the assessment of the type of free radicals involved in the oxidative damage.

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