Archives of Razi Institute, Vol. 76, No. 4 (2021) 975-983

DOI: 10.22092/ari.2021.355947.1745

Original Article

Effects of the Long-term Treatment of Proton Pump Inhibitors on the Function of Kidney and Liver in Laboratory Female Rats

AL-Hadrawy, S. M. J[1 *], Mahdi AL-Turfi, Z. S[2]

1. Department of Biology, Faculty of Sciences, University of Kufa, Iraq
2. Department of Biology, Faculty of Education for Girls, University of Kufa, Iraq

Received 3 September 2021; Accepted 19 September 2021
Corresponding Author: suaadm.alhadrawi@uokufa.edu.iq

Abstract

Proton pump inhibitors (PPIs) are a group of medications effectively used to inhibit gastric acid secretion and to treat many acid-related disorders, including gastroesophageal reflux disease and other gastric disorders. Recent studies recommended that they may be associated with the risk of chronic kidney disease and liver disease. Therefore, the current study aimed to investigate the effect of long-term treatment with PPIs on kidney and liver function in laboratory rats. Fifteen female albino white rats (*Rattusnorvigicus*) were randomly assigned to three groups of five animals. The control group was fed regular pellet, group PPI-2 received standard pellet diet and was given esomeprazole (10 mg/kg b.w.) via daily oral gavage in mornings for two weeks, and group PPI-3 was fed standard pellet diet and was given esomeprazole (10 mg/kg b.w.) via daily oral gavage in mornings for three months. Blood samples were taken after 2 weeks and 3 months by cardiac puncture for measuring serum creatinine, urea, total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). In addition, kidney and liver tissues were histopathologically evaluated. Serum creatinine, urea, ALT, total bilirubin, and ALP significantly increased in group PPI-3, compared to other groups. Histopathological study of the kidneys and liver revealed normal histology structure in the control group and the rats of the PPI-2 group, while some histological changes were observed in the liver and kidney of the animals in the PPI-3 group. The histological changes included the widening of Bowman's space and shrunken glomeruli, whereas the renal tubules had congested tubular cells. Furthermore, congestion in the blood vessels and hepatic cells degradation were observed in the liver. These data indicate that the long-term administration of PPIs has adverse effects on the structure and function of the kidney and liver.

Keywords: Proton pump inhibitor, Liver function, Kidney function, Long-term Treatment, Esomeprazole

1. Introduction

Proton pump inhibitors (PPIs) are very common prescriptions, and a high percentage (25%-70%) of these prescriptions have no suitable indication (1). On the other hand, the continuous use of PPIs beyond recommended guidelines may lead to the malfunction of the liver and kidney (2). Moreover, there is a trend in the usage of PPIs for children (3), and many patients are discharged from the hospital on a PPI with improper indications or use high doses for a long time (4). Since 1990 that this drug was introduced to the market, numerous studies have linked PPI use to uncommon consequences and adverse health effects, including acute kidney injury (AKI) (5), acute interstitial nephritis (AIN) (6), community-acquired pneumonia (7), *Clostridium difficile* infection (8), and hip fracture (9). Furthermore, the use of PPIs may be a risk factor for chronic kidney disease (CKD), possibly

mediated by repeated AIN (10) or hypomagnesemia, which have been linked with PPI (11) and CKD occurrence (12).

Based on previously published studies, long-term use of PPIs has a relationship with liver function. On the other hand, Mohajeri et al. (13) have clarified that PPIs may encourage modifications in the gut microbiota causing dysbiosis and damaged gut barrier (14). In addition, the administration of PPIs in cirrhosis patients is linked with an increased chance of hepatic encephalopathy and spontaneous bacterial peritonitis (15). The results of a study conducted by Dultz et al. (16) recommended that PPI usage may be related to the risk of mortality. They reported PPI utilization to be an independent predictor of mortality in patients with compensated and decompensated liver cirrhosis. Therefore, the current research aimed to investigate the relationship of long-term PPI use with kidney and liver function in laboratory rats.

2. Material and Methods

2.1. Experimental Animals

Healthy adult albino female rats (*Rattusnorvigicus*) weighing 250-300 g were prepared from the animal house in the Faculty of Science, University of Kufa. The animals were housed in the animal house in standard and controlled environmental conditions, including the temperature of 22°C-28°C. The animals were given standard laboratory commercial food (pellets) and water was provided throughout the experiment. None of the rats had any clinically evident infections.

2.2. Dosage Calculation and Preparation of the Stock Solutions of Proton Pump Inhibitors

Esomeprazole (Nexium® 20 mg tablets, AstraZeneca) was crushed into powder and was dissolved in normal saline. The dose of esomeprazole used in this study was 10 mg/kg body weight.

2.3. Experimental Design

Fifteen mature female rats were randomly assigned into three groups of 5 rats. The animals in the control group were fed a normal pellet diet, group PPI-2 received a normal pellet diet and was given esomeprazole (10 mg/kg b.w.) through oral gavage every day in the morning for two weeks, and group PPI-3 was fed a normal pellet diet and was given esomeprazole (10 mg/kg b.w.) via oral gavage every day in the morning.

2.4. Blood Sample Collection

At the end of the experiment (after 2 weeks and 3 months), animals were anesthetized by a mixture of ketamine 0.1 mL and xylazine 0.2 mL and were scarified. Each animal was located on the cork pin box and 5 ml of blood was taken directly from the heart by cardiac puncture. Blood specimens were collected in tubes without anticoagulants and were left for 30 min at room temperature followed by centrifugation at 6000 rpm for 5 min to obtain serum. Next, all the serum samples were biochemically analyzed.

2.5. Animal Dissection

The abdominal cavity of each animal was exposed and the kidneys and liver were eradicated. All the fat tissue was removed and the organs were placed in formalin solution 10% in a plastic container until evaluations.

2.6. Biochemical Testing

2.6.1. Kidney Function Test

Serum creatinine and urea were measured by kinetic colorimetric method and enzymatic colorimetric method, respectively. The measurements were performed according to the procedures provided by Linear Chemicals, Spain.

2.6.2. Liver Function Test

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were evaluated by UV enzymatic method. In addition, bilirubin was assessed by endpoint colorimetric method and alkaline phosphatase (ALP) was measured by kinetic colorimetric method. All these tests were completed according to the procedure provided by Linear Chemicals, Spain.

2.7. Histopathological Preparations of Samples for Light Microscopic Examination

The renal and hepatic specimens were taken and the following steps were taken for tissue preparation:

1. Fixation in 10% formal saline solution for 24 h;
2. Dehydration in the increasing concentrations of ethyl alcohol;
3. Clearance in twice changes of xylol for 30 min each time;
4. Impregnation in clean paraffin for 2 h at 60ºC;
5. Embedding in solid paraffin;
6. Obtaining sections of 5 µm by Micro-Tom;
7. De-waxing and hydrating the sections by graded alcohol;
8. Staining by Harris hematoxylin for 2-5 min;
9. Differentiation in 1% acid alcohol (1% HCL in 70% alcohol) for 5-10 sec;
10. Washing the sections well in tap water for 5 min and staining with 1% eosin for 1-3 min; and
11. Finally, dehydration by the ascending concentrations of ethanol alcohol, clearance by xylol, and mounting by using Distyrene Plasticizer Xylene (DPX).

2.8. Statistical Analysis

All data were expressed as mean ± SD. The statistical significance was assessed by the one-way analysis of variance (ANOVA) and the Tukey HSD posthoc test using the SPSS software version 26. Values were considered statistically significant when $P < 0.05$.

3. Results and Discussion

3.1. Effect of PPI (10 mg/kg) on Kidney Function in Rats

Our results revealed a significant increase ($P<0.05$) in serum creatinine (Figure 1-A) and urea (Figure 1-B) in the animals of the PPI-3 group, in comparison with the rats of PPI-2 and control groups.

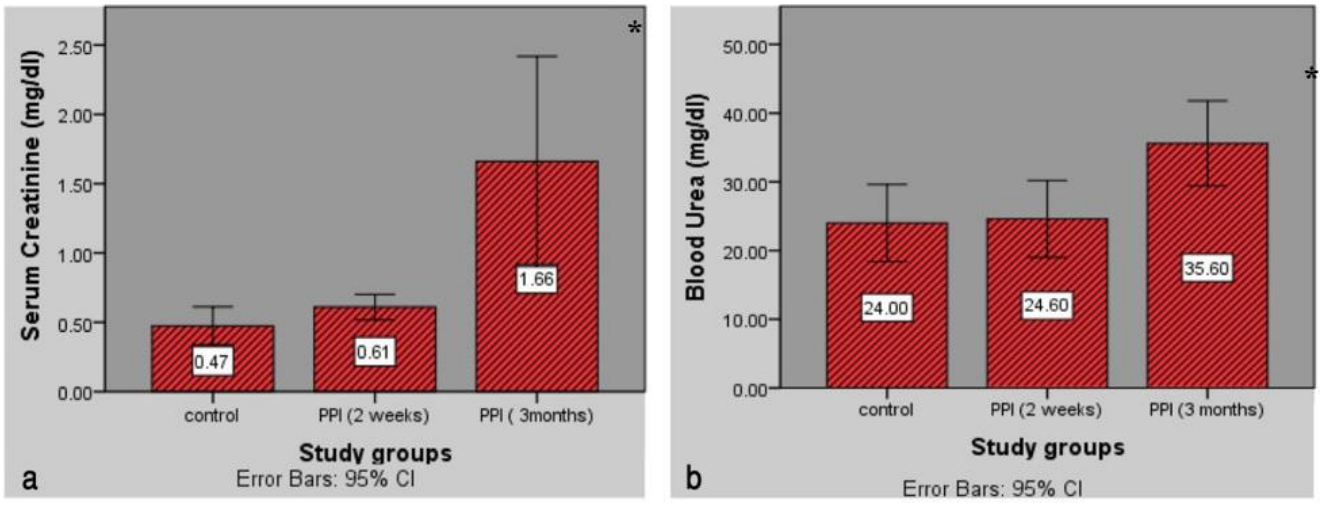

Figure 1. Comparison of serum levels of creatinine and urea in female rats treated with PPI (10 mg/kg) and control group (❋): significant difference in comparison with other groups.
* The mean difference is significant at 0.05 level

3.2. Effect of PPI (10 mg/kg) on the Histological Structure of Kidney in Rats

Histological sections were taken from the kidney tissues of rats and were examined histopathologically. Histopathological evaluation of kidneys revealed normal histology structure in the control (Figure 2-A) and PPI-2 groups (Figure 2-B). On the other hand, some histological changes were observed in the rats of the PPI-3 group as the widening of Bowman space, shrunken glomeruli, and congested tubular cells (Figure 2-C).

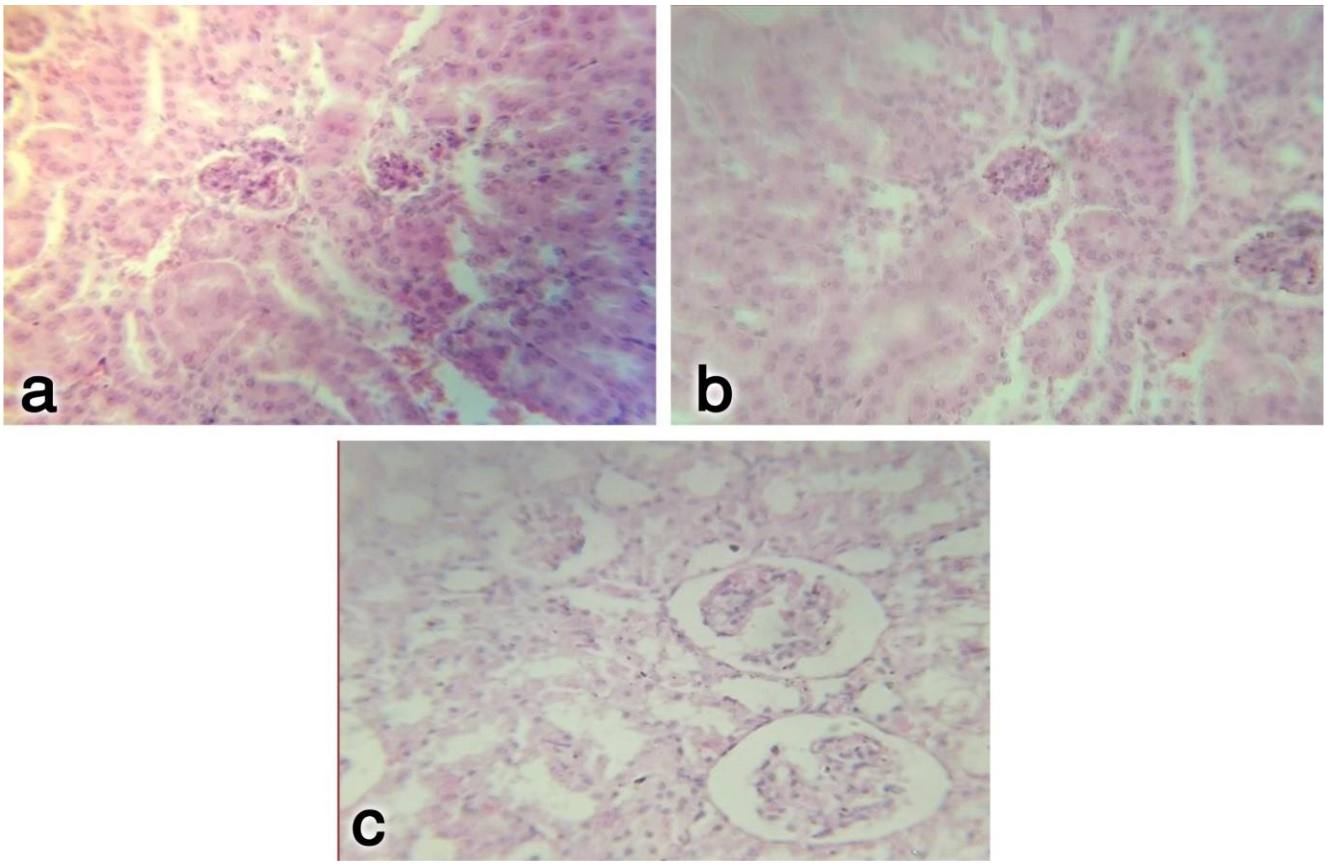

Figure 2. Photomicrograph of the kidney shows a normal histological structure of glomeruli, as well as proximal and distal convoluted tubules in the control group, A) in PPI-2 group, and B) in PPI-3 showing the widening of Bowman space, shrunken glomeruli, and congested tubular cells; C) H and E ×40 and ×10

The results of our study proved that prolonged treatment with PPIs has clear effects on kidney function in laboratory rats, which is consistent with the findings of other investigations that proved the impact of PPIs on kidney function. Many studies have demonstrated PPIs as one of the common causes of AIN, especially in older patients (17). However, the mechanism of causing AIN by PPIs is not well known. Several studies have proven PPI-induced AIN as a consequence of cell-mediated immune responses, which are probably idiosyncratic and dose-independent (18).

Several mechanisms clarify the link between the use of PPIs and the risk of exposure to adverse kidney function consequences. A new study by Yepuriand et al. explained that long-term PPIs usage might damage the endothelial function and hasten endothelial senescence leading to endothelial dysfunction, oxidative stress, vascular senescence, and kidney disease development (19). Moreover, the induction of hypomagnesemia by PPIs can justify the link between using PPIs and CKD because magnesium deficit can increase the risk of kidney disease through oxidative

stress, inflammation, and endothelial cell dysfunction (20). In recent years, many studies have shown that using PPIs is associated with kidney, neurological, and cardiovascular morbidity, which may support the likelihood of a mechanistic connection (21). The study by Lazarus et al. demonstrated that PPIs are an independent risk factor for kidney disease and AKI. As a result, additional studies are necessary to find whether PPIs cause kidney disease and what are the possible mechanisms (21).

It has been demonstrated that interstitial nephritis may occur in patients treated with PPIs, which could be attributed to an allergic reaction to the medication. However, the exact mechanism is unknown (18). In cases proven by biopsy, the results indicated that about 70% of AIN cases were described to be caused by the medicines, and approximately 14% of them were caused by PPIs (17). In addition to AKI, CKD is also described by several investigators to be associated with long-term PPI treatment based on glomerular filtration rate and serum creatinine concentration. However, the odds ratio is modest (1.1-1.5) and the effects are based only on observational studies (22). Histopathology of kidney tissue revealed widen Bowman space, shrunken glomeruli, and congested tubular cells. Geevasinga et al. reported eosinophils within the tubular interstitium of 88% of kidney patients treated with PPIs (23).

Salib et al. (24) explained the cause of histopathological findings in kidneys as the susceptibility of this organ to the toxic influences of diverse noxious chemicals due to its unique physiologic and anatomic structures. Functionally, kidneys obtain about 20% of the resting cardiac output and so any chemical material in the circulation will be supplied in high amounts to it. Physiologically, the processes of urine formation and concentration result in the accumulation of toxic materials in renal tubular cells and their lumen. Consequently, a non-toxic concentration of certain chemical materials in plasma might reach a poisonous concentration in the kidney (24). Therefore, we concluded that the long-term use of PPIs affects the histological structure of the kidney.

3.3. Effect of PPI (10 mg/kg) on Liver Functions in Rats

Results of the current study revealed a significant increase ($P<0.05$) in serum ALT (Figure 3-A) and total bilirubin (Figure 3-C) in the rats of the PPI-3 group, in comparison with those of PPI-2 and control groups. On the other hand, serum ALP significantly elevated ($P<0.05$) (Figure 3-D) in the rats of the PPI-3 group, in comparison with the control group only. In addition, AST level was not significantly different between the treated groups (Figure 3-B).

Histopathological study of the liver revealed normal histology structure in the control group (Figure 4-A) and the animals in the PPI-2 group (Figure 4-B), while some histological changes were observed in the rats of the PPI-3 group. These alterations included congestion in the blood vessels and degradation in the hepatic cells (Figure 4-C).

The results of liver function tests and the histological study revealed that long-term use of PPIs influences liver function. A study by Kinoshita et al. explained that many medications, including PPIs, phenytoin, and warfarin are at least partially degraded by the drug-metabolizing enzyme CYP2C19 in the liver. However, that enzyme is not adequately capable. Therefore, long-term treatment with PPIs might reduce the degradation of additional medicines amplifying their pharmacological properties. Alternatively, for the stimulation of clopidogrel, CYP2C19 enzyme activity is required. Consequently, the administration of PPIs in patients treated with clopidogrel might diminish its anti-thrombotic activity and augment the risk of cardiovascular disease (25).

In addition, other studies have indicated that PPIs are frequently described by many researchers to raise the risk of spontaneous bacterial peritonitis from an odds ratio of 1.4 to 5. However, there are some discrepancies in the results of different studies. Spontaneous bacterial

peritonitis is a bacterial infection of the abdominal cavity occurring in patients with ascites and liver cirrhosis. Because of the increased penetrability of the intestinal mucosa in patients with cirrhosis, intestinal bacteria might penetrate the intestinal wall and proliferate in the ascites fluid without macroscopic intestinal impairment (26). Recently, treatment with PPIs has also been reported to be associated with hepatic encephalopathy in cirrhosis patients (27). The PPIs induced hypomagnesemia and vitamin B12 deficiency with gut microbial flora being considered as the possible link between hepatic encephalopathy and PPIs. However, the exact mechanism is not yet explained (25).

Gastric hydrochloric acid is bactericidal and is a defense mechanism against digested microorganisms. Therefore, the increased incidence of hepatic complications following PPIs usage and elevated mortality in patients with liver cirrhosis could be attributed to the suppression of intestinal acid and restriction of this defense (28, 29). Furthermore, in patients with liver cirrhosis, hepatic clearance of PPIs declines (30), which results in increased overall exposure to PPIs. Finally, PPIs also affect the intestinal microenvironment by changing pH in the small intestine and stomach leading to gut dysbiosis. Dysbiosis can cause inflammasome-deficiency-related changes through microbiome metabolites deteriorating liver inflammation and producing endotoxins that worsen intestinal penetrability and inflammation (31).

Yepuri et al. demonstrated that long-term exposure to PPIs, particularly esomeprazole damages enzyme activity and lysosomal acidification, which cause protein accumulation, augment the generation of reactive oxygen species, and exacerbate oxidative stress (19). According to the results of the present study, long-term administration of PPIs caused adverse effects on kidney and liver function in laboratory rats.

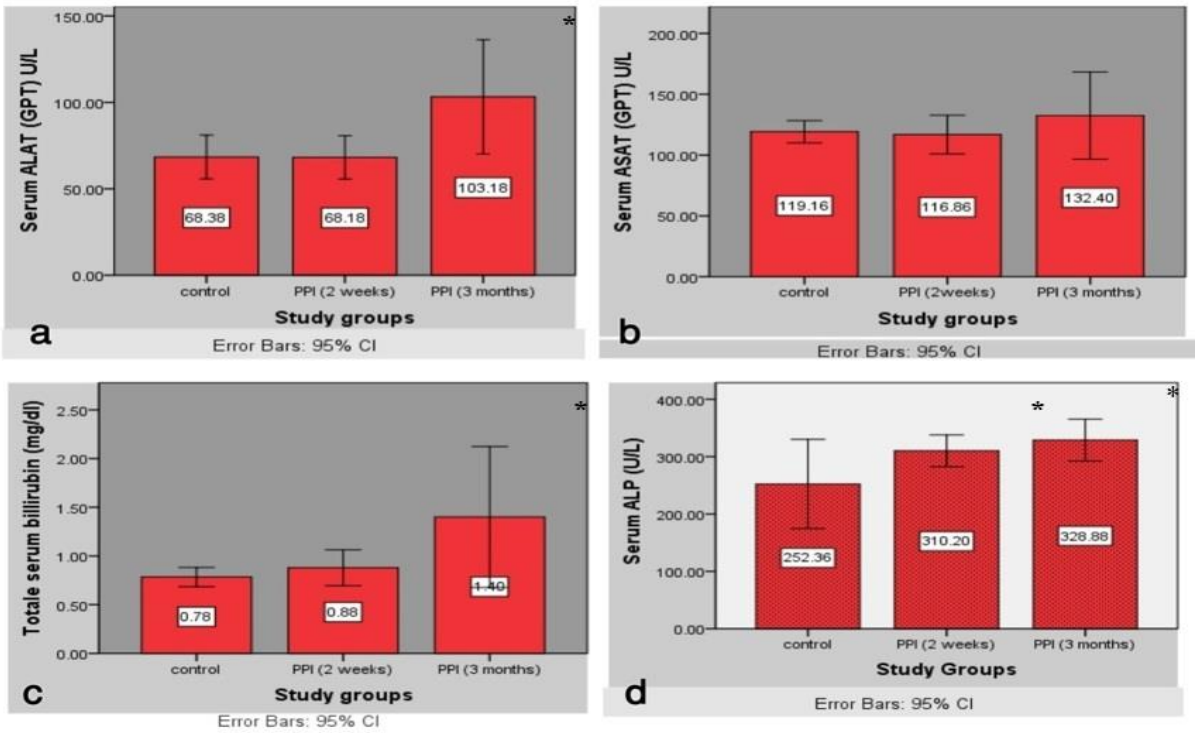

Figure 3. Comparison of the serum levels of ALT (a), AST (b), total serum bilirubin (c), and ALP (d) in rats treated with PPI (10 mg/kg) and control group
(❋): significant difference in comparison with other groups
*The mean difference is significant at 0.05 level

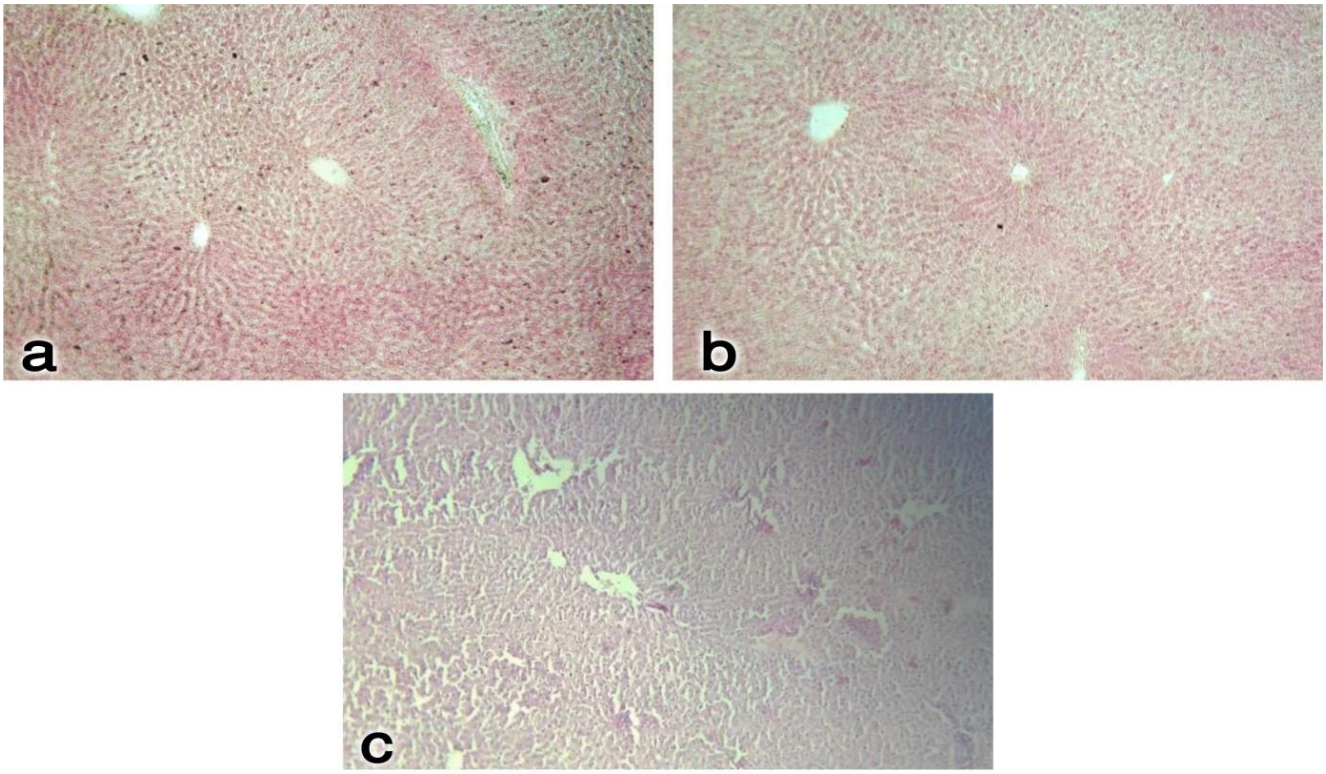

Figure 4. Photomicrograph of the liver shows the normal histological structure in the control group, A) in the rats of the PPI-2 group, while B) the liver in the animals of the PPI-3 group represented congestion in the blood vessels and degradation in hepatic cells; C) H and E ×40 and ×10

Authors' Contribution

S. M. J. A. was the main investigator in this study. Z. S. M. A. participated in preparing the final draft of the manuscript, reviewing the manuscript. S. M. J. A. and Z. S. M. A. have read and agreed to the content of the manuscript and have confirmed the accuracy or integrity of all parts of the work.

Ethics

This experimental procedure was carried out according to the guidelines of the Institutional Animal Care and Use Committee of the University of Kufa. Moreover, animals were transported, cared for, and used in accordance with the Animal Act 1953 (revised 2006), the Wildlife Conservation Act 2010, applicable federal laws, other government legislation and policies, as well as the code of practice for the care and use of animals for scientific purposes.

Research ethical issues, including plagiarism, data fabrication, and double-publishing were fully noted by the authors.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Forgacs I, Loganayagam A. Overprescribing proton pump inhibitors. Br Med J; 2008; 336(7634):2-3.
2. Wilhelm SM, Rjater RG, Kale-Pradhan PB. Perils and pitfalls of long-term effects of proton pump inhibitors. Expert Rev Clin Pharmacol. 2013;6(4):443–51.
3. De Bruyne P, Christiaens T, Vander Stichele R, Van Winckel M. Changes in prescription patterns of acid-suppressant medications by Belgian pediatricians: analysis of the national database,[1997–2009]. J Pediatr Gastroenterol Nutr. 2014;58(2):220–5.

4. Ahrens D, Chenot J-F, Behrens G, Grimmsmann T, Kochen MM. Appropriateness of treatment recommendations for PPI in hospital discharge letters. Eur J Clin Pharmacol. 2010;66(12):1265–71.
5. Klepser DG, Collier DS, Cochran GL. Proton pump inhibitors and acute kidney injury: a nested case–control study. BMC Nephrol. 2013;14(1):1–7.
6. Blank M-L, Parkin L, Paul C, Herbison P. A nationwide nested case-control study indicates an increased risk of acute interstitial nephritis with proton pump inhibitor use. Kidney Int. 2014;86(4):837–44.
7. Lambert AA, Lam JO, Paik JJ, Ugarte-Gil C, Drummond MB, Crowell TA. Risk of community-acquired pneumonia with outpatient proton-pump inhibitor therapy: a systematic review and meta-analysis. PLoS One. 2015;10(6):e0128004.
8. Dial S, Alrasadi K, Manoukian C, Huang A, Menzies D. Risk of Clostridium difficile diarrhea among hospital inpatients prescribed proton pump inhibitors: cohort and case–control studies. Cmaj. 2004;171(1):33–8.
9. Yang Y-X, Lewis JD, Epstein S, Metz DC. Long-term proton pump inhibitor therapy and risk of hip fracture. Jama. 2006;296(24):2947–53.
10. Coca SG, Singanamala S, Parikh CR. Chronic kidney disease after acute kidney injury: a systematic review and meta-analysis. Kidney Int. 2012;81(5):442–8.
11. Lazarus B, Chen Y, Wilson FP, Sang Y, Chang AR, Coresh J, et al. Proton pump inhibitor use and the risk of chronic kidney disease. JAMA Intern Med. 2016; 176 (2): 238–46.
12. Tin A, Grams ME, Maruthur NM, Astor BC, Couper D, Mosley TH, et al. Results from the Atherosclerosis Risk in Communities study suggest that low serum magnesium is associated with incident kidney disease. Kidney Int. 2015;87(4):820–7.
13. Mohajeri MH, Brummer RJM, Rastall RA, Weersma RK, Harmsen HJM, Faas M, et al. The role of the microbiome for human health: from basic science to clinical applications. Eur J Nutr. 2018;57(1):1–14.
14. Brandl K, Schnabl B. Is intestinal inflammation linking dysbiosis to gut barrier dysfunction during liver disease? Expert Rev Gastroenterol Hepatol. 2015;9(8):1069–76.
15. Weersink RA, Bouma M, Burger DM, Drenth JPH, Harkes-Idzinga SF, Hunfeld NGM, et al. Safe use of proton pump inhibitors in patients with cirrhosis. Br J Clin Pharmacol. 2018;84(8):1806–20.
16. Dultz G, Piiper A, Zeuzem S, Kronenberger B, Waidmann O. Proton pump inhibitor treatment is associated with the severity of liver disease and increased mortality in patients with cirrhosis. Aliment Pharmacol Ther. 2015;41(5):459–66.
17. Muriithi AK, Leung N, Valeri AM, Cornell LD, Sethi S, Fidler ME, et al. Biopsy-proven acute interstitial nephritis, 1993-2011: a case series. Am J Kidney Dis. 2014;64(4):558–66.
18. Berney-Meyer L, Hung N, Slatter T, Schollum JBW, Kitching AR, Walker RJ. Omeprazole-induced acute interstitial nephritis: A possible Th 1–Th 17-mediated injury? Nephrology. 2014;19(6):359–65.
19. Yepuri G, Sukhovershin R, Nazari-Shafti TZ, Petrascheck M, Ghebre YT, Cooke JP. Proton pump inhibitors accelerate endothelial senescence. Circ Res. 2016;118(12):e36–42.
20. Ghebremariam YT, LePendu P, Lee JC, Erlanson DA, Slaviero A, Shah NH, et al. Unexpected effect of proton pump inhibitors: elevation of the cardiovascular risk factor asymmetric dimethylarginine. Circulation. 2013;128(8):845–53.
21. Lazarus B, Chen Y, Wilson FP, Sang Y, Chang AR, Coresh J, et al. Proton pump inhibitor use and the risk of chronic kidney disease. JAMA Intern Med. 2016;176(2):238–46.
22. Nochaiwong S, Ruengorn C, Awiphan R, Koyratkoson K, Chaisai C, Noppakun K, et al. The association between proton pump inhibitor use and the risk of adverse kidney outcomes: A systematic review and meta-Analysis. Nephrol Dial Transplant. 2018;33(2):331–42.
23. Geevasinga N, Coleman PL, Webster AC, Roger SD. Proton pump inhibitors and acute interstitial nephritis. Clin Gastroenterol Hepatol. 2006;4(5):597–604.
24. Salib YS, Kandeel S, El-Mehey KA, Zamzam AEEF. Histological study of the effect of proton pump inhibitor (esomeprazole) on the renal cortex of adult male albino rats. Egypt J Histol. 2019;42(3):624–34.
25. Kinoshita Y, Ishimura N, Ishihara S. Advantages and disadvantages of long-term proton pump inhibitor use. J Neurogastroenterol Motil. 2018;24(2):182–96.
26. Kim JH, Lim KS, Min YW, Lee H, Min B, Rhee P, et al. Proton pump inhibitors do not increase the risk for recurrent spontaneous bacterial peritonitis in patients with cirrhosis. J Gastroenterol Hepatol. 2017;32(5):1064–70.
27. Tsai C-F, Chen M-H, Wang Y-P, Chu C-J, Huang Y-H, Lin H-C, et al. Proton pump inhibitors increase risk

for hepatic encephalopathy in patients with cirrhosis in a population study. Gastroenterology. 2017;152(1):134–41.

28. Freedberg DE, Kim LS, Yang Y-X. The risks and benefits of long-term use of proton pump inhibitors: expert review and best practice advice from the American Gastroenterological Association. Gastroenterology. 2017;152(4):706–15.

29. Scarpignato C, Gatta L, Zullo A, Blandizzi C. Effective and safe proton pump inhibitor therapy in acid-related diseases–A position paper addressing benefits and potential harms of acid suppression. BMC Med. 2016;14(1):1–35.

30. Lodato F, Azzaroli F, Di Girolamo M, Feletti V, Cecinato P, Lisotti A, et al. Proton pump inhibitors in cirrhosis: tradition or evidence based practice? World J Gastroenterol WJG. 2008;14(19):2980.

31. Chen P, Stärkel P, Turner JR, Ho SB, Schnabl B. Dysbiosis-induced intestinal inflammation activates tumor necrosis factor receptor I and mediates alcoholic liver disease in mice. Hepatology. 2015;61(3):883–94.