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Original Article

Histological Evaluation and Liver Function after Administration of Omeprazole on Fisher Male Rats

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Abstract

Omeprazole is a member of the substituted benzimidazoles class, that inhibits the protons pump of the gastric parietal cells. On the other hand, taking omeprazole for more than a year may increase the chances of developing other side effects such as bone fractures, gastrointestinal infections, and vitamin B12 deficiency, this work aimed to determine and estimate the effect of Omeprazole drug on the histology and enzyme function of the liver in the laboratory rat (Fischer) by measuring the concentration of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), enzymes of the liver, and their histology. We conducted a comparative cross-sectional study between March 2024 and July 2024. 20 male Fischer rats (Albino) aged two months, weighting (200-250g) was obtained from the animal house located in The Libyan Centre for Medical Research, in the city of Alzawia, and were enrolled in this study. Rats were divided into two groups, the first group was the control (Normal saline group) (n=10), second was administered 40mg/kg omeprazole via IP route (n=10) once daily. The statistical processes calculated for the results of this study are using Jamovi (Version 2.3) and the variance analysis test (ANOVA) at (p 0.05). Alkaline phosphatase (ALT) ($p<0.001$) and aspartate aminotransferase (AST) ($p<0.01$) levels were elevated in long-term rats treated with omeprazole. In contrast, no significant change was found in the level of alanine aminotransferase (ALP) ($p>0.05$). In the omeprazole group, minimal diffuse hepatocyte was observed, within the portal spaces, damage, and congestion in sinusoids around the central vein (CV) hepatocyte destruction, and showed a mildly congested central vein. Prolonged use of omeprazole might result in disturbances in liver enzyme levels and severe histopathological changes such as bleeding, lining endothelium damage, sinusoid congestion around the CV, and hepatocyte destruction

Key words: *Omeprazole, Liver Enzymes, Albino Rats, Liver Histopathology, Central Vein***.**

Introduction

Omeprazole is classified as a drug belonging to the group of proton pump inhibitors, which plays an important role in the short-term treatment of ulcer infections; reducing acidity of the stomach, and in the treatment of Helicobacter pylori infection (1). Although this type of medicine, known as PPI (Proton Pump Inhibitors), as mentioned earlier, is very effective and has a high safety ratio, its long use may cause some problems (2). After oral administration, Omeprazole is absorbed by the gastrointestinal tract, and the drug's metabolism processes occur in the liver, which in turn converts the drug into simple substances that can be excreted by the urine (3). For these reasons, taking omeprazole for a long period can lead to serious liver dysfunction (4).

The liver is one of the largest organs in the body and its function is metabolisms through liver enzymes, which are one of the important terms in biochemistry, the importance of these enzymes is that in the case of liver infections (hepatitis) note the rate level (5). High rates of liver enzymes caused by cirrhosis of the liver, hepatitis, hepatic and liver cancer, obesity, and drugs lead mainly to liver diseases and an imbalance of enzymes among these enzymes are: Transaminases; there are two types of A- Glutamate pyruvate Transaminase (GPT) or Alanine Transaminase (ALT) B-Glutamate Transaminase (GOT) or Aspartate Transaminase (AST) (6). These enzymes are present in the liver as well as in other organs of the body, GPT and GOT tests can help to identify diseases and infections that affect the liver where the rate of the enzymes much higher according to damage of liver (7).

Despite their wide use, omeprazole and esomeprazole have only rarely been associated with hepatic injury. In large-scale, long-term trials, serum ALT elevations occurred in less than 1% of patients and at rates similar to those with placebo or comparator drugs (8). Liver biopsy typically shows prominent centrilobular necrosis, suggesting an acute, toxic hepatic injury (acute hepatic necrosis); however, recurrence upon rechallenge has been documented in several cases. In some instances, other organ involvement is prominent including rhabdomyolysis, lactic acidosis, renal insufficiency, or Stevens-

Johnson syndrome (9). In large case series of drug-induced liver injury, omeprazole has accounted for few instances of symptomatic acute liver injury and rare instances of acute liver failure (10).
As a result of all of this, the long-term safety and must tolerability of OME be appraised and confirmed again as a call to action to prevent potential adverse events associated with its long-term use and based on the warnings issued by the United States FDA on the use of OME (and other proton pump inhibitors) (11). Therefore, this study was designed to contribute to medical and academic knowledge on the safety or potential dangers of long-term administration of OME. This study evaluated the effect of subchronic administration of OME on liver function enzymes and their histopathological parameters in male rats.

Methods

Experimental design

20 male Fischer rats (Albino) aged two months, weighting (200-250g) were obtained from the Animal House located in The Libyan Centre for Medical Research, in the city of Alzawia, Rats were housed in a temperature of (22±2cº) and light (12:12h) light: dark and feed on commercial standard pellets diet, Rats were divided into two groups, the first group was the control (Normal saline group) (n=10), second was administered 40mg/kg omeprazole via IP route (n=10) once daily, .and diluted with 20 ml of water for injection, with 35 units given to each rat. The experiment lasted 90 days.

Biochemical Measurements

After the animals were sacrificed, a sterile syringe withdrew 5ml of blood from the animal after anesthetizing and stabilizing it on the anatomy site. The blood was then placed in test tubes inside the centrifuge at a speed of 3000 cycles per minute for 15 minutes.
Then, keep the serum at (º 20-) until use. The serum was used to measure the level of liver function (alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate aminotransferase (AST), AST/ALT ratio, and total bilirubin) were measured using biochemical analysis tools.

Histological examination

Water and food were withdrawn from the experimental animals 24 hours before the end of the experiment. The animals were then euthanized, and the liver weights of each rat were recorded. Liver samples were placed in a 10% formalin solution until further use. The samples were subsequently processed in a tissue processor for 16 hours through the following stages: 70% ethanol for 6 hours, followed by a series of increasing concentrations of ethanol (80%, 90%, and 95%) for 1 hour each, and finally 3 hours in 100% ethanol. The tissues were then passed through three changes of xylene, each for 1 hour. Finally, they were immersed in paraffin wax. The paraffin blocks were kept until sectioning. Paraffin sections (4 µm) were prepared, stained with H&E, and examined under a light microscope. Analyze the tissue architecture, cellular morphology, and any pathological changes.

Data analysis

The statistical processes calculated for the results of this study are using Jamovi (Version 2.3) and the variance analysis test (ANOVA) at ($p< 0.05$).

Results

Effect of OMP on liver enzymes

The data in Table 1 illustrate the levels of liver enzymes in two distinct groups: Treatment and Control. The treatment group exhibited a higher mean ALT level (51.73 U/L) than the Control group (37.51 U/L), although both groups displayed variability. Furthermore, the Treatment group displayed a significantly higher mean AST level (182.99 U/L) than the control group (84.10 U/L), with substantial variability in both groups. The control group had a higher mean ALP level (58.39 U/L), but the standard deviations were relatively similar. Additionally, the treatment group had a higher mean AST/ALT ratio (4.01) compared to the control group (3.19), with larger standard deviations in the treatment group (2.53) compared to the control group (0.73). Total bilirubin levels were similar between the treatment and control groups (0.06 U/L and 0.05 U/L), with minimal variability and small standard deviations (0.03 U/L for both).

Table 1. Comparison of the level of the serum marker of hepatic functions in the control and omeprazole group (Data expressed as mean±SD)

Parameter	Treatment (Omeprazole) Group (mean ± SD)	Control Group (mean ± SD)	P-value (*<0.05 is significant)
ALT U/L	51.73 ± 7.10	37.51 ± 3.96	0.001*
AST U/L	182.99 ± 37.46	84.10 ± 10.77	0.001*
ALP U/L	35.60 ± 6.58	58.39 ± 7.73	0.001*
AST/ALT	4.01 ± 2.53	3.19 ± 0.73	0.337
TOTAL BILIRUBIN	0.06 ± 0.03	0.05 ± 0.03	0.465

Histological examination

In order to emphasize the results obtained from biochemistry parameters, our results have been supported by histological sections. The experiment was designed to establish whether hepatotoxicity resulted from the effects of sub-chronic omeprazole administration on biochemical and histological parameters in the liver of Fischer male rats. The liver tissue sections were divided into four main structural units which are: the central vein area, portal area, hepatic acinus (metabolic zones), and hepatocytes, which were deeply looked at. Histological examination of hepatic tissues from the control group (Figure 1) showed no significant change in findings, showing a normal rounded central vein, normal lining endothelial, no bleeding observed, normal hepatocytes radiating arrangement from the central vein, and blood sinusoids appearing between the hepatocytes. The omeprazole treatment group (Figure 2), shows a mild congested central vein showing severe histopathological change such as bleeding, damage in lining endothelium minimal diffuse hepatocyte was observed, within the portal spaces. Damage, congestion in sinusoids around the CV, and hepatocyte destruction.

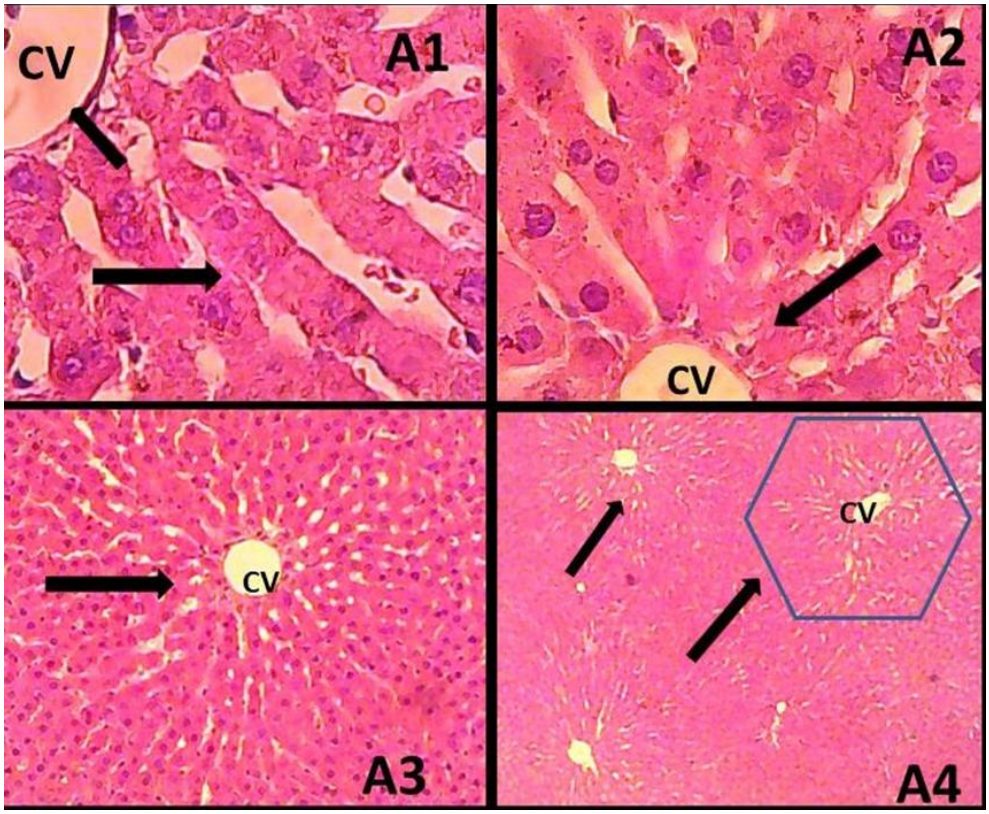

Figure 2. Micrographs of central vein zone collected from control group rats. A1; section from control group showing normal rounded central vein (black arrow) A2, normal lining endothelial, no bleeding observed, A3 normal hepatocytes radiating arrangement from the central vein and blood sinusoids appear between the hepatocytes, A4 normal structure. The snapshot sections of H&E dye were taken with a light microscope, magnification (400X).

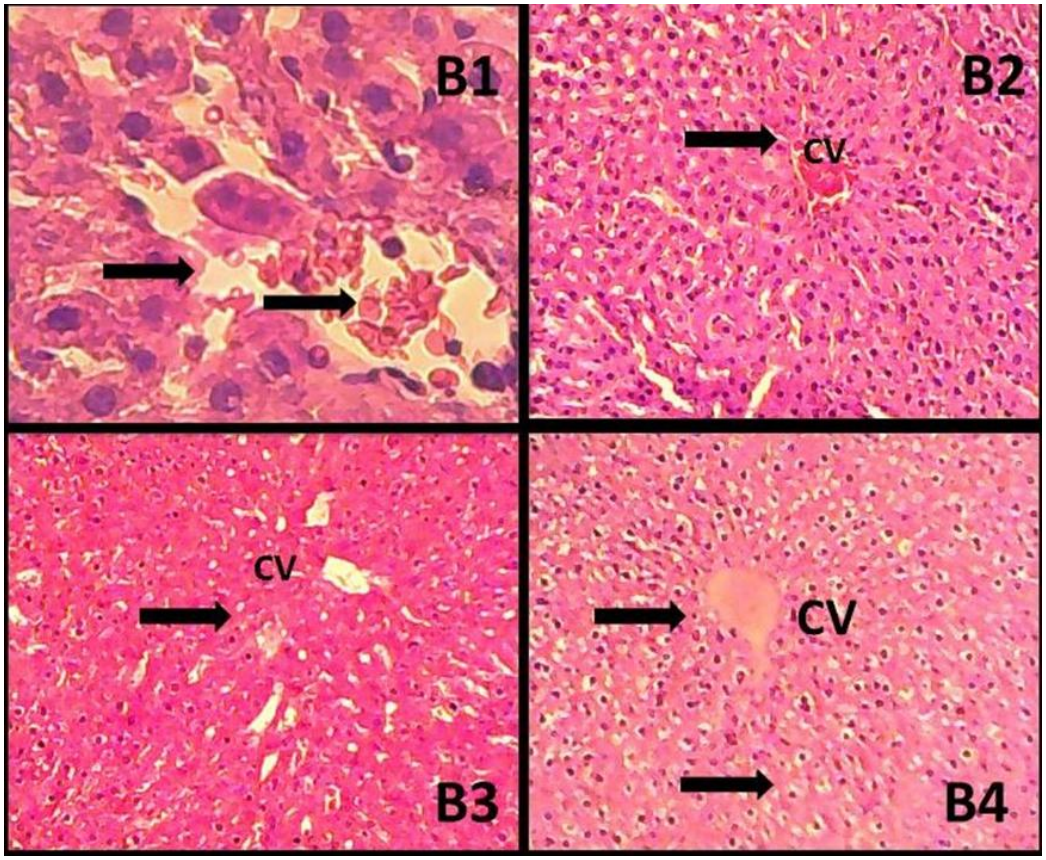

Figure 2. B1, The Omeprazole-treated group section shows a mildly congested central vein (black arrow) and damage in the lining endothelium. B2 a section of the treated group shows a marked injury in the central vein with hemorrhage (black arrow). B3, the central vein area section (CV) harvested from the OMP group showed severe histopathological changes such as bleeding, lining endothelium (black arrow) damage. B4, sinusoid congestion around the CV, and hepatocyte destruction. showing marked injury in CV and perivenous zone (black arrow), hepatic necrosis associated with inflammatory cells (head arrow) and congestion in the periportal zone. Light microscope a magnification is (100) H&E stain.

Discussion

Over recent years, the focus on the adverse effects of using PPI medications for long-term therapy has gained increasing concerns. Omeprazole is commonly used for treating multiple acid-dependent gastrointestinal disorders. The present study was planned to detect the adverse effects of sub-chronic use of omeprazole on liver enzymes, and their histology.

The result of this study demonstrated that measurement of liver function biomarkers revealed a marked rise in serum AST and ALP levels in the treatment group compared to the control group, with significant changes in ALT and the total bilirubin levels were similar between the treatment and control groups, with minimal variability and small standard deviations therefore aminotransferases and alkaline phosphatases are enzymes that exist primarily in the hepatic parenchymal cell. So Increased levels of these enzymes in the bloodstream are indicators of tissue damage to the liver. In Comparison with Literature: Cameron et al. (2003) both reported increased ALT and AST levels in rodents treated with omeprazole (12). Additionally, Borges et al. (2003) found a similar pattern in humans, where liver enzymes such as ALT and AST were elevated following long-term omeprazole therapy, indicating hepatocellular damage (13). On the other hand, total bilirubin levels were similar between the treatment and control groups (0.06 U/L and 0.05 U/L), with minimal variability and small standard deviations (0.03 U/L for both). This is consistent with Literature: According to Pechlivanoglou et al. (2017), itraconazole can impair bilirubin conjugation, leading to its accumulation in the blood (14). This is consistent with findings in Fisher male rats, where cholestasis and hepatic dysfunction lead to elevated serum bilirubin levels.

However, hepatocellular damage and inflammation: studies in animal models, particularly rats, have demonstrated that omeprazole administration can lead to histological changes in the liver. In our study histological examination of hepatic tissues from the control group (Figure 1) showed no significant change in findings. In the omeprazole group (Figure 2), minimal diffuse hepatocyte was observed, within the portal spaces. damage, congestion in sinusoids around the CV, and hepatocyte destruction, and shows a mildly congested central vein in a study by Al Ali et al. (2023), rats administered high doses of omeprazole showed signs of hepatocellular necrosis and steatosis (fatty liver), similar to other findings in PPI-related liver injury (15). This finding is consistent with the results observed in Fisher male rats, where omeprazole-induced hepatotoxicity is marked by the accumulation of lipid droplets in hepatocytes and inflammatory infiltrates, particularly in the periportal and mid-zonal regions of the liver. Comparison with Literature: Research by Zimmerman (2000) discusses how hepatocellular necrosis and steatosis can result from metabolic alterations or oxidative stress induced by drugs like

omeprazole (16). This oxidative damage is commonly linked with liver injury due to cytochrome P450 enzyme induction, as observed in rodent models.

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