IJBPAS, July, 2022, 11(7): 3122-3145

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

THE POTENTIAL IMPACT OF PROTON PUMP INHIBITORS AND HISTAMINE-2 BLOCKER ON THE BONES OF ADULT AND AGED FEMALE RATS

DINA SALEM[1], ELHAMY EL-KHOLY[1], HALA ABDEL MALAK[1], ABDELHADI M. SHEBL[2], HANY A. ELKATTAWY[*4, 5] AND MAHMOUD ABDALLA[3]

1: Department of Clinical Pharmacology, Faculty of Medicine, Mansoura University, 35516, Mansoura, Egypt

2: Department of pathology, Faculty of Medicine, Mansoura University, 35516, Mansoura, Egypt

3: Department of Clinical Pharmacology, Faculty of Medicine, Mansoura University and Qunfudh Medical College, Umm Al-Qura University, Makkah, Saudi Arabia,

4: Department of basic medical Sciences, College of Medicine, Almaarefa University, P.O. Box 71666, Riyadh, Saudi Arabia

5: Department of Medical Physiology, College of Medcine, Zagazig university, Egypt

***Corresponding Author: Hany A. Elkattawy: E Mail: hmohammed@mcst.edu**

Received 5th July 2021; Revised 9th Aug. 2021; Accepted 16th Oct. 2021; Available online 1st July. 2022

https://doi.org/10.31032/IJBPAS/2021/11.7.6214

ABSTRACT

Background: Acid-suppressive drugs usage is widespread in patients with osteoporosis. An association between gastric acid suppressants and increased fractures risk has been found, denoting the possible effects of chronic use of these acid suppressants, as omeprazole, pantoprazole (PPIs) and famotidine (H_2 receptor antagonist).

Purpose of the study: it aimed to investigate the potential impact of proton pump inhibitors and histamine-2 blocker on the bones of adult and aged female rats.

Methods: Forty-eight (48) female Sprague–Dawley rats were used; they were divided into two main groups. Group A (7-week old) and group B (7-month old). Each main group was subdivided into four subgroups. Control group and omeprazole, pantoprazole, famotidine

treated-groups and received drugs daily (successive 3 months). Then, blood was collected for serum calcium, alkaline phosphatase, estradiol, and osteocalcin, and femurs were processed for histopathology.

Results and conclusion: Omeprazole or pantoprazole administration produced bone loss (low serum calcium, elevated serum alkaline phosphatase, and osteocalcin and decrease in cortical and trabecular bone thickness). These drugs have no effect on serum estradiol level. Their effects on bone tissue were more prominent in old rats. On the other hand, famotidine produced bone changes only in older rats.

Keywords: H_2 receptor antagonists; proton pump inhibitors; bones; aged; rats

1. INTRODUCTION

Osteoporosis is a chronic progressive degenerative systemic skeletal disease, which leads to bone fragility associated with a risk of low trauma fractures of all bones **[1]**. Osteoporosis fractures are a serious health problem among the elderly **[2, 3]**. Acid-suppressive drugs (ASDs) represent the second leading medication worldwide in terms of sales **[4]**. Proton pump inhibitors (PPIs) and H_2 receptor antagonists (H_2RAs) are the most popular ASDs available **[5]**. These drugs are indicated in the management of several acid-related gastrointestinal disorders **[6]**. Omeprazole and pantoprazole are the most frequently used PPIs clinically. These drugs can reduce gastric acid secretion by up to 98%, irreversibly deactivating the proton pump (H+/K+ ATPase) of the gastric parietal cells **[7]**. H_2RAs (cimetidine, ranitidine, famotidine) competitively inhibit H_2 receptors, have similar effects to PPIs, although they are less potent, blocking only 70% of gastric acid production **[8, 9]**. The use of ASDs is widespread in osteoporotic patients to counteract inflammation and ulceration of esophagus and stomach caused by prolonged use of anti-resorptive medications especially bisphosphonates **[10]**. It has been suggested a possible association between PPIs use and increased fracture risk **[11-14]**. While, others have not observed any fracture risk with the use of PPIs **[15-19]**. The data on the effects of H_2R. As are conflicting too **[5, 12, 20]**. Several mechanisms of this association have been proposed in theory, such as the possibility that PPIs decrease calcium-absorption, leading to bone mineral density (BMD) loss **[11]**, or they decrease magnesium absorption, which is important to bone health **[21]**, other studies suggest that these agents can cause hyperparathyroidism by acid suppression and lead to decrease in BMD **[22]**. This study

was aimed to clarify the possible effects of chronic use of omeprazole, pantoprazole and famotidine on adult and old female rat.

2. MATERIALS AND METHODS

Ethical considerations

This study was performed at faculty of medicine -Mansoura University. The Animal Research Ethical Committee approved the experimental protocol. All animals received care according to the National Institutes of Health guide for the use and care of laboratory animals.

Experimental animalsand protocol

This study used forty-eight (48) female Sprague–Dawley rats, 24 of them are the adult (7- week old, each of them weighing 120-150 g), the other 24 are old (7-month old, each of them weighing 250-300 g). Animals were obtained from the medical experimental research center (MERC) at Mansoura Faculty of Medicine; they were put in similar optimum housing conditions with free access to food and water. Animals were kept in cages at a room with controlled temperature 26 °c and on a 12-h light–dark cycle. The local animal ethics committee has approved all experimental procedures. Rats were randomly divided into 8 groups (n=6 per group). Group (A) represents adult rats, Group (B) represent old age rats. Each main group was subdivided into four subgroups. Control subgroups in which rats were given oral carboxy methylcellulose, Omeprazole-treated subgroups. Rats received omeprazole (Healsec40 mg capsules from BORG pharmaceutical Inc.) at a dose of 10 mg/kg of body weight per day **[23]**, Pantoprazole-treated subgroups. Rats received pantoprazole (Pantoloc 40 mg tablets, Medical Union Pharmaceuticals "MUP") at a dose of 3 mg/kg of body weight per day **[23]**, Famotidine-treated groups. Rats received famotidine (Famotin 40 mg tablets, Memphis Co., Pharm. & Chem. Ind.) at a dose of 3 mg/kg of body weight per day **[24]**. These drugs were dissolved in 0.5% carboxy methylcellulose solution and given daily for successive 3 months by oral gavage.

After acclimatization for two weeks, the animals were divided into 4 equal groups (n=10 rats). Group I: the control adult group (CA); the 6-month-old rats. Old, aged animals were divided into 3 groups: Group II: Aged vehicle-treated group (AG). Group III: Aged testosterone-treated rats (AGT); received subcutaneous testosterone propionate (TP) injection (2mg/kg daily at 5:00 to 6:00 PM) **[17]** for 6 weeks. Group IV: Aged humanin-treated rats (AGH); received daily intraperitoneal (IP) injection of 50 mcg HNG for 6 weeks **[18]**. The CA and AG groups received a daily

subcutaneous sesame oil injection as a vehicle for 6 weeks **[17]**.

Blood sampling and biochemical analysis

At the end of the experimental period, animals were sacrificed by thiopental high dose (50mg/kg). The blood was collected from carotid arteries. Then, centrifuged at 4000 rpm for 15 min at 4°C where the clear sera were separated then stored at –20°C until measurement of serum calcium, alkaline phosphatase, estradiol, and osteocalcin levels. Femurs were collected and processed for histopathological examination. Calcium colorimetric kits (Spinreact, S.A. / S.A.U. Ctra measured calcium level. Santa Coloma, Spain). Alkaline phosphatase kits (AGAPPE Diagnostics Switzerland Gmbh) measured alkaline phosphatase level. Estradiol ELISA kits (Biosource Europe S.A, Nivelles, Belgium) determined estradiol (E2) level. Rat osteocalcin ELISA kits were used in this study for measurement of serum osteocalcin level.

Bone Histomorphometrical Analysis.

Femurs were removed, dissected free of soft tissue, fixed with 10 % buffered neutral paraformaldehyde for 24 hours at 4°C then decalcified in EDTA (Ethylenediamine-tetraacetic acid) solution for 2 weeks. Once decalcified, the specimens took after routine histological handling and were embedded in paraffin. Paraffin sections (5 µm thick) from the metaphysis and the diaphysis of the femurs were deparaffinized then processed for hematoxylin eosin staining (H/E) and viewed under the light microscope **[25]**. Osteoporosis changes in the bones were graded from 0 to 3 as indicated by the following schedule **[26]**. Grade 0. Bone with normal structure, Grade 1. Slight osteoporosis, bone showed early osteoporosis, namely osteocytic activation (hypertrophy of osteocytes and enlargement of their lacunae) and hypertrophy of endosteal cells, Grade 2. Moderate osteoporosis, beside the above changes, appearance of resorption cavities was in the compact bone, Grade 3. Severe osteoporosis, many resorption cavities were in the diaphysis with the larger cavities containing bone marrow spaces and the compact bone became significantly thinner.

Image Analysis Procedure.

Slides were digitalized using Olympus® digital camera installed on Olympus® microscope with1/2 X photo adaptor, using 40X objective. The resulted images were analyzed on Intel® Core I3® based computer using Video Test Morphology® software (Russia) with a specific built-in routine for distance measurement. Five images were taken for each sample, five measurements for

cortical and trabecular bone thickness were taken by using line measurement tool.

Statistical Analysis.

Data were tabulated, coded then analyzed using the computer program SPSS (Statistical package for social science) version 17.0. Descriptive statistics were calculated in the form of Mean ± SD (Standard deviation) for quantitative parametric data and in the form of median and range (Minimum – maximum) for quantitative non-parametric data. In the statistical comparison between different groups, the significance of difference was tested using ANOVA (Analysis of variance) to compare between more than two groups of numerical parametric data followed by post-hoc turkey test for comparison between two groups. Kruskal-Wallis test was used to compare between more than two groups of non- parametric data followed by mann-whitney test for comparisons between two groups. P value <0.05 was considered statistically significant in all analyses and P value <0.001 was considered highly significant in all analyses.

RESULTS

Biochemical results.

Effect of omeprazole, pantoprazole, and famotidine on serum calcium level in adult and old age female rats.

Omeprazole and pantoprazole administration to adult and old age female rats produced highly significant decrease ($p< 0.001$) in serum calcium level as compared with control group but serum calcium level in pantoprazole groups is still much lower than omeprazole groups while famotidine administration to adult female rats produced non-significant change ($P = 0.69$) in serum calcium level as compared with control group but administration to old female rats produced moderate significant decrease ($P < 0.01$) in serum calcium level as compared with control group, and is still much higher than omeprazole group ($p= 0.001$) and pantoprazole group($p < 0.001$) (table 1, 2).

Effect of omeprazole, pantoprazole, and famotidine on serum alkaline phosphatase level in adult and old age female rats.

Omeprazole and pantoprazole administration to adult and old age female rats produced highly significant increase ($p< 0.001$) in serum alkaline phosphatase level as compared with control group but serum alkaline phosphatase level in pantoprazole groups is still much higher than omeprazole groups while famotidine administration to adult female rats produced non-significant change ($P = 1$) in serum alkaline phosphatase level as compared with control group but administration to old female rats produced

moderate significant increase (P < 0.01) in serum alkaline phosphatase level as compared with control group and is still much lower than omeprazole group (p= 0.001) and pantoprazole group (p < 0.001) **(Table 1, 2)**.

Effect of omeprazole, pantoprazole, and famotidine on serum estradiol level in adult and old age female rats.

Omeprazole, pantoprazole, and famotidine administration to adult and old age female rats produced non-significant change (p = 0.2) in serum estradiol level as compared with control group **(Table 1, 2)**.

Effect of omeprazole, pantoprazole, and famotidine on serum osteocalcin level in adult and old age female rats.

Omeprazole and pantoprazole administration to adult and old age female rats produced highly significant increase (p < 0.001) in serum osteocalcin level as compared with control group but serum osteocalcin level in pantoprazole groups is still much higher (p = 0.01) than omeprazole group while famotidine administration to adult female rats produced non-significant change (p=0.99) in serum osteocalcin level as compared with control group but administration to old female rats produced moderate significant increase (P < 0.01) in serum osteocalcin level as compared with control group and is still much lower than omeprazole group (p= 0.029) and pantoprazole group (p < 0.001) **(Table 1 , 2)**.

Histopathology and bone histomorphometric changes after administration of omeprazole, pantoprazole, and famotidine.

Omeprazole and pantoprazole groups showed signs of osteoporosis (pantoprazole more than omeprazole) as compared with control groups. On the other hand, famotidine groups revealed signs of osteoporosis only in old rats but less than omeprazole and pantoprazole groups **(Table 3, 4 and 5)**.

Table 1: Effect of omeprazole, pantoprazole, and famotidine on serum calcium, alkaline phosphatase, estradiol and osteocalcin levels in adult female rats

		Control group	Omeprazole group	Pantoprazole group	Famotidine group
Serum calcium (mg/ dl)	Mean ± SD	11.00 ± 0.89	8.50 ± 0.55[a]	6.5 ± 0.54[a b]	10.50 ± 1.05[b c]
Serum alkaline phosphatase (IU/L)		130.00 ± 20.76	164.5 ± 12.88 [a]	198.00 ± 8.69[a b]	130.50 ± 12.76 [b c]
Serum estradiol (Pg/ ml)		60.50 ± 15.91	47.50 ± 11.57	51.3 ± 5.15	60.00 ± 12.41
Serum osteocalcin (ng/ml)		12.00 ± 2.00	19.66 ± 2.8[a]	26.5 ± 5.08 [a b]	12.50 ± 2.66[b c]

Data are presented as means ± SD and analyzed by one-way ANOVA followed by post-hoc tukey test for comparison between groups

Superscript letters indicate significant differences between groups at (P < 0.05); a: significance in relation to control group; b: significance in relation to omeprazole group; c: significance in relation to pantoprazole group

Table 2: Effect of omeprazole, pantoprazole, and famotidine on serum calcium, alkaline phosphatase, estradiol and osteocalcin levels in old female rats

		Control group	Omeprazole group	Pantoprazole group	Famotidine group
Serum calcium (mg/ dl)	Mean ± SD	10.00 ± 1.41	6.92 ± 0.21[a]	5.52 ± 0.55 [a b]	8.50 ± 0.55 [a b c]
Serum alkaline phosphatase (IU/L)		103.50 ± 8.55	145.67 ± 5.79[a]	163.50 ± 8.41[a b]	125.17 ± 8.08 [a b c]
Serum estradiol (Pg/ ml		32.50 ± 8.38	27.50 ± 6.16	24.50 ± 2.74	30.00 ± 5.33
Serum osteocalcin (ng/ml)		5.00 ± 0.89	10.17 ± 2.56[a]	14.33 ± 0.82[a b]	7.50 ± 1.05[a b c]

Data are presented as means ± SD and analyzed by one-way ANOVA followed by post-hoc tukey test for comparison between groups

Superscript letters indicate significant differences between groups at (P < 0.05):

a: significance in relation to control group.

b: significance in relation to omeprazole group.

c: significance in relation to pantoprazole group.

Table 3: Histomorphometric parameters of the femur of adult female rats after administration of omeprazole, pantoprazole, and famotidine

		Control group	Omeprazole group	Pantoprazole group	Famotidine group
Hypertrophy of osteocytes & endosteal cells*	Present %	0.0% (n=0/6)	66.7% (n=4/6)	83.3% (n=5/6)	0.0% (n=0/6)
Resorption cavities at compact bones*		0.0% (n=0/6)	0.0% (n=0/6)	83.3% (n=5/6)	0.0% (n=0/6)
Significant thin cortical bone*		0.0% (n=0/6)	0.0% (n=0/6)	0.0% (n=0/6)	0.0% (n=0/6)
Grade ♦	Median (Minimum-Maximum)	0.00 (0.00-0.00)	1.00 (0.00-1.00)[a]	2.00 (1.00-2.00)[a b]	0.00 (0.00-0.00)[b c]

* Test used: Chi-square

♦Test used Kruskal Wallis test followed by mann-whitney for pairwise comparisons.

Superscript letters indicate significant differences between groups at (P < 0.05):

a: significance in relation to control group.

b: significance in relation to omeprazole group.

c: significance in relation to pantoprazole group.

Table 4: Histomorphometric parameters of the femur of old female rats after administration of omeprazole, pantoprazole, and famotidine

		Control group	Omeprazole group	Pantoprazole group	Famotidine group
Hypertrophy of osteocytes & endosteal cells*	Present %	0.0% (n=0/6)	83.3% (n=5/6)	83.3% (n=5/6)	66.7% (n=4/6)
Resorption cavities at compact bones*	Present %	0.0% (n=0/6)	66.7% (n=4/6)	100% (n=6/6)	0.0% (n=0/6)
Significant thin cortical bone*	Present %	0.0% (n=0/6)	0.0% (n=0/6)	100 % (n=6/6)	0.0% (n=0/6)
Grade ♦	Median (Minimum-Maximum)	0.00 (0.00-0.00)	1.50 (1.00-2.00)[a]	3.00 (2.00-3.00)[a b]	1.00 (0.00-1.00)[a b c]

* Test used: Chi-square

♦Test used Kruskal Wallis test followed by mann-whitney for pairwise comparisons.

Superscript letters indicate significant differences between groups at (P < 0.05):

a: significance in relation to control group.

b: significance in relation to omeprazole group.

c: significance in relation to pantoprazole group.

Table 5: Effect of omeprazole, pantoprazole, and famotidine on cortical and trabecular thickness of the femoral bones of adult female rats by image analysis

Groups (n=6)	Cortical bone thickness (µm) Mean ±SD	Trabecular bone thickness (µm) Mean ±SD
Control group	65 ± 10.3	28± 6.4
Omeprazole group	46 ± 7.4[a]	16± 3.9[a]
Pantoprazole group	30 ± 6.7 [a b]	7± 2.6 [a b]
Famotidine group	59 ± 8.1[b c]	25± 3.6 [b c]

Data are presented as means ± SD and analyzed by one-way ANOVA followed by post-hoc tukey test for comparison between groups.
Superscript letters indicate significant differences between groups at (P < 0.05):
a: significance in relation to control group.
b: significance in relation to omeprazole group.
c: significance in relation to pantoprazole group.

Table 6: Effect of omeprazole, pantoprazole, and famotidine on cortical and trabecular thickness of the femoral bones of old female rats by image analysis

Groups *(n=6)*	Cortical bone thickness (µm) Mean±SD	Trabecular bone thickness (µm) Mean ±SD
Control group	59.6 ± 12.6	26.4± 4.3
Omeprazole group	31.3 ± 6.3 [a]	12.9± 3.2[a]
Pantoprazole group	16.4 ± 4.7 [a b]	6.2 ± 1.8 [a b]
Famotidine group	46.2 ± 7.3[a b c]	19.3± 3.9 [a b c]

Data are presented as means ± SD and analyzed by one-way ANOVA followed by post-hoc tukey test for comparison between groups.
Superscript letters indicate significant differences between groups at (P < 0.05):
a: significance in relation to control group.
b: significance in relation to omeprazole group.
c: significance in relation to pantoprazole group.

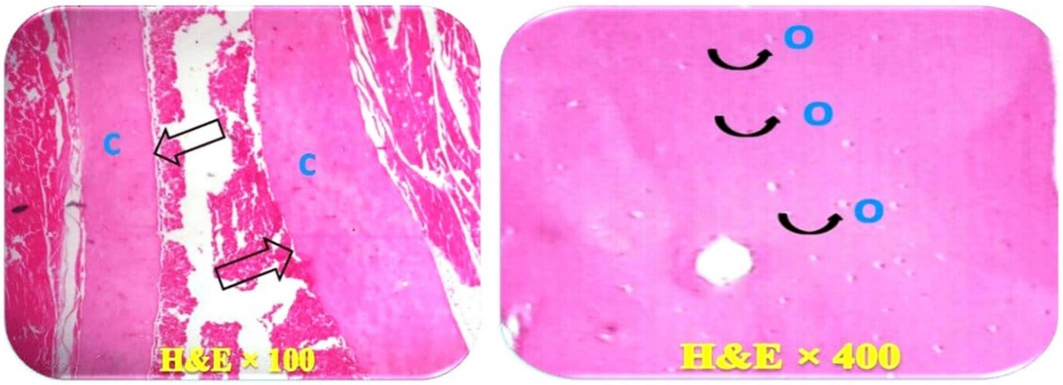

Fig. 1(A) Fig. 1(C)

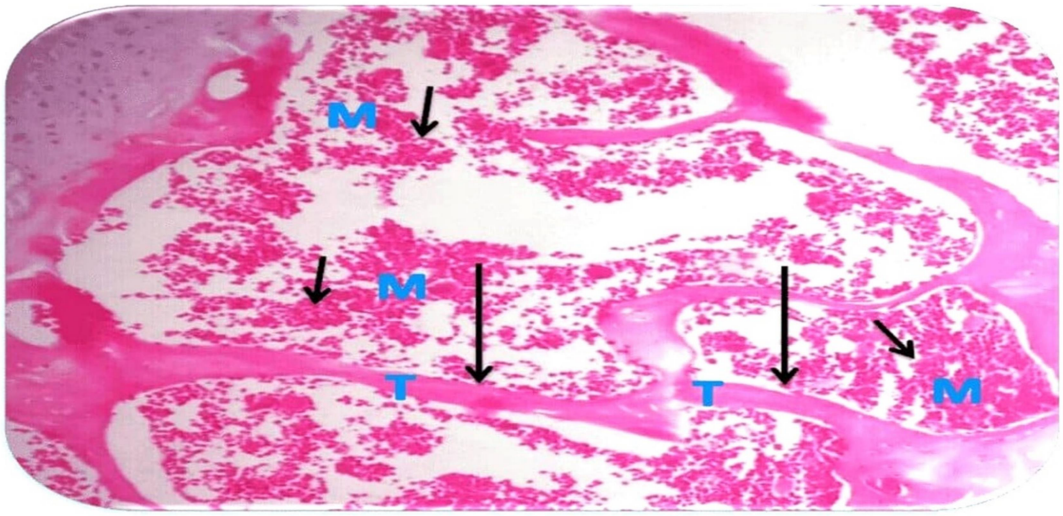

Fig. 1(B)

Figure 1: Normal histological appearance of the femur of adult female rats (Grade 0)
The proximal metaphysis of the femur formed of an outer shell of cortical bone and inner trabecular bone. A: Cortical (C) bone (empty arrows) consisted of bone lamellae that are covered by periosteum and lined with endosteum. B: Trabecular (T) bone composed of regular bone lamellae (long arrows) enclosing average thickness marrow spaces (M) (short arrows).C: Average size osteocytes (O) resided in their lacunae in between the lamellae (curved arrows)

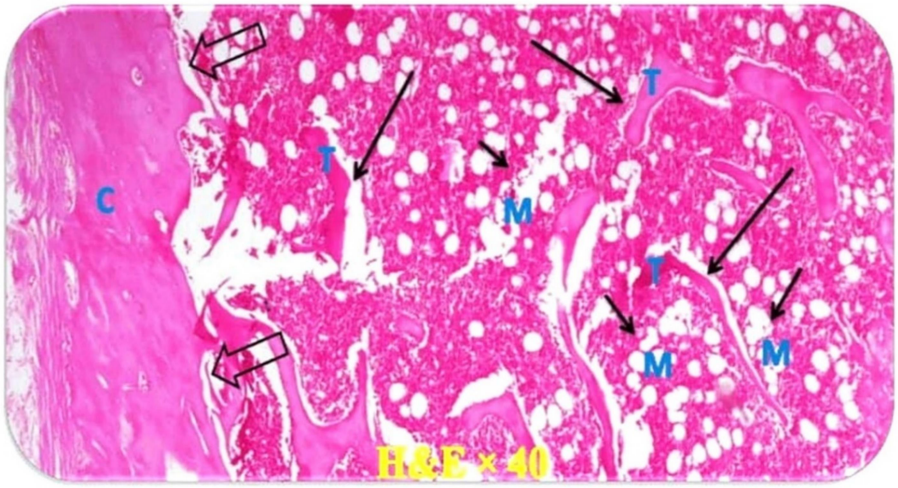

Fig. 2(A)

Fig. 2(B)

Figure 2: Histopathological findings in omeprazole treated adult female rats (Grade 1)
A: Significant decrease in cortical (C) bone thickness (empty arrows). Significant decrease in trabecular (T) bone thickness (long arrows) with wide marrow spaces (M) filled with fat tissue (short arrows).B: Hypertrophy of osteocytes (O) and enlargement of their lacunae (curved arrows) with hypertrophy of endosteal cells (E) (stars)

Fig. 3(A)

Fig. 3(B)

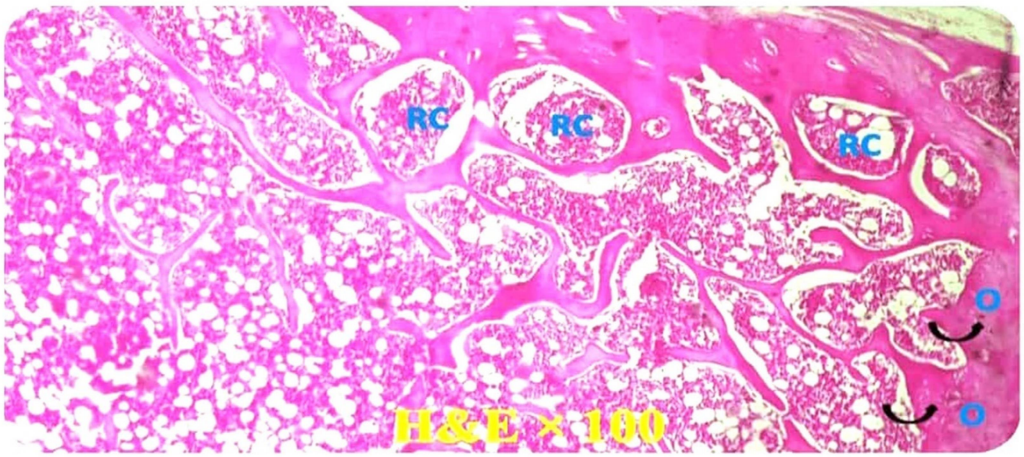

Fig. 3(C)

Figure 3: Histopathological findings in pantoprazole treated adult female rats (Grade 2)
A: Significant decrease in cortical (C) bone thickness (empty arrows) with loss of continuity.
B: Significant decrease in trabecular (T) bone thickness (long arrows) with wide marrow spaces (M) filled with fat tissue (short arrows).C: Hypertrophy of osteocytes (O) and enlargement of their lacunae (curved arrows). Resorption cavities (RC) filled with bone marrow spaces are detected

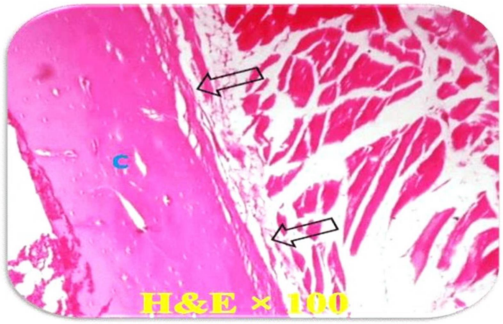

Fig. 4(A)

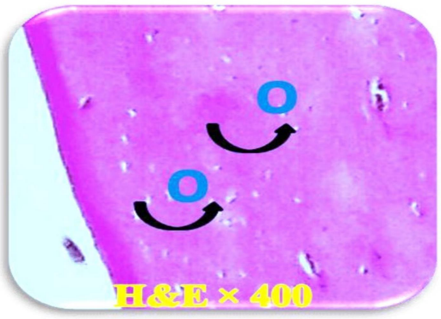

Fig. 4(C)

Fig. 4(B)

Figure 4: Histopathological findings in famotidine treated adult female rats (Grade 0)
A: Average cortical (C) bone thickness (empty arrows).B: Average trabecular (T) bone thickness (long arrows) with average size marrow spaces (M) (short arrows).C: Average size osteocytes (O) resided in their lacunae (curved arrows).

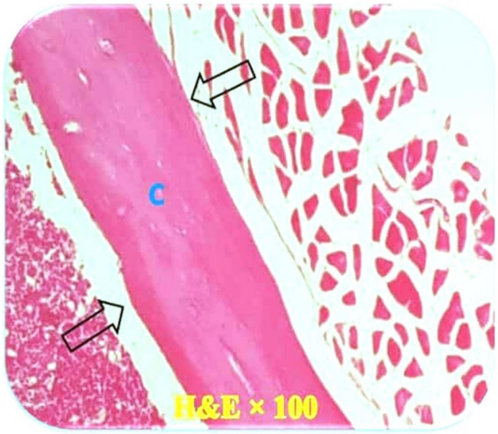

Fig. 5(A)

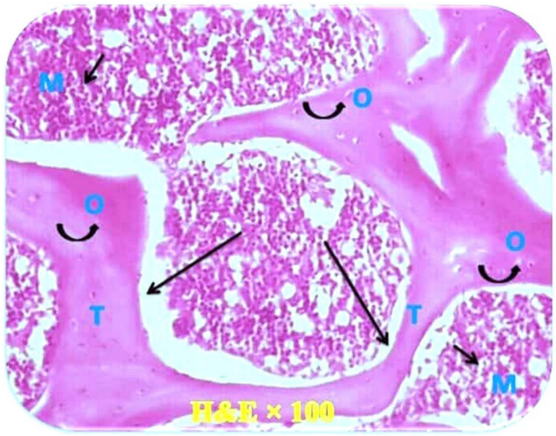

Fig. 5(B)

Figure 5: Normal histological appearance of the femur of old female rats (Grade 0)
A: Cortical (C) bone (empty arrows) consisted of bone lamellae that are covered by periosteum and lined with endosteum. B: Trabecular (T) bone composed of regular bone lamellae (long arrows) enclosing average thickness marrow spaces (M) (short arrows). Average size osteocytes (O) resided in their lacunae in between the lamellae (curved arrows).

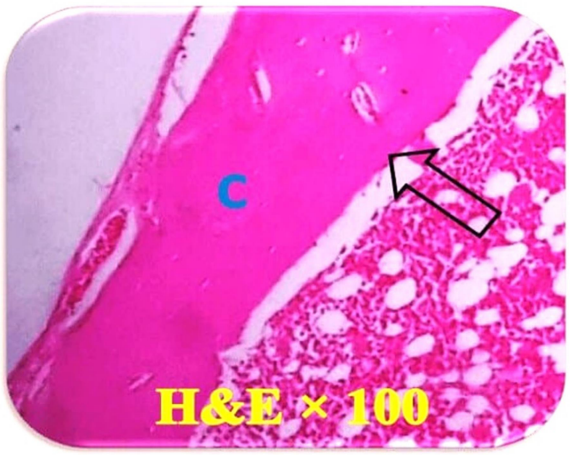

Fig. 6(A)

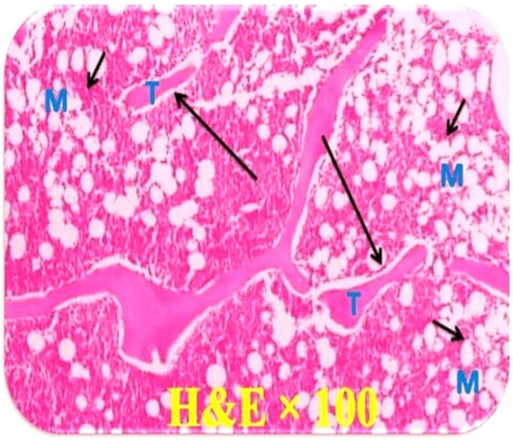

Fig. 6(B)

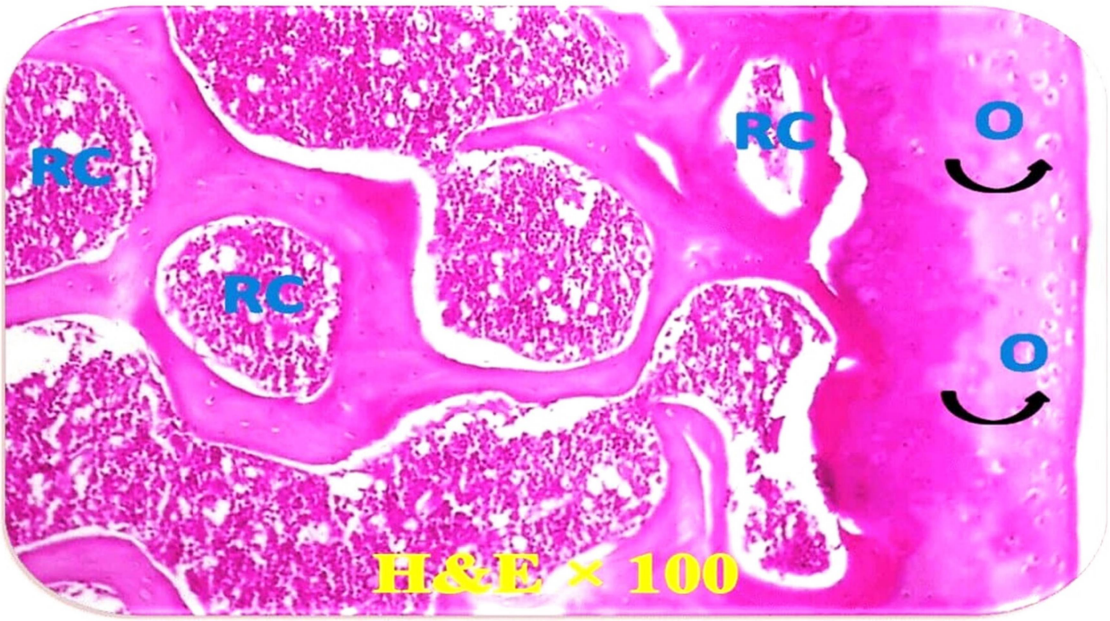

Fig. 6(C)

Figure 6: Histopathological findings in omeprazole treated old female rats (Grade 2)
A: Significant decrease in cortical (C) bone thickness (empty arrows). B: Significant decrease in trabecular (T) bone thickness (long arrows) with wide marrow spaces (M) filled with fat tissue (short arrows). C: Hypertrophy of osteocytes (O) and enlargement of their lacunae (curved arrows) with appearance of resorption cavities (RC) filled with bone marrow spaces.

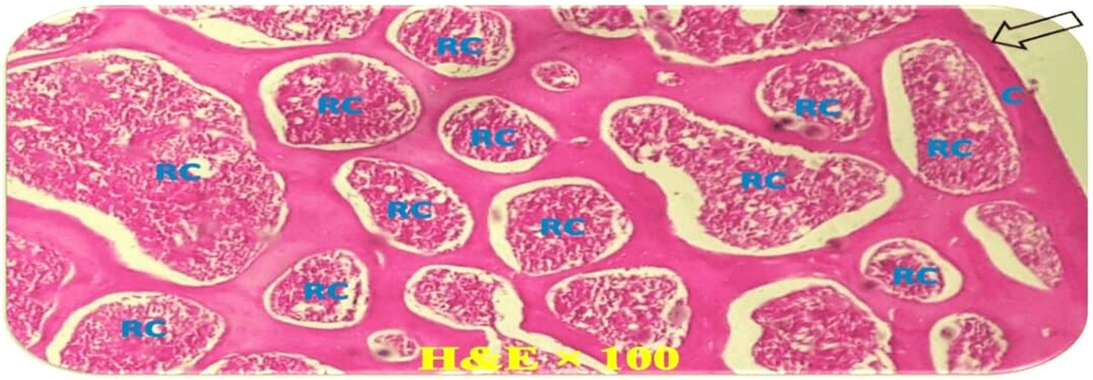

Fig. 7(A)

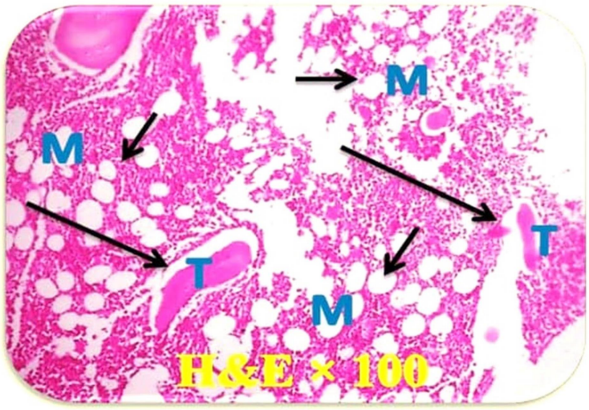

Fig. 7(C)

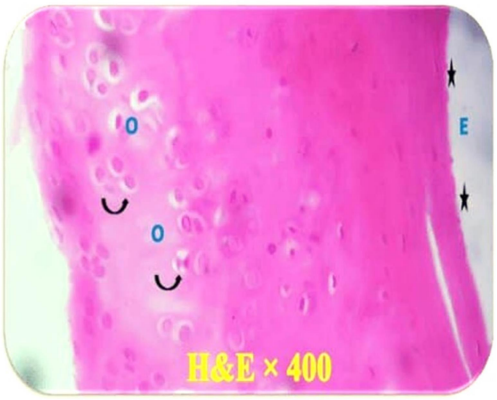

Fig. 7(B)

Figure 7: Histopathological findings in pantoprazole treated old female rats (Grade 3)
A: Significant decrease in cortical (C) bone thickness (empty arrows) with appearance of resorption cavities (RC) filled with bone marrow spaces. B: Significant decrease in trabecular (T) bone thickness (long arrows) with wide marrow spaces (M) filled with fat tissue (short arrows). C: Hypertrophy of osteocytes (O) and enlargement of their lacunae (curved arrows) with hypertrophy of endosteal cells (E) (stars)

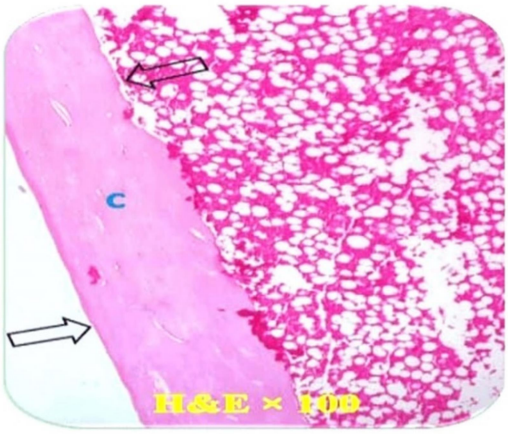

Fig. 8(A)

Fig. 8(C)

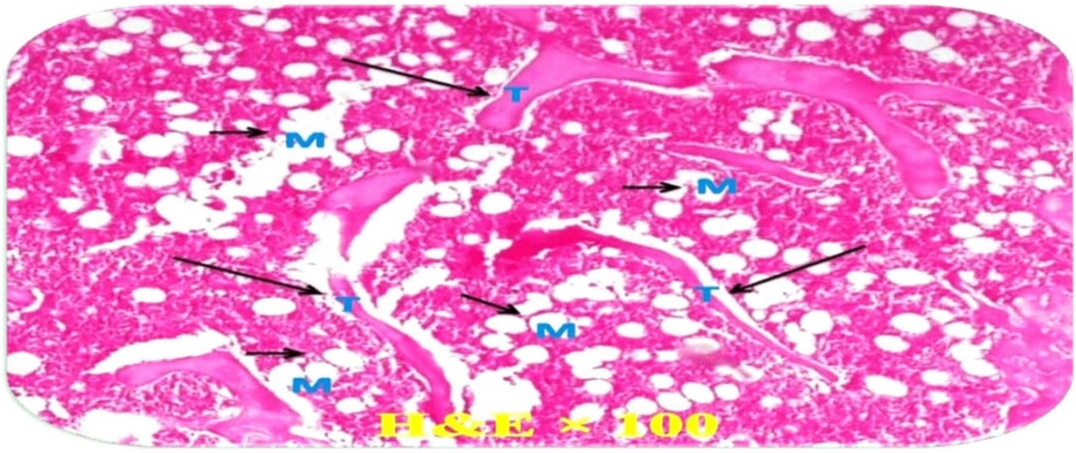

Fig. 8(B)

Figure 8: Histopathological findings in famotidine treated old female rats (Grade 1)
A: Significant decrease in cortical (C) bone thickness (empty arrows). B: Significant decrease in trabecular (T) bone thickness (long arrows) with wide marrow spaces (M) filled with fat tissue (short arrows). C: Hypertrophy of osteocytes (O) and enlargement of their lacunae (curved arrows) with hypertrophy of endosteal cells (E) (stars)

3. DISCUSSION

Osteoporosis is a common chronic progressive degenerative systemic skeletal disease, which leads to increased bone fragility that is associated with increased risk of low trauma fractures of all bones **[1]**. Many risk factors are related to fractures including medication classes that have been associated with increased fracture risk include antidepressants, antipsychotics, antidiabetic agents, and opiate agonists **[27-29]**. Proton pump inhibitors (PPIs) and histamine-2 receptor antagonists (H2RAs) are the most popular acid suppressive drugs (ASDs) **[5]**. These drugs are indicated in the management of several acid- related gastrointestinal disorders, including. Duodenal ulcer, gastric ulcer, and gastroesophageal reflux disease (GERD) **[6]**. The use of ASDs is widespread in osteoporotic patients to counteract inflammation and ulceration of esophagus and stomach either caused by prolonged use of anti-resorptive bisphosphonates **[10]** or by nonsteroidal anti-inflammatory drugs (NSAIDs) used for pain management in fractures **[30]**. There is a possible association between PPIs use and increased fracture risk **[11, 13]**. Although another studies have not observed any risk in hip fracture **[15, 19]**. In this study, the aim was to clarify the possible effect of chronic use of PPIs (omeprazole, pantoprazole) and H2RAs (famotidine) on adult and old female rat bones for successive 3 months. In the present study, omeprazole or pantoprazole administration to adult or old age female rats induced bone lesions that are explained by a significant biochemical and histopathological changes as compared with control groups. On the other hand, famotidine administration produced mild changes only in old rats. In the present study, serum calcium level in both adult and old female rats was much decreased by omeprazole or pantoprazole administration as compared with control groups (pantoprazole produced greater changes more than omeprazole). On the other hand, famotidine caused mild decrease in serum calcium level only in old age female rats. There are several explanations for the risk of fractures with PPIs, which seems to be more prominent than with H2RAs **[12]**. Some suggested that PPIs have deleterious effects on calcium absorption leading to increased risk of bone fractures. In addition, secondary hypergastrinemia due to acid suppression by PPIs may induce hyperparathyroidism and result in increased bone resorption **[31]**. Prolonged PPIs use has been shown to worsen vitamin B12 absorption, followed by hyperhomocysteinemia and interfering with

collagen crosslinking, and bone strength **[32]**. Histamine may also be involved in bone metabolism regulation. Histamine receptors are expressed on osteoblastic and osteoclastic cells **[33]**. Studies had been reported that cimetidine is shown to prevent osteoclast differentiation and showed anti-resorptive effect in estrogen-deficient rats **[34-35]**. Calcium is absorbed into the small intestine and is dissociated from its complexes by the acidic environment in the stomach **[36]**. Impaired calcium absorption because of reduced gastric acid leads to compensatory physiologic responses including secondary hyperparathyroidism. PTH increases the rate of osteoclastic bone resorption. Over time, this would lead to an increase in the rate of skeletal turnover and increase the risk of fracture **[31, 37 - 41]**. In the present study, serum alkaline phosphatase level in both adult and old female rats was much increased by omeprazole or pantoprazole administration as compared with control groups (pantoprazole produced greater changes more than omeprazole). On the other hand, famotidine caused mild increase in serum alkaline phosphatase only in old female rats. Alkaline phosphatase (ALP) is the most commonly used biomarker of bone formation and a sensitive marker of increased bone turnover in osteoporosis **[42-45]**. In the present study, omeprazole, pantoprazole, and famotidine administration to either adult or old rats produced non-significant change in serum estradiol level as compared with control groups **[34, 46]**. Estradiol level is decreased with aging. This decline with aging leads to increased bone remodeling rate, both bone resorption and formation, with the balance moved in favor of bone resorption, causing progressive loss of bone mass and strength **[47 -51]**. Osteocalcin (OC), an osteoblast- specific protein, is released from the bone matrix into blood during bone resorption, suggesting that osteocalcin is a marker of bone turnover **[52-53]**. In the present study, serum ostocalcin level in adult and old female rats was much increased by omeprazole or pantoprazole administration as compared with control groups (pantoprazole produced greater changes more than omeprazole). On the other hand, famotidine caused mild increase in serum osteocalcin only in old rats **[54-56]**. Omeprazole and pantoprazole groups showed signs of osteoporosis (pantoprazole more than omeprazole) as compared with control groups. On the other hand, famotidine groups revealed signs of osteoporosis only in old rats but less than omeprazole and pantoprazole groups. Increased bone resorption in the present study is explained by enlargement of

the resorption area on trabecular surface and cortical thinning, primarily by enhancing osteoclast lifespan and decreasing osteoclast apoptosis **[50]**. In addition, the contraction or even the loss of some trabeculae is produced by resorption of some connecting trabeculae **[57]**. Hypertrophy of endosteal cells suggests increased activity of these cells which is related to the resorptive process **[58-62]**. In the present data, the unfavorable effect of pantoprazole on rat bones was stronger than that of omeprazole **[59, 63]**. The possible explanation is that pantoprazole demonstrates a much faster onset of action **[64]**, higher bioavailability **[65]**, and slower restoration of the proton pump activity than omeprazole results in prolonged and potent suppression of gastric acid secretion **[66]**. The effect of omeprazole or pantoprazole on old female rat bones was stronger than that of young Rats, most probably is due to the associated risk factors of osteoporosis that are more evident with age such as atrophy of gastric mucosa and calcium malabsorption as a result of estradiol decline with age. Moreover, there are some mechanisms of compensation for PPIs effects on bone metabolism especially in young age **[67-68]**. In the present data, the unfavorable effect of PPIs on rat bones was stronger than famotidine that caused bone changes only in old female rats. This is in agreement with a previous study that reported that the risk fracture was greater with PPIs use than H2RAs **[11]**. Acid suppression in the stomach caused by PPIs is significantly greater and lasts longer compared with H2RAs since their effect is irreversible. Thus, if the impaired calcium absorption caused by acid suppression is associated with an increased risk of fracture, this should be most abundant with PPIs use. Perhaps, prolonged exposure is necessary to see effects on fracture risk with less potent acid inhibitors such as H2RAs, and the risk is increased with advancing age due to the associated risk factors **[69-70]**.

CONCLUSION

The present study suggested that omeprazole and pantoprazole administration to either adult or old female rats for successive 3 months produced bone loss, these effects are more prominent in old rats. On the other hand, famotidine administration to either adult or old female rats for 3 months produces bone loss in old rats.

Disclosure Statement

The authors declare no conflicts of interest related to this article.

Funding

This work has been supported by the Researchers Supporting Program (TUMA-

Project-2021-35), Almaaefa University, Riyadh, Saudi Arabia.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Acknowledgments:

The authors deeply acknowledge the Researchers Supporting Program (TUMA-Project-2021-35), Almaaefa University, Riyadh, Saudi Arabia for supporting the steps of this research work.

REFERENCES

[1] Geusens P, Dinant G, Integrating a gender dimension into osteoporosis and fracture risk research, Gender Medicine, 2007, 4, 147-161.

[2] Woolf A D, Akesson K, Preventing fractures in elderly people. BMJ.2003, 327, 89–95.

[3] Budhia S *et al*. Osteoporotic fractures. a systematic review of US healthcare costs and resource utilization, Pharmacoeconomics, 2012, 30,147–170.

[4] Roughead E E *et al*. Proton-pump inhibitors and the risk of antibiotic use and hospitalisation for pneumonia, Med J Aust, 2009, 190(3), 114-116.

[5] Eom C S, *et al*. Use of Acid-Suppressive Drugs and Risk of Fracture. A Meta-analysis of Observational Studies. Annals of Family Medicine, 2011, 9(3), 257–267.

[6] Lacy C.F *et al*, Drug Information Handbook, 20th ed. Hudson, Ohio, Lexi-Comp, Inc 2011, 1143-1147.

[7] Schuler A. Risks versus benefits of long-term proton pump inhibitor therapy in the elderly. Geriatr Nurs, 2007, 28(4), 225-229.

[8] Colin-Jones D G, The role and limitations of H2-receptor antagonist in the treatment of gastro-esophageal reflux disease, Alimentary pharmacology & therapeutics, 1995, 9(s1), 9-14.

[9] Freston JW. Overview of medical therapy of peptic ulcer disease. Gastroentrol, Clin North Am, 1990, 19, 121–140.

[10] Roughead E E, *et al*, Bisphosphonate use and subsequent prescription of acid suppressants. Br J Clin Pharmaco l.2004, 57, 813-816.

[11] Yang Y X *et al*, Long-term proton pump inhibitor therapy and risk of hip fracture, JAMA 2006,296 (24), 2947- 2953.

[12] Corley, DA, Kubo A, Zhao, W, and Quesenberry C, Proton pump inhibitors and histamine-2 receptor antagonists are associated with hip fractures among at-risk patients, Gastroenterology, 2010, 139, 93–101.

[13] Gray SL *et al*, Proton pump inhibitor use, hip fracture, and change in bone mineral density in postmenopausal women – results from the women's health initiative. Arch Intern Med, 2010, 170(9), 765–771.

[14] Zhou B *et al*, Proton-pump inhibitors and risk of fractures. an update meta-analysis. Osteoporos. 2015, Int, 1-9.

[15] Kaye JA, Jick H, Proton pump inhibitor use and risk of hip fractures in patients without major risk factors, Pharmacotherapy, 2008, 28(8), 951–9.

[16] Yu EW *et al*. Acid suppressive medications and risk of bone loss and fracture in older adults. Calcified tissue international, 2008, 83(4), 251.

[17] Roux C *et al*, Increase in vertebral fracture risk in postmenopausal women using omeprazole. Calcif Tissue Int. 2009, 84, 13–19.

[18] Pouwels S *et al*. Use of proton pump inhibitors and risk of hip/femur fracture. a population based case-control study, Osteoporos Int, 2011, 22(3) ,903-910.

[19] Reyes C*et al*. Use of proton pump inhibitors and risk of fragility hip fracture in a Mediterranean region, Bone, 2013, 52(2), 557-561.

[20] Kwok CS *et al*, Meta-analysis. Risk of fractures with acid-suppressing medication. Bone, 2011, 48,768–76.

[21] Kuipers MT *et al*. Hypomagnesaemia due to use of proton pump inhibitors--a review, Neth J Med, 2009,67,169-172.

[22] Vestergaard P *et al*, Proton pump inhibitors, histamine H2 receptor antagonists, and other antacid medications and the risk of fracture. Calcif Tissue Int, 2006, 79, 76-83.

[23] Takeuchi K*et al*. Effects of pantoprazole, a novel H+/K+ ATPase inhibitor, on duodenal ulcerogenic and healing responses in rats. A comparative study with omeprazole and lansoprazole. Journal of gastroenterology and hepatology, 1999, 14(3), 251-257.

[24] Shikama N *et al*, Different effects of two types of H2-receptor antagonists, famotidine and roxatidine, on the mucus barrier of rat gastric mucosa. Biomedical Research, 2012, 33(1), 45-51.

[25] Bancroft JD, Theory and Practice of Histological Techniques, Fifth edition, London (UK), Churchill Livingstone, 2002.

[26] Ornoy A *et al*. Structure of long bones of rats and mice fed a low calcium diet. Calcified tissue research, 1974, 15(1), 71-76.

[27] Cummings S R, Melton L J. Epidemiology and outcomes of osteoporotic fractures, Lancet, 2002, 359, 1761–1767.

[28] Kanis J A*et al*, Assessment of fracture risk, Eur J Radiol2009, 71.392–397.

[29] Woolcott JC *et al*. Meta-analysis of the impact of 9 medication classes on falls in elderly persons, Arch Intern Med, 2009, 169, 1952–1960.

[30] Prause M *et al*. Pantoprazole increases cell viability and function of primary human osteoblasts in vitro, Injury, 2014, 45(8), 1156-1164.

[31] O'Connell M B *et al*, Effects of proton pump inhibitors on calcium carbonate absorption in women. a randomized crossover trial. The American journal of medicine, 2005, 118(7), 778-781.

[32] Saito M, Marumo K. Degree of mineralization-related collagen crosslinking in the femoral neck cancellous bone in cases of hip fracture and controls, Calcif Tissue Int, 2006, 79,160–8.

[33] Biosse-Duplan M *et al*., Histamine promotes osteoclastogenesis through the differential expression of histamine receptors on osteoclasts and osteoblasts, Am J Pathol, 2009, 174, 1426–34.

[34] Lesclous P*et al*. Short-term prevention of osteoclastic resorption and osteopenia in ovariectomized rats treated with the H 2 receptor antagonist cimetidine, Bone, 2002, 30(1), 131-136.

[35] Lesclous P *et al*. Histamine mediates osteoclastic resorption only during the acute phase of bone loss in ovariectomized rats, Exp Physiol, 2006, 91,561–70.

[36] Ivanovich P*et al*. The absorption of calcium carbon- osteopenia

appeared within three weeks after gastrectomy in ate, Ann Intern Med, 1967, 66, 917–923.

[37] Recker R R., Calcium absorption and achlorhydria. N Engl J Med.1985, 313.70–73.

[38] Graziani G *et al*. Calcium and phosphate plasma levels in dialysis patients after dietary Ca-P overload, Nephron, 2002, 91(3), 474-479.

[39] Yanagihara G *et al*, "Effects of long-term administration of omeprazole on bone mineral density and the mechanical properties of the bone." Revista Brasileira de Ortopedia (English Edition), 2015, 50(2), 232-238.

[40] Hansen K E, Do proton pump inhibitors decrease calcium absorption? J Bone Miner Res, 2010, 25, 2786–95.

[41] Sharara A I *et al*, Proton pump inhibitors have no measurable effect on calcium and bone metabolism in healthy young males. a prospective matched controlled study. Metabolism, 2013, 62(4), 518-526.

[42] Delmas P D *et al*, The Use of Biochemical Markers of Bone Turnover in osteoporosis. Osteoporosis Int, 2000, 11 (6), S2–S17.

[43] Mukaiyama K *et al*., Elevation of serum alkaline phosphatase (ALP) level in postmenopausal women is caused by high bone turnover, Aging clinical and experimental research ,2014, 1-6

[44] Joo M K *et al*, The effect of a proton pump inhibitor on bone metabolism in ovariectomized rats, Molecular medicine reports, 2013, 7(4), 1267-1272.

[45] Petrakov AV *et al*, Experimental osteoporosis and its correction, Bulletin of experimental biology and medicine. 2014, 157(1), 99-102.

[46] Müller P *et al*, [4 weeks' admistration of omeprazole. effect on acid behavior and basal hormone levels], Zeitschrift fur Gastro-enterologie. 1984, 22(5), 236-240.

[47] Jazbutyte V *et al*. Aging reduces the efficacy of estrogen substitution to attenuate cardiac hypertrophy in female spontaneously hypertensive rats, Hypertension, 2006, 48(4) , 579-586

[48] Marosi K*et al*, Are the neuroprotective effects of estradiol and physical exercise comparable

during ageing in female rats? Biogerontology,2012, 13(4), 413-427

[49] Riggs B L. Sex steroids and the construction and conservation of the adult skeleton, Endocr Rev, 2002, 23, 279–302

[50] Weitzmann M N, Pacifici R, Estrogen deficiency and bone loss. An inflammatory tale. J Clin Invest, 2006, 116, 1186–1194.

[51] Manolagas SC, Parfitt AM, what old means to bone. Trends Endocrinol Metab.2010, 21, 369–374.

[52] Dogan E, Posaci C. Monitoring hormone replacement therapy by biochemical markers of bone metabolism in menopausal women, Postgrad Med J, 2002, 78, 727-731.

[53] Ivaska *et al*, Release of intact and fragmented osteocalcin molecules from bone matrix during bone resorption in vitro, J Biol Chem.2004, 279, 18361-9.

[54] Mizunashi K *et al*, Effect of omeprazole, an inhibitor of H+, K+ -ATPase, on bone resorption in humans, Calcif Tissue Int, 1993, 53, 21-25.

[55] Hyun JJ *et al*, Effect of omeprazole on the expression of transcription factors in osteoclasts and Osteoblasts, Int J Mol. Med. 2010, 26, 877–883.

[56] Lim E H*et al*, The Effect of Pantoprazole on Bone Turnover in Ovariectomized ICR Mice, The Korean Journal of Medicine, 2011, 80(1), 56-62.

[57] Marcu F *et al*, The histopathological study of osteoporosis. Rom J Morphology & Embryology, 2011, 52(1), 321-325.

[58] Salomon M C D, Osteoporosis following calcium deficiency in rats, calcified tissue research, 1971, 8(1), 320-333.

[59] Pytlik M, Bone remodeling after administration of proton pump (H+/K+-ATPase) inhibitors and alendronate in ovariectomized rats, Acta poloniae pharmaceutica ,2012, 69(1) ,113-20.

[60] Dobrowolski P *et al*, can 2-oxoglutarate prevent changes in bone evoked by omeprazole?, Nutrition, 2013, 29(3), 556-561.

[61] Lauretani F *et al*, Use of proton pump inhibitors is associated with lower trabecular bone mineral density in older individuals,

European Geriatric Medicine, 2013, (4), S44.

[62] Folwarczna J *et al*, Modifications of histamine receptor signaling affect bone mechanical properties in rats, Pharmacological Reports, 2014, 66(1), 93-99.

[63] Pytlik M, Proton pump (H+/K+-ATPase) inhibitors weaken the protective effect of alendronate on bone mechanical properties in estrogen-deficient rats, Pharmacol Rep, 2012, 64,625–34.

[64] Burkhardt D, Pantoprazole versus omeprazole. Influence on meal-stimulated gastric acid secretion. Eur J Gastroenterol Hepatol, 1999, 11, 1277–1282.

[65] Mohamed A H, Hunt R H, The rationale of acid suppression in the treatment of acid-related disease, Aliment Pharmacol Ther,1994, 8(1), 3–10.

[66] Shin J M, Sachs G, Differences in binding properties of two proton pump inhibitors on the gastric H＋, K ＋ -ATPase in vivo, Biochem Pharmacol, 2004, 68, 2117-2127.

[67] Jo Y*et al*, A Proton Pump Inhibitor's Effect on Bone Metabolism Mediated by Osteoclast Action in Old Age. A Prospective Randomized Study, Gut and liver, 2014, 9(5), 607.

[68] Freedberg D E *et al*, Use of proton pump inhibitors is associated with fractures in young adults, a population-based study, Osteo-porosis International. 2015, 1-7.

[69] Richter J, Gastrooesophageal reflux disease. Best Pract Res Clin Gastroenterol, 2007, 21, 609–631.

[70] Grisso J, 786-793. A *et al*, Risk factors for hip fracture in men, American Journal of Epidemiology, 1997, 145(9).