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RESEARCH ARTICLE

POTENTIAL OSTEOPOROTIC EFFECT OF TYPE 2 DIABETES MELLITUSON FEMALE RATSIN COMPARISON TO OVARIECTOMIZED MODEL OF OSTEOPOROSIS.

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Abstract

Background:- Type 2 diabetes mellitus (T2D) is a metabolic disease with extensive illness. Additionally, osteoporosis (OP) is a silent disorderwithdecreased bone mineral density (BMD).

Objective:- to explore the possible relationship between OP and T2D. Also, to declare the possible mechanisms involved.

Design:- A total number of 24adult healthy female albino rats were used. the 1st group was the control (sham operated) group (n=8), the 2nd group was the ovariectomized (OVX) group (n=8) and the 3rd group was the type 2 diabetic (T2D) group (n=8). At the end of procedures, rats were sacrificed after 12h overnight fasting. Blood samples were collected in dry clean test tubes for separation of serum. Serum was separated and stored at -20^{o}C for measurement of the fasting blood glucose and serum insulin levels. Calculation of HOMA-IR (homeostasis model assessment insulin resistance) as an index of insulin resistance was done.Also, serum bone specific alkaline phosphatase (AP), and serum osteocalcin (OC) concentration wereestimated.In addition, hydroxyproline (HP) was measured and histopathology of femur was studied in all the groups.

Results:- there was a significant decrease in body weight in T2D final group (325±10.1) in comparison to the T2D initial group (376.5±7.6). Also, there was a significant increase in fasting blood glucose(290.88±8.99),seruminsulin(5.2±0.23)and HOMA-IR (3.75±0.25)in T2D group in comparison to those of control (sham operated) group (104.13±2.29), (2.15±0.08) and (0.55±0.02) respectively.Moreover, there was a significant increase in serum AP(30.92±1.1) and urinary HP (24.12±0.36) in T2D group in comparison to that in control group (17.4±0.38) and(13.54±0.28) respectively. On the other hand, there was a significant decrease in serum OC in T2D group (7.51±0.18) in comparison to that inthe control group (9.34±0.07).

Conclusion:- to our knowledge, this study is one of the few studies that confirmed the osteoporotic changes occurring in T2D and explained their possible mechanisms which may be the decreased body weight, insulin sensitivity and OC level in T2D. Further studies should be done to confirm these results and to study the effect of T2D therapy on these osteoporotic changes.

Introduction:-

Osteoporosis (OP) is a debilitating disorder with a multifactorial etiology characterized by a generalized loss of bone mass leading to an increased risk of fracture(Abdulameer, Sulaiman, Hassali, Subramaniam, & Sahib, 2012; Roglic et al., 2005).Diabetes mellitus (DM) is a metabolic disease that affects about 4% of the world population (J. Zhou et al., 2009). Like OP, DM is also a long-lasting metabolic disorder that is characterized by an increase in blood glucose level(Blonde & Russell-Jones, 2009; Rodbard et al., 2007).Though the relationship between type 2 diabetes mellitus (T2D) and OP has been widely investigated, it remains controversial. For patients with T2D, some authors have reported an elevated bone mineral density(BMD)(Barrett-Connor & Holbrook, 1992; Bauer et al., 1993; De Leeuw & Abs, 1977; G. C. Isaia et al., 1999; Johnston, Hui, & Longcope, 1985). On the contrary, some other studies have reported a decreased BMD (Gregorio, Cristallini, Santeusanio, Filipponi, & Fumelli, 1994; G. Isaia et al., 1987; Ishida et al., 1985; Levin, Boisseau, & Avioli, 1976) and some studies have reported unaltered bone density (Giacca et al., 1988; Wakasugi et al., 1993; Weinstock et al., 1989).In addition, in one study in Saudi Arabia, the frequency of OP in diabetic postmenopausal women was higher than normal group (Al-Maatouq et al., 2004), but, in another study in Japan, no difference was found between diabetic and normal people in terms of their bone density(Majima et al., 2005). On the contrary, Sahin et al. (2001)have detected a higher bone density in lumbar spine and femoral neck in diabetics than in normal people.On the other hand, Roy (2013) stated that osteocalcin (OC) is a peptide positively regulates osteogenesis and DM limitedits production through the negative regulation of osteoblast by decreased synthesis of insulin and amylin.Serum alkaline phosphatase (AP) and serum OC are associated with bone formation, while urinary hydroxyproline (HP)is associated with bone resorption. These are useful in measuring bone turnover in OP(Frolik, Bryant, Black, Magee, & Chandrasekhar, 1996). The purpose of this study was to examine the effects of T2D on biochemical markers and histopathological changes of bone and to compare these effects with those occurred in ovariectomized rats which were used as a model for estrogen deficiency-induced OP in humans.

Materials and methods:-

Animals' preparations and experimental protocol:-

24 adult femalealbino rats, weighting 200–250 g, were purchased from the animal house of Zagazig University andwere housed at 20-22°C on a 12-h light-dark cycle. They were separated into three groups: the 1st group was the control(sham operated) group (n=8), the 2nd group wasthe ovariectomized (OVX) group (n=8) and the 3rd group was the type 2 diabetic (T2D) group (n=8). The rats in the T2D group initially fed with a high-fat diet for 8 weeks for induction of animal obesity with varying degrees of insulin resistance and beta cell failure and then, given intraperitoneal injection of streptozotocin (STZ, at a dose of 30 mg/kg, Sigma-Aldrich, USA, dissolvedin 0.1 M sodium citrate buffer, pH 4.5) (J. Liu, Liu, Chen, Wang, & Li, 2013)to be modeled to T2D.Three days after STZ injection, fasting blood glucose was measured (H. W. Zhang, Jiang, & Xu, 2013) after 12 h fasting by one-touch glucose auto analyzer (ACCU-CHEK Advantage II Test Strips; Roche Diagnostics, Mannheim, Germany) between 8:30 am and 9:30 am from the tail vein. The second drop of blood was used for testing after cleaning the tail vein by ethyl alcohol cotton swab and removing the first drop of blood (Manaer, Yu, Zhang, Xiao, & Nabi, 2015). The rats with fasting blood glucose levels above 200 mg/dl were considered T2D.In the control (sham operated) group, rats were exposedto the same procedure of ovariectomy without removal of the ovaries, then fed for 8 weeks with a regular chow and then, were given intraperitoneal injection ofcitrate buffer in a dose volume of 1ml/kg.The rats of the secondgroup were ovariectomized (were modeled to the OP by OVX). Eight weeks after the operation, the rats in the secondgroup were given also an intraperitoneal injection of citrate buffer in a dose volume of 1 ml/kg. Five weeks after the injections, rats were sacrificed after 12h overnight fasting. Blood samples were collected in dry clean test tubes for separation of serum. Serum was separated by centrifugation at 3000 rpm for 15 min and was stored at -20^{o}C for measurement of the following fasting blood glucose level, fasting serum insulin that was assessed by Enzyme-linked immunosorbent assay (ELISA), Calculation of HOMA-IR (homeostasis model assessment insulin resistance) as an index of insulin resistance from the product of fasting plasma glucose and insulin (Ascaso et al., 2001), serum bone specific alkaline phosphatase as a marker for bone formation was determined using a rat bone alkaline phosphataseELISA kit (CUSABIO BIOTECH CO., Ltd), and serum osteocalcin (OC) concentration was determined using a rat OC ELISA kit (San Clemente, CA, USA). HOMA-IR was calculated as follows: HOMA-IR = [fasting glucose (mg/dl) × fasting insulin (μIU/ml)] × $(405)^{-1}$(Nayak, Hillemane, Daroji, Jayashree, & Unnikrishnan, 2014).There is a direct relation between the value of HOMA–IR and insulin resistance(Bonora et al., 2000).

Ovariectomy (OVX):-

In the OVX group, the rats were fasted overnight prior to surgery. All animal dissections were conducted by surgical procedures with aseptic technique. Rats were anesthetized by ether and were injected intramuscularly with antibiotics (penicillin G procaine 40,000 U/kg). After anesthesia, the ventral part of the abdominal region was shaved and then cleaned with ethanol. One small incision (1 cm) was made through the skin and the muscle wall on the center of peritoneal area. The ovaries were then located, and removed. The wound was closed in two layers, i.e. muscle and skin using sterile sutures (Behr, Schnorr, & Moreira, 2012). After surgery, rats were housed individually for some hours to allow recovery and then re-grouped in their home cages.In the control group (sham operated), rats were exposed to the same procedure of ovariectomy without removal of the ovaries.

Collection of 24h urine:-

Twenty-four hours before scarification each rat was kept in a special metallic cage with a perforated plate form to calculate urine output starting from 8 a.m. to 8 a.m. next day. A glass funnel was fixed under each cage fitting to the area of its plate form. From this funnel the urine passed to a collecting bottle. Urine samples were collected in dry test tubes and kept at -20^{o}C until being analyzed. Urine samples were collected from all animals to measure urinary hydroxyproline level as a marker for bone resorption (Gallo et al., 2005) using Hydroxyproline Assay Kits (Sigma-Aldrich Co. LLC).

Femur histopathology:-

After the rats were sacrificed, all femurs were immediately sampled. The femur, including the femoral head, was cut and fixed in 4% paraformaldehyde solution for 72 h, and then decalcified with a formalin-nitric acid solution for 3 days. The samples embedded in paraffin were sliced into 6 μm sections, stained with Hematoxylin-Eosin, and then photographed using a PM-10AD optical microscope (Olympus, Japan)(Yange et al., 2015).

Statistical analysis:-

Results were expressed as mean ± standard error (SEM). Statistical differences among groups were evaluated by one way of analysis of variance (ANOVA) using SPSS V23 for windows. Differences betweengroups were estimated using Tukey HSD multiple comparison post hoc test when necessary. P value of less than 0.05 indicated a significant difference.

Results:-

Table (1) and figure (1) showed body weight changes among different groups. There was a significant increase in body weight in OVX final (267±5.6), T2D initial (376.5±7.6) and T2D final (325±10.1) groups in comparison to the control initial group (234.8±6.1) but, there was an insignificant change in body weight in control final (258.3±5.1) and OVX initial (230.5±6.4) groups in comparison to the control initial group (234.8±6.1). Also, there was a significant increase in body weight in the OVX final group (267±5.6) in comparison to the OVX initial group (230.5±6.4). On the other hand, there was a significant decrease in body weight in T2D final group (325±10.1) in comparison to the T2D initial group (376.5±7.6). Table (2) and figure (2) showed changes in insulin resistance parameters in all studied groups. There was a significant increase in blood glucose level in OVX group (123.88±2.14) and T2D group (290.88±8.99) in comparison to the control group (104.13±2.29). Also, there was a significant increase in serum insulin level in OVX group (2.78±0.16) and T2D group (5.2±0.23) in comparison to the control group (2.15±0.08). Moreover, there was a significant increase in HOMA-IR in T2D group (3.75±0.25)but, an insignificant increase inHOMA-IR in OVX group (0.85±0.05) in comparison to the control group (0.55±0.02).Table (3) and figure (3) showed changes in bone markers in all studied groups. There was a significant increase in serum APin OVX group (35.74±0.44) and T2D group (30.92±1.1) in comparison to the control group (17.4±0.38). On the other hand, there was a significant decrease in serum AP in T2D group (30.92±1.1) in comparison to that in the OVX group (35.74±0.44).Also, there was a significant increase in urinary HP in OVX group (28.98±0.33) and T2D group (24.12±0.36) in comparison to that in the control group (13.54±0.28). On the other hand, there was a significant decrease in urinary HP in T2D group (24.12±0.36) in comparison to that in the OVX group (28.98±0.33).Moreover, there was a significant increase in serum OCin OVX group (12.55±0.11) in comparison to that in the control group (9.34±0.07). On the other hand, there was a significant decrease in serum OC in T2D group (7.51±0.18) in comparison to that inthe control group (9.34±0.07). Also, there was a significant decrease in serum OCin T2D group (7.51±0.18) in comparison to that in the OVX group (12.55±0.11). Figure (4) showed Photomicrographs of paraffin-embedded H&E–stained rats' femur sections. In figure (4A), rat femur section (H&E, 630X) from the control group showed normal bone with normal distribution of bone forming cells osteoblast and osteocytes with normal deposition of bone matrix and the periosteum appeared normal. In figure (4B), rat femur section (H&E, 630X) from the OVXgroup showed osteoclasts in Howships lacunae were started to appear with thinned, hypocellular bone with irregular disturbed trabeculae and some include bone marrow spaces. The bone forming cells were decreased in number. In figure (4C), rat femur section (H&E, 630X) from the T2D group showed that the bone forming cells osteoblasts and osteocytes were few in number with decreased bone matrix which appeared hypodense. The bone trabeculae were disturbed with decreased number of Volkmans canals and the Haversian systems and multicavities started to appear.

Table 1: Body weight changes among different groups (in gm).

Group		**Mean±SEM**
Control	**Initial weight**	**234.8±6.1**
	Final weight	**258.3±5.1**[a]
OVX	**Initial weight**	**230.5±6.4**[a& d]
	Final weight	**267±5.6**[b, d& f]
T2D	**Initial weight**	**376.5±7.6**[c, e, g& h]
	Final weight	**325±10.1**[c, e, g, h &i]

Data was expressed as Mean ± SEM. [a] P>0.05, [b] P<0.05 while [c] P<0.001 in comparison to the control initial group. [d] P>0.05 while [e] P<0.001 in comparison to the control final group. [d] P>0.05 while [e] P<0.001 in comparison to the control final group. [f] P<0.01 while [g] P<0.001 in comparison to the OVX initial group. [h] P<0.001 in comparison to the OVX final group.[i] P<0.001 in comparison to the T2D initial group.Control was the sham operated group.OVX was the ovariectomized group. T2D was the diabetic group.

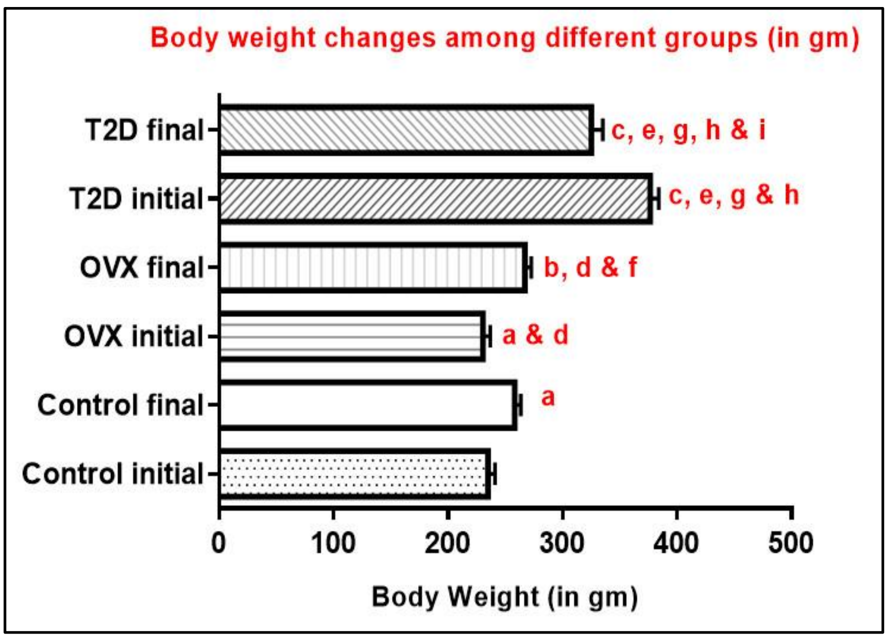

Figure-1: Body weight changes among different groups.

Data was expressed as Mean ± SEM.[a] P>0.05, [b] P<0.05 while [c] P<0.001 in comparison to the control initial group. [d] P>0.05 while [e] P<0.001 in comparison to the control final group. [d] P>0.05 while [e] P<0.001 in comparison to the control final group. [f] P<0.01 while [g] P<0.001 in comparison to the OVX initial group. [h] P<0.001 in comparison to the OVX final group. [i] P<0.001 in comparison to the T2D initial group. Control was the sham operated group. OVX was the ovariectomized group. T2D was the diabetic group

Table 2: Insulin resistance parameters in all studied groups.

	Control	OVX	T2D
Glucose (mg/dL)	**104.13±2.29**	**123.88±2.14[a]**	**290.88±8.99[b&c]**
Insulin (µIU/mL)	**2.15±0.08**	**2.78±0.16[a]**	**5.2±0.23[b&c]**
HOMA-IR	**0.55±0.02**	**0.85±0.05[d]**	**3.75±0.25[b&c]**

Data was expressed as Mean±SEM. [a] $P<0.05$ while [b] $P<0.001$ in comparison to the control group. [c] $P<0.001$ in comparison to OVX group.[d]$P>0.05$ in comparison to the control group. Control was the sham operated group.OVX was the ovariectomized group while, T2D was the diabetic group.

Table-3: Bone markers in all studied groups.

	Control	OVX	T2D
Serum bone specific alkaline phosphatase (AP) in U/L	**17.4±0.38**	**35.74±0.44[a]**	**30.92±1.1[a& b]**
Urinary hydroxyproline (HP) in mg/dl	**13.54±0.28**	**28.98±0.33[a]**	**24.12±0.36[a&b]**
Serum osteocalcin (OC) concentration innmol/L	**9.34±0.07**	**12.55±0.11[a]**	**7.51±0.18[a&b]**

Data was expressed as Mean±SEM. [a] $P<0.001$ in comparison to the control group. [b] $P<0.001$ in comparison to OVX group.Control was the sham operated group. OVX was the ovariectomized group while, T2D was the diabetic group.

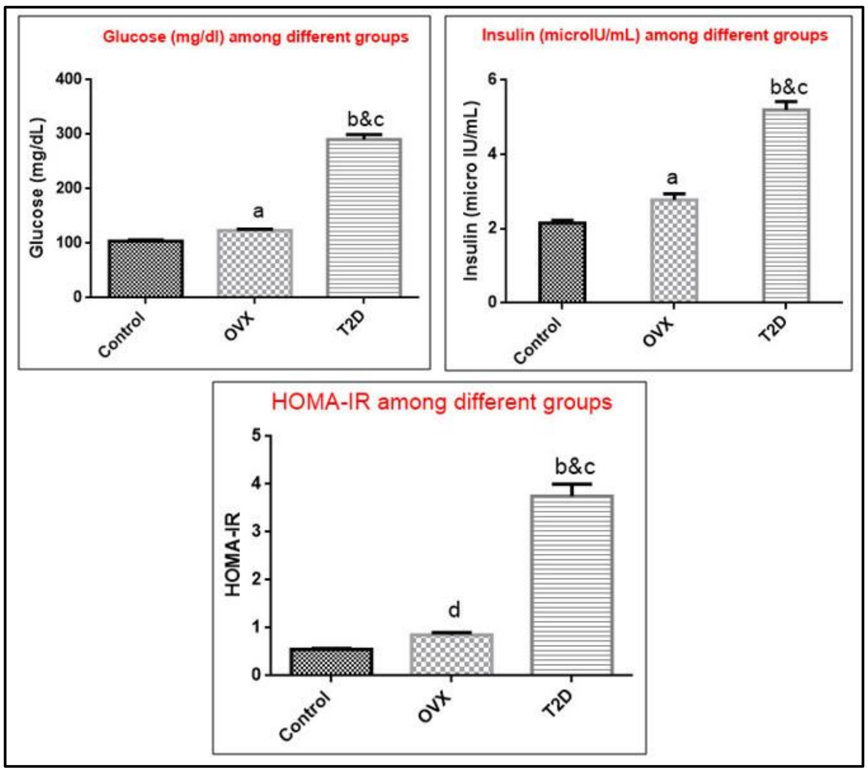

Figure-2: Glucose (mg/dl), Insulin (µIU/mL) and HOMA-IR among different groups.

Data was expressed as Mean±SEM. [a] $P<0.05$ while [b] $P<0.001$ in comparison to the control group. [c] $P<0.001$ in comparison to OVX group. [d] $P>0.05$ in comparison to the control group. Control was the sham operated group.OVX was the ovariectomized group. T2D was the diabetic group.

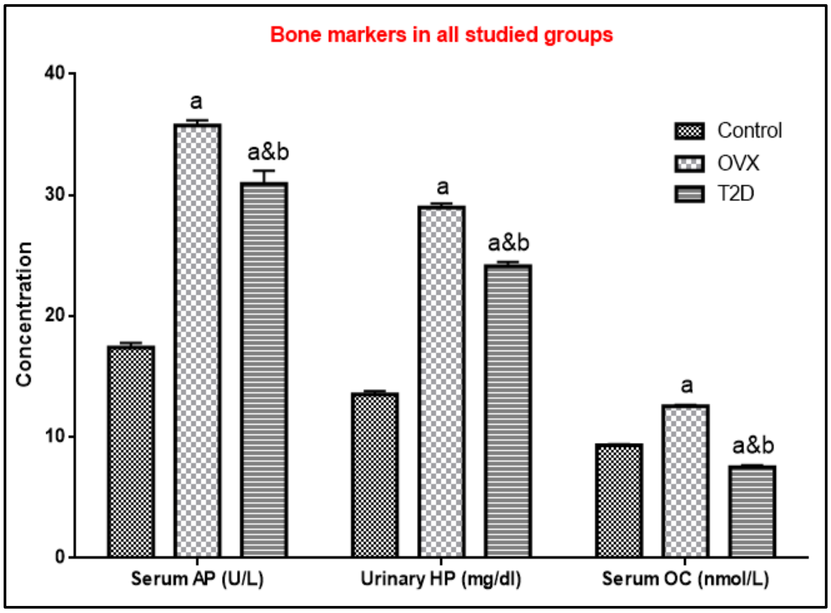

Figure 3:- Bone markers in all studied groups.
Data was expressed as Mean±SEM. [a]P<0.001 in comparison to the control group.[b]P<0.001 in comparison to OVX group.Control was the sham operated group,OVX was the ovariectomized group while, T2D was the diabetic group.

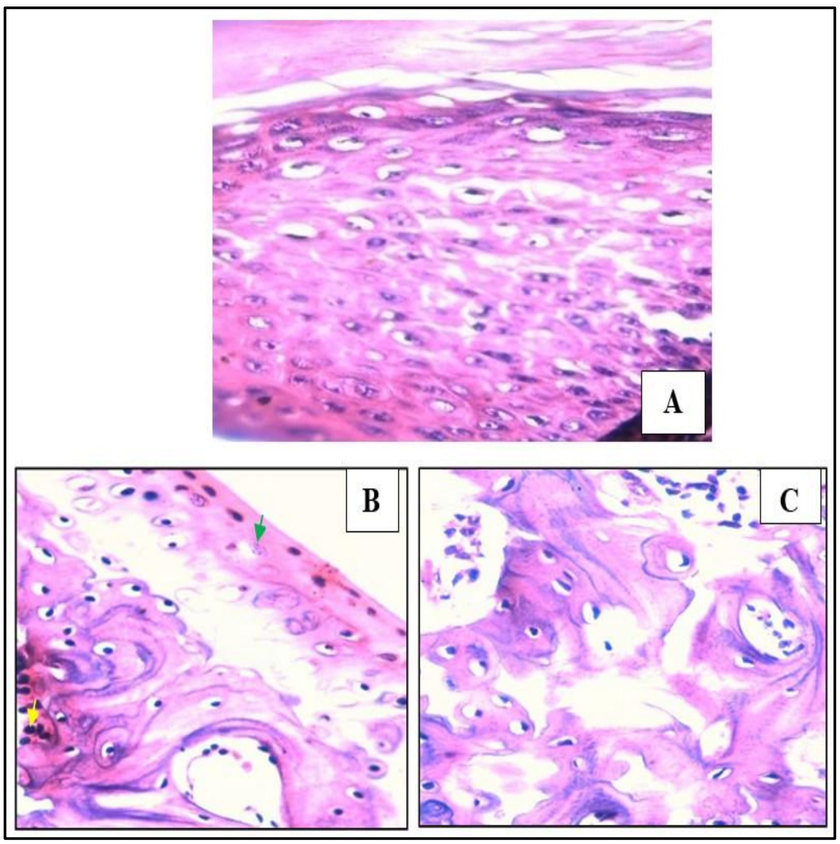

Figure 4: Photomicrographs of paraffin-embedded H&E–stained rats' femur sections.
(A) Rat femur section (H&E, 630X) from the control (sham operated) group showingnormal bone with normal distribution of bone forming cells osteoblast and osteocytes with normal deposition of bone matrix and the periosteum appeared normal. **(B)** Rat femur section (H&E, 630X) from the ovariectomized (OVX) group showingosteoclasts in Howships lacunae are started to appear (green arrow head) with thinned, Hypocellular bone with irregular disturbed trabeculae and some include bone marrow spaces (yellow arrow head). The bone forming

cells are decreased in number. **(C)** Rat femur section (H&E, 630X) from the diabetic (T2D) group showing that the bone forming cells osteoblasts and osteocytes are few in number with decreased bone matrix which appeared hypodense. The bone trabeculae are disturbed with decreased number of Volkmans canals and the Haversian systems and multicavities started to appear.

Discussion:-

OP is a metabolic skeletal disease, characterized by reduction in bone mass and microarchitectural deterioration of bone tissue which causes increased bone fragility and susceptibility to fractures (Y. Zhang et al., 2016). In this study, ovariectomy was used as a rat model of OP to which changes in T2D rats were compared.In this study, there was a significant increase in body weight in the OVX final group in comparison to the OVX initial group. Many studies supported this result (Devlin & Ferguson, 1989; El Habachi, Maklad, Sharara, Allam, & Fawzy, 2014; Manolagas, O'Brien, & Almeida, 2013; McElroy & Wade, 1987; Shuid, Ping, Muhammad, Mohamed, & Soelaiman, 2011) as they explained the increased body weight in ovariectomy by fat deposition caused by estrogen deficiency. On the other hand, the results of this study showed a significant decrease in body weight in T2D final group in comparison to the T2D initial group. This was supported by Alselami, Noureldeen, Al-Ghamdi, Khan, and Moselhy (2015), Drincic, Armas, Van Diest, and Heaney (2012) and (Musumeci, Loreto, Clementi, Fiore, & Martinez, 2011).Alselami et al. (2015) found an inverse association between vitamin D level and anthropometric measures of body size in T2D.The decreased body weight may be one of the causative factors for osteoporosis in T2D which was supported by some studies whichfound increased bone formationwith increased body weight and stated that this protected against osteopenia as it was accompanied by high estradiol levels (Kalu, 1991; Notomi, Okimoto, Okazaki, Nakamura, & Suzuki, 2003; Ziegler, 1992).This study declared that there was a significant increase in blood glucose and serum insulin levels in OVX group in comparison to those in the control group. On the other hand, there was an insignificant increase in HOMA-IR in OVX group in comparison to the control group. Theseresults indicated a decrease in insulin sensitivity, which is in agreement with some other studies (El Habachi et al., 2014; M.-L. Liu, Xu, Rang, Li, & Song, 2004; Saengsirisuwan, Pongseeda, Prasannarong, Vichaiwong, & Toskulkao, 2009).The results of this study also, showed that there was a significant increase in blood glucose, serum insulin and HOMA-IR in T2D group in comparison to that in the control group. These results confirmed the incidence of insulin resistance among T2D rats.These results were supported by Jouad, Haloui, Rhiouani, El Hilaly, and Eddouks (2001). The hyperglycemia that was present in T2D was found to be related a decrease in density of bone as confirmed by Sharifi, Ahmadimoghadam, and Mousavinasab (2006). In addition, Bauer et al. (1993) stated that hyperglycemia increased collagen advanced glycation end-products reducing bone strength. Moreover, Gregorio et al. (1994) declared that glycosuria that occurred with hyperglycemia caused hypercalciuria, leading to a decrease in blood calcium level and poor bone quality, increased bone loss.Furthermore, (Cosentino, Hishikawa, Katusic, & Lüscher, 1997) and (Martín-Gallán, Carrascosa, Gussinyé, & Domínguez, 2003) stated that bone turnover dysregulation in diabetes was caused by hyperglycemia-induced changes on synthesis and activity of nitric oxide. On the other hand, Reid, Evans, Cooper, Ames, and Stapleton (1993) declared that bone changes occurred in T2D may be due to insulin resistance as normally, insulin increases bone formation.On studying changes in bone markers, the results of this study showed a significant increase in serum AP, urinary HP and serum OCin OVX group in comparison to the control group. These results indicated an increase in bone turnover; both resorption (urinary HP) and formation (serum AP and OC). This wassupportedbyISMAIL, EPSTEIN, FALLON, THOMAS, and REINHARDT (1988), Williams, Paul, and Black (1991), Mitra et al. (2001), Mukherjee et al. (2006), Puel et al. (2006), Zhao et al. (2011) and Y. Zhang et al. (2016). Also, it was in agreement with Vural, Akgul, and Canbaz (2006) who stated that estrogen deficiency as in OVX rats up-regulatedseveral cytokines production and action such as tumor necrosis factor alpha (TNFα)which increasedresorption of bone. Moreover, the resultsof this study were supported by Löfman, Magnusson, Toss, and Larsson (2005) who explained the increased risk of osteoporosis in OVX rats on the fact that the increased formation of bone, to fill in the higher number of resorption cavities, was lower than the quantity of bone resorbed causing a net bone loss.In addition, in T2D group, there was a significant increase in serum AP and urinary HPbuta significant decrease in serum OCin comparison to that in the control group. These results were supported by Musumeci et al. (2011)who stated that osteopenic changes with diabetes were due to oxidative stress, hyperglycemia, and body weight loss. OC deficiency in T2D may be explained by the deficiency of estrogen which was found by Cushman, Kim, Hoyt, and Traish (2009) to be decreased in diabetes. In addition, Lee et al. (2007) stated that OC is secreted by osteoblasts and enhanced insulin sensitivity. This could explain the relation between T2D and OP as T2D decreased insulin sensitivity and OC levelwhich was confirmed by (Shu, Pei, Chen, & Lu, 2016), (Tan et al., 2011), (Kanazawa et al., 2009), (M. Zhou et al., 2009) and (Kindblom et al., 2009). On the contrary, Aoki et al. (2011) found that serum OC concentration is increased in early-stage T2D subjects. The discrepancy between the results of this study and our results may be due to the duration of T2D and the time of estimation of OC.The photomicrographs of paraffin-embedded H&E–stained rats' femur sections in OVX showed osteoclasts in Howships lacunae started to appear with thinned, hypocellular bone with irregular disturbed trabeculae and some include bone marrow spaces. The bone forming cells decreased in number. These results were supported byChen et al. (2016),Y. Zhang et al. (2016), Fang, Yang, Zhang, Zhu, and Wang (2015), Ferreri, Talish, Trandafir, and Qin (2011) and Mosekilde et al. (2000) who found that OVX was associated with alterations in bone microarchitecture. In addition, these results were in agreement with Al-Maatouq et al. (2004) who stated that the frequency of OP in diabetic postmenopausal women was higher than normal group.On the other hand, rat femur section from the T2D group showed that the bone forming cells osteoblasts and osteocytes were few in number with decreased bone matrix, which appeared hypodense. The bone trabeculae were disturbed with decreased number of Volkmans canals and the Haversian systems and multicavities started to appear. These results were supported by D'Amelio et al. (2008) and Ginaldi, Di Benedetto, and De Martinis (2005)who explained the osteoporotic changes seen in T2D to be due to the deficiency of estrogen. Also, these results were supported byMusumeci et al. (2011),Hamada et al. (2007), Genant et al. (2007)andHeinemann (2000) who stated that OP is accompanied by deterioration of the micro-architecture of bone tissue, with increased bone frailty and fractures.Moreover, the results of this study were supported by some other studieswhich reported a decrease in BMD and an increase in the risk for fracture with T2D(Adami, 2009; Ahren, 1998; Choi et al., 2016; Dytfeld & Michalak, 2016; Gregorio et al., 1994; G. Isaia et al., 1987; Ishida et al., 1985; Levin et al., 1976; New, 1999; Notarnicola et al., 2016).The osteoporotic changes in T2D may be explained by the decrease in serum estradiol and the increase in parathyroid hormone and cortisol levels(Sieradzki, Trznadel-Morawska, & Olszanecki, 1998). In addition, Roy (2013) and Roglic et al. (2005)stated that T2D affected bone cells, the osteoblast and osteoclast.On the contrary, some studies have reported an elevated BMD and a decrease in the fracture ratein T2D (Barrett-Connor & Holbrook, 1992; Bauer et al., 1993; De Leeuw & Abs, 1977; G. C. Isaia et al., 1999; Johnston et al., 1985; Leidig-Bruckner & Ziegler, 2001; Piepkorn et al., 1997; Sahin et al., 2001; Ziegler, 1992).On the other hand, some more studies have reported unaltered BMD and the fracture rateinT2D(Giacca et al., 1988; Majima et al., 2005; Wakasugi et al., 1993; Weinstock et al., 1989). The cause of the discrepancy between the results of our study and these results was not clear but may be due to species differences or the duration of T2D. From the discussed results, it was clear that there were osteoporotic changes as detected in histopathological study of femurs from rats of T2D group. To our knowledge, this study is one of the few studies that confirmed the osteoporotic changes occurring in T2D and explained their possible mechanisms which may be the decreased body weight, insulin sensitivity and OC level in T2D. Further studies should be done to confirm these results.

Conclusion:-

To our knowledge, this study is one of the few studies that confirmed the osteoporotic changes occurring in T2D and explained their possible mechanisms which may be the decreased body weight, insulin sensitivity and OC level in T2D. Further studies should be done to confirm these results and to study the effect of T2D therapy on these osteoporotic changes.

Conflict of Interest:-

The authors declared that there were no conflicts of interest.

Acknowledgement:-

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