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Histological Characterization of Small Intestinal Tissue Changes Resulting from Chloroquine Treatment in Wistar Rats

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Abstract: This study investigated the histological effects of chloroquine on the small intestine of Wistar rats, with emphasis on chloroquine-induced alterations in intestinal structure and function. Twenty adult Wistar rats (190–250g) were randomly allocated into four groups of five. The control group received distilled water, while the experimental groups were administered oral chloroquine at doses of 10mg/kg, 25mg/kg, and 50mg/kg body weight for 21 days. Body weight was recorded throughout the study. At the end of the exposure period, the rats were sacrificed, and their small intestines were dissected, measured, and processed for histological assessment using hematoxylin and eosin staining. Data were expressed as mean, and expressed in chat using Microsoft Excel version 16. Chloroquine-treated rats demonstrated a clear dose-dependent reduction in body weight, intestinal length, and intestinal weight compared with controls. Histological evaluation revealed progressive structural deterioration characterized by villous atrophy, epithelial degeneration, mucosal erosion, ulceration, and inflammatory cell infiltration, with these alterations becoming more pronounced at higher chloroquine doses. These findings highlight the importance of cautious chloroquine use and the need for further studies into its long-term effects on the gastrointestinal system.

Keywords*: Chloroquine, Wistar Rats, Small Intestine, Histology, Toxicity.*

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I. INTRODUCTION

Chloroquine, a 4-aminoquinoline derivative, has served as a cornerstone therapy for malaria and remains widely used in the management of autoimmune conditions such as rheumatoid arthritis and systemic lupus erythematosus (Cooper and Magwere, 2020; White, 2017). Despite its longstanding therapeutic significance, accumulating evidence indicates that prolonged or high-dose administration may result in multisystem toxicity, with documented adverse effects on the hepatic, cardiac, and neurological systems (Izunya et al., 2019; Cooper and Magwere, 2020). More recently, concerns have emerged regarding chloroquine's potential to induce structural and functional disturbances within the gastrointestinal tract, particularly the small intestine, a region essential for nutrient assimilation, mucosal immunity, and metabolic regulation (Li et al., 2022a).

The small intestine's intricate mucosal architecture—comprising villi, crypts, and specialized epithelial cell populations—underpins its absorptive efficiency and barrier functions (Hryn et al., 2019). Disruption of these structures, including villus atrophy, epithelial degeneration, or crypt disorganization, can compromise nutrient uptake and predispose to broader systemic dysfunctions (Sood et al., 2021). Although several pharmacological agents have been

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implicated in drug-induced intestinal injury in experimental models (Li et al., 2022b; Hussain et al., 2024), investigations specifically addressing the histopathological consequences of chloroquine exposure remain comparatively limited. Mechanistic insights suggest that chloroquine's accumulation within lysosomes and its interference with intracellular processes may precipitate oxidative stress, autophagic dysregulation, and subsequent tissue damage (Cooper and Magwere, 2020). Reports of jejunal thinning and villus architectural distortion following chloroquine administration (Li et al., 2022a) further underscore the necessity of focused histological studies using Wistar rats, a well-validated model for toxicological and gastrointestinal research (Hryn et al., 2019).

II. MATERIALS AND METHODS

➢ *Ethical Approval*

Ethical approval was obtained from the Research and Ethics Committee, Faculty of Basic Medical Sciences, Federal University of Technology, Owerri. All procedures followed the guidelines of the NIH (2011) and the NHREC of Nigeria (2014) for laboratory animal care.

➢ *Animal Procurement*

Twenty (20) male Wistar rats aged 8 weeks and weighing 190–250g were procured from an accredited laboratory animal facility. The animals were housed in standard plastic cages under controlled environmental conditions, including a temperature of 22 ± 2 °C, a 12-hour light/dark cycle, and unrestricted access to clean water and standard rat chow. All animals were allowed a two-week acclimatization period before the commencement of the experiment.

➢ *Drug Procurement*

Pharmaceutical-grade chloroquine was purchased from Octovia Pharmacy, Owerri, Imo State, Nigeria. The drug was verified to be within its expiry date and stored according to manufacturer recommendations until use.

➢ *Drug Administration*

Following acclimatization, the rats were randomly assigned to four groups of five animals each. Group A served as the control and received distilled water only. Groups B, C, and D received chloroquine orally via gavage at doses of 10mg/kg, 25mg/kg, and 50mg/kg body weight, respectively, once daily for 21days consecutive days. Body weight was monitored throughout the study to assess treatment-related physiological changes.

Table 1: Experimental Grouping and Treatment Schedule.

Group	Number of Rats (n)	Treatment	Dose (mg/kg)	Route of Administration	Duration (Days)
A	5	Rat chow and Distilled water	0	Oral gavage	21
B	5	Rat chow, distilled water, and chloroquine	10	Oral gavage	21
C	5	Rat chow, distilled water, and chloroquine	25	Oral gavage	21
D	5	Rat chow, distilled water, and chloroquine	50	Oral gavage	21

➢ *Determination of Body Weight*

The animals were weighed before administration (initial weight) and on sacrifice day (final weight) using an electronic weighing balance. Their body weight difference was calculated and recorded as: Body weight difference (g) = Final weight (g) - Initial weight (g).

➢ *Animal Sacrifice and Tissue Preservation*

After treatment, animals were fasted overnight (with water access), euthanized by cervical dislocation, and the small intestines were excised, rinsed with normal saline, and fixed in 10% neutral buffered formalin for 48 hours for histological analysis.

➢ *Histological Analysis*

The fixed tissues were subsequently processed for histological analysis, which included dehydration, clearing, embedding, sectioning, and staining with hematoxylin and eosin (H&E) for microscopic examination. The tissues were dehydrated through a graded series of ethanol (70%, 80%, 90%, and 100%), cleared in xylene, and embedded in paraffin wax. Sections of 5 µm thickness were cut using a rotary microtome and mounted on clean glass slides. The slides were stained with hematoxylin and eosin (H&E) for general histological evaluation and periodic acid-Schiff (PAS) to assess goblet cell distribution and mucosal integrity. Histological evaluations were performed under a light microscope by a pathologist. Photomicrographs were captured using a digital camera attached to the microscope for documentation (Bancroft and Gamble, 2008).

➢ *Data Analysis*

Data were analyzed using SPSS version 26 (IBM Corp., Armonk, NY, USA). The results were presented as a mean and expressed in chat using Microsoft Excel version 16.

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III. RESULTS

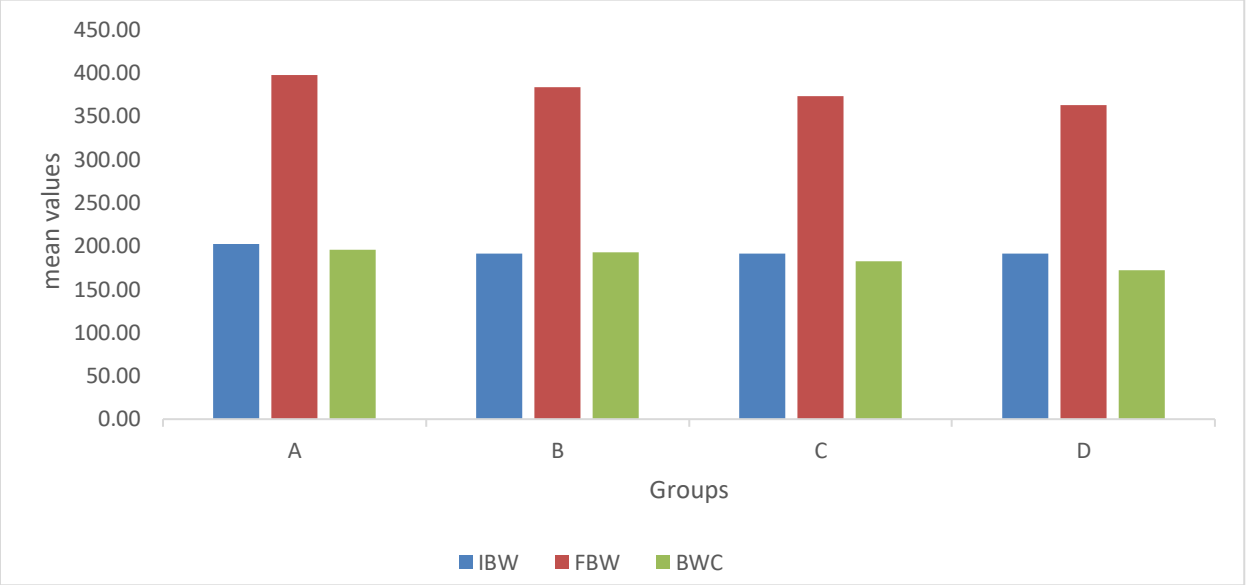

Fig. 1: Mean Value of Rats' Body Weight Among Groups

All groups (A–D) begin with relatively similar Initial Body Weight (IBW) values, indicating that baseline body mass was comparable before treatment**.** Group A (control) displays the highest Final Body Weight, while Groups B, C, and D show progressively lower FBW values. Body Weight Change (BWC) follows the same downward pattern: Group A shows the greatest weight gain, while Groups C and D show much lower gains. This confirms that chloroquine impairs normal weight progression, likely due to toxicity, reduced feed efficiency, and impaired intestinal absorption.

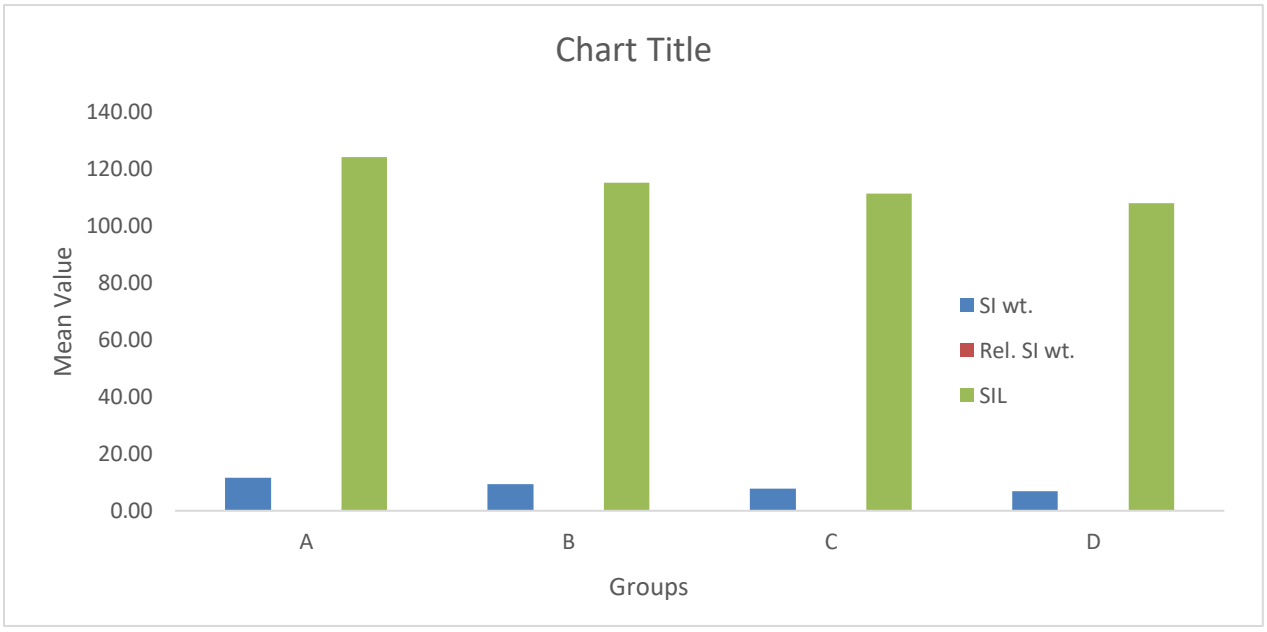

Fig 2 Shows the Mean Value for Small Intestine Weight, Relative Small Intestine Weight, and Small Intestine Length Among Groups.

Small Intestine Weight among groups shows that Group A exhibits the highest intestinal weight, while Groups B–D exhibit gradual reductions. This trend indicates chloroquine-induced atrophy and loss of intestinal tissue, consistent with mucosal degeneration. Relative Small Intestine Weight also decreases across chloroquine-treated groups. This suggests that the intestine becomes lighter even when normalized to total body weight, confirming a direct toxic effect on intestinal tissue. Small Intestine

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Length (SIL) is highest in Group A and decreases progressively from Groups B to D. Reduced intestinal length implies villus atrophy, mucosal shrinkage, and impaired regenerative activity resulting from chloroquine exposure.

- *Microscopic Findings of the Intestinal Tissues*

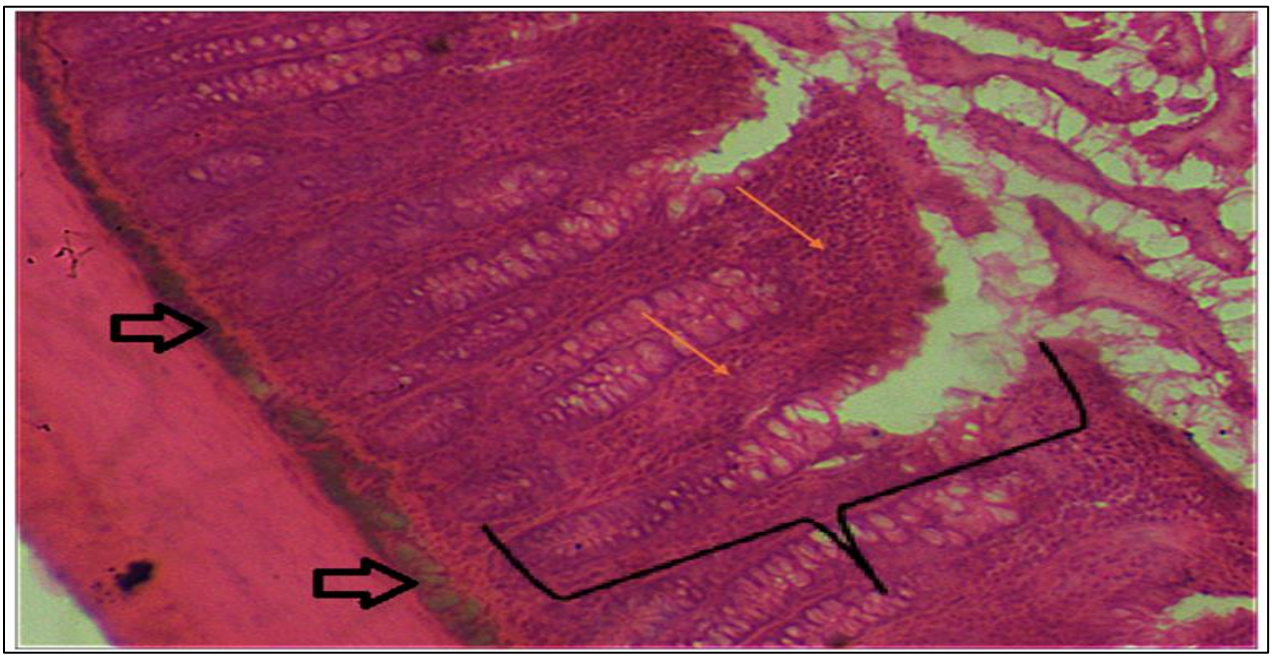

Fig 3: The Photomicrograph of a Control Intestinal Tissue (x100). The Submucosal Architecture is Almost Intact (Marked with Black Arrows), with No Sign of Epithelial Damage. The Mucosal Layer (Marked with a Bracket) has a Well-Arranged Lamina Propria with the Presence of Immune Cell Infiltrates Such as Neutrophils or Lymphocytes (Marked with Yellow Arrows).

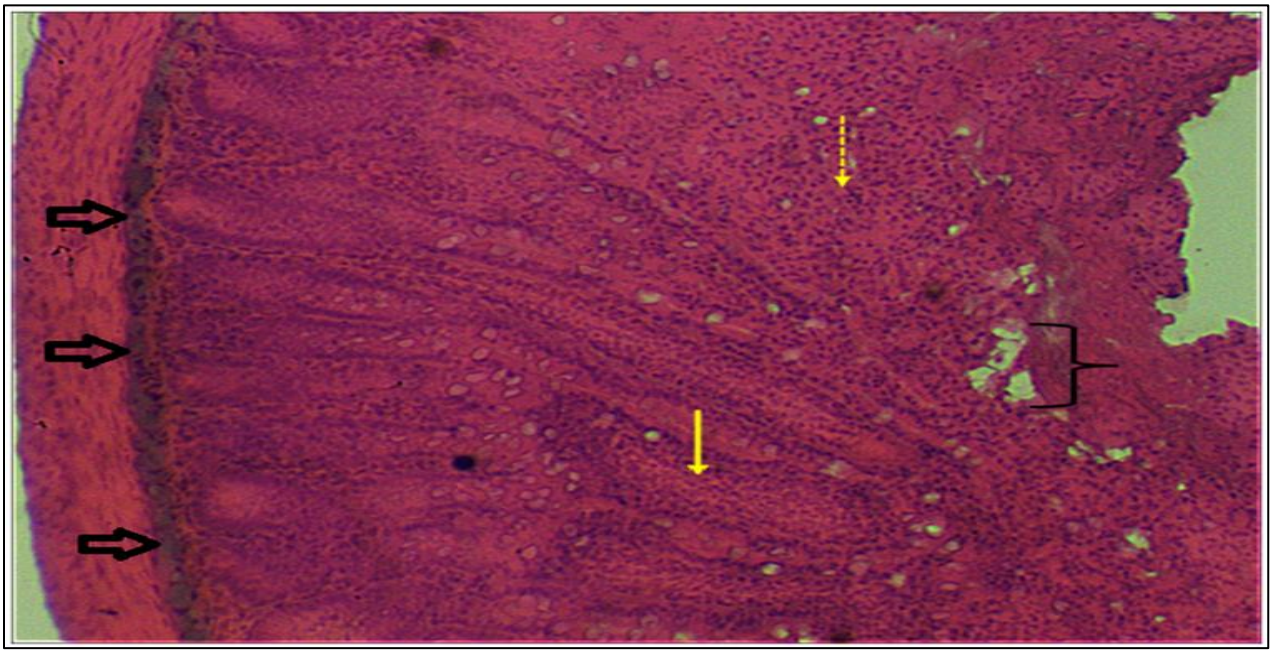

Fig 4: The Photomicrograph of a Low-Dose-Treated Intestinal Tissue (x100). The Submucosal Architecture is Almost Intact (Marked with Black Arrows), with Mild or no form of Hyperplasia. However, within the Mucosal Layer is a Mildly Irregular Lamina Propria Where Some Areas Show an Arranged Aggregation of Immune Cell Infiltrates (Yellowish Solid Arrows) While Other Areas Show Distorted Immune Cell Infiltrates (Yellowish Dotted Lines), and Evidence of Mild Ulcerations (Brackets).

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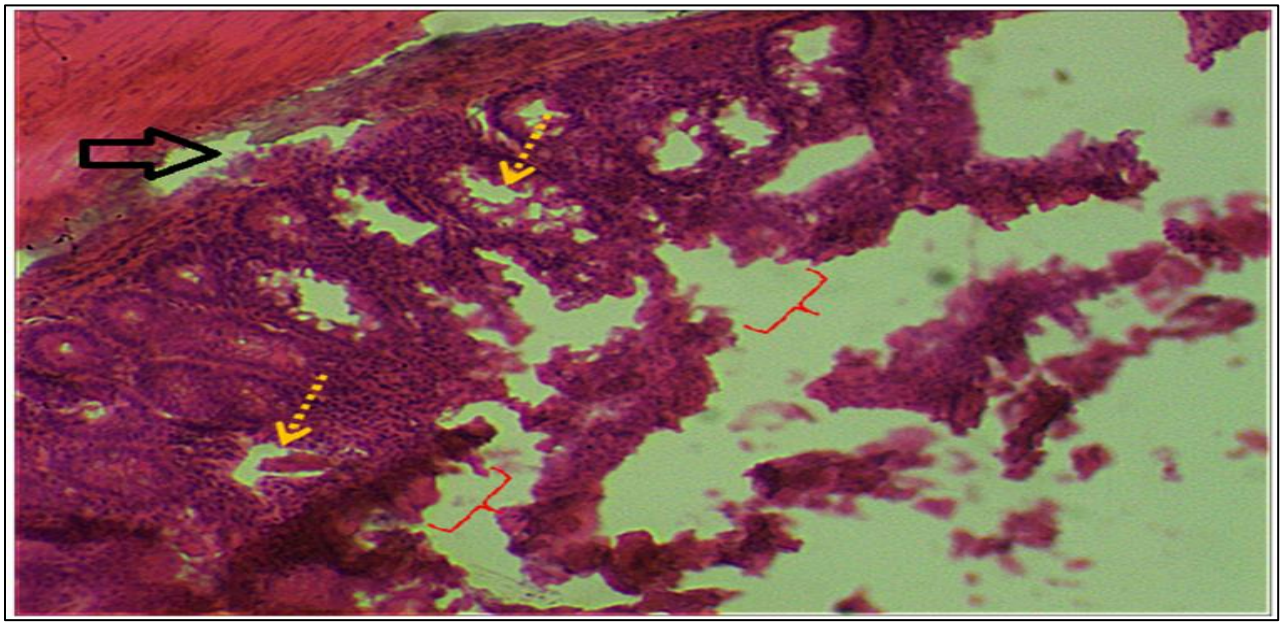

Fig 5: The Photomicrograph of a Medium-Dose-Treated Intestinal Tissue (x100). In Plate E, the Histological Changes are not that Different from Those of the Low-Dose Group. The Submucosal Epithelial Lining Shows Mild Hyperplasia (Marked with Black Arrows). The Mucosa has an Irregular Lamina Propria Architecture with Distorted Immune Cell Infiltrates (Yellowish Dotted Lines), and Mild Mucosal Ulcerations (Red Brackets.

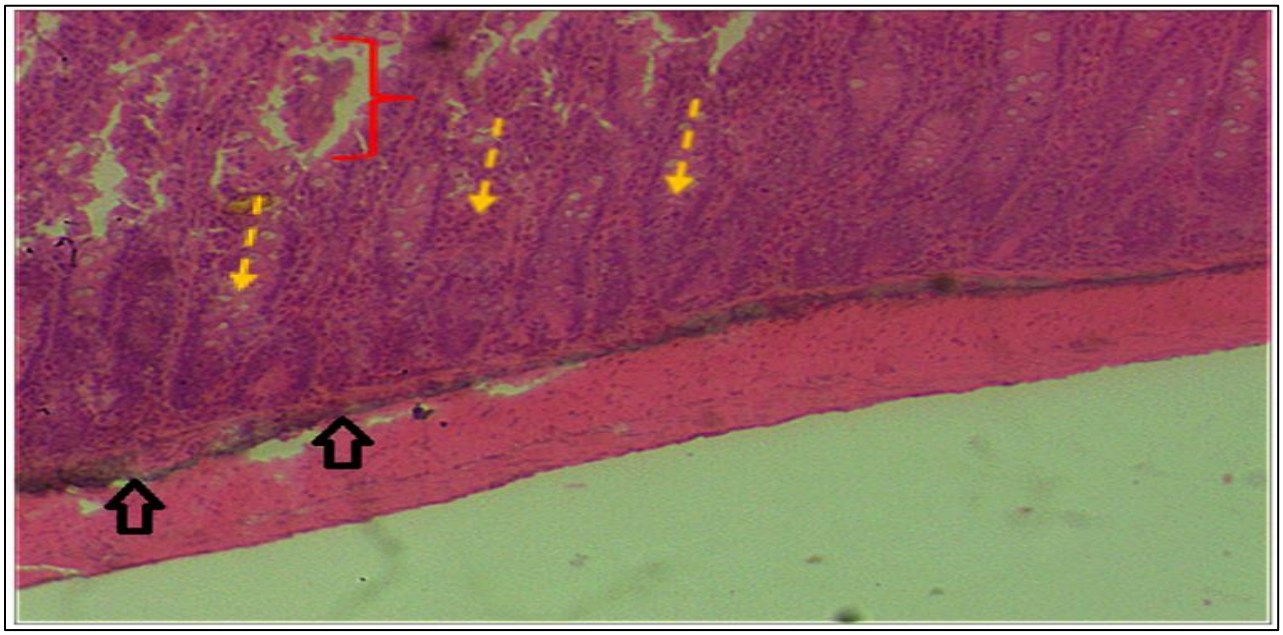

Fig 6: The Photomicrograph of a High-Dose-Treated Intestinal Tissue (x100). In Plate G, the Submucosal Epithelial Lining Depicts Mild to Moderate Hyperplasia (Marked with Black Arrows). The Mucosa is Largely Made up of Irregular Lamina Propria – Mostly Showing Distorted Immune Cell Infiltrates (Yellowish Dotted Lines), Moderate Mucosal Ulcerations (Red Brackets), and Signs of Villous Atrophy.

IV. DISCUSSION

The present study demonstrated that chloroquine administration led to significant reductions in body weight, body weight change, intestinal weight, and intestinal length in all treated groups compared with the control. These findings indicate that chloroquine induces dose-dependent toxic and degenerative effects on the small intestine of Wistar rats, with more severe alterations occurring at higher doses.

The observed decline in body weight and body weight change may be linked to chloroquine's adverse influence on

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gastrointestinal function and metabolic pathways. Chloroquine has been shown to impair normal digestion and nutrient absorption, ultimately reducing feed efficiency and limiting weight gain. This aligns with the findings of Wang *et al*. (2022), who reported notable weight loss and decreased feed intake in chloroquine-treated rats, attributing these effects to systemic toxicity and disrupted metabolic activity. Additionally, chloroquine interferes with lysosomal function and autophagy, processes essential for cellular maintenance and energy regulation, thereby contributing to tissue damage and reduced energy utilization (Mauthe *et al*., 2018; Hussain *et al*., 2024). These mechanisms collectively explain the weight reduction observed in the present study.

The reduction in absolute and relative intestinal weight may result from mucosal atrophy and epithelial degeneration (Gallo *et al*., 2024; Biegler *et al*., 2023; and Shaw *et al*., 2012). Similarly, studies have documented villus atrophy, epithelial necrosis, and inflammatory infiltration in chloroquine-treated animals, supporting the likelihood that such structural damage decreases intestinal mass (Liao *et al*., 2018; Nwodo *et al*., 2021; Ogunshola *et al*., 2021; and Hussain *et al*., 2024). Consistent with these findings, Wang *et al*. (2022) reported jejunal wall thinning, vacuolization, and disrupted villus architecture.

The shortened intestinal length observed may reflect chloroquine-induced destruction of the intestinal wall and mucosa. Villus atrophy, diminished mucosal elasticity, and reduced regenerative activity can all contribute to intestinal shortening (Muff *et al*., 2022; Ameen *et al*., 2020; and Shaw *et al*., 2012). Chronic exposure may further impair motility and limit the mechanical stimuli required for intestinal growth. These pathological alterations correspond with the observations of Wang *et al*. (2022), Sachdev and Pimentel (2021).

Overall, the study confirms that chloroquine exerts dose-dependent structural and functional damage to the small intestine, compromising absorption and contributing to systemic effects, including weight loss and nutritional deficiencies.

V. CONCLUSIONS

This study showed that chloroquine administration produces significant, dose-dependent histological alterations in the small intestine of Wistar rats. The drug caused reductions in body weight, intestinal weight, and intestinal length, alongside clear degenerative changes in the mucosa. These effects suggest that chloroquine disrupts normal intestinal structure and function, likely through oxidative stress, lysosomal dysfunction, and impaired autophagy. The associated decline in intestinal dimensions indicates reduced absorptive capacity, contributing to malnutrition and weight loss. Overall, the findings demonstrate that prolonged or high-dose chloroquine exposure can damage gastrointestinal tissues, emphasizing the need for careful dosing and monitored therapeutic use.

- *Conflict of Interest*

The authors guarantee responsibility for all the data published in this manuscript. The authors confirm the absence of a conflict of interest and the absence of their financial interest in conducting this research and articulating this manuscript. This manuscript is extracted and written from an original research work and has never been published, nor is it under consideration for publication elsewhere.

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