OXYTOCIN HORMONE AS A PREVENTIVE STRATEGY FOR TREATING OSTEOPOROSIS IN OVARIECTOMIZED RATS

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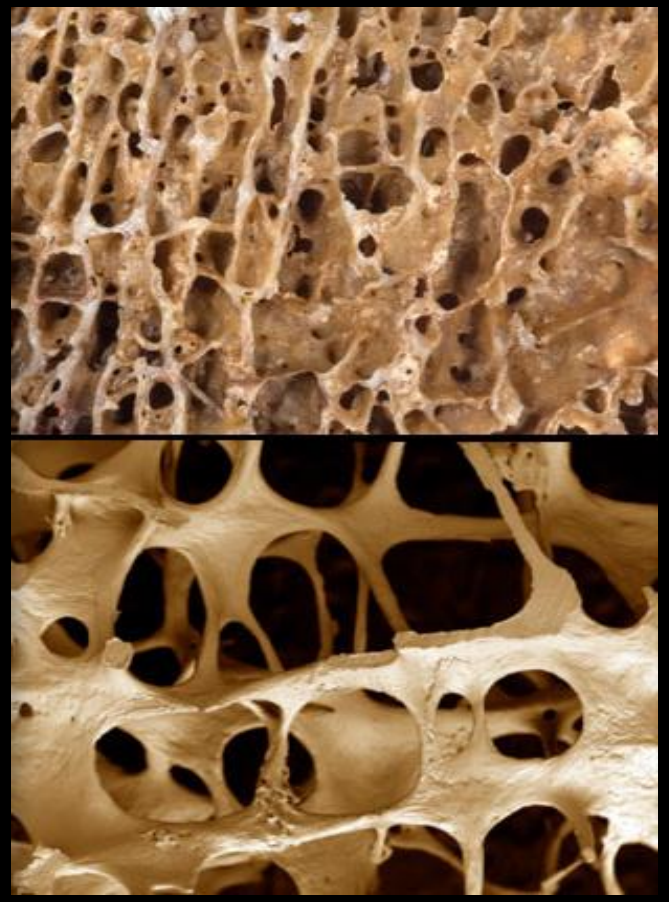
INTRODUCTION

- Osteoporosis (OP) constitutes a major worldwide public health burden characterized by enhanced skeletal fragility. Bone metabolism is the combination of bone resorption by osteoclasts and bone formation by osteoblasts. Whereas increase in bone resorption is considered as the main contributor of bone loss that may lead to osteoporosis.

- With aging, the composition of bone marrow shifts to favor adipocyte formation, increase in osteoclast activity, and decrease in osteoblast functions.



INTRODUCTION

- A limited number of approved therapeutic molecules capable of activating bone formation and increasing bone mass and strength has been available. It is hoped that providing more options for developing efficient therapeutic strategies targeting bone formation will allow prevention and restoration of age-related bone strength.

- Recently, it has been shown that oxytocin (OT), a hormone produced in the neurohypophysis and also by osteoblasts, is involved in bone metabolism.

INTRODUCTION

- Osteoblasts in bone marrow produce abundant OT, suggesting that locally released OT may be an autocrine regulator of bone formation and bone mass. In this local circuit OT produced from osteoblasts in response to estrogen acts upon the OT receptors to stimulate further OT release, which amplifies estrogen action.

- Plasma OT levels could represent a novel diagnostic marker for osteoporosis and that OT administration holds promise as a potential therapy for this disease

THE AIM OF THE WORK

- **The aim of this work was to clarify the possible protective effect of oxytocin hormone in ovariectomized osteoporotic rats.**

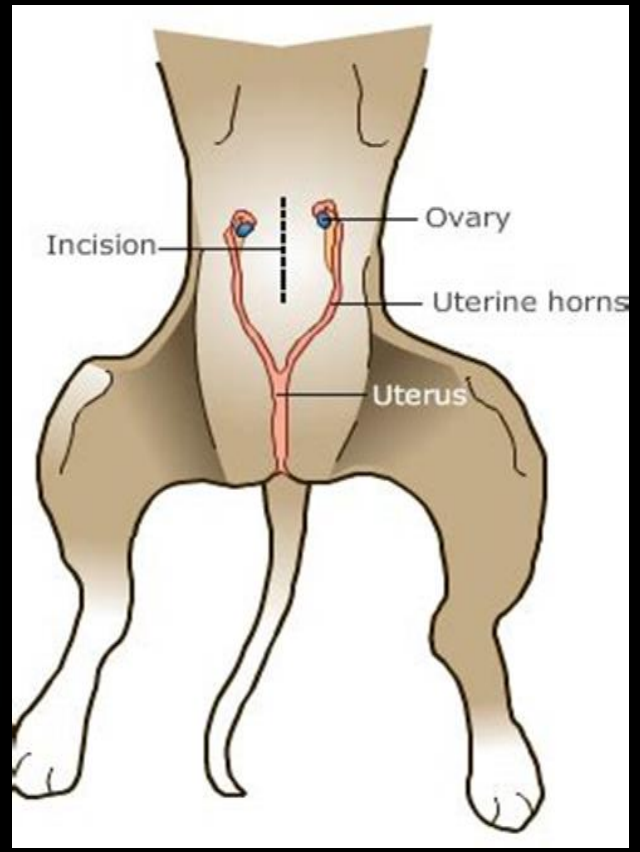
MATERIALS AND METHODS

Starting from January 2017 to June 2017 in the physiology department, Sohag University. This study was carried out in accordance with the guidelines of the University Animal Ethics and approved by Research Ethics Committee considering care and use of laboratory animals.

30 adult female albino rats about, (200-gm250) weight, (75-90) days age, were maintained in room temperature a nand in ormal light-dark cycle, they were fed a standard diet of commercial rat chow and tap water. Animals were left one week for acclimatization prior to inclusion in the experiment.

MATERIALS AND METHODS

- All rats were subjected either to bilateral ovariectomies (OVX) or sham surgery.
- Under intraperitoneal anesthesia, a midline incision (about 2 cm) was made. The ovary was withdrawn out and was tied with a silk ligature. The ovary of the other side was similarly removed. The sham operated control group will have the same previous incision, but with no excision of the ovaries.
- All animals were given oral antibiotic with postoperative care of the surgical incision.



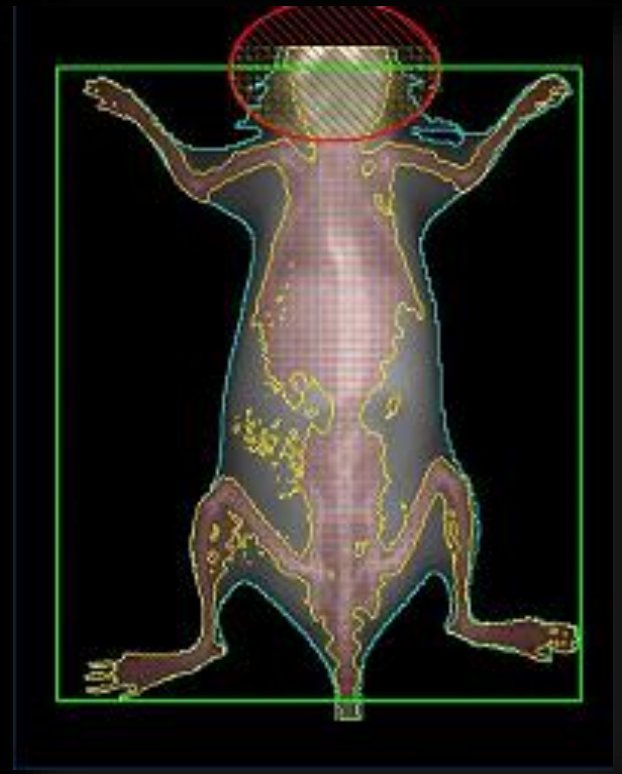
MATERIALS AND METHODS

- ***The Rats were divided into three groups (n= 10):***

  - **Group I, (the control group) after sham surgery, rats were received intraperitoneal injection (Ip) of physiological saline 0.1 mg/kg/day Nacl 0.9 % for 7 weeks.**
  - **Group II, after being ovariectomized, rats were received Ip of physiological saline 0.1 mg/kg/day Nacl 0.9 % for 7 weeks.**
  - **Group III, two weeks after ovariectomy, rats were injected Ip with (0.1 mg/kg/day of OT according to the manufacture instruction) for 7 weeks.**

MATERIALS AND METHODS

Prior to the scarification, BMD was assessed using DEXA machine and calibrated according to the manufacturer's instruction protocol. Measurements were obtained by positioning the rat in a prone position with knee flexed and extended hips.



MATERIALS AND METHODS

Biochemical measurements:

- Serum concentration of OT (OT EIA kit) was measured using commercially available kits (WKEA Med. supplies, Changchun, China, Cat. No. WAR-671).
- Moreover, ALP was determined by ALP assay kits (DALP-250, Bioassay Systems, CA, USA).

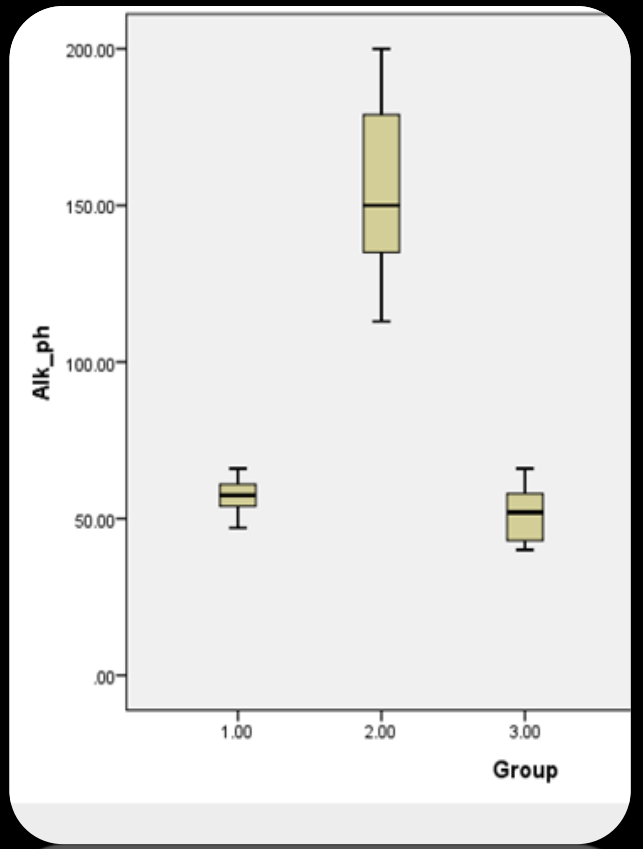
Histological measurement :

- Head of the tibia were surgically removed with a sharp blade, followed by fixation and decalcification using EDTA 10% for 2–3 weeks.
- The specimens were impregnated in soft Paraffin wax. Measurements were obtained, using the image analyzer (Leica version; 3.7.2005-2010) counting the numbers of osteoclast and osteoblast lining the endosteum under x 400 magnification in three sections from each slide.

RESULTS

a) Measures of serum level of ALP:

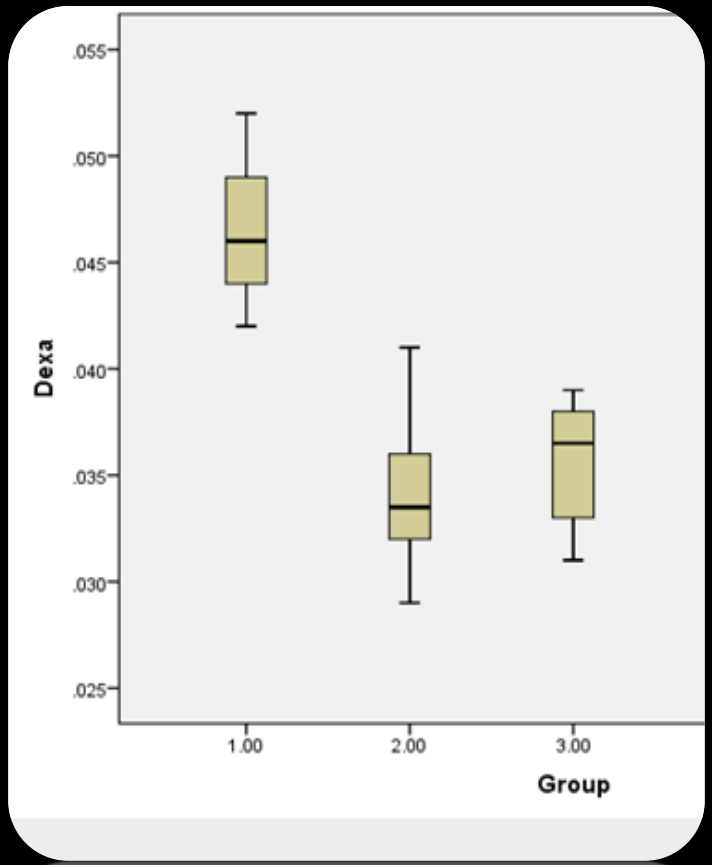
- In this work, serum ALP was increased in the ovariectomized group II and was noticeably decreased in group III after being treated with OT.
- The highest level of ALP was observed in group II compared to other groups. Marked increase was noticed in group II compared to group I (154.3±30.3 vs 57.0±6.0, P=0.002, respectively).
- When OT therapy was introduced 2 weeks after ovariectomy in group III, it resulted in a decrease in the serum ALP in this group compared with group II, (51.1±9. 2 vs, 154.3±30.3 P=0.009, respectively).



RESULTS

b) DEXA measurements:

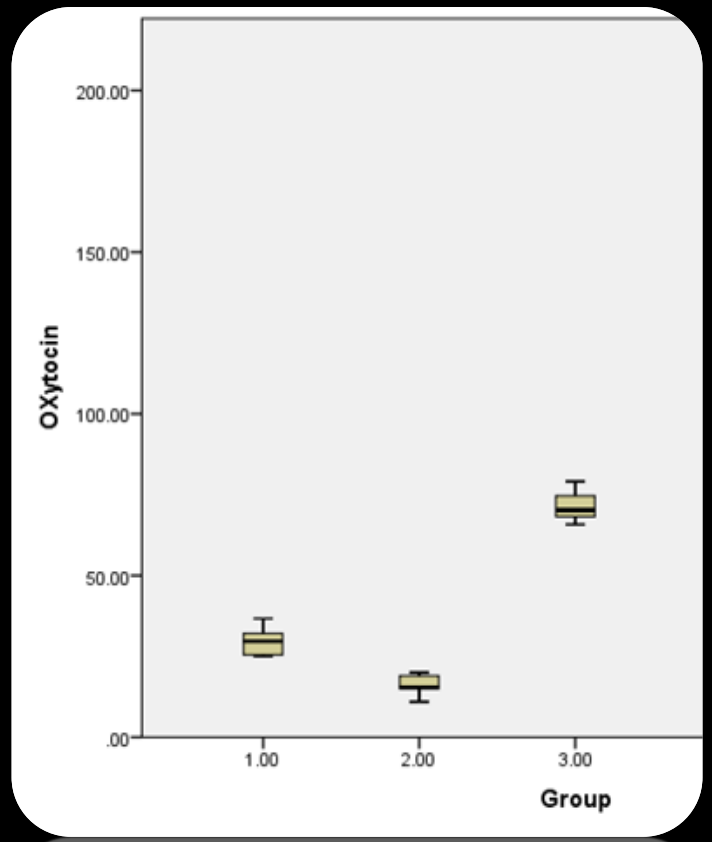
- BMD measures was decreased in the ovariectomized group II and was noticeably increased in groups III after being treated with OT.
- Marked deterioration was noticed in group II in the DEXA measurements, when compared between group II and group I (0.009 ± .002 vs 0.0675±0.007, P=0.000, respectively).
- A noticed improvement in the DEXA measurements showed in group III on comparing between group II (0.0492±0.009 vs 0.009±0.002, P=0.003, respectively).



RESULTS

c) *Measures of serum Oxytocin level:*

- Serum OT was increased in group III after being injected by OT for 7 weeks. There was a noticed decrease in the serum level of OT in group II compared to group I (16.3±2.7 vs 29.4±3.9, P=0.313, respectively).
- There was a high significant increase in the OT level in group III in comparison to group II (71.5±4.4 vs, 16.3± 2.7 P=0.000, respectively.

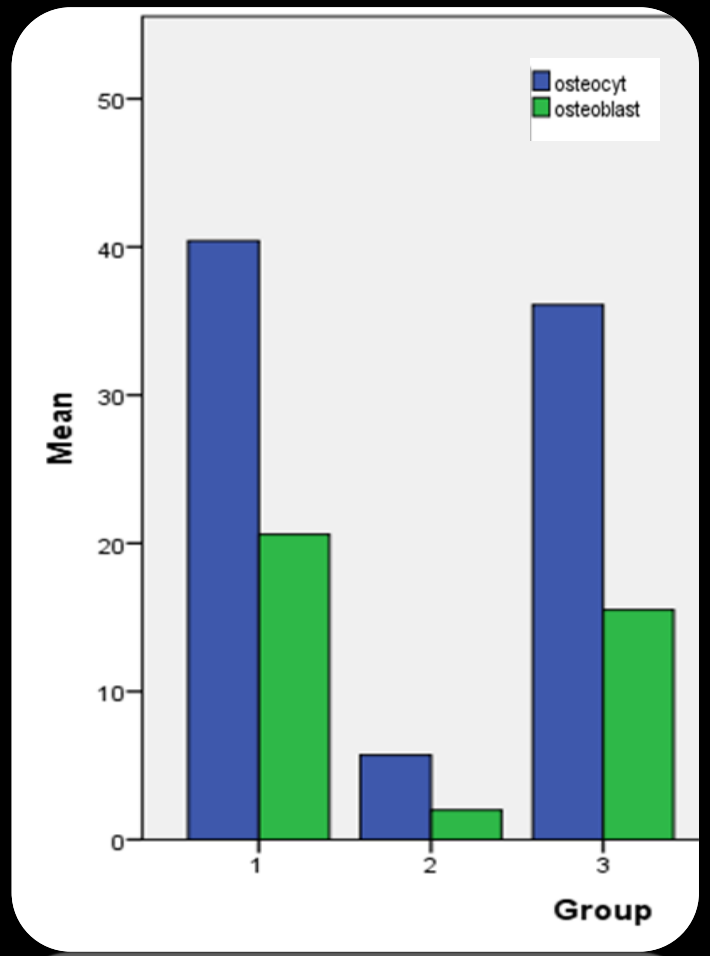


RESULTS

d) Morphometric and histological results:

- **There was a significant decrease in the number of osteocyte and osteoblast between group II and group I (5.7±1.4 vs 40.4±4.8, P=0.028), (2.0±0.8 vs 20.6±1.9, P=0.048) respectively.**

- **Supportive results were observed with significant increase in osteocyte and osteoblast number, group III vs group II (P=0.003 and P=0.03) respectively. No significant difference was obtained on comparing group III with group I as regard the number of osteocyte and osteoblast (P=0.897 and P=0.654) respectively.**



MORPHOMETRIC AND HISTOLOGICAL RESULTS

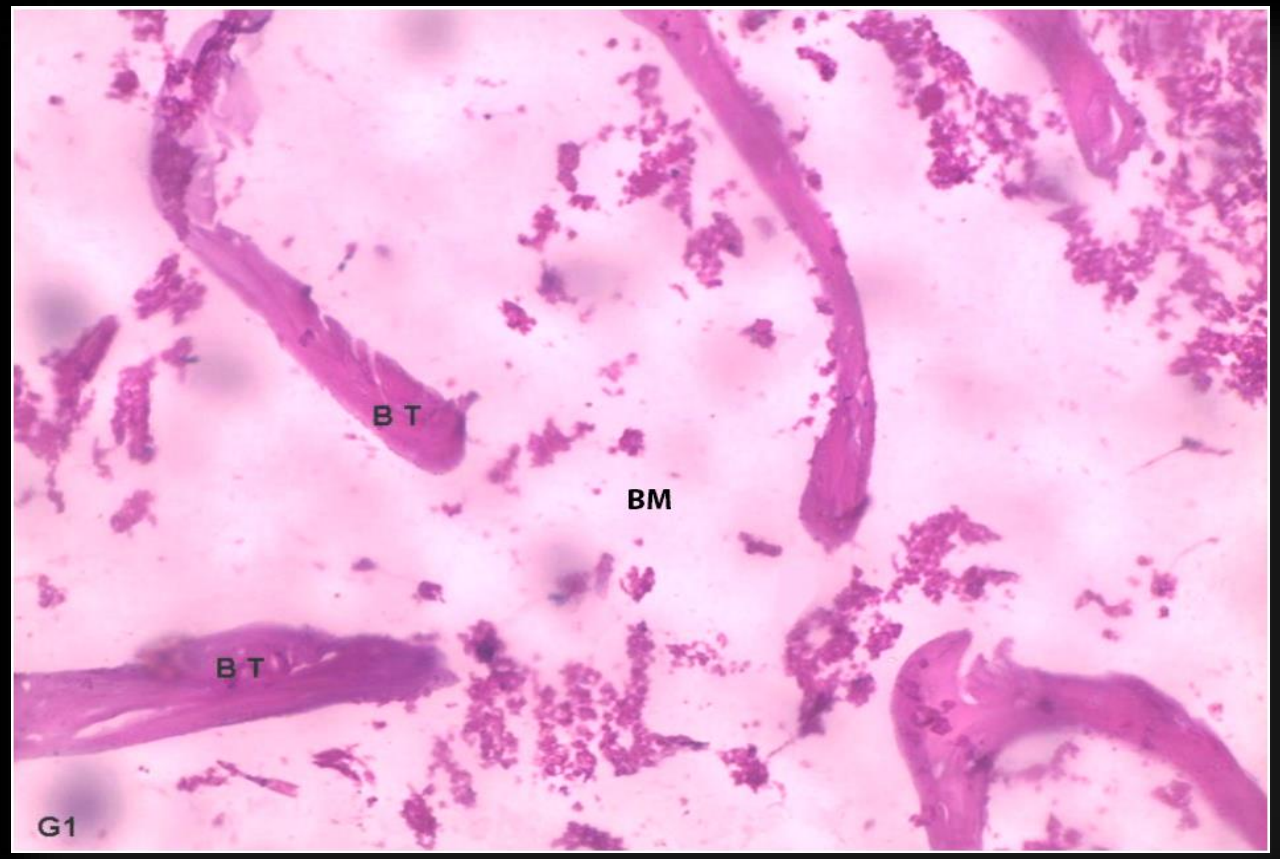


Fig. 1: A photomicrograph of a section of a control group (G I) at the head of the tibia showing: Irregular cancellous bone trabeculae (BT) of the metaphysis and bone marrow spaces in between the trabeculae. Osteocytes seen in the bone lamella (↑). H&E (X 200)

MORPHOMETRIC AND HISTOLOGICAL RESULTS

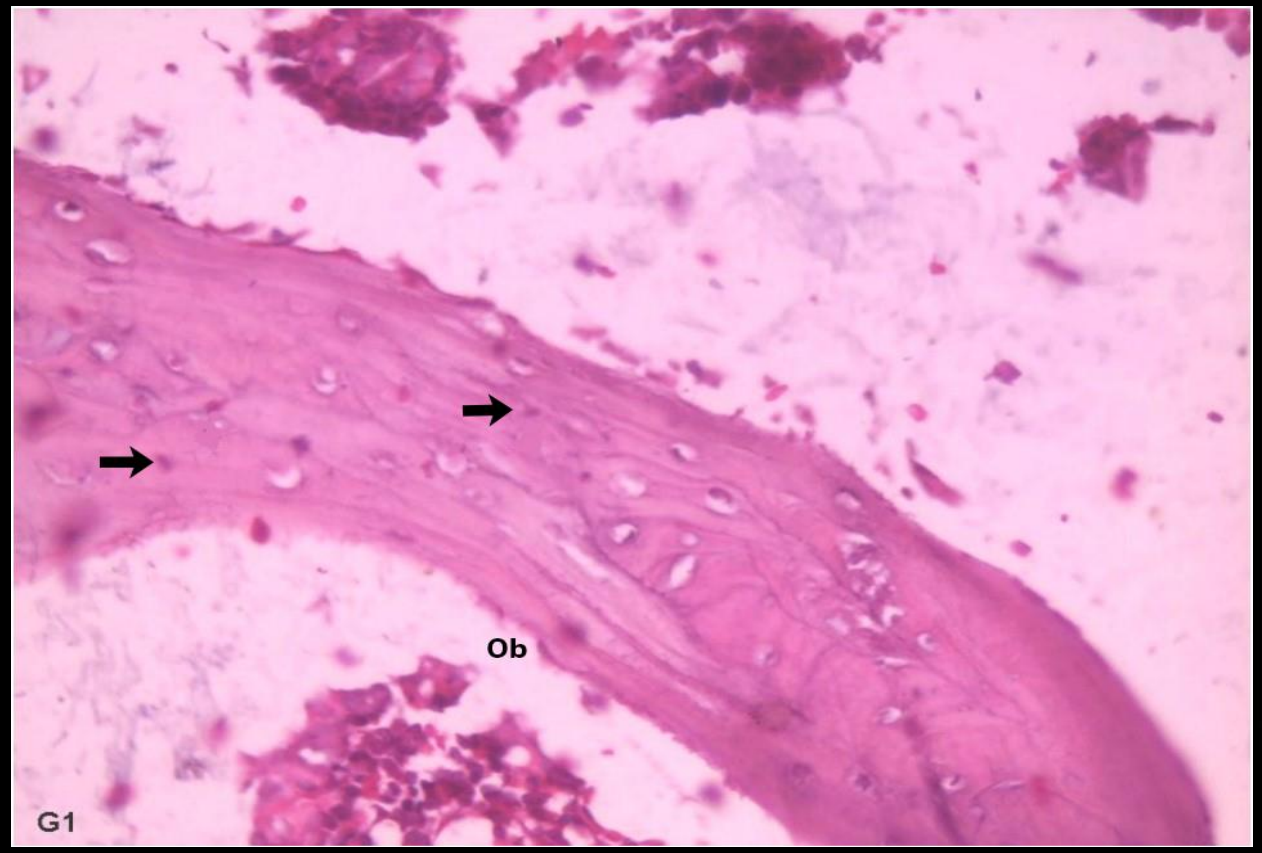


Fig. 2: A higher magnification of a section of a control group (G I) at the head of the tibia showing: Osteocytes seen inside their lacunae in the bone lamella (↑). Osteoblast (Ob) are seen in the outer surface of the lamellae. H & E (X400)

MORPHOMETRIC AND HISTOLOGICAL RESULTS

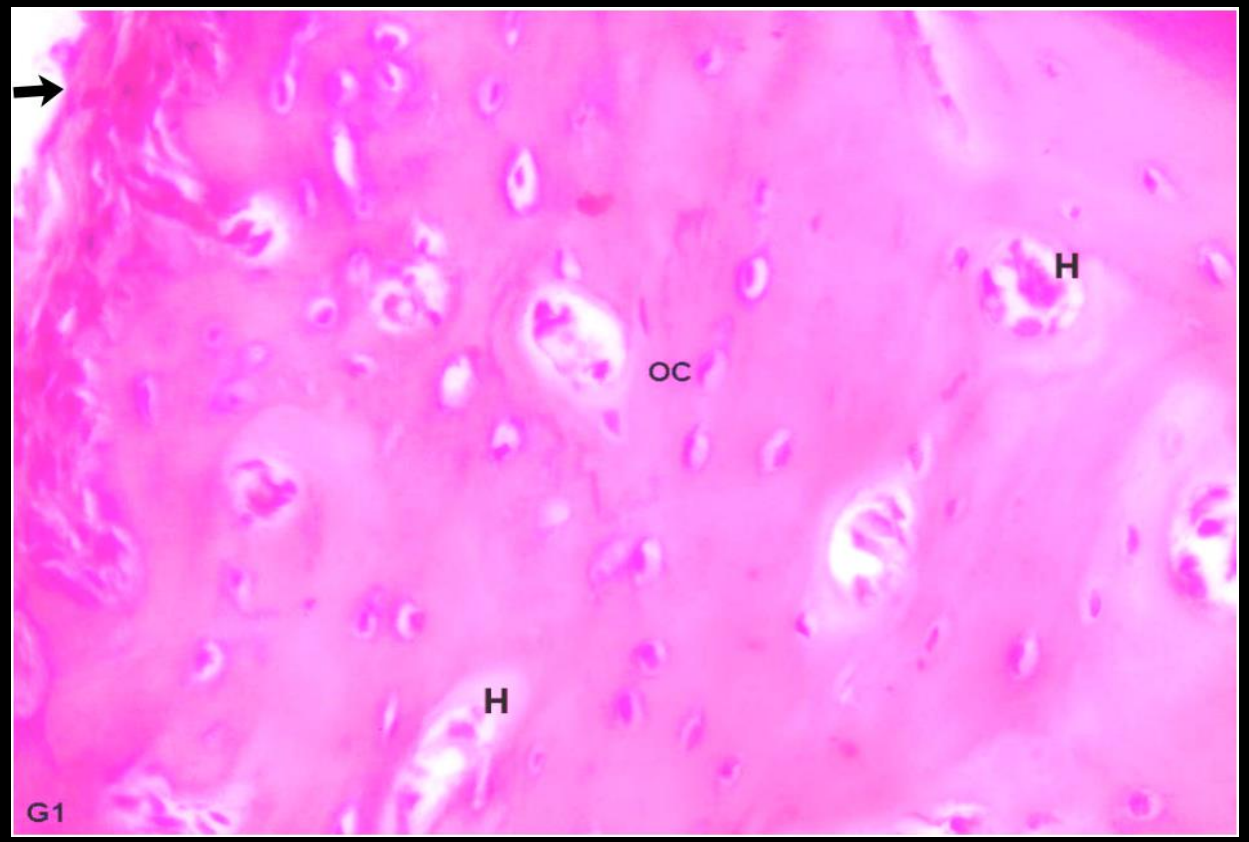


Fig. 3: A photomicrograph of a section from a control rat (G I) showing the outer part of the cortex of tibia: The bone is covered from outside by the periosteum (↑). The compact bone tissue is well organized, showing concentric lamellae arranged around the Haversian canals (H). Osteocytes (OC) inside the lacunae are shown in between the bone lamellae. (H & E X 400)

MORPHOMETRIC AND HISTOLOGICAL RESULTS

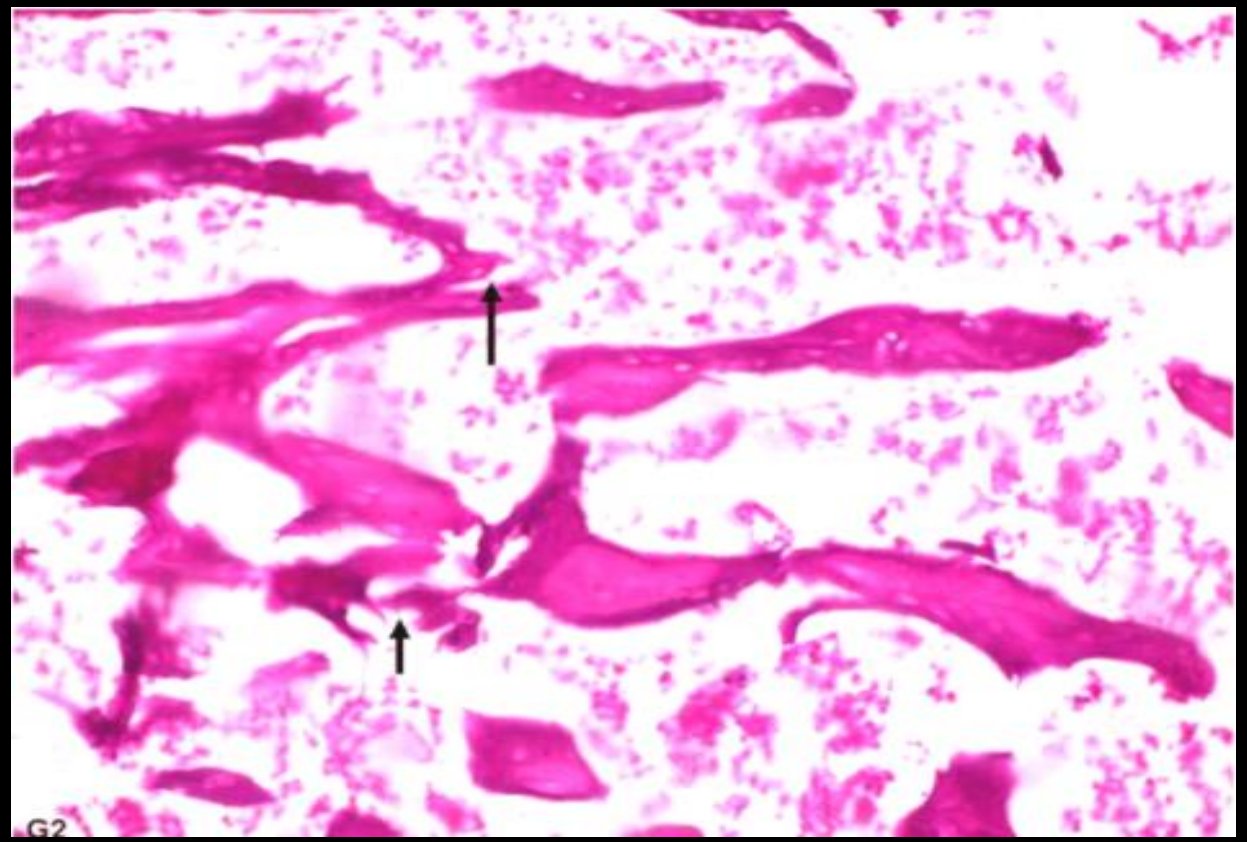


Fig. 4: A photomicrograph of a section of ovariectomized group (G II) at the head of the tibia showing: Bone trabeculae appeared thin and fragmented (↑). H&E (X200)

MORPHOMETRIC AND HISTOLOGICAL RESULTS

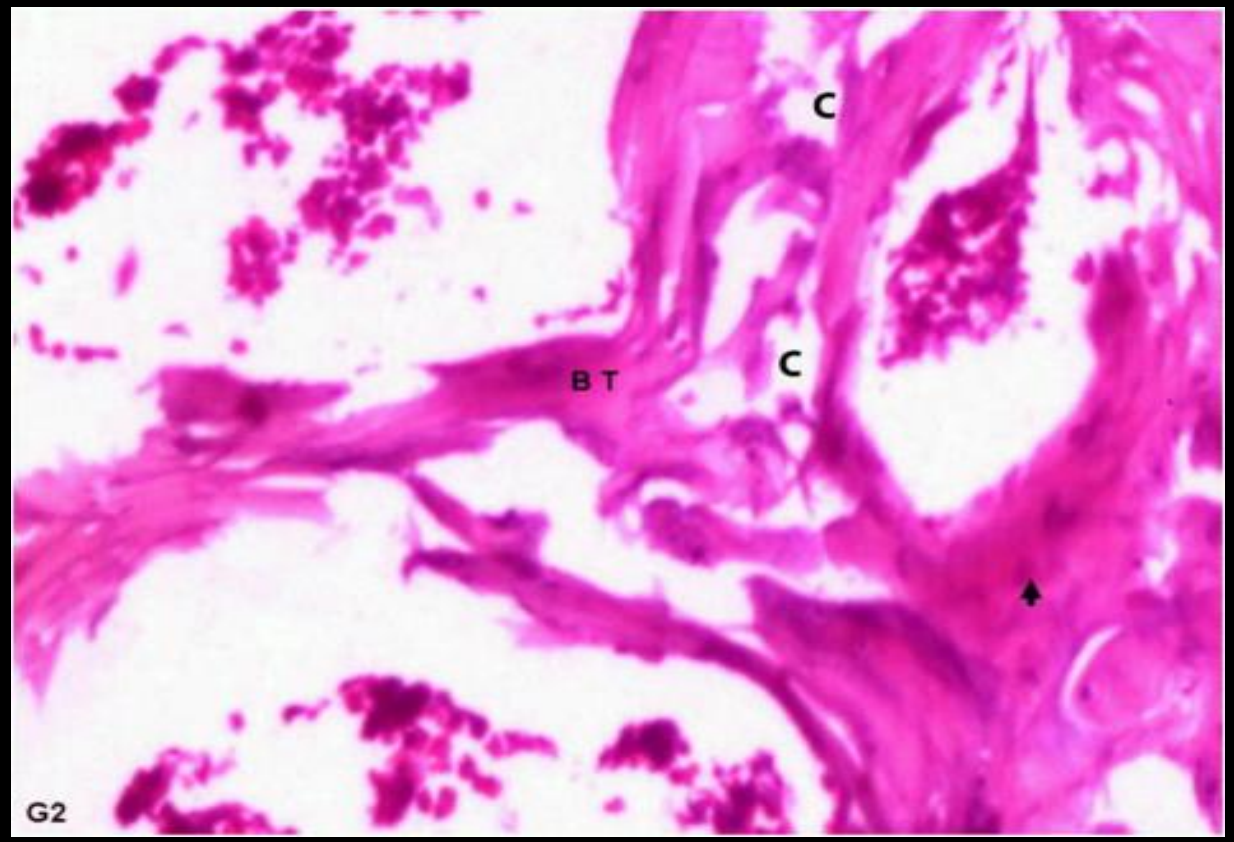


Fig. 5: A higher magnification of a section of ovariectomized group (G II) at the head of the tibia showing: Appearance of cavities (C) in the bone trabeculae near the medullary cavity. There is also apparent decrease in number of the osteocyte (↑). H&E (X400)

MORPHOMETRIC AND HISTOLOGICAL RESULTS

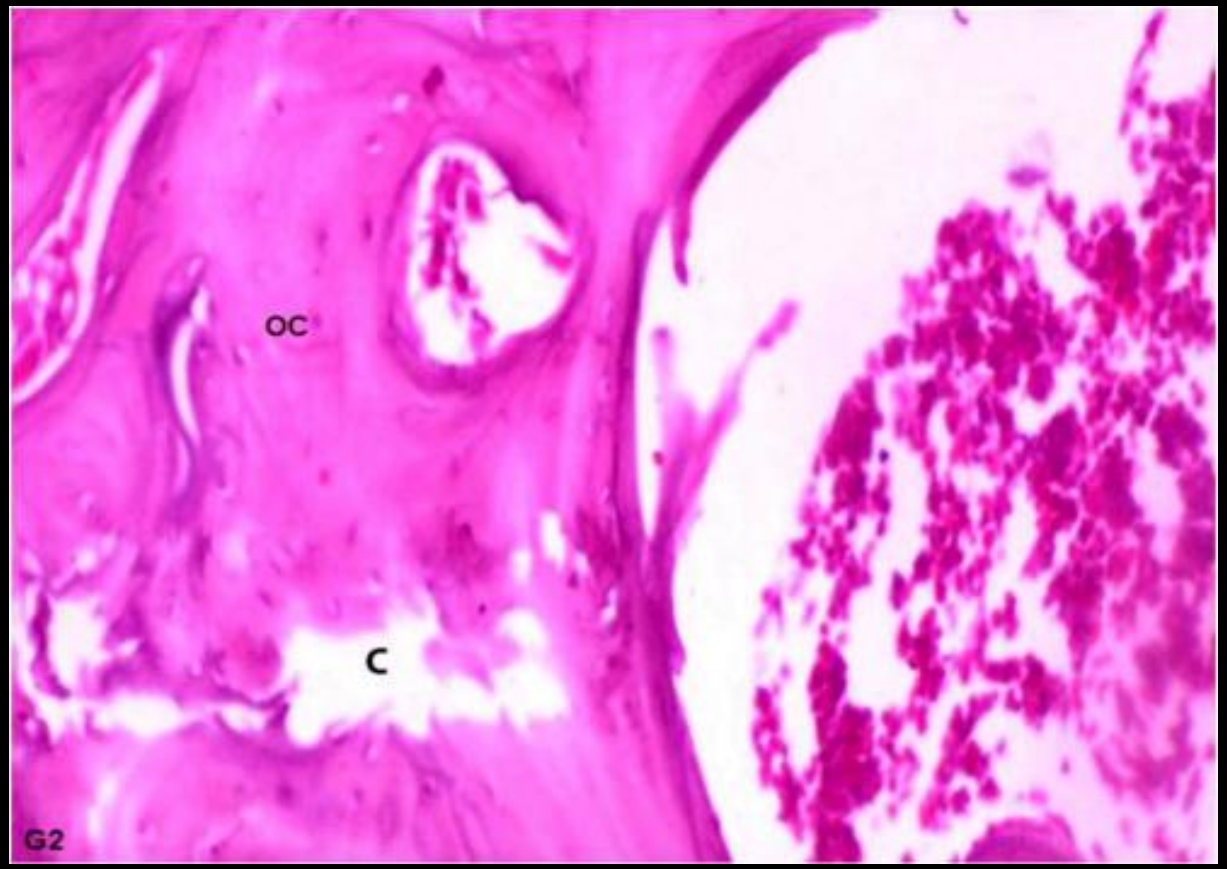


Fig. 6: A photomicrograph of a section of ovariectomized group (GII) at the head of the tibia showing: Areas of bone loss that appeared in the form of cavities (C) in the compact bone. osteocytes showing apparent decrease in number (OC). H&E X400

MORPHOMETRIC AND HISTOLOGICAL RESULTS

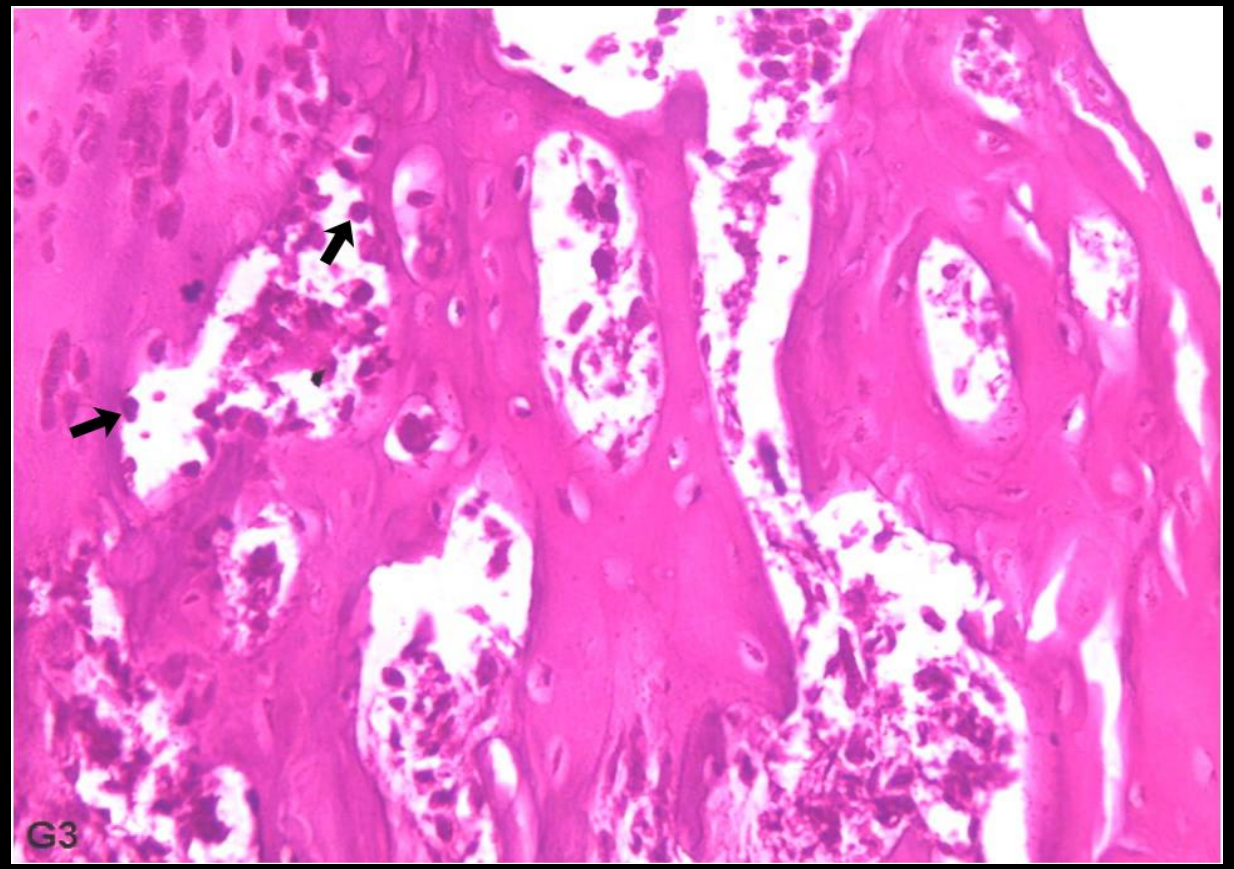


Fig. 7: A photomicrograph of a section of group III at the head of the tibia showing: Apparent thickened bone trabeculae. The osteoblasts were seen covering the trabeculae (↑). H&E X 400

MORPHOMETRIC AND HISTOLOGICAL RESULTS

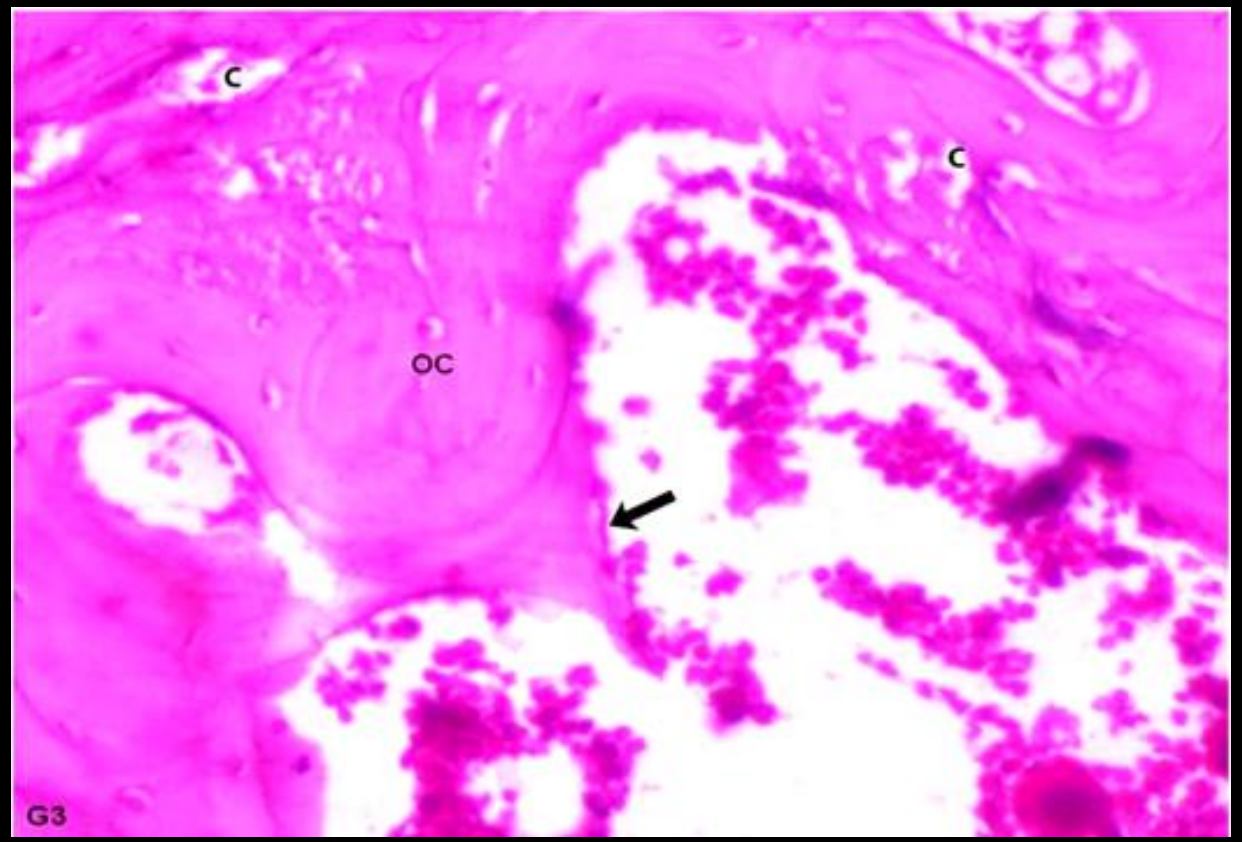


Fig. 8: A photomicrograph of a section from group III rat showing: The outer part of the cortex of tibia bone in which the number of osteocytes (OC) and osteoblasts (↑) showed improvement. The matrix appeared homogenous with less widening of marrow spaces with small cavities appeared (C). H&E (X400)

CONCLUSION

- Up till now, there is no efficient treatment free from side effects that can restore bone health. Current therapies for OP mainly consist of anti-resorptive treatments, such as bisphosphonates, estrogen, selective estrogen receptor modulators, calcitonin and the only currently available anabolic treatment for osteoporosis is PTH.
- For most of these treatments, if not all, side effects have been reported, that is, osteonecrosis, dysphagia, esophagitis, headache, nausea, arthralgia, dizziness, and others.

- One of the most meaningful results recently obtained in bone research has been that the posterior pituitary hormone OT have profound effect on bone.
- The possible mechanism by which OT can protect against postmenopausal OP is that the action of OT on the skeleton is mainly mediated not only through its stimulation of osteoblast differentiation but also through a modulation of osteoclast formation and function.

CONCLUSION

These beneficial observations showed that OT is involved in regulation of bone balance and OT may provide a protective role against postmenopausal OP. Further studies are needed to support these results.

THANK YOU