

# Comparative Evaluation of Haematoxylin Stain Uptake in Prostate Glands of Cadmium Chloride-Exposed Wistar Rats

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## ABSTRACT

Haematoxylin stains are widely used in histopathology for visualizing tissue architecture, yet variations in preparation and uptake can influence diagnostic accuracy. This study compared the staining outcomes of different haematoxylin formulations on prostate glands of Wistar rats exposed to cadmium chloride across Nigeria's six geopolitical zones. Thirty-five adult male rats were grouped and administered cadmium chloride (2 mg/kg, subcutaneously), while controls received distilled water. Prostate glands were harvested, weighed, and processed histologically using Harris and Cole's haematoxylin stains sourced from the respective zones. Microscopic assessment showed that most groups retained normal histoarchitecture, including acini lined by ductal epithelium, eosinophilic secretions, papillary infoldings, and apical vacuoles. In the Southwest and South-south zones, staining was intensely dark with poor nuclear differentiation, whereas the North Central and Southeast zones demonstrated relatively clearer outcomes with occasional dehydration artifacts. The Northeast slides showed atrophic and poorly differentiated changes, while mild hyperplastic alterations appeared in the Northwest and Southeast

zones. Overall, cadmium chloride exposure did not significantly disrupt prostate histoarchitecture, but distinct differences in haematoxylin stain uptake were observed across regions. Harris stains generally produced darker, less differentiated sections, while Cole's stains yielded comparatively clearer images. These findings highlight the importance of stain quality and preparation in ensuring reliable histopathological interpretation and adherence to standards.

**Keywords:** Haematoxylin stain; Cadmium chloride; Prostate histology; Histopathology; Staining variability; Nigeria Geopolitical zones

## INTRODUCTION

Haematoxylin is one of the most widely used stains in histopathology, valued for its ability to delineate nuclear and tissue architecture with clarity [1]. Different formulations such as Harris, Cole's, Mayer's, and Weigert's vary in composition, staining intensity, and affinity for cellular structures, which may influence diagnostic outcomes [2]. While Harris haematoxylin is widely applied in routine practice, alternatives like Cole's and Mayer's have been reported to provide enhanced nuclear definition and tissue contrast [3].

Global studies have documented significant variability in histological staining outcomes even when using the same staining protocols. For example, a multi-laboratory study of hematoxylin and eosin (H&E) staining across various labs found large inter-laboratory variation in stain appearance, which can reduce reproducibility and diagnostic consistency [4]. A more recent method proposed quantitative assessment tools for H&E stain uptake to help standardize variability and improve quality assurance in pathology labs [5].

In Nigeria, there is limited published evidence addressing the regional variability of stain uptake itself; most histopathology studies focus on lesion prevalence or diagnostic patterns rather than the quality of staining per se. For instance, studies on prostate specimens report frequency of benign and malignant lesions using H&E stains, but do not compare how stain uptake differs depending on the source or preparation of haematoxylin across regions [6].

The prostate gland is clinically important due to its predisposition to hyperplasia, inflammation, and malignancy [7]. Heavy metals such as cadmium have been linked to oxidative stress and cellular injury within the prostate, making cadmium chloride a relevant experimental agent for evaluating tissue response [8]. Assessing how haematoxylin stains perform [9] in cadmium-exposed prostate tissue offers an opportunity to examine both the quality of staining and the diagnostic accuracy achievable under variable laboratory conditions [8].

This study therefore aimed to compare haematoxylin stain uptake in the prostate glands of Wistar rats exposed to cadmium chloride across Nigeria's six geopolitical zones. By evaluating differences in nuclear clarity, tissue differentiation, and histoarchitectural preservation, the study contributes to efforts toward improved standardization and quality assurance in histopathology practice.

## **MATERIALS & METHODS**

### **Animals and Ethical Approval**

Thirty-five adult male Wistar rats (180-200 g) were obtained from the University of Benin animal facility. Animals were housed under standard laboratory conditions (12-hour light/dark cycle,  $25 \pm 2$  °C, free access to food and water) and acclimatized for one week before experimentation. All protocols followed the guidelines of the University Ethical Review Committee (approval no. URDC 3035).

### **Experimental Design**

Rats were randomly assigned into experimental and control groups. Experimental groups received cadmium chloride (2 mg/kg body weight; Sigma-Aldrich, USA; analytical grade,  $\geq 99\%$  purity) via subcutaneous injection for 21 days at 3 days interval, while control animals received equal volumes of distilled water. Cadmium chloride administration at this dosage and duration has been validated in previous studies as sufficient to induce oxidative stress and histological alterations in rat prostate tissue [10,11].

### **Organ Collection and Processing**

At the end of exposure, rats were anesthetized with light chloroform and sacrificed by cervical dislocation. Prostates were excised, trimmed of adherent fat, blotted, and weighed. Organs were fixed in 10% neutral buffered formalin for 24 hours, dehydrated in ascending grades of ethanol, cleared in xylene, and embedded in paraffin wax.

### **Histological Staining**

Paraffin blocks were sectioned at 5  $\mu\text{m}$  using a rotary microtome (Leica RM2235, Germany). Sections were stained with hematoxylin and eosin (H&E) following standard procedures [9]. Harris and Cole's haematoxylin formulations were sourced from suppliers in each of Nigeria's six geopolitical zones. However, sections from the control group were stained with Harris and Cole's haematoxylin imported from

Leica Biosystems (21440 W. Lake Cook Road, Deer Park, IL 60010, USA), a leading manufacturer whose H&E reagents are used by multiple reference histology laboratories across the United States and Europe with proven consistency in staining quality. Slides were mounted with DPX and examined under a light microscope (Olympus CH20, Japan).

### Scoring System

Histological quality was assessed semi-quantitatively using parameters of nuclear clarity, cytoplasmic contrast, background staining, and overall histoarchitecture. Scoring was independently performed by two blinded histopathologists.

### STATISTICAL ANALYSIS

Data were expressed as mean  $\pm$  standard deviation (SD). One-way analysis of variance (ANOVA) was performed, followed by Tukey's post-hoc test for multiple comparisons. Statistical significance was set at  $p < 0.05$ .

### RESULT

#### General Observations

All animals tolerated cadmium chloride exposure without mortality. Experimental rats demonstrated reduced activity, mild piloerection, and decreased food intake compared to controls.

#### Relative Prostate Weights

There were no statistically significant differences in relative prostate weights between cadmium-exposed groups and controls ( $p > 0.05$ ) (Table 1).

#### Histological Scoring

Semi-quantitative scoring of histological quality revealed marked regional differences

in nuclear clarity, cytoplasmic contrast, background staining, and overall histoarchitecture (Table 2).

### Histological Observations

Microscopic examination of control prostate sections showed normal histoarchitecture, characterized by acini lined with columnar epithelium, papillary infoldings, eosinophilic secretions, and apical vacuolation (Figure 1).

In cadmium-exposed groups, prostate sections generally preserved normal architecture, though regional variations in haematoxylin uptake were observed across the six geopolitical zones.

- Southwest & South South: Sections stained intensely dark with Harris haematoxylin, leading to poor nuclear differentiation and obscured cytoplasmic detail.
- North Central & Southeast: Sections displayed relatively clearer staining, with well-defined nuclei and mild dehydration artifacts.
- Northeast: Slides showed atrophic glands with weak staining and poor differentiation.
- Northwest & Southeast: Mild hyperplastic changes were observed in some sections, with moderate clarity of nuclear details.

### Comparison of Haematoxylin Formulations

Harris haematoxylin generally produced darker sections with reduced nuclear clarity, while Cole's haematoxylin yielded more balanced staining with better tissue differentiation. Regional differences in stain preparation contributed to variability in staining outcomes (Figure 1).

**Table 1. Relative prostate weights of Wistar rats exposed to cadmium chloride across Nigeria's geopolitical zones**

Group	Mean prostate weight (g) $\pm$ SD	Relative prostate weight (%) $\pm$ SD	p-value versus Control
Control	0.41 $\pm$ 0.05	0.16 $\pm$ 0.02	–
South West (CdCl <sub>2</sub> )	0.39 $\pm$ 0.04	0.15 $\pm$ 0.02	>0.05
South South (CdCl <sub>2</sub> )	0.40 $\pm$ 0.06	0.15 $\pm$ 0.02	>0.05

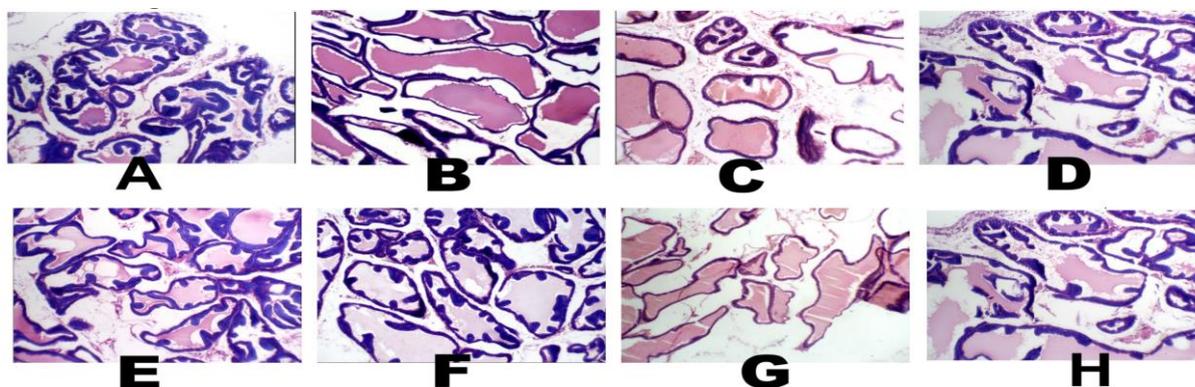
North Central (CdCl <sub>2</sub> )	0.38 ± 0.05	0.14 ± 0.01	>0.05
South East (CdCl <sub>2</sub> )	0.42 ± 0.07	0.16 ± 0.03	>0.05
North East (CdCl <sub>2</sub> )	0.37 ± 0.05	0.14 ± 0.02	>0.05
North West (CdCl <sub>2</sub> )	0.41 ± 0.06	0.16 ± 0.02	>0.05

**Legend:** Values are mean ± standard deviation (SD), n = 5 rats per group. Relative prostate weight calculated as (organ weight/body weight × 100). No statistically significant differences compared to control (ANOVA, p > 0.05).

**Table 2. Histological scoring of haematoxylin stain uptake in prostate sections of Wistar rats across Nigeria's geopolitical zones**

Group	Nuclear clarity (0–3)	Cytoplasmic contrast (0–3)	Background staining (0–3)	Overall histoarchitecture (0–3)
Control	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0
South West (CdCl <sub>2</sub> )	1.2 ± 0.4	1.5 ± 0.3	1.0 ± 0.5	1.4 ± 0.3
South South (CdCl <sub>2</sub> )	1.3 ± 0.5	1.6 ± 0.4	1.1 ± 0.4	1.5 ± 0.4
North Central (CdCl <sub>2</sub> )	2.4 ± 0.3	2.3 ± 0.3	2.2 ± 0.4	2.3 ± 0.2
South East (CdCl <sub>2</sub> )	2.2 ± 0.4	2.1 ± 0.5	2.0 ± 0.4	2.2 ± 0.3
North East (CdCl <sub>2</sub> )	1.0 ± 0.5	1.1 ± 0.3	1.0 ± 0.4	1.1 ± 0.3
North West (CdCl <sub>2</sub> )	2.0 ± 0.3	1.9 ± 0.4	1.8 ± 0.3	1.9 ± 0.4

**Legend:** Scoring scale: 0 = poor, 1 = fair, 2 = good, 3 = excellent. Values represent mean ± SD from two independent blind observers (n = 5 rats per group). Harris haematoxylin generally showed darker staining with reduced nuclear clarity, while Cole's haematoxylin produced clearer differentiation.



**Figure 1.** Photomicrographs of prostate sections stained with H&E using Harris or Cole's haematoxylin (×400). Variability in nuclear clarity and cytoplasmic contrast was observed across geopolitical zones.

(A) Harris haematoxylin-stained section from the control group showing normal histoarchitecture with acini lined by columnar epithelium, papillary infoldings, eosinophilic secretions, and apical vacuolation. (B) Southwest (CdCl<sub>2</sub>, Harris haematoxylin): intensely dark staining with poor nuclear differentiation. (C) South-south (CdCl<sub>2</sub>, Harris haematoxylin): similar dark uptake obscuring nuclear detail. (D) North-central (CdCl<sub>2</sub>, Cole's haematoxylin): clear nuclear staining with mild dehydration artifacts. (E) Southeast (CdCl<sub>2</sub>, Cole's haematoxylin): relatively balanced staining and preserved histoarchitecture. (F) Northeast (CdCl<sub>2</sub>, Cole's haematoxylin): weak uptake, poorly differentiated and atrophic glands. (G) Northwest (CdCl<sub>2</sub>, Harris haematoxylin): mild hyperplastic changes with moderate nuclear clarity. (H) Control section stained with Cole's haematoxylin, showing well-defined nuclear detail and preserved glandular architecture.

## DISCUSSION

This study evaluated the uptake of different haematoxylin formulations in the prostate

glands of cadmium chloride-exposed Wistar rats across Nigeria's six geopolitical zones. The findings demonstrated that although

prostate histoarchitecture was generally preserved, there were marked regional variations in staining quality, particularly in nuclear clarity and tissue differentiation.

The absence of significant differences in relative prostate weights between exposed and control groups (Table 1) indicates that the experimental dose of cadmium chloride induced histological rather than gross morphological alterations. This observation aligns with previous reports that subacute cadmium exposure produces subtle microscopic lesions instead of overt organ hypertrophy [10,11]. In human studies, disturbances in trace metals such as cadmium and zinc have been implicated in prostate pathology, reinforcing the biological plausibility of cadmium-induced changes [12]. Thus, organ weight measurements alone may underestimate toxicological effects, highlighting the need for histological evaluation as a more sensitive endpoint.

Semi-quantitative histological scoring (Table 2) revealed that while control samples achieved maximum scores across all parameters, cadmium-exposed groups showed region-specific differences. The Southwest and South-south zones had the lowest nuclear clarity and background scores, consistent with the overly dark staining patterns observed in Figure 1B and 1C. Conversely, the North-central and Southeast samples demonstrated higher scores, with clearer nuclear details and preserved morphology (Figure 1D and 1E). The weakest outcomes were recorded in the Northeast, where poor nuclear definition and glandular atrophy were observed (Figure 1F). Northwest samples presented moderate scores with some hyperplastic changes (Figure 1G). These findings reflect the impact of inconsistent stain preparation on diagnostic clarity, a challenge also highlighted in previous Nigerian work documenting dye-tissue variability in human histological sections [13].

Globally, variability in H&E staining has been recognized as a major barrier to reproducibility. Dunn et al. report

significant inter-laboratory differences that compromise diagnostic accuracy [4], and later work introduced quantitative methods to improve consistency [5]. In Nigeria, however, histopathology research has focused largely on lesion prevalence rather than stain quality [6]. The current study therefore provides novel data showing that variability exists not only between countries but also within a single nation, raising important implications for diagnostic reliability.

The photomicrographs (Figure 1A-G) further underscore these findings. Control tissues displayed intact glandular structure with clear acini, while cadmium-exposed tissues showed staining outcomes ranging from overly dark to poorly differentiated. These results are consistent with earlier animal studies that demonstrated cadmium-induced histological alterations in prostate tissues, including hyperplasia and inflammatory changes [14]. More recently, environmental exposure studies have suggested that cadmium may increase prostate cancer risk in Nigerian men, adding human relevance to the present experimental findings [15].

The strength of this study lies in its multi-regional design and use of blinded histopathologist scoring, which reduces observer bias. However, limitations must be acknowledged. Chemical analysis of the haematoxylin formulations was not performed, leaving the exact causes of variability undetermined. In addition, the modest sample size per region (n=5) may have limited statistical power. Despite these weaknesses, the consistent trends observed across Tables 1 and 2, supported by Figure 1, reinforce the validity of the findings.

In other words, this study demonstrates that while cadmium chloride exposure did not significantly affect prostate weights, substantial regional differences in haematoxylin stain uptake were observed across Nigeria. These differences may result from variations in dye composition, preparation, and storage, which are often influenced by local laboratory practices and

resource availability [11]. To improve reproducibility and diagnostic accuracy, harmonized protocols, regular quality assurance, and capacity building for pathology services in Nigeria and globally by extension are urgently required [11].

## CONCLUSION

This study demonstrated that while cadmium chloride exposure did not significantly alter prostate weights, there were clear regional differences in haematoxylin stain uptake. The strength of this work lies in its multi-regional design and blinded histological assessment, which provided robust evidence of variability in staining quality. However, the absence of chemical analysis of the stains and the modest sample size are recognized limitations. Overall, the findings underscore the need for standardized staining protocols and quality assurance measures to ensure diagnostic reliability in histopathology laboratories.

## Declaration by Authors

**Ethical Approval:** Approved

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**Conflict of Interest:** The authors declare no conflict of interest.

## REFERENCES

1. Kiernan JA. Histological and Histochemical Methods: Theory and Practice. 5th ed. Bloxham, UK: Scion Publishing; 2015.
2. Fischer AH, Jacobson KA, Rose J, Zeller R. Hematoxylin and eosin staining of tissue and cell sections. CSH Protoc. 2008 May 1;2008:pdb. prot4986. doi:10.1101/pdb. prot4986. PMID: 21356829.
3. Malatesta M. Histological and histochemical methods: theory and practice. Eur J Histochem. 2016;60(1):2639. doi:10.4081/ejh.2016.2639
4. Dunn C, Brett D, Cockroft M, et al. Quantitative assessment of H&E staining for pathology: development and clinical evaluation of a novel system. Diagn Pathol. 2024;19(1):42. doi:10.1186/s13000-024-01461-w
5. Dunn C, Brett D, Hodgson C, et al. An international study of stain variability in histopathology using qualitative and quantitative analysis. J Pathol Inform. 2025; 17:100423. doi: 10.1016/j.jpi.2025.100423
6. Adediran AO, Olatunbosun EI. Descriptive study of prostate lesions in largest hospital in Ondo State of Nigeria. Glob J Health Sci. 2020;5(2):18-39. Available from: <https://ideas.repec.org/a/bdu/ojgijhs/v5y2020i2p18-39id1131.html>
7. De Marzo AM, Platz EA, Sutcliffe S, et al. Inflammation in prostate carcinogenesis. Nat Rev Cancer. 2007;7(4):256–69. doi:10.1038/nrc2090
8. Waalkes MP. Cadmium carcinogenesis. Mutat Res. 2003;533(1-2):107-20. doi: 10.1016/j.mrfmmm.2003.07.011
9. Bancroft JD, Gamble M. Theory and Practice of Histological Techniques. 7th ed. London: Churchill Livingstone Elsevier; 2008.
10. Acharya UR, Mishra M, Patro J, Panda MK. Effect of vitamins C and E on spermatogenesis in mice exposed to cadmium. Reprod Toxicol. 2008;25(1):84–88. <https://doi.org/10.1016/j.reprotox.2007.10.004>
11. El-Demerdash FM, Yousef MI, Kedwany FS, Baghdadi HH. Cadmium-induced changes in lipid peroxidation, blood hematology, biochemical parameters, and semen quality of male rats: protective role of vitamin E and  $\beta$ -carotene. Food Chem Toxicol. 2004;42(10):1563-71. doi: 10.1016/j.fct.2004.05.001
12. Ogunlewe JO, Greer B, Willis J, Gosh C. Zinc and cadmium concentrations in indigenous blacks with prostate disorders. Cancer. 1989;63(7):1388-92. [https://doi.org/10.1002/1097-0142\(19890401\)63:7<1388::AID-CNCR2820630725>3.0.CO;2-M](https://doi.org/10.1002/1097-0142(19890401)63:7<1388::AID-CNCR2820630725>3.0.CO;2-M)
13. Onyije FM, Ngokere AA, Mgbere OO, Ligha AE. The preponderance and dye-tissue receptive variability analyses of malignant and benign lesions of the female genitalia. J Oncol Sci. 2017;3(1):12-7. <https://doi.org/10.1016/j.jons.2017.02.002>
14. Okorochi CM, Nifemi SM, Chibuikwe OE, et al. Comparative efficacy of prostate tumor

induction methods in Wistar rats. *Asian Pac J Cancer Biol.* 2025;10(3):623-8. <http://www.waocp.com/journal/index.php/apjcb/article/view/1866>

15. Bede-Ojimadu O, Orisakwe OE. Cadmium exposure and the risk of prostate cancer among Nigerians. *Environ Toxicol Pharmacol.* 2023; 98:103713. DOI: 10.1016/j.jtemb.2023.127168

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