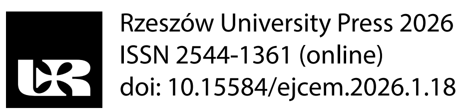
Rzeszów University Press 2026
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2026.1.18

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2026; 24 (1): 104–111

ORIGINAL PAPER

Histochemical evaluation of ethanol extracts of *Senecio biafrae* leaves in mercury chloride-induced hepatic injury in adult male Wistar rats

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ABSTRACT

Introduction and aim. Mercury chloride is a potent hepatotoxin that disrupts liver architecture, glucose metabolism, and nuclear integrity. To our knowledge, no previous study has evaluated the histochemical effects of the ethanol extract of *Senecio biafrae* leaves (EESBL) on mercury chloride-induced hepatic injury. This study presents new evidence for the glycogen stabilizing and genoprotective properties of its compounds.

Material and methods. Forty-nine adult Wistar rats were randomly assigned to seven groups (n=7 per group). Except for the control, all received 4 mg/kg mercury chloride orally for 21 days. Group II rats were sacrificed immediately after exposure, while group III underwent a 21-day recovery. Group IV received 2 mg/kg silymarin, and also Groups V–VII received 200, 400, and 600 mg/kg EESBL, respectively, for 21 days. Liver tissues were harvested for histochemical evaluation using periodic acid-Schiff (PAS) and Feulgen staining.

Results. Mercury chloride significantly depleted liver glycogen stores (PAS-positive area: control 75.00±0.56% vs toxic 20.00±1.09%). EESBL restored glycogen storage in a dose-dependent manner (200 mg/kg: 52.02±0.56%; 400 mg/kg: 60.00±0.57%; 600 mg/kg: 72.06±0.57%), approaching silymarin (68.00±0.57%). Nuclear DNA integrity was markedly affected by $HgCl_2$ (Feulgen-positive area: control 16.20±0.19% vs toxic 9.00±0.33%). EESBL improved nuclear morphology and DNA intensity (200 mg/kg: 11.11±0.12%; 400 mg/kg: 13.20±0.44%; 600 mg/kg: 14.06±0.33%), comparable to silymarin (14.00±0.25%) (all $p<0.001$).

Conclusion. EESBL demonstrated protective effects against mercury chloride-induced hepatotoxicity by stabilizing hepatic glycogen metabolism and nuclear structure, underscoring its therapeutic potential in mitigating heavy metal-induced liver injury.

Keywords. DNA integrity, Feulgen staining, glycogen storage, hepatic injury, mercury chloride, PAS reaction

Introduction

Mercury chloride ($HgCl_2$), a highly toxic inorganic compound, is commonly encountered through environmental and occupational sources such as mining, industrial effluents, battery manufacturing and pharmaceutical waste disposal.[1,2] Exposure in humans and animals occurs primarily through the consumption of tainted food or water, breathing in airborne contaminants, or contact with the skin. Once absorbed, mercury ions bind to sulfhydryl groups in proteins and also accumulate in vital organs, particularly the liver and kidneys.[3,4] In the liver, mercury disrupts key cellu-

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Received: 29.06.2025 / Revised: 30.09.2025 / Accepted: 28.10.2025 / Published: 30.03.2026

Ibitoye AO, Adeyemi DO. Histochemical evaluation of ethanol extracts of *Senecio biafrae* leaves in mercury chloride-induced hepatic injury in adult male Wistar rats. *Eur J Clin Exp Med.* 2026;24(1):104–111. doi: 10.15584/ejcem.2026.1.18.

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lar processes, including glucose metabolism and DNA stability.[5,6] The liver is essential for metabolic control, encompassing glycogen storage, detoxification, and maintenance of genomic integrity.[7,8] Its highly organized architecture rich in hepatocytes and a structured extracellular matrix (ECM) supports these vital physiological functions. As the primary site for glycogen synthesis and storage, the liver maintains the systemic energy balance.[7-9] Furthermore, the nucleus safeguards genetic material, making DNA integrity essential for liver regeneration and function.[10]

Mercury chloride doses in rodent models are typically classified as low, moderate, or high based on toxicological and experimental data. Low doses (1 mg/kg/day) mimic chronic environmental exposure and generally result in mild biochemical or histological changes without overt tissue damage.[11] Moderate doses (2–7 mg/kg) are associated with oxidative stress, DNA damage, and mild to moderate liver or renal lesions.[12] High doses (7 mg / kg) induce acute toxicity, characterized by hepatocellular necrosis, nuclear condensation, and widespread tissue degeneration.[13] These dose classifications are critical to modeling human exposure and evaluating the efficacy of therapeutic interventions.

To evaluate alterations in liver tissue, histochemical staining techniques offer a precise visualization of intracellular biochemical changes at the microscopic level.[14] The periodic acid-Schiff reaction (PAS) detects glycogen deposits, while the Feulgen reaction identifies nuclear DNA, providing sensitive markers of cytoplasmic and nuclear integrity, respectively. Together, these staining methods enable a comprehensive histochemical assessment of hepatocyte injury and recovery in experimental models of hepatotoxicity.[15]

In the search for safe and effective interventions, the therapeutic value of medicinal plants has drawn interest in mitigating toxicant-induced tissue damage.[16,17] *Senecio biafrae*, a leafy vegetable widely consumed in West Africa (including Nigeria, Benin and Ghana), is traditionally valued for its nutritional and therapeutic properties.[18] Phytochemical studies have identified flavonoids, phenolics, alkaloids, saponins, tannins, and vitamins as key bioactive constituents of *S. biafrae*.[19] These substances are recognized for their anti-inflammatory, antioxidant, and also cytoprotective activities. However, the specific effects of these constituents on liver histoarchitecture after mercury chloride-induced hepatotoxicity remain largely underexplored.

Aim

This study aims to investigate the histochemical effects of the ethanol extract of *S. biafrae* leaves (EESBL) on mercury-chloride-induced hepatic injury, specifically its ability to restore glycogen reserves and maintain nuclear DNA integrity. To our knowledge, this is the first study to provide histochemical evidence of the protective effects of *S. biafrae* on mercury-induced alterations in hepatic metabolism and nuclear architecture.

Material and methods

Chemicals and drugs

Mercury chloride (white crystalline form; British Drug Houses Ltd., Poole, England) was used as a hepatotoxic agent. Silymarin tablets (Silybon-70® 70 mg; Micro Labs Ltd., India) served as the reference hepatoprotective agent. Diethyl ether (≥ 99.8%; British Drug Houses Ltd., Poole, England) was utilized for anesthesia.

Gathering and authentication of plant materials

For this investigation, green *Senecio biafrae* leaves were collected and authenticated at Obafemi Awolowo University's Department of Botany in Ile-Ife. For future use, a sample from the voucher (IFE/18215) was placed in the departmental herbarium.

The preparation of ethanol extract of Senecio biafrae leaf

The collected leaves were air dried, pulverized, and extracted three times with 80% ethanol at standard temperature under continuous magnetic stirring every 24 hours. The combined extracts were filtered using Whatman No. 1 filter paper, condensed in a low pressure rotary evaporator and then lyophilized. Before being used, the drained extract was kept in a drying device.[20]

Experimental animals

The Animal Holding, College of Health Sciences, Obafemi Awolowo University, Ile-Ife, provided 49 adult male Wistar rats weighing between 180 and 200 grams. The animals were kept in conventional laboratory settings (12 hours light/dark cycle, temperature, and relative humidity) with unlimited access to clean water and standard rat food (Ace Feeds, Osogbo, Nigeria). The Institute of Public Health, Obafemi Awolowo University, Ile-Ife's Health Research and Ethics Committee (HREC) granted ethical approval (IPH/OAU/12/2543). In accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals, the rats were treated humanely.[21]

Experimental design

Animals were randomly assigned to seven groups (n=7 per group)

- Group I (Control): Distilled water (2 mL/kg, through oral) for 42 days.
- Group II (Toxic control): Mercury chloride (4 mg/kg/day, orally) for 21 days; sacrificed 24 h after the last dose.
- Group III (Recovery): Mercury chloride (4 mg/kg/day, orally) for 21 days; observed without treatment for another 21 days.

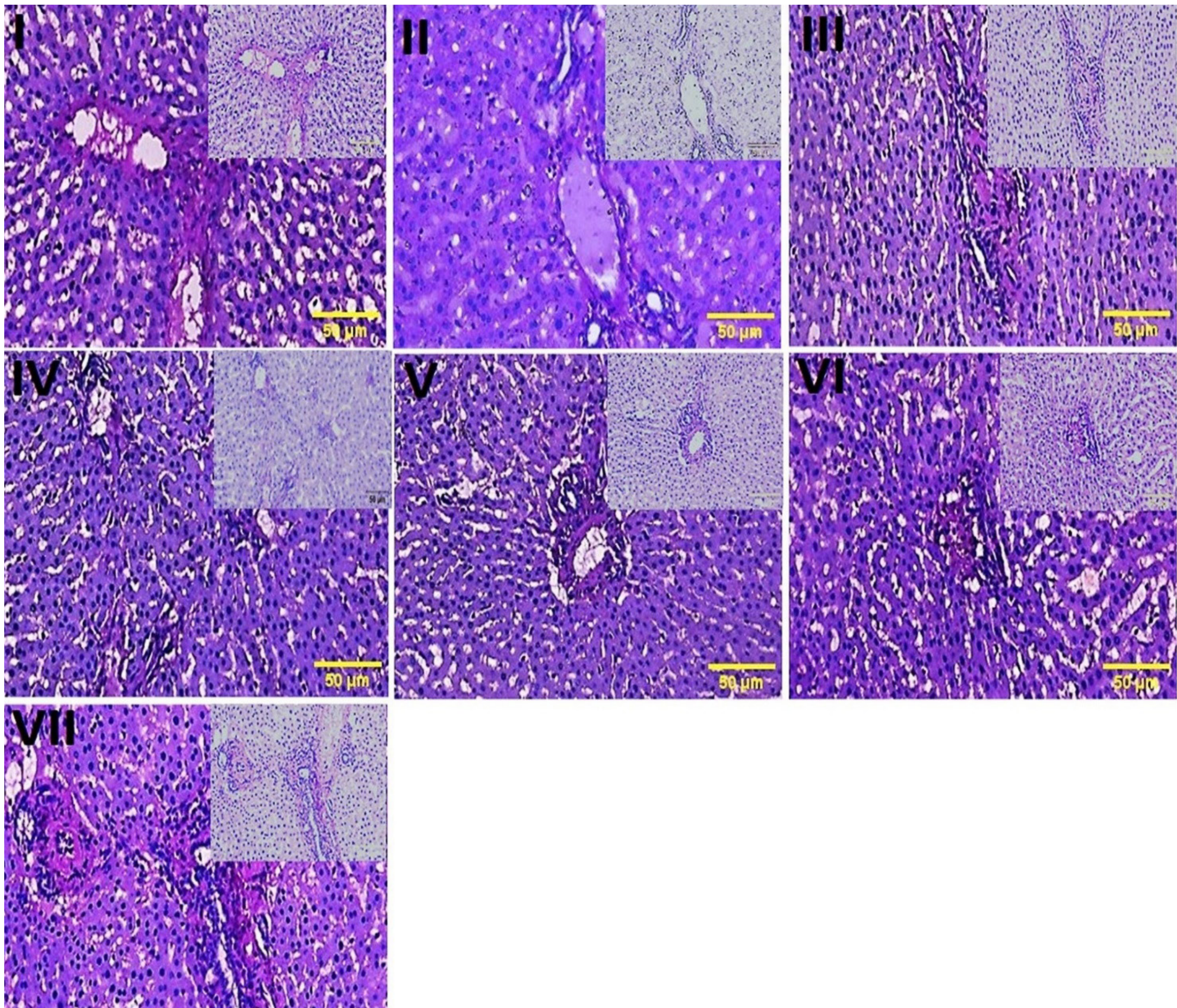

Fig. 1. Representative microscopy images of PAS-stained liver sections, I: Diffuse, severe PAS signal staining with uniform distribution throughout the lobule (normal glycogen storage), II: Markedly reduced PAS positivity with an irregular staining pattern, III, V: Partial restoration of PAS reactivity with improved, yet nonuniform staining, IV, VI, VII: Moderate PAS positivity indicates restored glycogen accumulation in hepatocytes. Insets: Diastase-digested sections serving as negative control (scale bar=50 µm)

- Group IV (Standard treatment): Mercury chloride (4 mg/kg/day, orally) for 21 days; followed by silymarin (2 mg/kg, orally 12-hourly) for 21 days.
- Group V-VII (Test treatment): Mercury chloride (4 mg/kg/day, orally) for 21 days; followed by ethanol extract of EESBL at 200, 400, and 600 mg/kg/day, respectively, for 21 days.

Tissue collection and processing

All rats were sacrificed under diethyl ether anesthesia twenty-four hours after the last treatment. The entire liver was excised and weighed and the median lobe was harvested uniformly from all animals to eliminate lobe-specific variability in response to toxic insult.[22]

Histochemical procedure

10% neutral buffered formalin (NBF) was used to fix liver samples by total immersion for 48 hours for the histochemical demonstration of glycogen and DNA. The Drury and Wallington paraffin wax embedding technique was used to process tissues for light microscopic examination.[23]

Process for periodic acid-Schiff (PAS) Staining

The PAS reaction was used for the demonstration of (carbohydrate) glycogen deposition. Deparaffinized tissue sections were rehydrated through descending alcohol concentrations and then treated with a 1% aqueous periodic acid solution for 10 minutes; This oxidation step creates aldehyde groups from tissue carbohydrates, which then react with Schiff reagent for 30 minutes,

turning the tissue sections a light pink color. After being washed in lukewarm water, the sections darken to vibrant pink. In contrast, the tissue was counterstained with Harris hematoxylin, turning the nuclei blue. The glycogen and other carbohydrates are stained with magenta, allowing their identification in the tissue. In PAS with diastase digestion (PAS-D), tissue sections were pretreated with amylase enzyme to digest glycogen, leaving only other carbohydrate deposits visible, helping to identify glycogen content.[23, 24]

Feulgen staining procedure

The Feulgen reaction was used to demonstrate the contents of deoxyribonucleic acid (DNA) contents. The procedure was carried out as described by Feulgen and Rossenbeck (1924).[23] This method relies on the acid hydrolysis of DNA to expose aldehydes on deoxyribose sugar, which are then detected by Schiff's reagent. Deparaffinized tissues were rehydrated in descending concentrations of alcohol for 2 minutes each, followed by mild hydrolysis in prewarmed 1N hydrochloric acid at 60ºC for 8 minutes. After rinsing, Schiff's reagent was applied for 1.5 hours, which binds to exposed aldehydes, resulting in a magenta color. The sections are then treated with sulfurous acid and washed with running water before being counterstained with light green for contrast. DNA is stained magenta, while the cytoplasm is stained green. At the same time, as the test sections were hydrolyzed, the control sections were also left in distilled water for the same time for hydrolysis. Both the test and control sections go through the same procedure and process. The control section was negative, showing no trace of magenta coloration, confirming the specificity of the reaction.[23,25]

Photomicrography and image analysis

Permanent pictures of stained sections were captured using a LEICA research microscope (DM750) attached to a digital photography device (LEICA ICC50). On every micrograph, the scale bars were combined. Using a computer that runs image analysis software (ImageJ® NIH, US) according to the manufacturer's instructions, PAS and Feulgen stained micrographs were measured for staining intensity and nuclear damage using the approach described by Amber.[26] To analyze particular regions of the micrographs, ImageJ® region of interest (ROI) management tool was used. After obtaining average gray values for each of the three ROI, the means were calculated and examined.

Statistical analysis

GraphPad Prism (version 9.3, GraphPad Software, San Diego, CA, USA) was used to analyze the data, and the results were presented as mean±standard error of the mean (SEM). One-way analysis of variance (ANOVA) with the Tukey post hoc test was used to evaluate whether there were significant differences between the means of the group. A 0.05 alpha threshold was considered significant.

Results

Effect of ethanol extract of S. biafrae leaves on hepatic glycogen

As depicted in Figure 1, a photomicrograph of liver sections subjected to PAS staining revealed that group I rats showed normal glycogen storage within the cytoplasm of their hepatocytes, evident by the intense and diffuse PAS staining, while in group II rats, mercury chloride caused abnormal glycogen storage, exhibited by a marked reduction in PAS positivity, reflecting glycogen depletion. However, EESBL-treated groups (V-VII) showed dose-dependent restoration of PAS staining, demonstrated by the consistent glycogen storage, with the 600 mg/kg dose showing near-normal glycogen distribution. This was comparable to the silymarin-administered rats, which had considerable glycogen reserves. The digested region of the glycogen store was disclosed by diastase control among the set (Insets, Fig. 1).

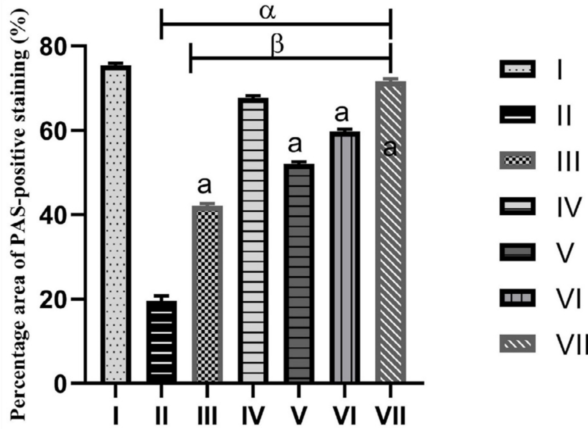

Fig. 2. Percentage area of PAS-positive staining for glycogen granules of the liver of adult Wistar rats exposed to $HgCl_2$ toxicity. Each bar represents Mean±SEM. The bars with superscript (α) are significantly different from I; the groups with alphabet (a) are significantly different from IV, however, the bars with superscript (β) are significantly different from II, using one-way ANOVA, Tukey test at $p<0.05$

Glycogen store morphometry

As shown in Figure 2, the percentage area of PAS-positive staining within the Region of Interest (ROI) in the seven experimental groups. Group I rats showed high PAS positivity (75.00±0.56), which indicates abundant glycogen stores compared with group II rats with very low PAS positivity (20.00±1.09) due to glycogen depletion caused by mercury chloride. There is a partial resto-

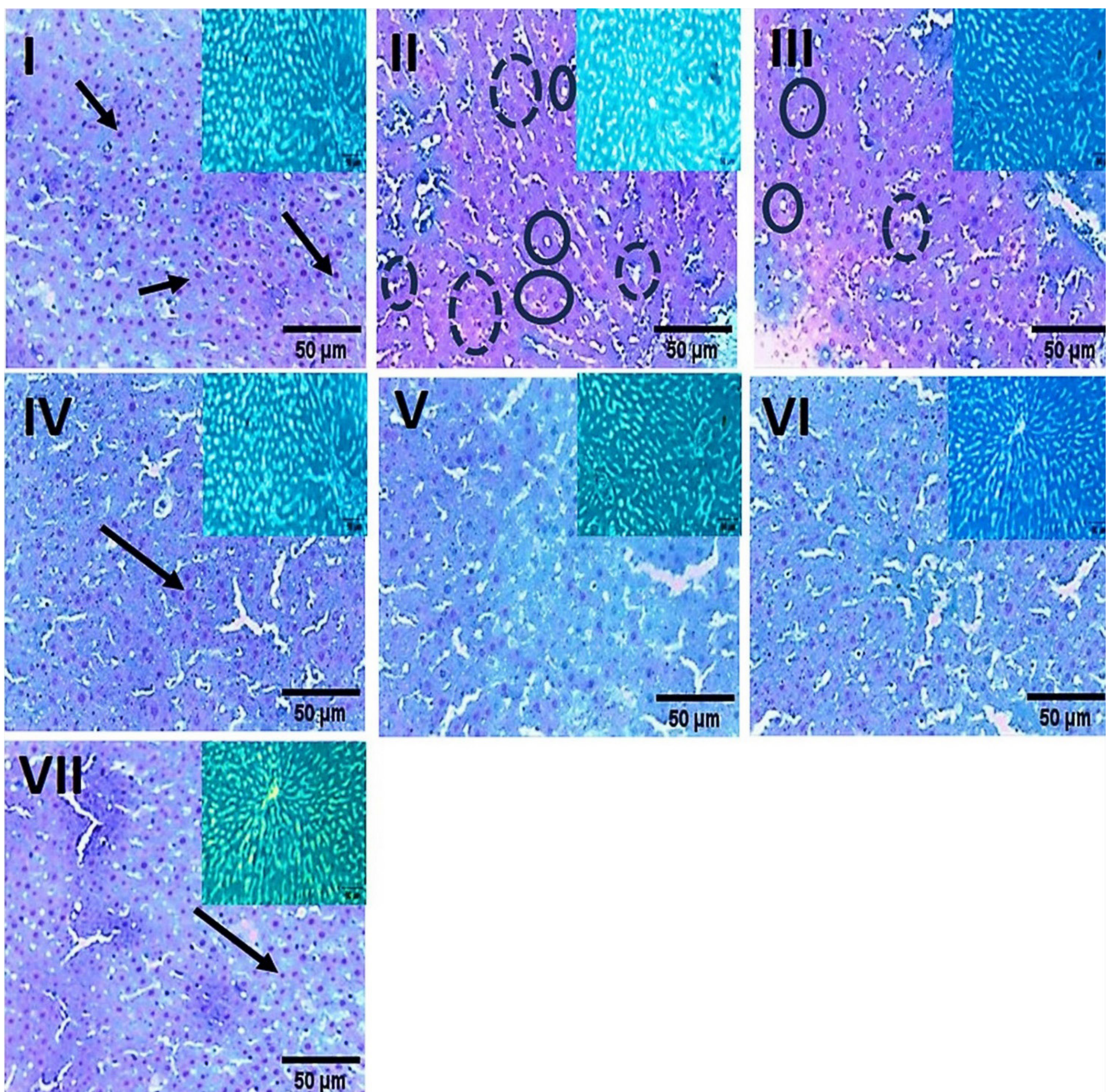

Fig. 3. Representative photomicrographs of liver sections stained with the Feulgen reaction for DNA, I: uniform magenta staining of nuclei, with regular nuclear size, shape, and even chromatin distribution (normal), II: Irregular nuclear morphology, chromatin fading (rounded circles), and fragmentation (dotted circles); prominent pyknosis, III, V: Reduced chromatin condensation, fewer pyknotic nuclei, and improved nuclear morphology, IV, VI, VII: Moderately restored nuclear features with reduced chromatin condensation. Insets: Unhydrolyzed sections showing the absence of DNA-specific staining (scale bar=50 µm)

ration of glycogen stores in group III rats (42.10±0.57), in contrast to the control group and the treatment groups. Silymarin-treated group IV rats displayed near-normal glycogen stores (68.00±0.57) in contrast to EESBL-treated rats and group I rats. EESBL-treated rats (groups V-VII) dose-dependently restored glycogen stores (52.02±0.56, 60.00±0.57, 72.06±0.57, respectively) compared with group II rats (20.00±1.09). 200 and 400 mg/kg EESBL showed moderate recovery of glycogen stores, in contrast to the control group, although 600 mg/kg EESBL almost matched the control group ($F=833.00$; $p<0.001$).

Effect of the ethanol extract of S. biafrae leaves on hepatic nuclear DNA

Photomicrograph Representative sections of liver exposed to Feulgen staining, as shown in Figure 3, revealed the following observations: group I rats exhibited well-defined Hepatocyte nucleoplasm has euchromatic nuclei with unique nuclear DNA, while mercury chloride caused DNA fragmentation and lightly stained nuclei, chromatin condensation, and pleomorphism, indicating DNA degradation as observed in group II rats. Groups III and V rats showed reduced chromatin condensation, improved nuclear morphology, and few-

er pyknotic nuclei. However, EESBL treatment markedly restored nuclear DNA staining, especially in the 400 600 mg/kg groups, preserving nuclear morphology similar to that of the silymarin-treated group IV rats. Unhydrolyzed sections (control) showed no specific nuclear staining (Insets, Fig. 3).

Nuclear DNA morphometry

As shown in Figure 4, the percentage area of Feulgen stained nuclear DNA in the seven experimental groups revealed that mercury chloride significantly distorts nuclear morphology, indicating nuclear damage and DNA loss in group II rats (9.00±0.33) when compared to group I rats (16.20±0.19), which revealed intact nuclear DNA and normal nuclear morphology. There is a slight improvement in group III rats (11.00±0.31) when compared to group II rats, which suggests partial spontaneous recovery, but is still insufficient to restore DNA integrity. However, EESBL demonstrated a dose-dependent improvement in the restoration of nuclear DNA integrity, evident by a significant increase ($p < 0.001$) in the nuclear DNA in rats given EESBL (11.11±0.12, 13.20±0.44, 14.06±0.33, respectively) compared with group II rats (9.00±0.33). Silymarin-treated group IV rats had a substantial rise in area covered by nuclear DNA (14.00±0.25) when contrasted with the toxic group (9.00±0.33); this is comparable to 600 mg/kg EESBL (group VII), suggesting that there is an increased restorative effect at a higher dose of EESBL comparable to the control group ($F=55.36$; $p<0.001$).

Discussion

Mercury-induced hepatotoxicity remains a major toxicological concern due to its deleterious effects on hepatic nuclear integrity and metabolic regulation. The liver, as a vital organ in detoxification and glucose homeostasis, is highly susceptible to $HgCl_2$ toxicity. In this study, $HgCl_2$ exposure resulted in marked hepatic glycogen depletion and nuclear damage, evidenced by reduced PAS and Feulgen staining. These findings align with the established role in inducing oxidative stress, altering glycogen metabolism, and promoting DNA strand breaks.[2,3] Under normal physiology, the liver stores glycogen postprandially and mobilizes it during fasting via glycogenesis and glycogenolysis.[27,28] Mercury disrupts this balance by inhibiting key enzymes such as glycogen synthase, glucose-6-phosphatase, and phosphoenolpyruvate carboxykinase (PEPCK), leading to hypoglycemia and energy deficits.[29,30] Cytologically, this manifests as reduced PAS positive hepatocytes and cytoarchitectural disarray. This finding aligns with a study of Mohamed et al.[31] who observed weakened PAS reactions in rats treated with $HgCl_2$, confirming the toxic impact of mercury on liver glycogen stores. In particular, the administration of the EESBL ethanol extract at 400 and 600 mg/kg preserved glycogen stores, restored PAS reactivity, and maintained hepatocyte structure, comparable to silymarin treatment. This cytoprotective effect is likely due to the antioxidative and enzyme-stabilizing properties of EESBL.

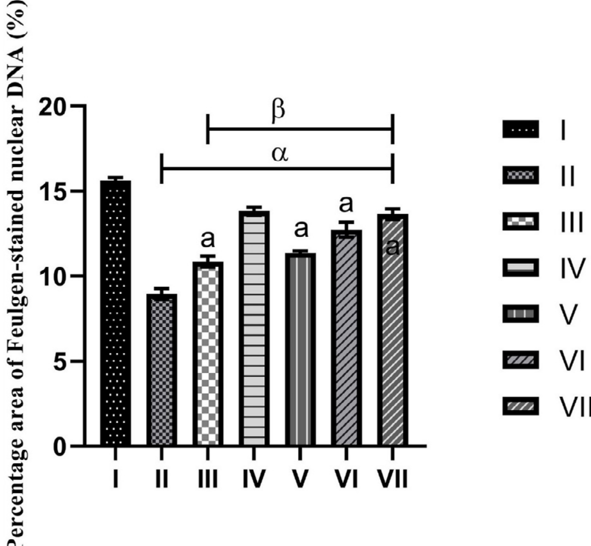

Fig. 4. Percentage area of Feulgen stained by nuclear DNA of adult Wistar rats exposed to $HgCl_2$ toxicity. Every line shows the mean±SEM the bars with superscript (α) are noticeably distinct from I; the groups with alphabet (a) are substantially different from IV, however, the bars with By one-way ANOVA and the Tukey test at $p<0.05$, superscripts (β) are substantially distinct from II

Similarly, mercury-induced genotoxicity, marked by chromatin condensation, pyknotic nuclei, nuclear fragmentation, and nucleolar disruption, was improved in EESBL-treated groups, which showed improved Feulgen staining and fewer nuclear abnormalities. Mercury chloride-induced oxidative stress is known to trigger DNA fragmentation and suppress DNA repair pathways,[32] including base and nucleotide excision repair.[33,34] Beyond direct DNA damage, mercury chloride impairs the cellular capacity for DNA repair.[34] Its exposure has been shown to downregulate the expression of critical repair base excision enzymes are among the DNA healing enzymes, and also nucleotide excision repair pathways.[35,36] The observed nuclear restoration suggests EESBL mitigates genomic instability and promotes hepatocellular regeneration in a dose-dependent manner comparable to the Silymarin-treated group. By eliminating free radicals, chelating mercury ions, and boosting antioxidant enzymes, phytochemicals in EESBL, especially flavonoids, may prevent mercury toxicity and maintain nuclear integrity and the breakdown of glycogen.

This research shows, for the first time, the histochemical evidence of *S. biafrae's* cytoprotective, hepato-

protective, and genoprotective effects in $HgCl_2$-induced toxicity, reinforcing its therapeutic potential beyond traditional antioxidant models.

Conclusion

Histochemical analyzes using PAS and Feulgen staining, in this study, have provided compelling evidence of the metabolic and nuclear disturbances caused by mercury chloride in the liver. These findings highlight the potential effect of the ethanol extract of *S. biafrae* leaves on stabilizing hepatic metabolic and nuclear architecture, reinforcing its relevance in mitigating mercury chloride-induced disruptions in liver structure and function.

Further studies should investigate the molecular pathways through which EESBL exerts its effect, particularly its influence on Nrf2/ARE signaling, mitochondrial biogenesis, apoptosis regulation (eg, Bcl-2, Bax, Caspase-3), and DNA repair gene expression, to elucidate its mechanisms at transcriptional and proteomic levels.

Acknowledgements

The authors thank Mr. M.S. Ige of Obafemi Awolowo University, Department of Anatomy and Cell Biology, for his technical help in preparing the histochemical slides.

Declarations

Funding

No particular grant from governmental, private, or charity funding organizations was obtained for this study.

Author contributions

Conceptualization, A.O.I. and D.O.A.; Methodology, A.O.I. and D.O.A.; Software, A.O.I.; Validation, D.O.A.; Investigation, A.O.I.; Writing – Original Draft Preparation, A.O.I.; Writing – Review & Editing, A.O.I. and D.O.A.; Visualization, D.O.A.; Supervision, D.O.A.

Conflicts of interest

No competing goals are disclosed by the writers.

Data availability

The appropriate author can provide the datasets created and examined for this study upon adequate request.

Ethics approval

The Health Research and Ethics Committee of the Institute of Public Health at Obafemi Awolowo University in Ile-Ife granted ethical approval (IPH/OAU/12/2543). The National Institutes of Health's Guide for the Care and Use of Laboratory Animals was followed in providing rats with proper care.

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