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Toxicological evaluation of repeated administration of povidone iodine in cockerels

D. Sani [a,b,*], P.A. Abdu [c], M. Mamman [a], K.O. Jolayemi [a]

[a] Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria
[b] Clinical Pharmacology Specialty, Medicine Faculty, Ahmadu Bello University Study Center, College of Veterinary Surgeons, Nigeria
[c] Department of Veterinary Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria

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ABSTRACT

Povidone-iodine (Polidine®) is a synthetic broad-spectrum antiseptic being applied topically to treat wounds and prevent their infection. It has been however reported with the assertions that it is effective in the treatment of infectious bursa disease (IBD) when administered orally by practicing Veterinarians and other poultry handlers. Acute kidney injury has been reported also with povidone iodine ingestion. Hence, in this study, graded dose administration was conducted to ascertain its safety profile. Forty chicks were obtained from a poultry hatchery in Ibadan, Oyo State. They were randomly divided into four (4) groups of ten chicks each. Group I served as negative control, groups II, III and IV were administered Polidine® at 1 mL/50 L, 1 mL/25 L, and 1 mL/10 L of water respectively for 7 days. Blood samples were collected on Days 3 and 7 post administration for determination of haematological and biochemical parameters. Liver and Kidney tissues were harvested following termination of the experiment and processed for histopathological examination. Results revealed no significant ($p > 0.05$) effect in the haematological and biochemical parameters of cockerels treated with Povidone iodine at 1 mL/50 and 25 L of water. On histopathological examination no lesion was also observed in the liver and kidney tissues of groups I, II and III (normal control, 1 mL/50 and 25 L respectively) when compared to group IV (1 mL PI /50 L of water) where lesions were recorded. Hence, this study has shown the relative safety of povidone iodine at different doses in cockerels.

1. Introduction

Povidone-Iodine is an iodophor solution containing a water-soluble complex of iodine and polyvinylpyrrolidone [1]. Free iodine, slowly liberated from the polyvinylpyrrolidone iodine complex in solution, kills eukaryotic or prokaryotic cells through iodination of lipids and oxidation of cytoplasmic and membrane compounds. This agent exhibits a broad range of microbicidal activity against bacteria, viruses, protozoa and fungi [1]. Povidone iodine is a substitute antiseptic to alcohol when it's not available, commonly used in clinical settings, including presurgical or postsurgical skin disinfection. Povidone iodine is usually applied to the skin to treat current infections and prevent the spread of opportunistic pathogens [2]. Povidone iodine has been reported to be used in the treatment of viral disease such as infectious bursa disease in chickens. Moreover, with povidone iodine, there have been concerns about allergy, ineffective penetration, toxic effects on host cells and acute kidney injury [3,4]. There is dearth of information with regards to the use of povidone iodine in cockerels, thus, necessitating the need for documentation of research findings. Therefore, the aim of this study was to evaluate the toxicological effect of repeated administration of povidone iodine in the drinking water of cockerels.

2. Materials and methods

2.1. Source of povidone iodine

Povidone iodine (Polidine®) was obtained from Kattle Care Limited (JAWA GROUPS), Nigeria.

2.2. Experimental chicken

Ethical clearance for the use of cockerels was obtained from the Ahmadu Bello University Committee on Animal Use and Care (ABU-CAUC). A total of forty (40) chicks were obtained from a poultry

* Corresponding author at: Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria.
E-mail address: dsani@abu.edu.ng (D. Sani).

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hatchery in Ibadan, Oyo State, Nigeria. They were kept in cages at the Poultry Research unit of the Department of Veterinary Medicine, Ahmadu Bello University, Zaria. Prior to the arrival of the birds, the cages were disinfected, the feeding and drinking troughs were properly washed and disinfected to reduce contamination or the likelihood of infection. The birds had access to pelletized growers feed and water provided *ad libitum*. The brooding of the birds took two weeks after and they were then randomly allocated into groups I, II, III and IV consisting of ten chicks per group.

2.3. Median lethal dose of povidone iodine

The median lethal dose (LD_{50}) was determined according to the Up and Down Procedure [5]. Each bird was administered with a limit dose (2000 mg/kg body weight of cockerels) and monitored for 24 h for instant death, possible signs of toxicity and death. The birds were further monitored for 48 h after administration [6].

2.4. Experimental design

Forty cockerels were randomly divided into four group consisting of ten birds each. Group I served as negative control while groups II, III and IV were administered 1 mL/50 L, 1 mL/25 L, 1 mL/10 of Polidine® in their drinking water for 7 days, respectively.

2.5. Haematological analysis

Blood samples were obtained on Days 3 and 7 post administration and used for haematological analysis. The packed cell volume (PCV) was determined using standard laboratory technique involving non-heparinized capillary tube, TG12MX® Micro-haematocrit centrifuge machine, at 3000×*g* for 15 min and Hawksley® Micro-haematocrit reader. Haemoglobin (Hb) concentration was assayed using cyanohaemoglobinometer. Red Blood Cells (RBCs) and White Blood Cells (WBCs) counts were evaluated using Natt-Herrick solution (1:200 dilution) and the improved Neubauer haemocytometer [7]. The differential WBCs and total WBCs count were determined by Giemsa preparation on slides and cytometer, respectively.

2.6. Serum biochemical analysis

Total protein, blood urea nitrogen and creatinine were determined using Randox assay kit (Randox Laboratories Ltd, Ardmore, Antrim, UK) according to manufacturer's instruction.

2.7. Histopathological examination

The kidney and liver from the sacrificed birds were harvested, observed for gross lesions, fixed in 10% formalin solution and then processed for histopathologic examination using standard histologic technique [8].

2.8. Data analysis

All data generated were expressed as mean ± standard error of mean (SEM) and subjected to one-way repeated measure analysis of variance (ANOVA) followed by Tukey's *post hoc* using Graphpad Instat (San Diego, CA). Values of $p \leq 0.05$ were considered statistically significant.

3. Results

3.1. Effect of povidone iodine administered at different doses on the haematological parameters of cockerels

There was no significant difference ($p > 0.05$) recorded in the packed cell volume, haemoglobin concentration, red blood cell, white blood cell, lymphocytes, heterophils and monocyte counts of povidone iodine administered group when compared to the normal control by days 3 and 7 respectively (Table 1).

3.2. Effect of povidone iodine administered at different doses on the serum biochemical parameters of cockerels

There was no significant difference ($p > 0.05$) recorded in the total protein, blood urea nitrogen and creatinine level of povidone iodine administered group when compared to the normal control by days 3 and 7 respectively (Table 2).

3.3. Effect of povidone iodine administered at different doses on the histopathology of liver and kidney from cockerels

Histopathology of kidney showed normal control group with normal architectural structure (Fig. 1), group II showed normal glomeruli and tubules (Fig. 2), group III showed mild hyperemia in tubular epithelium (Fig. 3) while congested glomeruli and coagulation necrosis in tubular epithelium with severe lymphocytic cell infiltration in interstitial tissue and degeneration of the epithelial lining of the bowman capsule was observed in group IV (Fig. 4). There were no lesions observed in the liver

Table 1
Haematological parameters of cockerels administered different doses of povidone iodine.

Parameters	Groups	Days	
		3	7
PCV (%)	Normal Control	27.00 ± 2.00[a]	28.00 ± 0.80[a]
	1 mL/50 L	27.00 ± 1.73[a]	30.76 ± 0.50[a]
	1 mL/25 L	27.33 ± 4.7[a]	30.33 ± 1.81[a]
	1 mL/10 L	26.67 ± 2.5[a]	30.23 ± 1.85[a]
Haemoglobin (g/dl)	Normal Control	8.53 ± 1.36[a]	8.55 ± 1.30[a]
	1 mL/50 L	8.76 ± 0.58[a]	8.06 ± 1.28[a]
	1 mL/25 L	8.63 ± 0.65[a]	8.30 ± 1.47[a]
	1 mL/10 L	8.00 ± 1.2[a]	8.15 ± 1.62[a]
TRBC ($\times 10^6$/µl)	Normal Control	4.56 ± 0.82[a]	4.66 ± 0.54[a]
	1 mL/50 L	4.50 ± 0.53[a]	4.20 ± 0.49[a]
	1 mL/25 L	4.76 ± 0.61[a]	4.23 ± 0.92[a]
	1 mL/10 L	4.76 ± 0.36[a]	4.33 ± 0.74[a]
TWBC (10^3/µl)	Normal Control	12.50 ± 1.34[a]	12.80 ± 1.35[a]
	1 mL/50 L	11.30 ± 2.42[a]	12.76 ± 2.95[a]
	1 mL/25 L	11.76 ± 1.96[a]	12.67 ± 2.00[a]
	1 mL/10 L	12.76 ± 3.29[a]	11.70 ± 2.76[a]
Lymphocyte (%)	Normal Control	79.30 ± 10.69[a]	82.00 ± 10.44[a]
	1 mL/50 L	81.30 ± 5.48[a]	80.00 ± 7.22[a]
	1 mL/25 L	81.66 ± 7.03[a]	81.33 ± 6.65[a]
	1 mL/10 L	82.00 ± 5.17[a]	85.33 ± 4.04[a]
Heterophil (%)	Normal Control	15.33 ± 2.50[a]	16.00 ± 3.24[a]
	1 mL/50 L	16.66 ± 2.29[a]	16.00 ± 3.20[a]
	1 mL/25 L	17.00 ± 3.50[a]	16.33 ± 2.81[a]
	1 mL/10 L	17.33 ± 3.14[a]	16.38 ± 3.06[a]
Monocyte (%)	Normal Control	0.66 ± 0.11[a]	0.66 ± 0.14[a]
	1 mL/50 L	0.66 ± 0.50[a]	0.66 ± 0.21[a]
	1 mL/25 L	1.00 ± 0.20[a]	0.66 ± 0.16[a]
	1 mL/10 L	1.66 ± 0.57[a]	0.66 ± 0.50[a]

Key: PCV; packed cell volume, TRBC; total red blood cell, TWBC; total white blood cell. [a]Means with the same letters along columns are not statistically significant ($p > 0.05$).

Table 2
Biochemical parameters of cockerels administered different doses of povidone iodine.

Parameters	Groups	Days	
		3	7
BUN (g/dl)	Normal Control	0.50 ± 0.15[a]	0.50 ± 0.18[a]
	1 mL/50 L	0.53 ± 0.10[a]	0.60 ± 0.21[a]
	1 mL/25 L	0.61 ± 0.12[a]	0.63 ± 0.15[a]
	1 mL/10 L	0.93 ± 0.15[a]	1.20 ± 0.26[a]
Creatinine (g/dl)	Normal Control	27.20 ± 2.00[a]	28.00 ± 080[a]
	1 mL/50 L	27.00 ± 1.70[a]	30.70 ± 0.50[a]
	1 mL/25 L	28.30 ± 5.50[a]	30.30 ± 1.80[a]
	1 mL/10 L	25.30 ± 4.70[a]	30.40 ± 1.90[a]
Total protein (g/dl)	Normal Control	6.67 ± 0.70[a]	6.53 ± 0.50[a]
	1 mL/50 L	6.53 ± 0.46[a]	6.17 ± 0.31[a]
	1 mL/25 L	7.77 ± 2.21[a]	6.97 ± 1.57[a]
	1 mL/10 L	7.93 ± 2.39[a]	8.30 ± 0.87[a]

Key: BUN; blood urea nitrogen. [a]Means with the same letters along columns are not statistically significant ($p > 0.05$).

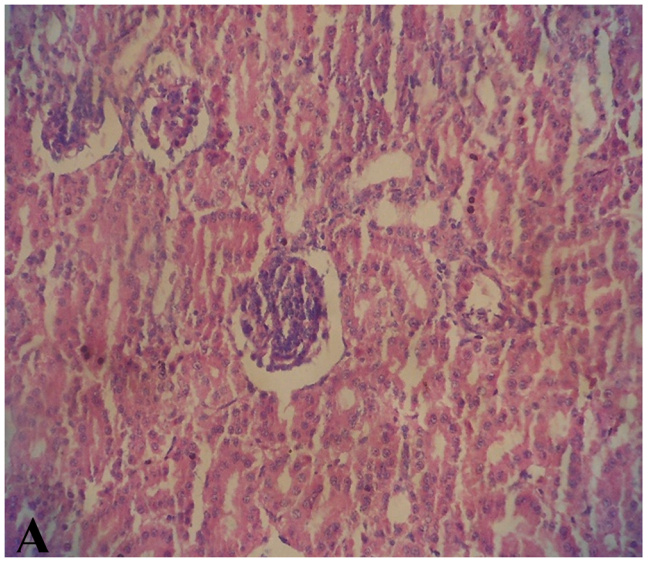

Fig. 1. Photomicrograph of the kidney from normal control chicken group showing kidney tissue with normal architectural structure. H and E × 200.

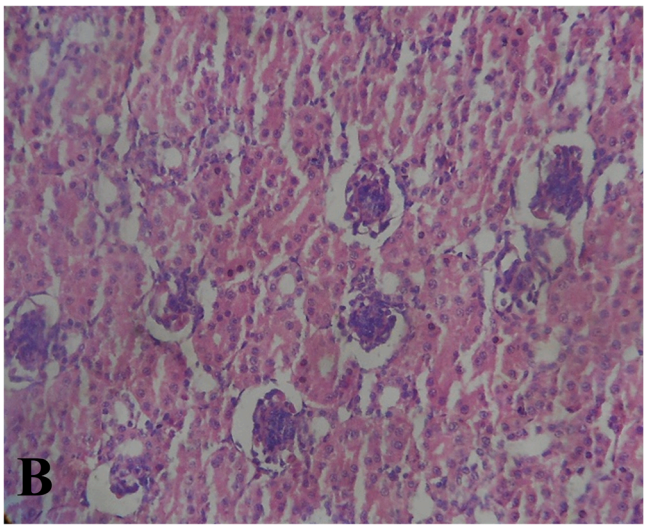

Fig. 2. Photomicrograph of the kidney from 1 mL/50 L of povidone iodine treated group showing normal glomeruli and tubules. H and E × 200.

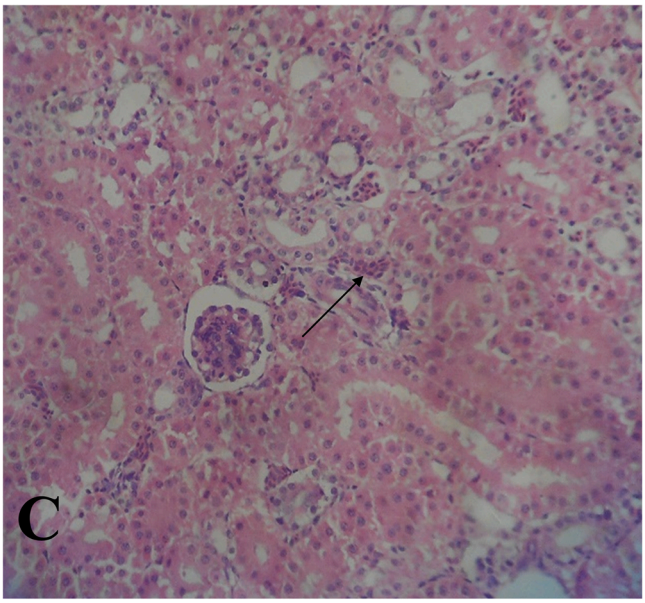

Fig. 3. Photomicrograph of the kidney from 1 mL/25 L of povidone iodine group showing close to normal histological structure and mild hyperemia in tubular epithelium (arrow). H and E × 200.

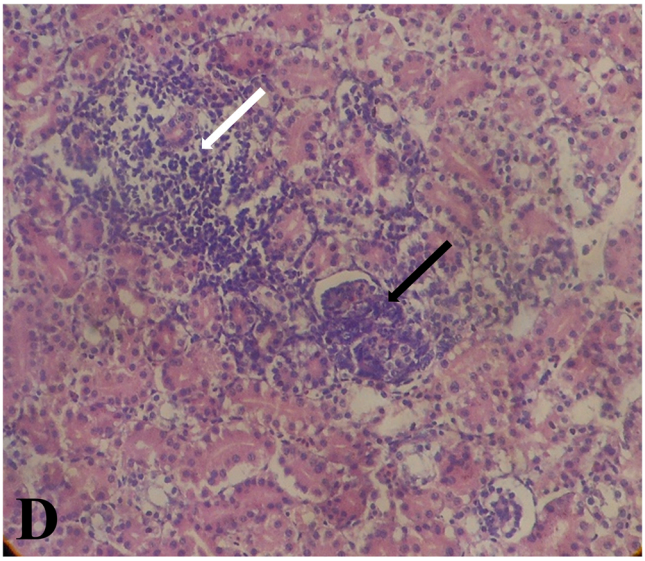

Fig. 4. Photomicrograph of the kidney from 1 mL/10 L of povidone iodine group showing congested glomeruli and coagulation necrosis in tubular epithelium (black arrow) with severe lymphocytic cell infiltration in interstitial tissue (white arrow) and degeneration of the epithelial lining of the Bowman capsule. H and E × 200.

of groups I (Fig. 5), II (Fig. 6) and III (Fig. 7) but disoriented central vein and severe congested portal vein was seen in group IV (Fig. 8).

4. Discussion

This study was carried out to evaluate the toxic effect of povidone iodine at different doses in cockerels. Povidone iodine have been shown to limit the impact and spread of infectious diseases with potent antibacterial, antiviral, and antifungal effects. In addition to lack of reported resistance, the benefits of povidone iodine include an excellent safety profile and a broad spectrum of effect due to its multimodal action [2]. This could account for the safety observed in the study by Sani et al. [6]

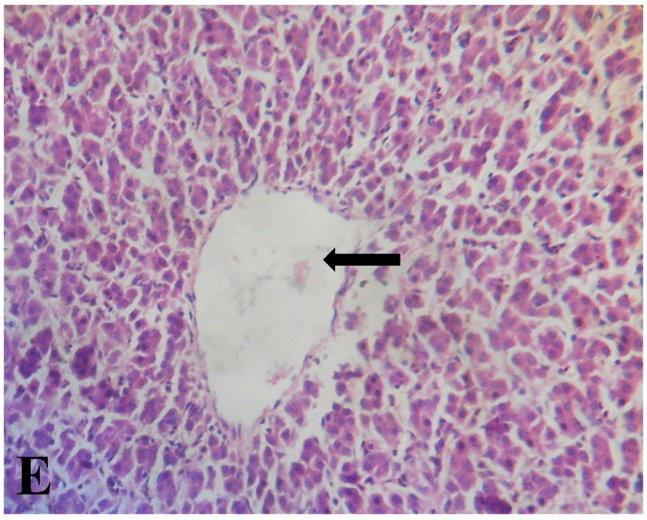

Fig. 5. Photomicrograph of the liver from normal control chicken group showing normal hepatic architecture with normal central vein (arrow). H and E × 200.

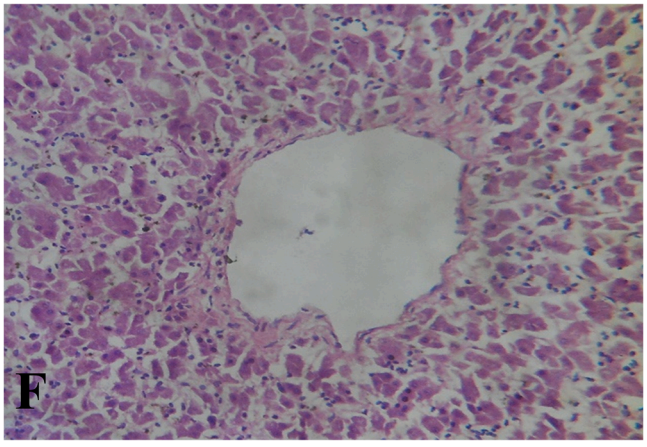

Fig. 6. Photomicrograph of the liver from 1 mL/50 L of povidone iodine treated group showing normal histological structures. H and E × 200.

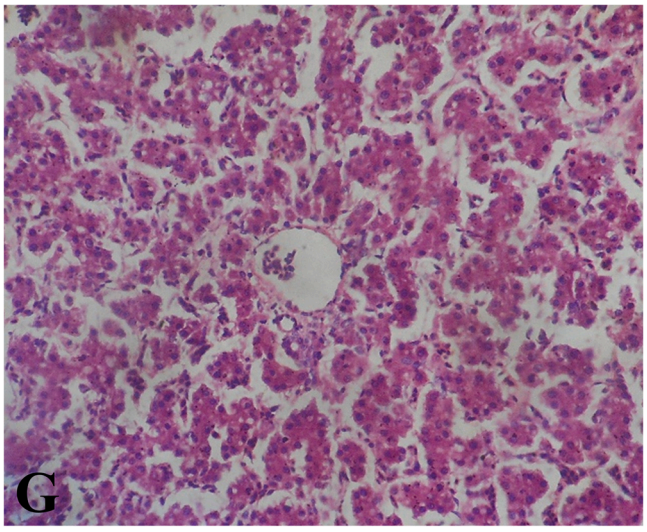

Fig. 7. Photomicrograph of the liver from 1 mL/25 L of povidone iodine group showing close to normal histological structure. H and E × 200.

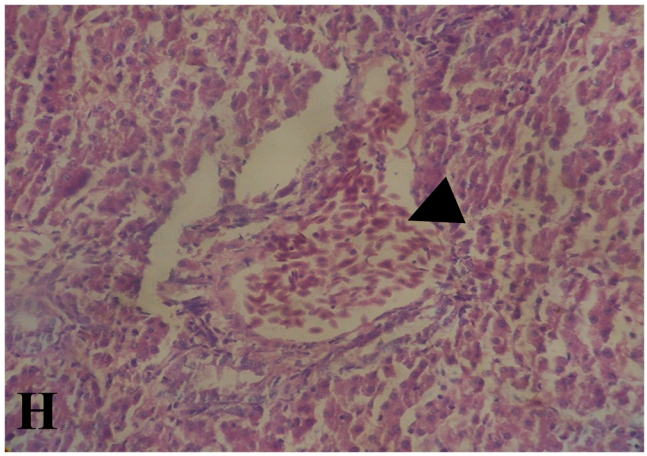

Fig. 8. Photomicrograph of the liver from 1 mL/10 L of povidone iodine group showing disoriented central vein and severe congested portal vein (arrow head). H and E × 200.

where the LD_{50} of povidone iodine was greater than 2000 mg/kg.

The effect of a toxic substance is evaluated through laboratory methods of haematological, biochemical, gross and histopathological examination to ascertain the degree of deviation from normal health status [9,10]. Changes in haematological parameters may be due to modifications in membrane permeability, cellular integrity, and metabolism, or even due to exposure to toxic chemicals [11]. The erythrocytic parameters showed no statistical differences although non-significantly increased after administration of povidone iodine by days 3 and 7 when compared to normal control. Previous report by Aletan [12] also showed no significant difference in the erythrocytes of Wistar rats treated with iodine. The non-significant alteration in the erythrocytic parameters observed in this study might be due to the indirect effect of iodine released following metabolism of povidone iodine. Iodine has been documented to play a critical role in the synthesis of thyroxine (T3 and T4) which tend to enhance erythropoiesis [13]. These effects will subsequently cause an increase in erythroid mass manifested as increased packed cell volume, haemoglobin concentration and red blood cells count, though non-significant as observed in this study. Similarly, the non-significant increase in leucocytic parameters might be due to the indirect enhanced myelopoietic effect by iodine *via* the action of thyroxine. This suggests that povidone iodine have no toxic effect on haematology of cockerels at these doses.

Urea concentration has been documented to be an indicator of the extent of kidney damage while creatinine concentration has been postulated to be an indicator to the extent of muscle destruction [14]. However, the absence of alterations in urea and creatinine concentration observed in this study suggest no kidney damage and muscle destruction, respectively by the doses of the povidone iodine used in this study. In contrast, acute renal damage has been reported following cutaneous absorption of povidone iodine [15,16]. Also, it was observed that povidone iodine non-significantly increased the serum total protein concentration in this study. On histology, povidone iodine did not produce alteration in the architecture of the liver and kidney of cockerels at 1 mL/50 L and 1 mL/ 25 L. But the congestion in the liver and renal changes observed at 1 mL/ 10 L suggest possible toxic effect of povidone iodine at higher doses.

5. Conclusion

Povidone iodine at the of doses 1 mL/50 and 25 L of water respectively does not have deleterious effect on the haematological, blood urea nitrogen, creatinine, total protein and histopathology of cockerels in this study. Hence, the use of povidone iodine at recommended dose is relative safe.

CRediT authorship contribution statement

DS, PAA and MM conceived the study. PAA and MM supervised the study. DS, KOJ performed the research. DS collected the samples. DS and KOJ analyzed data. DS wrote the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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