Investigation on the Effects of Long-Term Treatment of Proton Pump Inhibitors on Kidney and Liver Functions in Laboratory Female Rats

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Abstract
Proton pump inhibitors (PPIs) are a group of drugs that are effectively used to inhibit gastric acid secretion and are used to treat many acid-related disorders including gastroesophageal reflux disease and other gastric disorders, recent studies recommend that they may be associated with the risk of chronic kidney disease and liver disease. Therefore the aim of this study was to investigate the effect of long-term treatment with proton pump inhibitors on kidney and liver function in laboratory rats. Fifteen female albino white rats (Rattus norvegicus) were randomly assigned to three groups of five animals. The three groups included a control (group I) were fed regular pellet, the second group (group PPI-2 weeks) were fed with standard pellet diet and given esomeprazole (10 mg/kg b.w.) via oral gavage daily in the morning for two weeks, and the third group (group PPI-3 months) were fed with standard pellet diet and given esomeprazole (10 mg/kg b.w.) via oral gavage daily in the morning for three months. Blood samples were taken after 2 weeks and 3 months by using cardiac puncture technique for measurement of serum creatinine, urea, alanine aminotransferase ALAT, aspartate aminotransferase ASAT, total bilirubin and alkaline phosphatase (ALP). In addition to the histopathological study of kidney and liver tissue. Serum creatinine and urea were significantly increased in group (group PPI-3 months) compared with other groups. Serum ALAT, total bilirubin and ALP were significantly increased in group (group PPI-3 months) compared with other groups. Histopathological study of the kidneys and liver revealed normal histology structure in the control group and females rats which treated with PPI (10 mg/kg) for 2 weeks, while some histological changes were observed in the liver and kidney in females rats which
treated with PPI (10 mg/kg) for 3 months represented by the appearance of some widening of Bowman's space and shrunken glomeruli, whereas the renal tubules showed congestion tubular cells in kidneys and appearance of some congestion in the blood vessels and degradation was observed in the hepatic cells in liver. These data indicate that long-term use of proton pump inhibitors has adverse effects on kidney and liver structure and function.

**Keywords:** Proton Pump Inhibitor, Liver Function, Kidney Function, Long Term Treatment, Esomeprazole

1. **Introduction**

Proton pump inhibitors (PPIs) are very common prescriptions, and high percentage (25% and 70%) of these prescriptions have no suitable indication (1). On the other hand, continues use of PPIs beyond the recommended guidelines are often see in the prescriptions which may lead to a malfunction of liver and kidney (2). Also, there is a trend in the use of proton pump inhibitors for children (3) and many patients are described to be discharged from hospital on a PPI for improper indications or stay in high doses for long term use (4). From the time that this drug was introduced to the market in 1990, numerous studies have linked PPI use to uncommon consequences but dangerous adverse health effects, including acute kidney injury (AKI) (5); and acute interstitial nephritis (AIN) (6); community- acquired pneumonia (7), Clostridium difficile infection (8), and hip fracture (9). Therefore, the use of PPIs may be a risk factor for chronic kidney disease (CKD), possibly mediated by repeated acute interstitial nephritis(10), or by hypomagnesemia, which has been linked with PPI usage (11) and with occurrence CKD (12).

Based on previously published studies there exist a relationship between long-term use of PPIs and liver function, on the other hand in a research conducted by Mohajeri and his colleagues it has been clarified that PPIs may encourage modifications in gut microbiota (13), causing dysbiosis and damaged gut barrier function (14), and PPIs usage in cirrhosis patients is linked with increased danger of hepatic encephalopathy HE and spontaneous bacterial peritonitis SBP (15). The results of a study conducted by Dultz et al. have recommended that PPI use may be related with a developed risk of mortality (16) and reported that PPI usage to be an independent predictor of mortality in patients with compensated and decompensated liver cirrhosis.

Therefore, the aim of the current research was to investigate the association between long term PPI use and kidney and liver function in the laboratory rats.

2. **Materials and Methods**
2.1. Experimental animals
Healthy adult albino female rats, (*Rattus norvigicus*) weighing between 250-300g, were gotten from animal house in the Faculty of Science, University of Kufa. The animals were housed in the animal house in a standard environment include (degree of temperature 22- 28 °C) and controlled condition to standard laboratory nourishment with commercial food (pellets) and water provided to animals through the periods of the experiment. None of the rats had any clinically evident infections.

2.2. Dosage calculation and preparation of stock solution of a proton pump inhibitors
Esomeprazole (Nexium (Esomeprazole 20mg) Tablets, Astra Zeneca) was crushed into powder and 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 distributed into three groups (5 rats in each group). The control group (group I) were fed with normal pellet diet, the second group (group PPI 2 weeks) were fed with normal pellet diet and given esomeprazole (10 mg/kg b.w.) through oral gavage every day in the morning for two weeks, the third group (group PPI 3 months) were fed with normal pellet diet and given esomeprazole (10 mg/kg b.w.) via oral gavage every day in the morning.

2.4. Collection of blood samples
At the end of experiment (after 2 weeks and 3 months), each animal was anaesthetized by a mixture of ketamine 0.1 ml and xylazine 0.2 ml and they were scarified. It was installed on a box of cork pin by then was drawing blood from the heart directly by Heart puncture to get a sufficient amount of blood 5 ml. Each blood sample was placed in a tube without anticoagulant and left for 30 minutes at room temperature and used to obtain serum via centrifugation at 6000 rpm for 5 minutes. Then, biochemical analysis were performed on serum samples.

2.5. Animal Dissection
The animal were exposed to open the abdominal cavity and eradicated the kidneys and liver of each rat and the remove all the fatty tissue and they placed in a plastic container on a formalin solution of 10% for the purpose of conducting study.
2.6. Laboratory Biochemical Testing

2.6.1. Kidney function test
Serum creatinine were measure by Kinetic colorimetric method, blood urea were measure by Enzymatic colorimetric method, according to procedure provide by the Linear Chemicals, Spain.

2.6.2. Liver function tests
Serum Alanine aminotransferase (ALT/GPT) and Aspartate aminotransferase (AST/GOT) were measure by UV enzymatic method Kinetic, Bilirubin were measure by Colorimetric method End Point and Alkaline phosphatase were measure by Colorimetric method Kinetic, All of these tests were done using the according to procedure provide by the Linear Chemicals, Spain.

2.7. Histopathological preparations of samples for light microscopic examination
The kidney and liver specimens were taken and directly the following steps for tissue preparations were made:
1. Fixed in 10% formol saline solution for (24 hours).
2. Then dehydrated in arising concentration of ethyl alcohol.
3. Clearance in twice changes of xylol for 30 minutes each time.
4. After that, impregnation was done in clean paraffin for 2 hours at 60ºC
5. Embedding in solid paraffin.
6. Lastly, sections of 5 um obtained by using microtom.
7. Sections were de-waxed and hydrated through graded alcohol
8. Stained by using Harris’ hematoxylin for 2-5 min.
9. Differentiation in 1% acid alcohol (1% HCL in 70% alcohol) for 5-10 seconds was done.
10. Then, sections washed well in tap water for 5 min, and stained with 1% eosin for 1-3 min.
11. Finally, dehydration by using ascending concentration of ethanol alcohol and clearance by xylol and mounting by using DPX (Distrene Plasticizer Xylene).

Ethical issues: This experimental procedure was carried out according to the guidelines of the institutional animal care and use committee University of Kufa. Moreover, animals have been transported, cared for, and used in accordance with the Animal Act 1953 (revised 2006), the Wildlife Conservation Act 2010, applicable federal laws, and other government legislation and
policies and according to the policy laws of the University of Kufa policy and code of practice for the care and use of animals for scientific purposes.

**Statistical Analysis:** All data were expressed as mean ± SD. The statistical significance was calculated by one-way analysis of variance (ANOVA) using the SPSS (version 26.0) program followed by post-hoc Tukey HSD test. Values were considered statistically significant when p < 0.05.

3. Results and Discussion

3.1. Effect of PPI (10 mg/kg) on the kidney functions in female rats

Our results in this study revealed a significant increase (P < 0.05) of serum creatinine (Figure 1-A) and urea (Figure 1-B) in the females rats group treated with PPI (10 mg/kg) for 3 months in comparison with females rats group treated with PPI (10 mg/kg) for 2 weeks and control group.

![Figure 1](image1)

Figure 1. Comparison of serum levels of creatinine and urea in females rats treated with PPI (10 mg/kg) and control group

(*): significant difference in comparing with other groups.

* The mean difference is significant at the 0.05 level.

3.2. Effect of PPI (10 mg/kg) on the histological structure of kidney in female rats

A histological section was taken from the kidneys tissue removed from the rats and examined histopathologically. Histopathological study of the kidneys revealed normal histology structure in the control group (Figure 2-A) and females rats which treated with PPI (10 mg/kg) for 2 weeks (Figure 2-B), while some histological changes were observed in the females rats which
treated with PPI (10 mg/kg) for 3 months represented by the presence of some widening of Bowman's space and shrunken glomeruli, whereas the renal tubules showed congestion tubular cells (Figure 2-c).

Figure 2. Photomicrograph of the kidney show: normal histological structure of glomeruli, proximal, and distal convoluted tubules in control group A) and in females rats treated with PPI (10 mg/kg) for 2 weeks, B) while females rats treated with PPI (10 mg/kg) for 3 months show: some widening of Bowman's space and shrunken glomeruli, the renal tubules showed congestion tubular cells, C) (H and E, ×40, ×10).

The results of our study proved that prolonged treatment with proton pump inhibitors has clear effects on kidney function in laboratory rats and this result is consistent with the results of other studies that proved the effect of proton pump inhibitors on kidney function. Many studies have proven that PPIs drugs are some of the greatest common causes of acute interstitial nephritis AIN, chiefly in aging patients (17). However, the mechanism of causing AIN by PPIs is not well known. Several studies have proven that PPI-induced AIN as a consequence of a cell-mediated immune response, perhaps idiosyncratic and probable characterized as dose independent (18). It is believed that there are several mechanisms that
clarify the link between the use of PPIs and the risk of exposure to adverse kidney function consequences. A new study by Yepuri and his Colleagues which explained that long-term PPIs use might damage endothelial function and hasten endothelial senescence and then increasing endothelial dysfunction, oxidative stress presses, and vascular senescence and favorable the pathogenesis of the development of kidney disease (19). Also, PPIs induced hypomagnesemia can clarify the link between PPIs usage and chronic kidney disease, since magnesium deficit can increase the danger of kidney disease through oxidative stress, inflammation, endothelial cell dysfunction, and oxidative stress (20). In recent years there have been many studies that have shown that use of PPIs is associated with kidney, neurological, and cardiovascular morbidity, which may support the likelihood of a mechanistic connection (21).

The study of Lazarus and his colleagues showed that PPIs usage is an independent risk factor for kidney disease and acute kidney injury, and additional study is necessary to explore whether PPIs use itself causes kidney disease and, if so, the essential mechanisms of this link is required (21).

It has been demonstrated that interstitial nephritis may occur in patients treated with PPIs, and it is likely that the cause is an allergic reaction to the drug, however the exact mechanism is unknown (18). And in cases that have been proven by biopsy, the results are proven that about 70% of acute interstitial nephritis was described to be caused by the drugs and as high as about 14% of them is caused by PPIs (17). Furthermore to an AKI, it is described by several investigators that long-term PPIs treatment is linked with chronic kidney disorder when the kidney functions were estimated by glomerular filtration rate and the serum creatinine concentration, however the danger ratio is actual modest (1.1-1.5) and the study effects are based only on observational studies (22).

Histopathology of kidney tissue reveals a widening of Bowman's space and shrunken glomeruli, the renal tubules showed congestion tubular cells. A study by Geevasinga and his colleagues showed that the major case series where biopsy findings were recorded documented eosinophils within the tubule interstitium of 88% of kidney patients treated with PPIs (23).

Salib and his groups explained that the cause of the histopathological effects on the kidneys due to the use of PPIs is the kidney was very susceptible organ to the toxic effects of diverse noxious chemicals which is credited to its unique physiologic and anatomic structures (24). 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 process of urine formation and concentration causes accumulation of the toxic material in the renal tubular cells and their lumen. Consequently, a non-toxic concentration of certain chemical
materials in the plasma might reach poisonous concentration in the kidney (24). Therefore, through these results, we conclude that the long-term use of proton pump inhibitors has an effect on the histological structure of the kidney.

3.3. Effect of PPI (10 mg/kg) on the liver functions in female rats
The results in our study revealed a significant increase (P< 0.05) of serum ALAT (Figure 3-A) and total serum bilirubin (Figure 3-C) in the females rats group treated with PPI (10 mg/kg) for 3 months in comparison with females rats group treated with PPI (10 mg/kg) for 2 weeks and control group, while there was significant increase (P < 0.05) of serum ALP (Figure 3-D) in the females rats group treated with PPI (10 mg/kg) for 3 months in comparison with control group only. In addition, ASAT levels did not show significant differences between the treated groups (Figure 3-B).

Histopathological study of the liver revealed normal histology structure in the control group (Figure 4-A) and females rats which treated with PPI (10 mg/kg) for 2 weeks (Figure 4-B), while some histological changes were observed in the females rats which treated with PPI (10 mg/kg) for 3 months represented by congestion in the blood vessels and degradation was observed in the hepatic cells (Figure 4-C).
Figure 3. Comparison of serum levels of serum ALAT (a), ASAT (b), total serum bilirubin (c) and ALP (d) in females rats treated with PPI (10 mg/kg) and control group.

(*) : significant difference in comparing with other groups.

* : The mean difference is significant at the 0.05 level.
Figure 4. Photomicrograph of the liver show: normal histological structure in control group A) and in females rats treated with PPI (10 mg/kg) for 2 weeks B), while the liver in females rats treated with PPI (10 mg/kg) for 3 months show: congestion in the blood vessels and degradation was observed in the hepatic cells C) (H and E, ×40, ×10).

Through the results of liver function tests and histological study, it is clear that long-term use of proton pump inhibitors has an effects on liver function.

A study of Kinoshita and his colleagues explained that many drugs including PPIs, phenytoin, and warfarin are at least partly degraded by drug metabolizing enzyme CYP2C19 in liver. But, the capability of that enzyme is not large adequate, therefore PPIs long term treatment might reduction the level of degradation of additional drugs, amplifying their pharmacological properties. Alternatively, for stimulation of clopidogrel, CYP2C19 enzyme activity is required. Thus, PPIs use in patients treated with clopidogrel might reduction its anti-thrombotic activity and increase the risk of cardiovascular disease (25).

In addition to that, other studies have indicated that PPIs treatment has been frequently described to raise the risk of spontaneous bacterial peritonitis from a danger ratio of 1.4 to 5.0 by many researchers, though there are some discrepancies in the study results. Spontaneous bacterial peritonitis is a bacterial infection of the abdominal cavity experiential in patients with ascites affected by liver cirrhosis. Because of the increased penetrability of the intestinal
mucosa in cirrhosis patients, intestinal bacteria might penetrate the intestinal wall and then proliferate in the ascites fluid without macroscopic intestinal impairment (26).

Lately, PPIs treatment is also reported to be associated with hepatic encephalopathy in cirrhosis patients (27). The PPIs induced hypomagnesemia, vitamin B12 deficiency and gut microbial are considered to be likely relations between hepatic encephalopathy and PPIs administration, however the exact mechanism is not yet explained (25).

The reason that could clarify increased incidence of hepatic events with proton pump inhibitors use and increase mortality in patients with liver cirrhosis was gastric hydrochloric acid is bactericidal and is a defense mechanism from consumed microorganisms (28). Though, PPI are strong intestinal acid suppressants, therefore restrictive this defense 46 (29). Also, in liver cirrhosis patients, there is decrease hepatic clearance of PPIs (30), which therefore increases the overall PPIs exposure. Latter and maybe most essentially, PPI also affect the intestinal microenvironment by changing pH in the small intestine and stomach and is established to cause gut dysbiosis. Dysbiosis in actual, can cause inflammasome-deficiency-related changes through microbiome resulting metabolites, which deteriorates liver inflammation and produces endotoxins that worsen intestinal penetrability and inflammation (31).

Also Yepuri and his colleagues demonstrated that the long-term exposure to PPIs particularly esomeprazole damages enzyme activity and lysosomal acidification which cause an accumulation of protein aggregates, increases the generation of reactive oxygen species and increase oxidative stress (19).

From the results of this study it is clear that long-term administration of proton inhibitors caused adverse effects on kidney and liver function in laboratory rats.

Authors’ contribution:
I was the main investigators in the study. The other researcher participated in making the final draft of the manuscript, reviewed the manuscript and have read and agree to the manuscript’s content and have confirmed the accuracy or integrity of any part of the work.

Ethical considerations:
This experimental procedure was carried out according to the guidelines of the institutional animal care and use committee University of Kufa. Moreover, animals have been transported, cared for, and used in accordance with the Animal Act 1953 (revised 2006), the Wildlife Conservation Act 2010, applicable federal laws, and other government legislation and policies.
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Ethical issues (including plagiarism, data fabrication, and double-publishing) were fully noted by the authors.

**References**


