

Research Article

Acute Oral Toxicity Study of Povidone-Iodine in Wistar Rats Using Up-and-Down Procedure

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ABSTRACT

This study was conducted to assess the acute oral toxicity of Povidone iodine in Wistar rats and determine its safety profile. The up and down method as per Organization for Economic Co-operation and Development guidelines using 15 adult male Wistar rats. They were randomly divided into three groups, given varying amounts of distilled water, 2000 and 5000 mg/kg to determine the median lethal dose (LD₅₀). Clinical signs and mortality were observed for 48 hours and 14 days for immediate and delayed toxicity. Blood samples were collected at the end of the experiment for haematological and serum biochemical analysis for kidney and liver functions. Also, the kidney and liver were harvested, preserved, and processed for histomorphological evaluation. The result from this study indicates that Mortality (60 %) was observed in the group administered with 5000 mg/kg of PV-I within 3 h of administration. In contrast, the group served with 2000 mg/kg of PV-I, and the control remained viable up to 14 d of the experiment, the median lethal (LD₅₀) dose of PV-I is less than 5000 mg/kg in Wistar rats. No significant (P> 0.05) difference was observed in the haematological parameters and serum biochemical markers for the kidney and liver function tests between the treatment and the control group. Furthermore, a histomorphological examination of the kidneys and liver shows normal kidneys with slight hepatic necrosis in the group administered with 2000 mg/ kg BW of PV-I. In conclusion, PV-I is safe for Wistar rats in doses below 2000 mg/kg.

Keywords: Acute toxicity. Povidone iodine, Limit dose, Mortality, Haematological parameters

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INTRODUCTION

Povidone-iodine (PV-I) is an iodophor, a compound made of water-soluble povidone polymer and iodine that infiltrates bacteria and kills cells by oxidizing fatty acids, proteins, and nucleotides (Raiyyan *et al.*, 2023). PV-I is effective against viruses, fungi, and gram-positive and gram-negative bacteria (Lepelletier *et al.*, 2020). According to earlier reports, when administered orally, PV-I was specified to be successful in the treatment and control of infectious bursal disease (IBD) by field

veterinarians, poultry farmers, and other animal health professionals (Aliyu *et al.*, 2016). PV-I is combined with other chemical agents to eradicate *Bacillus thuringiensis* endospores from surgical instruments, and as a result, it is recognized as an effective sterilizing agent (Tweij *et al.*, 2020). Pre-operative shower use of topical PV-I decontamination has been shown to reduce presurgical staphylococcal skin colonization in patients having elective plastic surgery (Veiga *et al.*, 2008). In orthopaedic surgery, it also lowers operative site

infection (Lepelletier *et al.*, 2020). Al-Kaisy and Salih (2005), report that the addition of PV-I ointment, either alone or in conjunction with daily systemic vitamins E and C, to a routine hospital antibiotic treatment enhanced markers of oxidative stress and wound healing. The use of PV-I as a highly effective topical virucidal antiseptic against SARS-CoV-2 is supported by a recent study to a 99 percent level (Jones, 2022). Although the acute oral toxicity of pure iodine in rats and mice has been recorded as 1,400 and 22,000 mg/kg respectively (Alexender and Armen, 2013), acute kidney injury (Mao *et al.*, 2011), time- and concentration-dependent apoptosis and necrosis in human cultured epithelial cells and rats' oral mucosal tissue (Satos *et al.*, 2014), and more than 2000 mg/kg in cockerels (Sani *et al.*, 2021) were among the studies in which other researchers reported the toxicity of PV-I. The liver and kidneys are vital organs that aid in metabolism, digestion, detoxification, and elimination of toxins to maintain optimal body function (Younes-Ibrahim, 2021; Kalra *et al.*, 2023). Measuring biomarkers such as alanine aminotransferase (ALT), Aspartate aminotransferase (AST), and Alkaline phosphatase (ALP) for liver function and Protein, blood urea nitrogen (BUN), creatinine (CREAT), and albumin (ALB) for kidney function is essential in assessing potential injury to these organs (Salih, 2013). Despite the apparent increase in PV-I use, there is a limited report on the assessment of its safety and systemic acute evaluation following oral administration of the agent in Wistar rats. Hence, this study aimed to examine the acute oral median lethal dose (LD₅₀) of PV-I in Wistar rats to ascertain its safety.

MATERIALS AND METHODS

Ethical clearance

The protocol for the use of Wistar rats in this study was sought and obtained from the Ahmadu Bello University Zaria Committee on Animal Use and Care with approval number ABUCAUC/2023/096

Source of chemical

Povidone iodine (Batiqon^(ix), POVIDON iYAT COZELTi %10, BIYOSIDAL, Turkey) was obtained from a commercial store in Zaria, Nigeria.

Experimental Animals

A total of 15 young adult male Wistar rats aged 12-14 weeks and weighing 120- 160g were sourced from the Department of Veterinary Pharmacology and Toxicology, Ahmadu Bello University Zaria animal house. The experiment was conducted under standard conditions at the same Departmental research animal house. The rats were kept in Aluminum cages with sawdust. They were fed with pelletized grower feed (Vital Feed Nigeria), clean water was provided

ad libitum, and allowed to stay for 10 days to acclimatize with the environment before being randomly assigned into 3 groups for the commencement of the experiment.

Acute oral toxicity study of PV-I

The "up-and-down" procedure as described by OECD (2022) test guidelines was used to determine the acute oral toxicity (LD₅₀) of PV-I, with a limit dose of 5000 and 2000 mg/kg in male Wistar rats. Feed was withdrawn for two hours before treatment. The rats were randomly divided into 3 groups containing 5 rats each per group as A, B, and C. A total of 3 rats from groups A and B were randomly selected and sequentially administered with an initial single oral dose of 3ml/kg BW of distilled water (control) and upper limit dose of 5000 mg/kg of PV-I before the completion of the same dose to the remaining rats in these groups for the determination of the subsequent dose in group C. Rats in group C were administered a lower limit dose of 2000 mg/kg of PV-I following the same protocol as described earlier. They were monitored within 24 - 48 h and then 14 days for immediate and delayed clinical signs and mortality. The feed and water were restored after 1 hour of treatment.

Haematological analysis

The rats were anesthetized with chloroform, a 5ml sterile syringe, and a 23G needle was used to take 3 ml of blood samples by cardiac puncture at terminal necropsy after the animals had fasted overnight. An equal volume (1.5ml) of the blood sample was divided and immediately transferred into Ethylenediaminetetraacetic acid (EDTA) and plain sample bottles for haematological and biochemical analysis respectively (Rahman, 2016). For hematology, non-heparinized capillary tubes (Palmatec^(R) ISO 9001 China) were slanted and inserted into the EDTA sample bottles and allowed to drain the blood, while the open end of the tube was sealed with flame. The tubes were then inserted into the Microhematocrit Centrifuge machine (SH120 Wincom Company Ltd, Shangai China) and allowed to spin at 7000 rpm for 5 minutes. Hawksley microhematocrit reader (Lancing Business Park, Lancing Sussex UK) was used to read and determine packed cell volume (PCV) from the capillary tubes. Red blood cells (RBCs) and white blood cells (WBCs) were evaluated using a 2% acetic acid solution and normal saline (1:200) and read with an improved Neubauer hemocytometer. The differential leukocyte counts were determined by making a blood smear and stained with Geimsa's stain solution (Blumenreich, 1990).

Serum Biochemical Analysis

The blood in the plain sample bottles was allowed to stay for 30 minutes, then transferred into a centrifuge machine (80- 3 Wincom Company Ltd, Shangai China)

and centrifuged at 3000 rpm for 15 minutes. After centrifuging, the clear segment of the serum at the top layer of the bottle was aspirated with a micropipette and sterile syringe, and the needle was transferred into serum sample bottles. Biochemical parameters including albumin (ALB), creatinine (CTREAT), blood urea nitrogen (BUN), total serum protein, Aspartate aminotransferase (AST) alanine aminotransferase (ALT), and alkaline phosphatase (ALP) values were evaluated using Randox assay kits (Randox laboratories Ltd, Ardmore, Antrim UK) as per the protocol as provided by the manufacturer.

Histomorphology of Kidney and liver

Immediately after sacrifice, vital organs including the kidney and liver were harvested from each rat in the respective group. These organs were fixed in a 10 % formalin (Sigma- Andrich. Inc, St Louis, MO) solution. These specimens were cleared in xylem, embedded in paraffin, sectioned at 4-6 microns thickness, and stained with haematoxylin and eosin (H and E) according to the method of Luna, (1968) and Akpantah *et al.* (2003) then processed followed by histomorphological examination under a light microscope and the image is captured for interpretation.

Data Analysis

Data were analyzed by GraphPad Prism version 8 statistical software using multiple unpaired t-tests to compare means between groups, Results are presented as Mean ± SEM where significant differences were considered at P ≤ 0.05.

RESULTS

The results for evaluating the acute toxicity test, hematological parameters, serum biochemical parameters, and histomorphology of the kidney and liver in this study were presented in Table 1, 2, and 3, plates I and II respectively. The Single oral administration of 5000 mg/kg of PV-I produced mortality of 60 % within 3 h of administration. In comparison, the lower dose of 2000 mg/kg did not within the study period of 14 d. There is no significant (P< 0.05) difference in the haematological and serum biochemical parameters between the control and the group administered with a limit dose of 2000 mg/kg of PV-I. Also, PV-I at 2000 mg/kg does not affect the histomorphology of the kidney, however, slight hepatic necrosis was observed in the liver of rats administered with the limit dose of 2000 mg/kg of PV-I.

Table 1. The median lethal dose (LD₅₀) of PV-I at the limit dose of 5000 and 2000 mg/kg in Wistar rats

Dose (mg/kg)	No. of Mortality	
	Immediate	Delayed
Control (3 ml DW)	0/5	0/5
5000	3/5	0/2
2000	0/5	0/5

Single oral administration of 5000 mg/kg of PV-I produced mortality at 60 % within 3 h of administration. In comparison, the lower dose of 2000 Mg/kg did not within the study period of 14 d.

Table 2. Haematological parameters of Wistar rats administered with PVI orally at the limit dose of 2000 mg/kg

Parameter	Group (Mean ± SEM)	
	Control (3 ml/kg DW)	Treatment (2000 mg/kg PV-I)
PVC (%)	45.50 ± 1.12	47.50 ± 1.28
HGB (g/dl)	15.16 ± 0.37	15.78 ± 0.42
TP (g/dl)	7.40 ± 0.26	7.13 ± 0.22
TWBC (10 ⁹ /l)	10.40 ± 0.56	14.63 ± 1.27
TRBC (10 ⁶ /l)	4.98 ± 0.12	4.65 ± 0.30
Neutrophil (%)	26.50 ± 2.54	23.75 ± 2.52
Lymphocyte (%)	72.50 ± 2.87	71.25 ± 2.37

There is no significant difference (P> 0.05) in all the haematological parameters between the control and the treatment group

Table 3. Serum biochemical parameters of Wistar rats administered with PVI orally at the limit dose of 2000 mg/kg

Parameter	Group (Mean ± SEM)	
	Control (3 ml/kg DW)	Treatment (2000 mg/kg PV-I)
ALB (g/dl)	3.96 ± 0.55	5.00 ± 0.36
CREAT (mg/dl)	1.02 ± 0.13	0.85 ± 0.11
BUN (mg/dl)	33.26 ± 2.37	36.00 ± 5.03
T.S Protein(g/l)	66.5 ± 3.65	72.75 ± 3.28
AST(u/l)	62.5 ± 15.03	83.5 ± 11.03
ALT(u/l)	84.26 ± 3.75	85.75 ± 2.37
ALP(u/l)	58.00 ± 1.48	60.50 ± 3.31

There is no significant difference ($P>0.05$) in all the serum biochemical parameters for the kidney and liver function test between the control and the treatment group

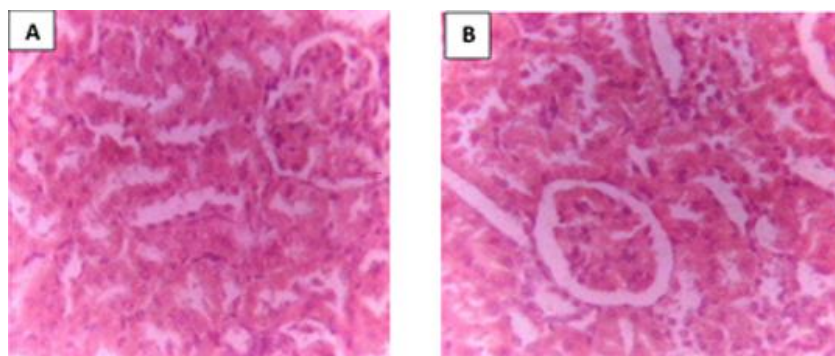


Plate I (A = Control; B = Treatment). Photomicrograph of the kidney showing normal histomorphology following oral administration of PV-I at the limit dose of 2000 mg/kg of BW in Wistar. H & E, X 400

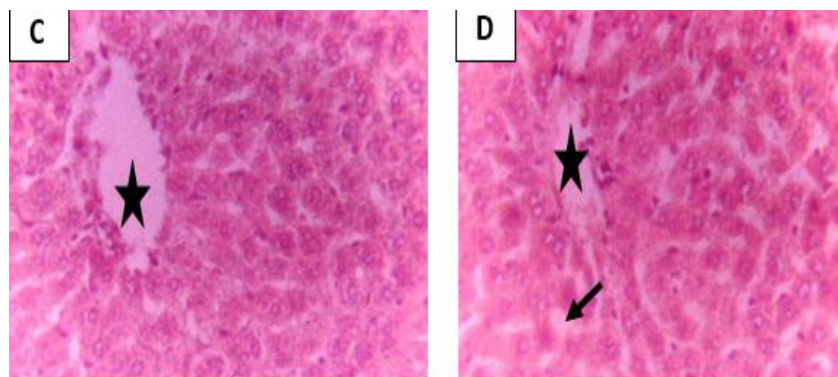


Plate II (C = Control; D = Treatment). Photomicrograph of the liver showing normal histomorphology in C (control) with central vein (star) and slight hepatic necrosis (arrow) following oral administration of PV-I at the limit dose of 2000 mg/kg of BW in Wistar. H & E, X 400

DISCUSSION

Over time, PV-I has been used more often to treat various illnesses (Eggers, 2019; Takeda *et al.*, 2024). Therefore, in-depth research is needed to assess its safety and efficacy application for human and animal uses. It was observed in this study that a single oral administration of an upper limit dose of 5000 mg/kg of PV-I produces mortality of 60 % within 3 h of

administration. In comparison, a lower limit of 2000 mg/kg did not up to 14 d of observation. This is an indication that LD_{50} of PV-I is greater than 2000 but less than 5000 mg/kg which is slightly toxic when administered once over 24 h in Wistar rats according to Loomis and Hayes (1996). This finding agrees with Sani *et al.* (2021) and Ibrahim *et al.* (2023) who report similar observations of PV-I in cockerels and mice.

Hematology is the study of the counts and morphologies of RBC, WBC, and platelets for diagnosis and monitoring of disease conditions in a biological system (Bullers, 2016). Haematological parameters are those parameters that are related to the blood and blood-forming organs, which are the indicators of the status of exposed animals to toxicants and other conditions (Bamishaiye *et al.*, 2009). After being released from povidone, iodine regulates haematological parameters by acting indirectly on haematopoiesis through thyroid hormones and prohormones (Oliveira *et al.*, 2018). In this study, PV-I at 2000 mg/kg does not elicit any toxic effect as per the haematological parameters. This finding agrees with the report of Sani *et al.* (2021) in cockerels and Ibrahim *et al.* (2023) in mice and disagrees with Aletan (2014) in Wistar rats. Changes in CREAT, BUN, Protein, and ALB markers of damage and impairment of the renal filtration mechanism can be used to measure renal function (Wasan *et al.*, 2001). The results from this study suggest that a single oral administration of PV-I at 2000 mg/kg has no negative effect on the kidney, while there was a slight increase in protein content in the PV-I group, it was not statistically significant ($p < 0.05$) when compared to the control group. This finding is in agreement with the report of Sani *et al.* (2021) in cockerels and disagrees with the report of Ibrahim *et al.* (2023) in mice which may be due to variation in species tolerance. Additionally, no notable variance was observed in ALT, AST, and ALP levels between the groups treated with PV-I and the control. Based on the histomorphological evaluation conducted in this study, there were no changes detected in the kidney. This may be because a single oral administration of 2000 mg/kg of PV-I does not elicit kidney injury as indicated in the kidney function test or might have resolved within 14 days. However, slight hepatic necrosis was observed in the liver, this may not be unconnected with the rise in AST level as a mark of liver injury, although not statistically significant.

Conclusion

This study has demonstrated that PV-I is safe in Wistar rats following a single oral administration of 2000 mg/kg. However, preliminary repeated oral administration needs to be conducted to evaluate its long-term effects in Wistar rats to ensure the safety of PV-I.

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