

## ORIGINAL ARTICLE

**Quantifying Renal Changes: A Study of Area of Bowman's Space Following Prolonged Itopride Hydrochloride Exposure in Albino Rats**Aasma Hashmi<sup>1\*</sup>, Hamd Syed<sup>1</sup>, Ayesha Irfan<sup>1</sup>, Ali Rabbani<sup>1</sup>, Amadah Mir<sup>2</sup>**ABSTRACT**

**Objective:** To see the effects of long-term administration of itopride hydrochloride on the histomorphology of kidney of the male Wistar albino rats.

**Study Design:** A Laboratory based experimental study.

**Place and Duration of Study:** The study was conducted at the Department of Anatomy, CMH Multan Institute of Medical Sciences Multan, Pakistan from September 2020 to March 2022.

**Methods:** The study involved two groups: Group A served as the control, and Group B functioned as the experimental group. Each group consisted of 30 adult male albino rats. The animals of group A were administered 2ml/100g of distilled water by oral gavage along with a diet for laboratory rodents daily for 15 days. The animals of group B were given 4.4mg/kg of itopride hydrochloride dissolved in 2ml/100g body weight of distilled water by oral gavage three times a day for 15 days. The kidney was processed for paraffin embedding and stained with Periodic Acid Schiff (PAS). The area of Bowman's space was measured by linear micrometer after calibration with a stage micrometer and image J software. The student's t-test was utilized to assess potential differences between the two groups, with significance set at a *p*-value of  $\leq 0.05$ .

**Result:** The area of Bowman's space was significantly increased in the experimental group as compared to the control group (*p*=0.045).

**Conclusion:** Long-term Itopride Hydrochloride administration appears to influence the area of Bowman's Space in Wistar albino rats.

**Keywords:** Albino Rats, Bowman's Space, Itopride Hydrochloride, Renal Changes.

**How to cite this:** Hashmi A, Syed H, Irfan A, Rabbani A, Mir A, Razzaq M. Quantifying Renal Changes: A Study of Area of Bowman's Space Following Prolonged Itopride Hydrochloride Exposure in Albino Rats. *Life and Science*. 2024; 5(2): 138-143. doi: <http://doi.org/10.37185/LnS.1.1.539>

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**Introduction**

Functional dyspepsia (FD) is indeed a chronic disorder characterized by various symptoms related to digestion, particularly in the upper digestive tract. The sensation referred to in FD includes abnormalities in gastric motility (peristalsis). Specifically, individuals with FD may experience

symptoms such as a burning sensation in the epigastrium, bloating, early satiety, postprandial fullness, and nausea.<sup>1</sup> One derivative of prokinetic benzamide is itopride hydrochloride. These medications have a gastrokinetic impact and inhibit the acetylcholine esterase and dopamine enzymes.<sup>2</sup> Itopride can be used to treat functional dyspepsia as well as other gastrointestinal conditions such as gastroparesis (slow stomach emptying), anorexia, heartburn, regurgitation, bloating, nausea, and vomiting.

Itopride inhibits acetylcholinesterase and dopamine D2 receptors, which raises acetylcholine concentrations. Increased acetylcholine levels promote gastric motility, quicken stomach emptying, raise the lower esophageal sphincter pressure, and enhance gastro-duodenal coordination.<sup>3</sup> Itopride is rapidly and extensively absorbed when taken orally,

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Funding Source: NIL; Conflict of Interest: NIL

Received: Nov 15, 2023; Revised: Mar 06, 2024

Accepted: Mar 20, 2024

reaching a peak in blood plasma levels in just 35 minutes.<sup>4</sup> Itopride is mainly excreted by the kidneys, where its half-life is roughly six hours.<sup>5</sup>

Dopamine is present in significant amounts in the gastrointestinal tract, and it has many inhibitory effects on motility. It can decrease lower esophageal sphincter pressure.<sup>4</sup> Dopamine receptor antagonists are effective as prokinetic agents.<sup>6</sup>

Renal dopamine is produced by the cells of the proximal convoluted tubules and is subsequently secreted through the apical and basolateral membranes. It expresses its hormonal and local paracrine actions on distal nephron segments with the help of circulation.<sup>6</sup> Dopamine signals via five known receptors. They are grouped into D1-like (D1 and D5 receptors) and D2-like (D2, D3, and D4) receptors.<sup>7</sup> D3 receptors are the predominant renal D2-like receptors and are expressed in proximal tubules, thick ascending limbs, and the cortical collecting duct.<sup>8</sup>

Dopamine is synthesized by the kidneys which is important in the regulation of renal function and blood pressure.<sup>8</sup> Additionally, dopamine controls inflammation and the generation of reactive oxygen species. Reactive oxygen species play a role in the progression of renal damage and, eventually, the development of hypertension. In mice with insufficient intrarenal dopamine, there is evidence of elevated oxidative stress as well as increased indicators of tubular injury and inflammation.<sup>9</sup> When there is decreased renal dopamine production, it aggravates angiotensin –II-mediated renal injury. Dopamine reduces renal Na<sup>+</sup> retention in the body when normal Na<sup>+</sup> intake occurs, negating the effects of the Renin-Angiotensin-Aldosterone System (RAAS).<sup>9</sup> In a study in which mice were generated with selective deletion of dopamine receptors, led to increased renal injury.<sup>10</sup>

Proximal tubules rely on aerobic metabolism and have more mitochondria with a more oxidized state. If there are reduced dopamine receptors, proximal tubules become vulnerable to mitochondrial dysfunction leading to an increase in renal microvascular loss, oxidative stress, and eventually renal failure.<sup>11</sup>

The majority of antipsychotic medications used to treat bipolar illness, schizophrenia, and other

psychoses are also dopaminergic antagonists belonging to the D2-like family; these are mainly D2 antagonists, while some are also D4 antagonists. While more recent antipsychotics like clozapine and olanzapine target D4 receptors, older antipsychotics like phenothiazine and haloperidol target D2 receptors.<sup>10</sup>

The term "acute kidney injury" refers to the abrupt loss of kidney function that results from antipsychotic medication use (APD).<sup>12</sup> Additionally, case reports exist demonstrating that clozapine-treated treatment-resistant schizophrenia patients developed interstitial nephritis, which ultimately resulted in acute renal failure.<sup>13</sup> The long-term use of olanzapine in laboratory animals also showed a focal increase in glomerular cellularity and cellular proliferation.<sup>14</sup>

As itopride hydrochloride is also a dopamine receptor(D2) blocker like antipsychotic agents, this study delves into the effects of its administration on the area of Bowman's Space in the kidneys of Wistar albino rats.<sup>15</sup>

## Methods

It was an experimental study and the animal model used in this study was a male Wistar rat. The study was conducted at the Anatomy Department, CMH Multan Institute of Medical Sciences Multan, Pakistan from September 2020 to March 2022 after getting approval from the Institutional Review Board and Ethical Committee held on 23<sup>rd</sup> February 2021 vide letter no: TW/25/CIMS. It was a non-probability convenience sampling. A total of sixty healthy male Wistar rats, aged 13-15 weeks and weighing between 200-300 grams each, were included in the study. The rats were divided into two groups. Group A, the control group, received 2ml/100g of distilled water via oral gavage three times a day for 15 days.<sup>16</sup> Group B, the experimental group, received 4.4mg/kg of itopride hydrochloride dissolved in 2ml/100g body weight of distilled water via oral gavage three times a day for the same 15-day period. The dose selection for Group B was based on converting the human dose of itopride to the Animal Equivalent Dose (AED) for rats, which was calculated to be 4.4 mg/kg/dose.<sup>17,18</sup> Itopride Hydrochloride used in the study was a product of a multinational pharmaceutical company.

After a week-long acclimation period, every rat was kept in a controlled environment. Their environment consisted of a 12-hour light-dark cycle,  $25 \pm 2$  °C temperature, and 50–70% humidity. Water was always available to the rats, along with a usual pellet diet. Rats with any physical abnormalities or those older than 15 weeks were excluded from the study. After 15 days of drug administration, all rats from both groups were sacrificed. Euthanasia was performed by chloroform inhalation, confirmed by the absence of respiratory movements and dilated pupils. Following euthanasia, the abdomen was opened, and the kidneys were dissected, trimmed, washed in 0.9% normal saline, and then weighed on an electrical balance. Subsequently, the organs were placed in 10% formalin for fixation. The selection of the 15-day duration for the study was informed by reference articles in which itopride hydrochloride was administered for a similar period.<sup>19</sup> The optimal dose and study duration were established through a pilot project before initiating the main study. The kidneys were placed in 10% formalin for 24 hours for fixation. After being cut transversely from the middle area of the right kidney, the organ was cleaned in xylene and put through an escalating series of alcohol concentrations from 70% to 100%. For penetration, paraffin wax with a melting point of 56–58 °C was utilized. To let the paraffin wax, solidify, the tissue was put into the mold and set over a tiny cooling tray. Group A's paraffin blocks were numbered 1–30, and Group B's were numbered 1–30. Approximately seven microns thick sections were cut with a rotary microtome. The sections were stained with Periodic Acid Schiff's reaction for light microscopy. The stained slide photographs were taken using the mobile camera installed with Adobe Photoshop Lightroom through the Olympus binocular microscope at 100X oil immersion lens.

The area of Bowman's space in the micrometer was measured using Image J software. Version 26 of the Statistical Package for Social Sciences (SPSS) computer program was used to statistically analyze the data. The results of the analysis showed that the area of Bowman's space ( $\mu\text{m}^2$ ) per unit area of the kidney was mean  $\pm$  standard deviation of mean. Student *t*-tests were applied to quantitative variables to detect any significant differences between experimental and control groups.

**Results**

The rat's area of Bowman's space from the control and experimental groups was calculated shown in Figure.1.

The Student's *t*-test showed that the area of Bowman's space of the experimental group was significantly higher than that of the control group ( $p=0.045$ ) (Table-1).

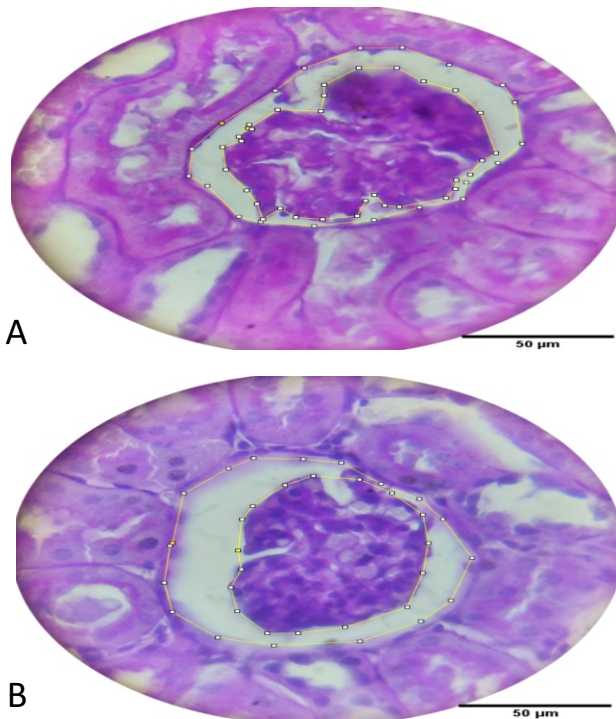
**Discussion**

The current study looked at the kidney's reaction to itopride hydrochloride. The area of Bowman's space varied statistically significantly between the two groups in the current investigation. Comparing the experimental group to the control group, there is a statistically significant increase in area. This is consistent with research done by A. Uyanik et al. The decrease in dopamine receptors leads to inflammatory reaction and retraction of glomerulus with a resultant increase in Bowman's space in the experimental group.<sup>14</sup>

One medication used to manage functional dyspepsia (FD) is Itopride Hydrochloride, a prokinetic benzamide derivative with known gastrokinetic effects. Itopride's mechanisms of action involve inhibiting dopamine and acetylcholine esterase enzymes, leading to increased acetylcholine concentrations, which promote gastric motility and enhanced coordination within the gastro-duodenal

**Table-1: Comparison of the histological variable of kidney between control (a) and experimental (b) groups of rats**

Variables	A (n=30) Mean $\pm$ SD	B (n=30) Mean $\pm$ SD	P-value
Area of Bowman’s Space ( $\mu\text{m}^2$ )	1721.2 $\pm$ 431.71	1933.57 $\pm$ 366.71	0.045*



**Fig.1: Photomicrograph of rat kidney showing comparison in area of Bowman's space between Control(A) and Experimental(B) groups (Periodic Acid Schiff stain, PAS)**

region. In this study, we explored the impact of prolonged Itopride Hydrochloride administration on the area of Bowman's Space in the kidneys of Wistar albino rats.

Itopride Hydrochloride's prokinetic properties, while beneficial in the management of gastrointestinal disorders, are associated with potential adverse effects, particularly in the renal system. Itopride Hydrochloride is a dopamine receptor blocker, and dopamine plays a critical role in gastrointestinal motility. Dopamine is involved in the regulation of renal function, blood pressure, and inflammation control.

Dopamine's inhibitory effects on motility have been documented, including its ability to decrease lower esophageal sphincter pressure. Moreover, dopamine receptor antagonists have been used as prokinetic agents. Our study revealed that Itopride Hydrochloride's action as a dopamine receptor blocker, like antipsychotic agents, may have implications for kidney health.

Renal dopamine, synthesized within proximal convoluted tubules, is crucial in regulating renal

function and blood pressure. This dopamine acts both hormonally and as a local paracrine agent on distal nephron segments. It signals through various receptors, primarily D1-like (D1 and D5 receptors) and D2-like (D2, D3, and D4) receptors. Among these, D3 receptors are the predominant renal D2-like receptors, expressed in proximal tubules, thick ascending limbs, and the cortical collecting duct. Reduced renal dopamine production can aggravate renal injury mediated by angiotensin-II and exacerbate oxidative stress, potentially leading to hypertension. Dopamine has been demonstrated to control inflammation and the generation of reactive oxygen species, which are implicated in renal damage progression.

Interestingly, D2 receptors are the target of several antipsychotic medications, including more traditional ones like phenothiazine and haloperidol. The main target of more recent antipsychotics like clozapine and olanzapine is the D4 receptor. Antipsychotic medications are known to impair renal function, and case studies have connected them to acute kidney damage. Additionally, long-term use of olanzapine in laboratory animals has shown increased glomerular cellularity and cellular proliferation, indicating potential renal damage. Given that Itopride Hydrochloride is a dopamine receptor blocker, like antipsychotic agents, our study aimed to investigate its effects on renal histology, specifically focusing on the area of Bowman's Space in the kidneys of Wistar albino rats.

Our study's results demonstrated a significant increase in Bowman's Space in the experimental group treated with Itopride Hydrochloride compared to the control group. This finding suggests that long-term Itopride Hydrochloride administration may have a substantial impact on renal morphology and, potentially, kidney function.

The observed increase in Bowman's space size in the experimental group suggests potential structural adaptations within the renal tissue. This morphological change may affect glomerular filtration efficiency and consequently kidney function.

The study's findings emphasize the need for further research to elucidate the underlying mechanisms and the clinical significance of these

histomorphological changes in the kidneys. Itopride Hydrochloride is primarily used to treat gastrointestinal disorders, and its impact on renal morphology may not have been thoroughly studied. Monitoring renal function and histomorphological alterations during Itopride Hydrochloride treatment is essential to ensure patient safety.

In conclusion, long-term Itopride Hydrochloride administration appears to influence the area of Bowman's Space in Wistar albino rats, suggesting potential effects on renal function. This study prompts further investigation into the mechanisms and clinical implications of these histomorphological changes in the kidneys, potentially contributing to a better understanding of the drug's safety and its impact on kidney health.

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**Authors Contribution**

**AH:** Idea conception, study designing, data collection, manuscript writing, and proof reading

**HS:** Manuscript writing, and proofreading

**AI:** Idea conception, manuscript writing, and proofreading

**AR:** Data analysis, results and interpretation

**AM:** Data analysis, results and interpretation

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