ORIGINAL ARTICLE

EFFECTS OF LONG-TERM ITOPRIDE HYDROCHLORIDE TREATMENT ON RAT PROXIMAL CONVOLUTED TUBULES AND SERUM GLUTATHIONE LEVELS

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Background: Functional dyspepsia is a chronic gastric motility disorder marked by upper gastrointestinal symptoms; itopride hydrochloride, a prokinetic benzamide derivative acting via acetylcholinesterase and dopamine D_2 receptor inhibition, is widely used, yet evidence on its long-term systemic effects remains limited. Objective of this study was to evaluate the effects of long-term administration of itopride hydrochloride on renal histomorphology and serum glutathione levels in male Wistar albino rats. **Methods:** This laboratory-based experimental study was conducted in Anatomy Department, CMH Multan Institute of Medical Sciences, Pakistan. Sixty adult male Wistar albino rats were divided into control and experimental groups (n=30 each). Controls received distilled water, while the experimental group received itopride hydrochloride (4.4 mg/Kg) orally three times a day for 15 days. Kidneys were processed, paraffin embedded, and stained with haematoxylin and eosin. Proximal convoluted tubule (PCT) height and luminal diameter were measured using image. Serum glutathione was assessed with ELISA. Data were analyzed using Student's *t*-test. **Results:** The experimental group showed a significant reduction in PCT epithelial height and a significant increase in luminal diameter compared to controls (p=0.001). Serum glutathione levels were also significantly decreased in experimental groups as compared to controls (p=0.001). **Conclusion:** Long-term administration of itopride hydrochloride adversely affects renal tubular histomorphology and reduces serum glutathione levels in Wistar albino rats.

Keywords: Functional dyspepsia, Glutathione, Luminal diameter, Proximal convoluted tubule

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INTRODUCTION

Functional dyspepsia (FD) is a chronic disorder that is characterized by various symptoms that are related to indigestion, especially 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.[1] One derivative of prokinetic benzamide is itopride hydrochloride. These medications have a gastroprokinetic effect and inhibit the acetylcholine esterase and dopamine enzymes.[2] Itopride can be used to treat functional dyspepsia as well as other gastrointestinal conditions such as gastroparesis (slow stomach emptying), anorexia, heartburn, bloating, regurgitation, nausea, and vomiting.

Itopride inhibits acetylcholinesterase and dopamine D2 receptors, which raises acetylcholine concentrations. Increased acetylcholine levels promote gastric motility, speed up stomach emptiness, raise the lower oesophageal sphincter pressure, and enhance gastro-duodenal coordination.[3] Itopride is rapidly and extensively absorbed when taken orally, reaching a peak in blood plasma levels in just 35 minutes.[4] Itopride is mainly excreted by the kidneys, where its half-life is roughly six hours.[5]

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

Renal dopamine is produced by the cells of the proximal convoluted tubules (PCTs) 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.[6] Dopamine signals via five known receptors. They are grouped into D1-like (D1 and D5 receptors) and D2-like (D2, D3, and D4) receptors.[7] D3 receptors are the predominant renal D2-like receptors and are expressed in PCTs, thick ascending limbs, and the cortical collecting duct.[8]

Dopamine is synthesized by the kidneys which is important in the regulation of renal function and blood pressure.[8] In addition to that, 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.[9] When there is decreased renal dopamine production, it aggravates angiotensin-II mediated renal injury. Dopamine reduces renal Na^+ retention in the body when normal Na^+ intake occurs, counteracting the effects of the Renin-Angiotensin-Aldosterone System (RAAS).[9] In a study in which mice were generated with selective deletion of dopamine receptors, led to increased renal injury.[10]

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.[11]

Glutathione is an important antioxidant. There are studies which prove the relationship between decrease serum glutathione levels and increase rate of multi morbidity development like diabetes mellitus, chronic liver injury and neurodegenerative diseases.[12] Hypoxia, DNA and mitochondrial damage and high levels of oxidative stress can lead to an imbalance either in the production of glutathione or there could be an increased consumption of this enzyme.

The effects on kidney after administration of itopride hydrochloride have a strong association with the production of reactive oxygen species (ROS). When there is an imbalance between the production of pro-inflammatory and anti-inflammatory markers, there will be an increase in the oxidative stress, and this can lead to production of ROS.

As itopride hydrochloride is also a dopamine receptor (D2) blocker, this study explores the effects of its administration on the height and luminal diameter of PCT as well as serum glutathione levels in the kidneys of Wistar albino rats.

METHODOLOGY

It was an experimental study, and the animal model used in this study was a male Wistar rat. The study was conducted at Anatomy Department, CMH Multan Institute of Medical Sciences, Multan, Pakistan from Sep 2020 to Mar 2022 after approval from the Institutional Review Board and Ethical Committee vide letter No. TW/25/CIMS.

It was a simple random sampling. A total of 60 healthy male Wistar rats, aged 13–15 weeks and weighing 200–300 grams each, were included in the study. The rats were divided into two groups. The control group, group A, received 2 mL/100 g of distilled water via oral gavage three times a day for 15 days.[13] The experimental group, group B, received 4.4 mg/Kg of itopride hydrochloride dissolved in 2 mL/100 g body weight of distilled water via oral gavage three times a day for the 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.[14,15]

After seven days of acclimatization period, every rat was kept in a controlled environment of a 12-hour light-dark cycle, 25±2 °C temperature, and 50–70% humidity. Water was available *ad libitum* 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. 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 after a reference articles in which itopride hydrochloride was administered for a similar period.[16] 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 cleared in xylene and put through an escalating series of alcohol concentrations from 70 to 100%. For embedding, paraffin wax with a melting point of 56–58 °C was utilized. To let the paraffin wax solidify, the tissue was put into the mould and set over a small cooling tray. Paraffin blocks were numbered 1–30 for each group. Approximately seven microns thick sections were cut with a rotary microtome. The sections were stained with haematoxylin and eosin for light microscopy. The stained slide photographs were taken using the mobile camera installed with Adobe Photoshop Lightroom through the Olympus binocular microscope at 100× oil immersion lens.

The luminal epithelium height and diameter of lumen of PCTs in the micro-meter were measured using Image J software. The SPSS-26 was used to statistically analyse the data. Student's *t*-test was applied to quantitative variables to detect any significant differences between experimental and control groups.

Blood samples were obtained by cardiac puncture after euthanizing of animals and collected in test tubes containing heparin as anticoagulant. Serum glutathione levels were measured using rat ELISA kit of Bioassay Technology Laboratory (BT LAB).

RESULTS

The rat's luminal epithelium height of proximal convoluted tubules and diameter of lumen of proximal convoluted tubules appeared to be different between control and experimental groups shown in Figure-1.

The student's *t*-test showed that the luminal epithelium height of proximal convoluted tubule of the control group was significantly higher than that of the experimental group ($p<0.001$). The Student's *t*-test showed that the luminal diameter of proximal convoluted tubule of the experimental group was significantly higher than that of the control group ($p<0.001$) (Table-1).

The serum glutathione levels were calculated shown in Table-2. The student's *t*-test showed that the Glutathione level of the control group was significantly higher than that of the experimental group (p<0.001).

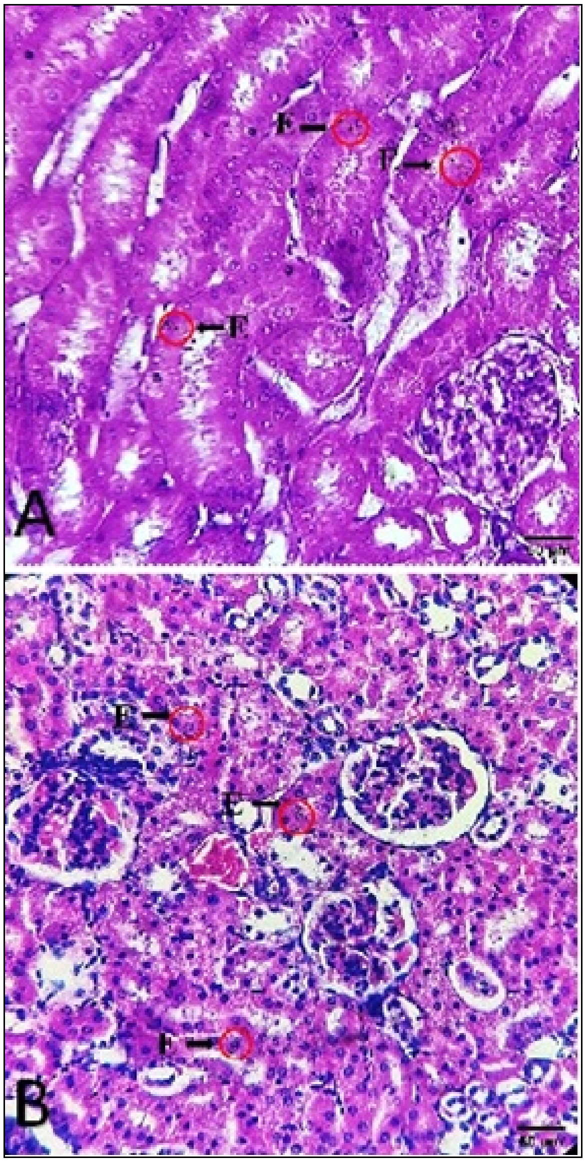

Figure-1: Photomicrograph of rat kidney showing epithelium height and luminal diameter in proximal convoluted tubules of control (A) and experimental (B) groups. (E=Epithelium, H & E stain)

Table-1: Comparison of histological variables of kidney between control and experimental groups

Variables	Mean±SD		p
	A (n= 30)	B (n= 30)	
Height of the Proximal Convoluted Tubule Luminal Epithelium (µm)	26.14±4.4	22.6±2.65	0.001*
Proximal Convoluted Tubule Lumen Diameter (µm)	15.53±2.69	28.09±5.43	0.001*

*Significant

Table-2: Comparison of serum glutathione levels between control (A) and experimental (B) groups

Variable	Mean±SD		p
	A (n=30)	B (n=30)	
Serum Glutathione (mg/L)	293.57±31.3	259.36±27.89	0.001*

*Significant

DISCUSSION

The current study evaluates the adverse effects of Itopride hydrochloride on histomorphology and biochemical analysis on kidney.

There was a statistically significant difference between control and experimental groups in the proximal convoluted tubule luminal epithelium and diameter. The luminal epithelium height of PCT of experimental group was significantly decreased as compared to control group. The diameter of the PCT lumen was considerably larger in experimental group as compared to the control group. This is in agreement with Zhang *et al*[17] and Nikolić-Kokić A *et al*[18].

Serum glutathione was decreased in experimental group as compared to the control and it is in agreement with other studies conducted by Nikolić-Kokić A and Chang GR *et al*.[18,19] Our study showed that deficient D2-receptors are a main cause of mitochondrial dysfunction and lead to increase in renal microvascular loss, oxidative stress, and renal failure.[11]

Itopride's mechanisms of action involve inhibiting dopamine and acetylcholine esterase, leading to increased acetylcholine concentrations, which promote gastric motility and enhanced coordination within the gastro-duodenal region.[3] Although Itopride hydrochloride is effective in treating gastrointestinal disorders due to its prokinetic properties, it may also lead to potential adverse effects, especially concerning the renal system.[8] As a blocker of dopamine receptors, Itopride hydrochloride affects gastrointestinal motility because dopamine is a key regulator of digestive processes. Dopamine plays a key role in managing renal function, regulating blood pressure, and controlling inflammation.[9]

Research has documented dopamine's role in decreasing motility, such as lowering the pressure of the lower oesophageal sphincter, that is why dopamine receptor antagonists are used to promote gastrointestinal movement.[3] Itopride hydrochloride, by blocking dopamine receptors, could impact kidney health.

Dopamine produced in the proximal convoluted tubules of the kidneys plays a vital role in regulating renal function and blood pressure. Dopamine acts in both hormonal and local paracrine capacities on the distal parts of the nephron. It engages with various receptors, particularly D1-like (D1 and D5) and D2-like (D2, D3, and D4) receptors. Of these, D3 receptors are the most prevalent renal D2-like receptors, located in the proximal tubules, thick ascending limbs, and the cortical collecting duct.[7]

A decrease in renal dopamine production can worsen kidney damage caused by angiotensin-II and increase oxidative stress, which may contribute to the development of hypertension. Dopamine has been shown to regulate inflammation and the production of reactive oxygen species, which play a role in the progression of renal damage.[17]

Considering that Itopride hydrochloride acts as a dopamine receptor antagonist, our research sought to explore its impact on renal histology, particularly examining luminal height and luminal epithelium of proximal convoluted tubules in the kidneys of Wistar albino rats. Our findings suggest that long-term use of Itopride hydrochloride may notably influence kidney structure and potentially alter the function of kidney.

The results emphasize the necessity for continued research to explore the mechanisms and clinical significance of the kidney's histomorphological changes observed with long-term use of Itopride hydrochloride exposure. Regular monitoring of kidney function is essential during Itopride hydrochloride therapy to safeguard patient health.

CONCLUSION

Extended use of Itopride hydrochloride appears to alter luminal epithelium height and luminal diameter of proximal convoluted tubules in the kidneys of Wistar albino rats. It also affects levels of serum glutathione suggesting potential implications for renal function.

RECOMMENDATIONS

The study highlights the need for more research to unravel the mechanisms and clinical effects of these histomorphological and biochemical changes in the kidneys, which could contribute to a deeper understanding of the drug's safety and its impact on renal health.

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MAR: Interpretation of data and proof reading
ZH: Analysis and discussion
HSS: Critical review and final approval
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