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Original article

Effect of Sub- Chronic Administration of Omeprazole on Hematological and Biochemical Parameters in Fischer Male Rats

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ABSTRACT

Long-term use of proton pump inhibitors (PPIs) is believed to have various potential adverse events. Omeprazole is a part of PPIs most commonly prescribed worldwide; it irreversibly binds to the H+-K+ ATPase enzyme system in the gastric parietal cells to reduce the secretion of H+ ions into the lumen of the stomach. The main objective of the current work is to assess the adverse effects of omeprazole medication on certain hematological and biochemical parameters in rats who were on treatment for three months. 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 center 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. Complete blood count and biochemical parameters were measured for both groups. The treatment group had remarkably significant reductions in the number of red blood cells (RBCs) ($p<0.001$) and the platelets indices. Omeprazole elevated the cholesterol level ($p<0.001$) and triglyceride ($p<0.001$) as well as low-density lipoprotein ($p<0.01$). However, no impact was found with high density lipoprotein (HDL) ($p>0.05$). Blood urea levels ($p<0.001$) were significantly increased in the treatment group treated with omeprazole medication. The results also showed that the treatment group had a significant decline in calcium levels ($p<0.001$) than that of the control group. Prolonged use of omeprazole might result in adverse effects on hematological profile, particularly RBCs and their indices leading to the development of anemia in patients on this medication. Furthermore, it might result in disturbances in biochemical profile, levels of minerals, and vitamins as consequences of affected absorption.

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INTRODUCTION

A popular proton pump inhibitor (PPI), omeprazole reduces the production of gastric acid by permanently blocking the H+/K+ ATPase enzyme in the stomach's parietal cells. Acid-related gastrointestinal conditions including GERD, peptic ulcers, and Zollinger-Ellison syndrome are frequently treated with it. Although omeprazole is effective in treating these problems, long-term or sub-chronic usage of the medication raises questions regarding possible negative effects, especially when it comes to haematological and biochemical markers. The drug's systemic effects and ability to change regular physiological functions outside of the gastrointestinal system have given rise to these worries [1].

Haematological indicators, such as hemoglobin (Hb) levels, platelet counts, white blood cell (WBC) counts, and red blood cell (RBC) counts, give crucial information about the blood's capacity to carry oxygen, fight infections, and stop excessive bleeding [1]. Biochemical indicators, including blood electrolytes, liver enzymes, and kidney function markers, provide information on how important organs like the liver and kidneys are performing and can be negatively impacted by long-term pharmaceutical usage [2]. Omeprazole's positive benefits in treating acid-related illnesses have been shown in studies, but nothing is known about how long-term or sub-chronic usage affects haematological and biochemical markers.

When evaluating the safety profile of pharmacological drugs, such as omeprazole, animal studies have proven to be quite helpful. Omeprazole has been shown in rodent studies, especially Fischer male rats, to have the ability to alter organ shape and function when given for prolonged periods of time [3]. However, the specific effects on haematological and biochemical markers are less well-documented, despite their critical role in evaluating drug-induced toxicity and overall health status [4]. The current study is to examine the effects of sub-chronic omeprazole administration on haematological and biochemical parameters in Fischer male rats, given the significance of these data in comprehending the systemic effects of medicines. The results of this study might assist define criteria for safer, long-term usage of this frequently prescribed drug and provide insight into the possible dangers connected to extended omeprazole therapy.

METHODS

Experimental design and grouping of animals

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±2C^{o}) 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, The experiment lasted 90 days during this, rats weights were measured three times during the experiment at the beginning, middle, and end of the experiment. Additionally, experimental rats were generally observed for behavioral changes.

Procedure of experiment

In this experiment, an intraperitoneal injection was used. Intraperitoneal (IP) injection is used widely in research involving laboratory rats and mice. Advantages include rapid absorption of substances, suitability for administering relatively large volumes, and technical simplicity when compared to the intravenous route. The needle is inserted into the abdominal cavity in the animal's lower right quadrant to avoid the cecum and urinary bladder. The needle should be directed towards the animal's head at an angle of 15 - 20 degrees from the skin and inserted approximately 5mm.

Preparation of injection substance

In this experiment, omeprazole was utilized at a 40 mg dosage and diluted with 20 ml of water for injection, with 35 units given to each rat.

Collection of blood samples

The equivalent of (5ml) of blood was withdrawn from the heart directly with a 5ml syringe after anesthetizing the animal and stabilizing it on the anatomy plate, and then discharged into anticoagulant tubes, The rest of the blood sample was collected immediately in a gel plain tube to prepare serum for performing further tests. Some haematological tests were measured in the laboratory such as haemoglobin concentration, counts of red and white blood cells, and the differential count of leukocytes which includes the proportion of both lymphocytes and neutrophils, the measure of these parameters was carried out based on laboratory standard methods.

Clinical biochemical tests

Clinical biochemical tests were done including kidney function tests (blood urea and serum creatinine), lipid profile (total cholesterol, triglycerides, LDL, HDL, and VLDL), and serum electrolyte level (calcium, magnesium, sodium, and potassium).

Statistical analysis

The data analysis was performed using Jamovi (Version 2.3). The findings are displayed as means and standard deviations along with various tables. A t-test was utilized to compare the two groups, with a p-value of <0.05 indicating statistical significance.

RESULTS

The study compares hematological parameters test levels and indices between a treatment group taking omeprazole and a control group. The omeprazole group has a significantly higher white blood cell count, suggesting an immune system activation. The omeprazole group has a higher absolute lymphocyte count and percentage, possibly related to the overall increase in WBC.

The omeprazole group has lower red blood cell and hemoglobin levels, indicating potential anemia. The omeprazole group has a lower mean corpuscular hemoglobin concentration, suggesting hypochromic red blood cells. The Omeprazole group significantly reduces platelet distribution width (PDW), indicating abnormal platelet production or function. No significant difference was found in other parameters such as monocytes, granulocytes, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, platelet count, mean platelet volume, and packed cell volume as illustrated in table 1.

Table 1. Comparison of hematological parameters tests between the treatment (Omeprazole) group and control group.

Parameter	Unit	Treatment (Omeprazole) Group (mean ± SD)	Control Group (mean ± SD)	P-value (*<0.05 is significant)
WBC	10^9/L	5.82±1.26	3.21±0.918	0.001*
Lymph	10^9/L	3.65±0.799	1.94±0.685	0.001*
Mono	10^9/L	0.17±0.082	0.1±0.0471	0.03
Gran	10^9/L	1.6±0.997	1.17±0.32	0.210
Lymph	%	67±7.12	59.6±7.84	0.040
Mono	%	4.09±0.725	3.77±0.672	0.319
Gran	%	32.9±10.2	36.6±7.91	0.376
RBC	10^{12}/L	8.22±2.09	9.89±0.515	0.024
HGB	g/dl	10.2±1.49	14.3±0.854	0.001*
HCT	%	46±9.73	49.3±3.04	0.319
MCH	Pg	14.1±0.408	14.4±0.509	0.163
MCHC	g/dl	28.4±0.781	28.9±0.471	0.001*
RDW	%	14.2±0.993	14.2±1.19	1.000
PLT	109/L	1332±326	1494±138	0.165
MPV	fL	5.12 ± 0.41	4.93±0.206	0.206
PDW	%	15.5±0.494	15.2±0.118	0.001*
PCT	%	0.507±0.207	0.658±0.0379	0.035

This difference is statistically significant, with a p-value of 0.001. However, the lack of a statistically significant difference in serum creatinine levels suggests minimal impact on kidney function based on this specific data set figure 1.

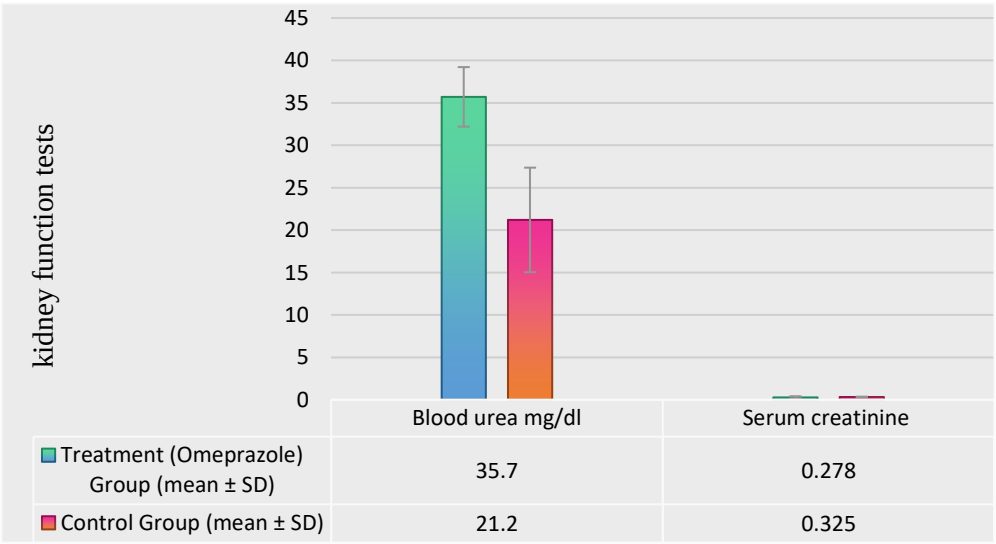

Figure 1. Comparison of kidney function tests between the treatment (Omeprazole) and control groups

Moreover, the blood lipid levels of patients were in two distinct groups: those undergoing treatment (with omeprazole) and those in the control group. The treatment group exhibited slightly elevated cholesterol levels (59.8 mg/dL) compared to the control group (58.1 mg/dL), yet this variance is not deemed statistically significant. Both the treatment and control groups displayed similar triglyceride levels (50.5 mg/dL and 49.8 mg/dL) with moderate variability. LDL cholesterol levels were comparable between the treatment and control groups (18.5 mg/dL), as were HDL cholesterol levels (31.4 mg/dL), although not statistically significant. VLDL cholesterol levels also showed no significant difference between the treatment and control groups (10.1 mg/dL and 9.94 mg/dL) as illustrated in Figure 4. Nevertheless, based on the data provided, it can be concluded that omeprazole treatment does not have a statistically significant impact on the major blood lipid levels (total cholesterol, triglycerides, LDL, HDL, and VLDL) in this particular study, as shown in figure 2.

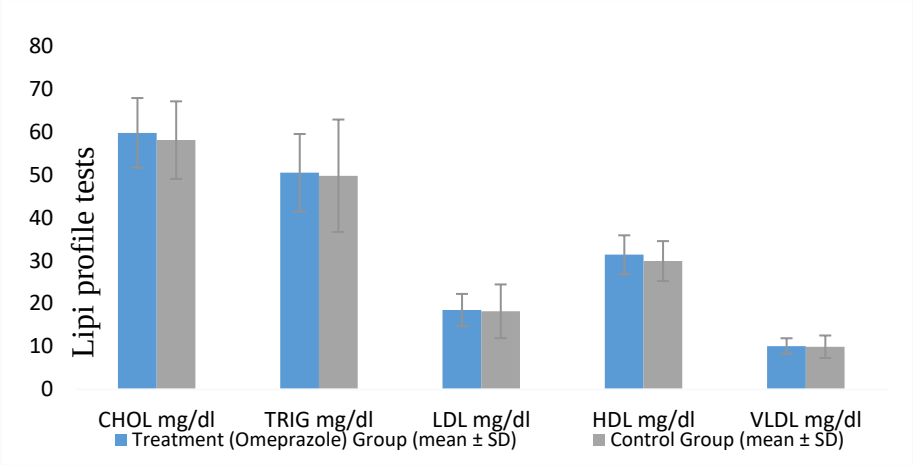

Figure 2. Comparison of lipid profile tests between the treatment (Omeprazole) and control groups.

The study analyzed blood electrolyte levels in patients taking omeprazole and compared them to a control group. The control group had a significantly higher mean calcium level (9.69 mg/dL) compared to the treatment group (8.48 mg/dL), with a p-value of less than 0.05. The mean magnesium levels were almost similar between the two groups, with a p-value of greater than 0.05. The treatment group had a significantly lower mean sodium level (119 mg/dL) than the control group (147 mg/dL), with a p-value of less than 0.05. The standard deviation was larger in the treatment group (15.5 mg/dL) compared to the control group (5.97 mg/dL). The treatment group had a higher mean potassium level (6.5 mg/dL) than the control group (4.48 mg/dL), with a p-value of less than 0.05. The standard deviations were similar for both groups. The study concluded that omeprazole treatment was associated with significant changes in blood sodium and potassium levels, with the treatment group having lower sodium and higher potassium levels as shown in table 2.

Table 2. Comparison of serum electrolyte level tests between the treatment (Omeprazole) and control groups.

Parameter	Treatment (Omeprazole) Group (mean ± SD)	Control Group (mean ± SD)	p-value (*<0.05 is significant)
Calcium mg/dl	8.48±1.2	9.69±0.622	0.011
Magnesium mg/dl	2.73±0.258	2.63±0.227	0.369
Na mg/dl	119±15.5	147±5.97	0.001*
K mg/dl	6.5±0.757	4.48±0.642	0.001*

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 prolonged use of omeprazole on haematological and biochemical parameters. The result of this study demonstrated that omeprazole might interfere with the blood profile in patients with long-term treatment. To reveal if long-term omeprazole use may exert an adverse effect on haematological indices or not, the blood test was performed for 20 Male Fischer Rats (Albino) 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. A retrospective cohort study has examined the impact of PPIs use on haematological indices among rats who received PPI medications for 3 months. The study revealed a significant reduction in values of haemoglobin, hematocrit, and mean corpuscular volume and suggested that the chronic use of PPIs may cause iron mineral deficiency, and long-term therapy may reduce the absorption of non-heme iron.8 Another study conducted in a group of patients using PPI

medications for long-term periods, Kaczmarczyk et al. showed that using PPIs might cause a reduction in the number of RBCs and levels of HGB and some serum micronutrients. This suggested that prolonged use of PPIs might give rise to iron deficiency anaemia [5]. Iron absorption usually occurs in the proximal small intestine, and this process is facilitated by gastric acid secretion which is necessary to convert the iron mineral from a ferric state to a ferrous state. [6]. Two biological mechanisms have been put forward that chronic use of PPIs causes anaemia. One of these mechanisms is the suppression of the absorption of iron in the small intestine due to the inhibition of H+-K+ ATPase and an increase in the pH of the stomach [7]. Another mechanism that contributes to the development of anaemia is the suppression of absorption of vitamin B12, food-bound vitamin B12 is liberated in the acidic medium and is bound to the glycoprotein haptocorrin for ready absorption in the ileum [8]. Proton pump inhibitors are powerful agents that inhibit the production of gastric acid, a reduction in gastric acid production as a result of PPIs use may influence the absorption of minerals and vitamins in the gastrointestinal tract. Means of MCV, MCH, and MCHC were no different in the treatment group in comparison to the control group. These patients likely developed iron deficiency anemia because omeprazole may suppress the secretion of gastric acid and thence inhibit the absorption of iron minerals. The numbers of white blood cells in the treatment group were not significantly affected by the chronic use of omeprazole medication in comparison to the control group. Omeprazole medication demonstrated a non-significant reduction in the number of WBCs. Although, this result differed from some published studies [5,9] it was consistent with those of other studies [10]. We also found statistical variation between the groups in the number of platelets. Our results show a reduction in the number of platelets due to the use of omeprazole.

Literature data regarding the influence of PPIs on platelet numbers are conflicting. The present findings are consistent with other research that found a decrease in the number of platelets between PPIs users and the control group [5,10]. On the other hand, only one case report has described the role of omeprazole medication in inducing thrombocytopenia [11]. A few numbers of studies over the past two decades have demonstrated thrombocytopenia with various types of PPIs therapy [12,13]. In the current study, significant elevations in serum urea and non-significant blood creatinine concentrations in the treatment group were observed in comparison to the control group. Similar findings were observed in previous research as well. Deterioration of kidney function tests was demonstrated in users of PPIs compared to nonusers with marked elevation of serum creatinine and blood urea levels [14,15]. Decreased serum creatinine clearance is not associated with H2-receptor blockers and other PPI nonusers [15]. Despite the results, these clinical markers are not optimal for detecting kidney diseases, as they are often used to find out whether patients have developed kidney diseases or not.

Reduction of glomerular filtration rate leads to accumulation of nitrogen waste products in circulation, evidenced by abnormal increase in serum creatinine and blood urea levels [16]. The precise mechanisms between PPIs and adverse kidney outcomes are unclear [15]. Our results contradict those of Mélo and colleagues who found that the level of serum creatinine did not change, while blood urea level was decreased in the group treated with omeprazole compared with the control group [17]. Omeprazole may be associated with the development of kidney diseases by increasing levels of serum creatinine and blood urea. Suppression of gastric acid secretion is linked with alterations in the digestion process of dietary lipids. It has been demonstrated that using omeprazole results in increased lipid absorption but our study contradicts this fact, so cholesterol level is no significant difference in rats with long term use of omeprazole medication compared to the control group, alongside no significant elevation in triglyceride plasma level and LDL in the treatment group. These results are in disagreement with what was reported by other researchers [18]. Plasma concentration of minerals must be maintained within a stable range so that cellular metabolism processes can work properly.

Our results showed that plasma concentration of calcium decreased in rats with long-term treatment of omeprazole. This finding is consistent with the reduction in intestinal calcium absorption [19,20]. The acidic environment is necessary for the absorption of intestinal calcium minerals, as this process is inhibited by omeprazole intake via blocking the gastric H+-K+ ATPase enzyme system that is located in the apical membrane of stomach parietal cells, which causes achlorhydria. Maintenance of low gastric acid reduces lipolysis which is essential for calcium absorption in the gastrointestinal region and hence reduces the absorption of calcium minerals in the gut causing hypocalcaemia. Additionally, dietary protein increases intestinal calcium solubility and absorption efficiency. Hypocalcaemia possibly mediates cardiovascular adverse events of omeprazole. It has been shown that hypocalcaemia was observed in patients with long-term treatment of PPI [21,22]. It may cause life-threatening arrhythmias and heart failure. Hypocalcemia is usually accompanied by hypomagnesemia and both these mineral abnormalities can give rise to cardiovascular instability [23]. A significantly lower mean sodium level and a higher mean potassium level of electrolytes. The findings of this study have a significant impact on how omeprazole and other PPIs are used clinically. Physicians should monitor patients by their awareness of the possible hematological and biochemical changes linked to long-term PPIs usage. The results point to the necessity of routinely checking lipid profiles, liver function tests, and blood parameters in patients

receiving long-term omeprazole therapy. In addition, the possibility of hypocalcemia emphasizes how critical it is to evaluate these individuals' bone health. Investigating the processes behind the noted hematological and biochemical alterations should be the main goal of future studies. A more thorough knowledge of omeprazole's long-term effects can be obtained by looking into the drug's impacts on both genders and various age groups. Studies should also look at whether these alterations may be reversed when omeprazole is stopped, as well as possible preventative measures to lessen negative effects.

CONCLUSION

In this study, the objective was to assess the effect of long-term omeprazole medication on blood parameters. Omeprazole is very effective in controlling gastric acid secretion. Accumulating data suggest that long-term use of omeprazole may reduce the numbers of circulating RBCs and their indices, ultimately leading to anaemia. Consequently, this results in hypocalcemia. Both hypocalcaemia and hypomagnesemia may affect the cardiovascular system, therefore, levels of serum magnesium should also be measured to evaluate any abnormality in the serum mineral levels and their relation with cardiovascular health. Omeprazole may be associated with the development of kidney functional disorders; therefore, physicians should be cautious when prescribing PPIs because of their adverse effects. A further study with a higher number of enrolled patients could assess the various long-term adverse effects of omeprazole medication on the organ systems by performing comprehensive blood and biochemical tests.

Conflict of interest. Nil

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تأثير الإعطاء المزمن لدواء أوميبرازول على المعايير الدموية والكيميائية الحيوية في ذكور فئران فيشر

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[2]المركز الليبي للبحوث الطبية، الزاوية، ليبيا.

المستخلص

يُعتقد أن الاستخدام طويل الأمد لمثبطات مضخة البروتون له العديد من الآثار الجانبية المحتملة. أوميبرازول هو جزء من مثبطات مضخة البروتون الأكثر شيوعًا في جميع أنحاء العالم؛ فهو يرتبط بشكل لا رجعة فيه بنظام إنزيم H+-K+ ATPaseفي الخلايا الجدارية المعدية لتقليل إفراز أيونات H+ في تجويف المعدة. الهدف الرئيسي من العمل الحالي هو تقييم الآثار الجانبية لدواء أوميبرازول على بعض المعايير الدموية والكيميائية الحيوية في الفئران التي كانت تتلقى العلاج لمدة ثلاثة أشهر. أجرينا دراسة مقارنة مقطعية بين مارس 2024 ويوليو 2024. تم الحصول على 20 فأرًا ذكرًا من نوع فيشر (ألبينو) بعمر شهرين، بوزن (200-250 جرامًا) من بيت الحيوانات الواقع في المركز الليبي للأبحاث الطبية بمدينة الزاوية، وتم تسجيلهم في هذه الدراسة. تم تقسيم الفئران إلى مجموعتين، المجموعة الأولى كانت المجموعة الضابطة (مجموعة المحلول الملحي العادي) (ن = 10)، المجموعة الثانية تم إعطاؤها 40 مجم / كجم من أوميبرازول عن طريق الحقن داخل الصفاق (ن = 10) مرة واحدة يوميًا. تم قياس تعداد الدم الكامل والمعايير الكيميائية الحيوية لكلا المجموعتين. أظهرت مجموعة العلاج انخفاضًا ملحوظًا في عدد خلايا الدم الحمراء (P <0.001) ومؤشرات الصفائح الدموية. رفع أوميبرازول مستوى الكوليسترول (P <0.001) والدهون الثلاثية (P <0.001) وكذلك البروتين الدهني منخفض الكثافة (P <0.01)ومع ذلك، لم يتم العثور على أي تأثير مع البروتين الدهني عالي الكثافة .(p>0.05) ارتفعت مستويات اليوريا في الدم (p<0.001) بشكل ملحوظ في مجموعة العلاج التي عولجت بدواء أوميبرازول. أظهرت النتائج أيضًا أن مجموعة العلاج شهدت انخفاضًا كبيرًا في مستويات الكالسيوم (p<0.001) مقارنة بمجموعة التحكم. قد يؤدي الاستخدام المطول لأوميبرازول إلى آثار ضارة على الملف الدموي، وخاصة خلايا الدم الحمراء ومؤشراتها مما يؤدي إلى تطور فقر الدم لدى المرضى الذين يتناولون هذا الدواء. علاوة على ذلك، قد يؤدي ذلك إلى اضطرابات في الملف الكيميائي الحيوي ومستويات المعادن والفيتامينات نتيجة لتأثر الامتصاص.

الكلمات المفتاحية. أوميبرازول، الإعطاء تحت المزمن، المعايير الدموية، المعايير الكيميائية الحيوية، فئران فيشر الذكور.