EFFECT OF FLUOXETINE ON THE STRUCTURE OF ALVEOLAR BONE IN RATS WITH INDUCED DEPRESSION

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ABSTRACT

INTRODUCTION: Depression is one of the most prevalent psychological disorders. It affects all body systems including endocrinal, neurological and immune system. Additionally, it is thought to affect bone homeostasis. Antidepressants are the most commonly prescribed drugs. They are classified into tricyclic and tetra cyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs) and selective serotonin-reuptake inhibitors (SSRIs). Fluoxetine, which is a selective serotonin-reuptake inhibitor, is used for the treatment of depression by selectively acting on serotonin (5-HT). Fluoxetine plays a major role in bone apposition and in maintaining the bone homeostasis.

OBJECTIVES: To investigate the effect of selective serotonin-reuptake inhibitor (fluoxetine) on the structure of alveolar bone in rats with induced depression.

MATERIALS AND METHODS: Thirty adult male rats were divided as follows: **Group I: (control group)** which consists of 10 rats, **Group II: (depression group)** 10 rats were exposed to chronic unpredictable stress (CUS) to induce depression, **Group III: (fluoxetine group)** 10 rats were exposed to chronic unpredictable stress (CUS) to induce depression and at the same time they were orally supplied with 10 mg/kg/day of fluoxetine. After 3 months, the mandibles were dissected out and prepared for histological analysis using light microscope, scanning electron microscope (SEM) and energy dispersive x-ray microanalysis (EDX).

RESULTS: In the control group, the alveolar bone surface showed a regular and smooth outline. In the depression group, there was a significant disturbance in the bone architecture. The bone surface showed an irregular outline with multiple osteoclasts lying in How ship's lacunae. Deeply stained incremental lines were also evident. In the fluoxetine group, the bone surface restored its regular outline with multiple osteoblasts and osteocytes. The bone showed incremental lines indicating bone formation.

CONCLUSIONS: Depression can lead to bone loss and osteoporosis. Fluoxetine is an effective drug in enhancing the bone condition and restoring the normal architecture of the alveolar bone.

KEYWORDS: serotonin, selective serotonin-reuptake inhibitor, alveolar bone, depression.

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INTRODUCTION

One of the most common psychological disorders is depression. Depression is a major health problem with high prevalence in females than males. It's a chronic, highly frequent disorder which is characterized by impaired intellectual functions and affects the quality of life (1). The exact causative factor for depression is not fully understood but mostly it's thought to be a multifactorial disorder that occurs due to a combination of neurotransmitter disturbances, genetic and psychosocial factors (2).

Studies show that depression has an effect on bone mineral density either due to debilitated life conditions resulting from pain and disturbed mood or may be influenced by factors as cytokines or cortisol which are dysregulated during depression (3). It is believed that there is a strong relationship between accelerated bone loss and depression leading to osteoporosis (4).

Since depression is a heterogeneous disorder, it was found that genetic factors are the causative factors for depression in early life while physical health, social and psychological risk factors are considered one of the major contributors to the development of depression late in life (5). It was also proposed that the serotonergic system plays a role in preventing feelings of fear, helplessness and depression. Evidences show that serotonin (5-HT) shows low concentrations in depressed patients (6).

Serotonin is a neurotransmitter (5- hydroxytryptamine 5HT) present in the nerve cell that helps in the transmission of signals between nerve cells. Serotonin is present in the central nervous system, digestive system and in the platelets. It is made of tryptophan amino acid which enters the body through our diet. Tryptophan deficiency can lead to disturbances in serotonin level which results in mood disorder. Serotonin is a chemical which affects digestion, mood and sleep (7). Platelets takes-up most of the body's serotonin which is produced peripherally by the gastrointestinal tract (8). The remaining serotonin circulates unbound in the serum where it diffuses into various tissues as the skeleton (9).

Antidepressants are the most commonly prescribed drugs. They are classified into tricyclic and tetra cyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs) and selective serotonin-reuptake inhibitors (SSRIs) (10).

Tricyclic and tetracyclic antidepressants (TCAs) are one of the oldest antidepressants used nowadays. It's not only used for the treatment of depression but also used for the treatment of anxiety disorders, eating disorders and chronic painful conditions. TCAs act by centrally blocking the reuptake of serotonin and nor-epinephrine and also they have the ability to bind to other receptors as histaminic (H1 and H2), adrenergic

and muscarinic receptors leading to a broad range of unfavorable side effects (11).

Monoamine oxidase inhibitors (MAOIs) are a group of antidepressants used since 1950 for the treatment of tricyclic antidepressant-resistant depression (12). It was found that MAOIs are useful in treating atypical depression, anergic bipolar depression and anxious/phobic associated depression (13).

Selective serotonin reuptake inhibitors (SSRIs) are used as the first-line of treatment for depression and anxiety disorders because they are considered safer and better tolerated than other antidepressant drugs as they decrease the adverse effects of other antidepressants. It's considered the most commonly used antidepressant among adults (14). SSRIs treat depression by increasing levels of serotonin in the brain. It blocks the reuptake of serotonin in the brain, making more serotonin available. The available SSRIs are fluoxetine, paroxetine, fluvoxamine, sertraline, citaloprarn, and escitalopram (15).

Selective serotonin-reuptake inhibitors (SSRIs), and among them fluoxetine, the active compound in Prozac are one of the major classes of antidepressants used. The elevated level of serotonin, produced by the administration of fluoxetine, is thought to be in charge for the bone phenotype. Serotonin is believed to increase bone mineral density and it also has a positive effect on bone formation (16).

Studies suggest that the administration of fluoxetine leads to enhancing the bone condition and increasing bone formation in case of bony defects (17). They also proposed that fluoxetine treatment can lead to enhancement in the bone regenerative properties under normal physiological conditions as well as in case of estrogen deficiency (18). Serotonin receptors and serotonin transporter are both expressed in the osteoclasts as well as in the osteoblasts and they are responsible for regulating the bone mass (19). However, other studies suggested that fluoxetine administration can lead to deleterious effects on the bone if it's used for a long period of time (20).

However, little research was done concerning the effect of fluoxetine in treating the deleterious effects of depression on the alveolar bone. Therefore, the present study investigates the effect of fluoxetine on the structure of alveolar bone in rats with induced depression.

MATERIALS AND METHODS

Experimental animals

Thirty adult male rats weighing 200-250 grams (approximately six months of age) were used in this study. These animals were obtained from the Institute of Medical Research, Alexandria University. Animals were caged in specially designed wire mesh cages. The animals were supplied with a regular diet throughout the whole experimental period.

The study was conducted after the approval of the research ethics committee, Faculty of Dentistry Alexandria University.

The animals were randomly divided into three equal groups as follows:

- **Group I (control group):** 10 rats were injected with vehicle to control the influence of any injection stress or buffer-induced effects on the animals.
- **Group II (depression group):** 10 rats were exposed to chronic unpredictable stress for 8 weeks (21).
- **Group III (fluoxetine group):** 10 rats were exposed to chronic unpredictable stress for 8 weeks and then they were orally supplied with fluoxetine for 1 months. A 240 mg/L fluoxetine solution was prepared so that the rats received 10 mg/kg/day orally (22).

1. Induction of depression

Rats of group II and III were exposed to chronic unpredictable stress paradigm (CUS) for 8 weeks to induce depression. CUS included the exposure to daily stressful stimuli, it included: **Restraint:** rats were placed in a plastic container with small openings for breathing for 1hr, **Shaking:** the cage containing the rats was placed on a shaker for 1hr, **Hot air stream**: rats were exposed to a source of hot air stream from a hair dryer for 10 min, **Overnight illumination**: rats were exposed to regular room light for 24hrs, **Inverted light cycle**: room light was turned on at night and turned off during daytime, **Tilted cage:** cages were tilted at 45° angle for 1hr (21).

2. Behavioral assessment

To measure the level of anxiety and depression, rats were exposed to behavioral tests in the following order: elevated plus maze and forced swimming test.

Elevated plus maze

Anxious-like behavior was tested by using the EPM test. This test consists of placing each rat in a plus-like apparatus elevated 72.4 cm from the floor, with two opposing open arms (50.8 cm × 10.2 cm) and two opposing closed arms (50.8 cm × 10.2 cm × 40.6 cm) and letting the animal freely explore it for 5 min. Time in the open arms and in the closed arms was used as a behavioral parameter of anxious-like behavior. Total entries in the closed arms is an index of anxiety and avoidance of the open arms is considered to be a result of the induction of higher levels of fear (23).

Forced swimming test

Depressive-like behavior was assessed through the FST. In the FST each rat was placed in an inescapable transparent cylindrical tank filled with water (±24°C), for 6 min. The activity of each rat was recorded, the latency (time to the first stop), mobility and immobility times were scored and used as a measure of behavioral despair (24).

3. Administration of fluoxetine:

Fluoxetine, which was used for this experiment, was purchased from Eli Lilly and co. It was available in the form of capsules, each containing 20 mg of fluoxetine hydrochloride. The powder content of each capsule was added aseptically to drinking water to prepare a 240 mg/L fluoxetine solution so that the rats received 10 mg/kg/day orally by using oral gavage syringe.

All rats were sacrificed after 3 months. The right first molar segments were prepared for light microscopic examination while the left first molar segments were prepared for Scanning Electron Microscope (SEM) and Energy Dispersive X-ray microanalysis (EDX).

Rats' dental formula is: I 1-1, C 0-0, P 0-0, M 3-3. Rats have 8 teeth on the lower jaw and 8 on the upper, a total of sixteen teeth.

Histological procedures (25)

Specimens were cut in a mesiodistal direction. They were labeled and fixed in 10% neutral buffered formalin. After fixation, specimens were decalcified in 8% tri-chloroacetic acid, washed, dehydrated in ascending concentrations of ethanol, cleared with xylene, infiltrated and embedded in paraffin wax blocks. Thin sections of 5 µm thickness were cut using a rotary microtome. Sections were stained with

Hematoxylin & Eosin stains (H&E) then examined by light microscope.

Scanning electron microscope (SEM) (26)

Specimens were fixed in 2.5% glutaraldehyde in phosphate buffer (PH 7.3) for 48 hours and rinsed in phosphate buffer for 10 minutes then dehydrated in the graded series of aqueous ethanol solution 50%, 70%, 90%, and 100% for two changes of one hour each to extract the water from the sample.

Then they were air-dried in a vacuum desiccator in which air was evacuated by a rotatory pump. After that the specimen was mounted on aluminum SEM stubs with silver paint and sputter coated with gold using an ion coater (sputter coater). This was achieved by the deposition of an evaporated thin layer of gold over the samples. After coating, the samples were ready for scanning electron microscopic examination.

Energy Dispersive X-ray (EDX) (26)

The specimens were washed under running water, dehydrated and air-dried. The surfaces of the mandibles of the study and control groups were exposed to x-ray analysis using EDX system to analyze the different percentages of calcium and phosphorus.

Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0**. (**Armonk, NY: IBM Corp**)**. The Kolmogorov-Smirnov test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Significance of the obtained results was judged at the 5% level. ANOVA test was used for normally distributed quantitative variables, to compare between more than two groups, and Post Hoc test (Tukey) for pairwise comparisons.

RESULTS

Histological results

Group I (control group)

Results obtained from sections prepared from the control specimens showed normal alveolar bone structure from the crest coronally to the apical part in the interdental region.

The alveolar bone showed normal trabeculation enclosing the bone marrow spaces. Osteocytes lacunae were regularly distributed. (Figure. 1)

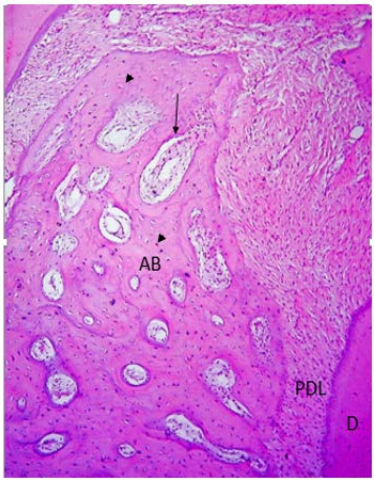

Figure 1: LM {control group} of the interdental bone showing a regular and smooth bone architecture from the crest coronally till the apical part. Osteocytes lacunae are regularly distributed (arrowhead). Normal trabeculation enclosing the bone marrow spaces are seen (arrow). The PDL exhibits a well organized fibers facing the alveolar bone. D: Dentine. AB: Alveolar bone. (H&E x100).

Group II (depression group)

The surface of the alveolar bone showed irregular outline indicating bone resorption. The bone trabeculae were thin and contained deeply stained reversal lines.

Osteoclast cells were evident along the borders occupying the How ship's lacunae. Osteoblastic cell layer showed a discontinuity along the surface of the alveolar bone with a reduced volume in comparison to the control group. (Figure. 2)

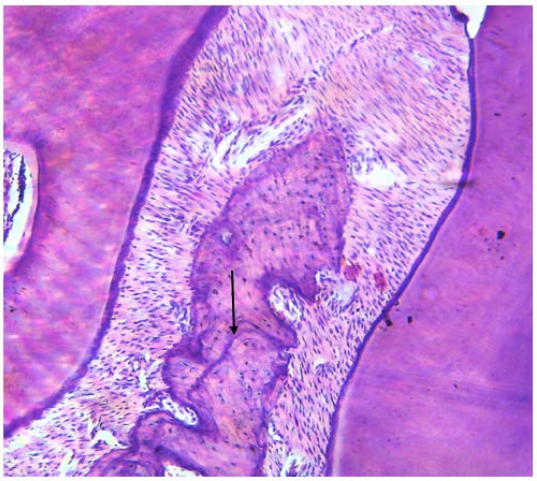

Figure 2: LM {depression group} of the interdental bone showing irregular alveolar bone surface. The bone trabeculae are thin with deeply stained reversal lines (arrow). Discontinuity of the osteoblasts on the bone surface is evident with reduced volume. D: dentine. AB: Alveolar bone. (H&E x100)

Group III (fluoxetine group)

A generalized appearance of reduced resorption of the alveolar bone was evident. The alveolar bone restored its normal architecture to a great extend near to the control group along all its borders.

The bony trabeculae are thick surrounding the bone marrow spaces. Incremental lines are clear indicating a high rate of bone remodeling. (Figure 3)

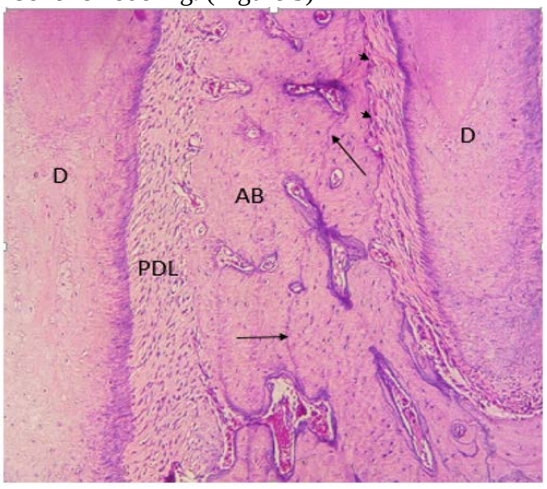

Figure 3: LM {fluoxetine group} of the interdental bone showing restoration of the normal bone architecture with a smoother surface. The incremental lines indicate bone remodeling (arrow). The bone trabeculae are well developed surrounding the bone marrow spaces. Osteoblasts are regularly distributed on the bone surface (arrowheads). D: Dentine. AB: Alveolar bone. (H&E x100)

Scanning electron microscope (SEM) results

Group I (control group)

The buccal cortical plate of the alveolar bone showed a regular smooth surface with a uniform surface topography. (Figure 4).

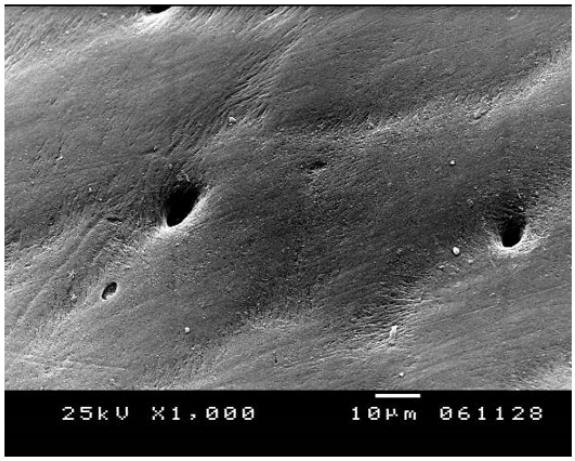

Figure 4: SEM {control group} of the buccal cortical plate showing smooth and regular bone surface with a regularly outlined nutritive canal. (x1000)

Group II (depression group)

Generalized roughness was observed. The buccal cortical plate showed an irregular pattern with osteoclasts lying in How ship's lacunae. (Figure 5)

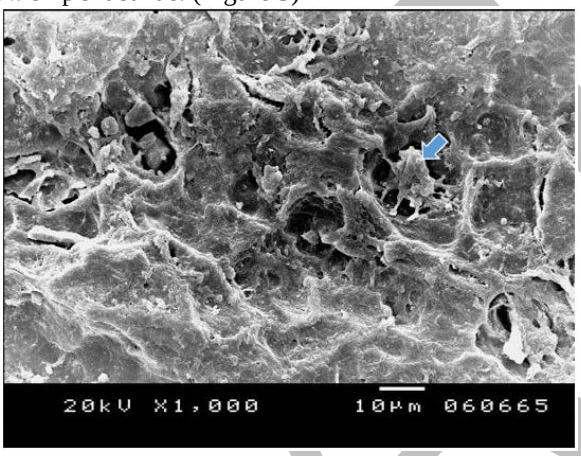

Figure 5: SEM {depression group} of the buccal cortical plate showing generalized roughened bone surface with irregularly outlined nutritive canal. Osteoclasts in Howship's lacunae (arrow) (x1000).

Group III (fluoxetine group)

The bone surface showed an enhanced surface topography than that seen in the depression group. The surface irregularities were reduced and bone formation was seen over the old bone. (Figure 6)

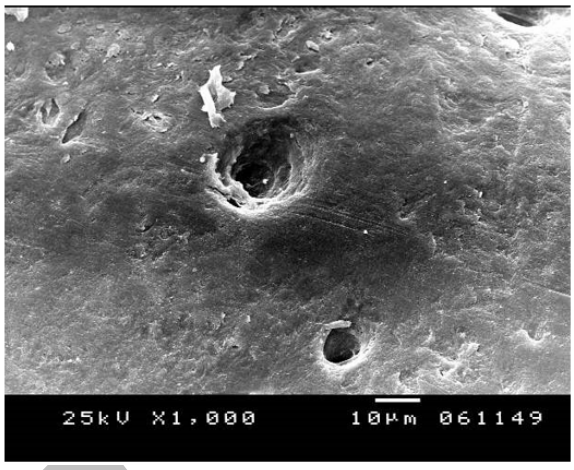

Figure 6: SEM {fluoxetine group} of the buccal cortical plate with less irregularities and smoother nutritive canals. (x1000)

Energy dispersive x-ray analysis (EDX)

The calcium and phosphorous levels in different groups are summarized by means and standard deviation. There were statistically significant decrease in calcium level and increase in phosphorous level in depression group (group II) in relation to control group (P1 for calcium<0.001) and (P1 for phosphorous<0.001). On the other hand, in the fluoxetine treated group (group III), the results weren't statistically significant in comparison to the control group as the values of calcium and phosphorous were more close to the control group (group I) than that of depression group (P2 for calcium=0.208) and (P2 for phosphorous =0.215). Moreover, the difference in calcium and phosphorous levels between fluoxetine and depression groups (group III and II respectively) were statistically significant (P3 for calcium <0.001) and (P3 for phosphorous <0.001). (Table 1)

Table (1): Comparison between the three studied groups regarding the different percentages of calcium and phosphorous:

	Group I (n = 10)	Group II (n = 10)	Group III (n = 10)	F	p
P					
Min. – Max.	31.40 – 35.70	38.90 – 44.20	33.30 – 38.30	25.573*	<0.001*
Mean ± SD.	33.22 ± 1.62	41.22 ± 2.44	35.27 ± 1.90		
Median	33.05	41.0	35.15		
Sig. bet. Grps	$p_1<0.001^*, p_2=0.215, p_3<0.001^*$				
Ca					
Min. – Max.	64.30 – 68.70	55.80 – 61.10	61.70 – 66.60	25.327*	<0.001*
Mean ± SD.	66.80 ± 1.64	58.80 ± 2.46	64.72 ± 1.88		
Median	66.95	59.0	64.85		
Sig. bet. Grps	$p_1<0.001^*, p_2=0.208, p_3<0.001^*$				

F: **F for ANOVA test**, Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (Tukey)**

p: p value for comparing between the studied groups

p_1: p value for comparing between group I and group II

p_2: p value for comparing between group I and group III

p_3: p value for comparing between group II and group III

*: Statistically significant at $p \leq 0.05$

DISCUSSION

Depression is categorized as a disabling disorder in which the person losses interest in all life activities. Symptoms of depression include loss of interest and pleasure, loss of appetite, feeling of worthlessness and despair (27).

Depression is thought to be correlated with low bone mineral density. This may be due to the debilitated quality of life and pain which causes inhibition of calcium absorption and bone cell proliferation. Additionally, bone metabolism may be disturbed due to the elevated cortisol level associated with depression (28).

Antidepressants are the most commonly prescribed drugs for the treatment of depressive disorders. They are classified into tricyclic and tetracyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), and selective serotonin reuptake inhibitors (SSRIs) (10).

Moreover, medications as fluoxetine, which is the first SSRIs to be approved in the United States, is used in the treatment of depressive disorders. It is used broadly and with high success rate due to its safety profile. Fluoxetine, under the brand name Prozac, is considered the most famous antidepressant drug used for depression (27). Additionally, it was proved that fluoxetine has a positive influence on correcting the adverse effects of depression on the bone (16).

However, little research was done concerning the effect of fluoxetine in treating the deleterious effects of depression on the alveolar bone.

The present study investigated the effect of selective serotonin-reuptake inhibitor (fluoxetine) on the structure of alveolar bone in rats with induced depression. This was done by using histological examination, SEM and EDX.

Induction of depression in laboratory animals can be done in several methods. Monteiro et al (2015) (21) suggested that chronic unpredictable stress paradigm is an effective method for the induction of depressive and anxiety- like disorders in mice. In this study the rats were placed under stressful conditions for 8 weeks which is estimated to be an enough period to cause depression complications in the bone.

Histological results of this study showed that the control group had a regular alveolar bone architecture. The osteocytes lacunae were regularly distributed. These results were supported by Nanci et al (2017) (29) who studied the normal structure of the alveolar bone and viewed its uniform appearance from the crest coronally to the apical end.

On the other hand, depression group showed irregular bone surface with multiple osteoclasts lying in How ship's lacunae. Marked bone resorption was evident with numerous deeply stained reversal lines indicating high degree of bone remodelling.

These results are in line with Wu et al (2009) (30) who suggested that depression has a negative effect on bone mineral density and increase the risk of fracture. Some literature proposes that the elevated level of glucocorticoids seen in depressive disorders leads to suppression in bone formation and reduced bone mass (31). Goshen et al (2008) (32) and Adler et al (2008) (33) suggested that inflammatory cytokines as interleukin-1 (IL-1) and interleukin-6 (IL-6) and tumor necrosis factor-α are expressed in both depression and osteoporosis. Also the disturbed diet and behaviour associated with depression can be a causative factor for osteoporosis.

Histological results of the fluoxetine group showed that the alveolar bone surface restored its regular, smooth outline to a great extend near to the control group, with deeply stained incremental lines indicating bone remodelling. The bone trabeculae are well developed surrounding the bone marrow spaces.

These results are consistent with Warden et al (2005) (34) who proposed that there is a relationship between the serotonergic system and the skeletal system. Gustafsson et al (2006) (35) suggested that the increase in serotonin, as a result of fluoxetine administration, has a regulatory effect on bone cells differentiation, proliferation and activation and it leads to increase in the bone mineral density.

Scanning electron microscope (SEM) results supported the histological findings as it showed that the control group revealed a regular and smooth surface topography. Unlike the depression group which showed an irregular, resorbed surface of the outer cortical plate of bone indicating the progressive bone resorption associated with depression. Yirmiya et al (2006) (36) used the Micro-CT scanning analysis to study the effect of depression on the bone. He found that the exposure to stressful and depressive behaviors leads to alteration in the bone architecture due to the stimulation of the sympathetic nervous system (SNS) which mediates depression-induced skeletal impairment.

On the other hand, SEM of fluoxetine group showed an enhancement in the surface topography with less irregularities in the outer cortical plate. These results were in line with Ortuño et al (2016) (37) who studied the effect of SSRIs on bone architecture and he concluded that short time use of fluoxetine increases the bone density and decrease bone resorption parameters.

The elemental microanalysis (EDX) revealed a decrease in calcium concentration in relation to phosphorous in the depression samples. The results of the elemental microanalysis coincide with scanning electron microscopic results.

Negareddy et al (2005) (38) mentioned that energy dispersive x-ray analysis is considered an efficient method to detect different percentages of minerals in the bone, also allows for region specific analysis of elemental composition and it is better than ash analysis which only provide information about total bone composition.

The results of EDX of the fluoxetine group revealed an increase in the calcium level and the relative regaining of the normal ratio between calcium and phosphorous in relation to the depression group. Appropriate balance of calcium and phosphorous levels is the key for maintaining healthy bone and both minerals are critical to support bone formation (39).

CONCLUSION

Depression has a deleterious effect on the bone which can be reversed by the administration of fluoxetine which has a stimulatory effect on osteoblast cells.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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