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Evaluation the Effect of Topical Application of Strontium Ranelate Gel on Bone Regeneration in The Tibia of Induced Diabetic Rats With Critical Size Bone Defects By Using Silver Stained Nucleolar Organizer Regions (Agnors).

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ABSTRACT: Aim: The aim of this study was to evaluate effect of topical application of Strontium Ranelate gel on bone regeneration in in the tibia of induced diabetic rats with critical size bone defects by quantitative and qualitative analysis of silver stained Nucleolar Organizer Regions (AgNORs). **Material and methods**: Sixty adult male albino rats were used in this study and divided into four groups equally, fifteen rats for each group. These groups were Normal control group (N), Diabetic control group (D), Normal experimental group treated with SrR gel (NSR) and Diabetic experimental group treated with SrR gel (DSR). Five animals from each group were sacrificed at 5, 10 and 30 days alternatively postoperatively. After termination of the experiment, the specimens were prepared and bone formation was evaluated by AgNOR stain as a proliferative marker. **Results:** In experimental groups treated with SrR gel, there was acceleration in the bone regeneration, while in diabetic control group there was obvious retardation in the bone regeneration than all other groups. **Conclusion:** The study showed that the topical application of SrR has positive regenerative effects in case of diabetic rats with critical size bone defects.

Keywords: Strontium ranelate, Bone regeneration, AgNOrs, Diabetes mellitus, Critical size defect.

I. INRTODUCTION

Bone undergoes different types of diseases and different forms of surgeries lead to problems in the continuity, normal architecture, and function of bone. In some clinical situations involving great bone loss or the presence of wide variety of diseases or tumor resection can resulted in delayed healing or even nonunion [1, 2].

Diabetes mellitus (DM) is a chronic endocrine disease that is characterized by excess levels of blood glucose. Poorly controlled diabetes is associated with adverse systemic sequelae including increased susceptibility to infection, delayed wound healing and micro vascular complications that lead to decreased immune response [3, 4] and increase bone fragility [5].

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DM reduces osteoblastic activity and bone mineralization [6]. In addition, it leads to reduced biomechanical properties of the healing fracture, reduced cellular proliferation in the early callus and reduced collagen synthesis and content [7, 8]. Many experimental and clinical studies have shown that diabetes impairs bone formation, weakens its mechanical strength and accelerates deterioration of bone architecture [9, 10, 11]. Moreover, Hyperglycemia produces deleterious effects on bone matrix and its components, and also affects adherence, growth and accumulation of extracellular matrix [12].

The local application of systemic bone modulating drug that is commonly used to treat bone disease could offer a plausible alternative to growth factors. The advantage of local drug delivery is releasing the drug directly into the site of infection for a sufficient period without systemic exposure preventing both bacterial resistance and drug-related systemic side effects [13]. There are many drugs can be used systemically for the stimulation of bone metabolism. Among these drugs, the Strontium Ranelate (SrR) which has received special attention from researchers in the past few years [14, 15].

Strontium ranelate (SrR), currently used for osteoporosis treatment, appears to be a promising alternative due to its ability to promote bone formation by both stimulating osteoblast function and inhibiting osteoclast activation [16,17]. This dual effect of SrR has been demonstrated in several in vitro studies in which SrR promoted osteoblast proliferation, survival, differentiation and function, while inducing apoptosis and the reduction of osteoclast differentiation and activity [18, 19]. In vivo, the systemic use of SrR has been shown to favors the regeneration of bone defects [20, 21].

Positive effects of SrR on Runx2 expression have been observed especially on mesenchymal stem cells. It was showed that SrR can drive aged osteopenic mesenchymal stem cells to the osteoblastogenesis pathway by upregulating Runx2 expression and matrix mineralization; while antagonizing the adipogenic differentiation by down regulating of peroxisome proliferator-activated receptor gamma 2 (PPAR-γ2) expression in these cells [22].

Nucleolar organizer Regions (NORs) are defined as nucleolar components containing a set of argyrophilic proteins, which are selectively stained by silver methods [23]. NORs are known to contain a number of acidic proteins that have a high affinity for silver (AgNOR proteins) [24]. Additionally, after silver-staining, the NORs can be easily identified as black dots exclusively localized throughout the Nucleolar area, and are called AgNORs [23].

AgNOR proteins accumulate in highly proliferating cells, whereas their expression is very low in non-proliferating cells. Some of these proteins remain associated with the nucleolar organizer regions (NORs) during mitosis. Thus, AgNOR analysis may provide further insights concerning the biological behavior of the cell and is simple and reliable method for evaluating the proliferative activity of the cell [25]. Several investigators study the qualitative and quantitative changes during the cell cycle and showed that the amount of AgNOR protein can be a marker of proliferation because the amount of AgNORs is related to cell cycle phases [26].

Animal models: This study was carried out on sixty adult male albino rats. The average age of these rats was around 2.5 months old, their weight approximately 250 gm. The procedures of management were carried out at animal house of Cairo University and approved by the local ethical committee.

Experimental design: The animals in this study were divided randomly into three main groups as the following:-

(I) Normal control group (N), 15 animals, which was not received SrR gel.
(II) Diabetic control group (D), 15 animals, which was not received SrR gel.
(III) Normal experimental group (NSR), 15 animals which treated with SrR gel.
(IV) Diabetic experimental group (DSR), 15 animals which treated with SrR gel.

Induction of diabetes: For groups (D) and (DSR), Diabetes was induced by a single intraperitoneal injection of 120 mg/kg monohydrated alloxan (Sigma, St. Louis, MO) dissolved in sterile 0.9% saline. Rats were made to fast before alloxan administration. After 12 hours, a 10% glucose solution was offered to the animals to prevent hypoglycemia. After 7 days, blood samples were collected from the caudal vein of the animals for evaluation of

plasma glucose levels. The animals that presented glycemic levels higher than 250 mg/dL were considered diabetic and used in the present study[27].

Preparation of SrR gel: SrR gel was prepared in pharmaceutical and industrial pharmacy department, Faculty of pharmacy, Boys, Cairo, Al-Azhar University as the following: - A 4.0% (w/v) methylcellulose (4,000 cps) gel, (Sigma chemicals Co., St. Louis, MO), which served as the vehicle for SrR was previously prepared, by adding the required amount of polymer to hot distilled water and cooling to gel at room temperature. Then 2.5 mg of SrR, (Sigma chemicals Co., St. Louis, MO), was dissolved in 1 ml of methylcellulose. The SrR gel was loaded into plastic insulin syringe until we can load it into the defects of the experimental groups [28].

Surgical procedures:- The animals were weighed, premedicated by atropine (IM 0.04mg/kg) and anaesthetized intramuscularly with a combination of 2% xylazine in a dose of 5 mg/kg (ADWIA, Egypt) and ketamine in a dose of 50 mg/ kg (ROTEXMEDICA, Germany). The overlying skin of the tibia was shaved and disinfected with iodate alcohol. An incision about 2 cm was then made in the medial aspect of the tibia, full thickness flap, the skin, subcutaneous tissue and muscular layer were reflected exposing the bone of the tibia. A circular 5-mm diameter defect created using carbide rose head surgical bur mounted in a dental hand piece connected to a micro-motor with 2000 rpm speed, a metal template with a round cavity 5 mm in diameter had been used to standardize the defect site and size. The process of defect creation was done under copious irrigation with saline to avoid bone burning and to maintain the vitality of bone cells around the defect. In group (N) and group (D) the defect was not received SrR, while in groups (NSR) and (DSR) SrR gel was placed in the defect, then the flap was repositioned, the muscular layer was sutured with resorbable (#4.0) catgut and the skin was sutured with interrupted (#4.0) silk sutures.

Postoperative care: Postoperative medications and periodical examination was carried out for the presence of signs of inflammation. Periodical monitoring of blood glucose level was carried out for exclusion of any rat with a blood glucose level less than 250 mg/dl. Moreover, any excluded rat was replaced by another one to maintain the total number of the diabetic rats until the day of scarification which determined for each rat.

SACRIFICATION OF ANIMALS: Five animals from each group were sacrificed after 5, 10, and 30 days alternatively post operatively. The specimens were prepared for examination in order to study the bone regeneration in each group by AgNOR stain as a proliferative marker.

Counting of AgNORs:- NORs appear as brown black dots inside the nucleus using silver stain. Counting of AgNORs were done according to the recommendations standard protocol using the morphometric method.

Statistical analysis:- Numerical data were entered to the statistical package for social science (SPSS) version 23 software for windows. The quantitative and qualitative data were presented as means (M) and standard deviations (SD). Analysis Of Variance (ANOVA) was used to compare between several means. Statistical analysis was performed by using **One Way ANOVA**. In addition, **Tukey's post hoc test** was performed when **ANOVA test** reveals significant difference. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as the following:-

P-value > 0.05: Non-significant.
P-value < 0.05: Significant.

II. RESULTS:

AgNORs statistical analysis (Quantitative & Qualitative)

(I) Quantitative statistical analysis for means of number of AgNOR dots per unit area.
(II) Qualitative statistical analysis for intensity of AgNOR dots.

1- Five days interval

(I) Quantitative statistical analysis for means of number of AgNOR dots per unit area:-

As shown in table (1) and figure (1), within 5 days NSR & DSR groups (120.51±3.03; 95.88±1.16 respectively) showed significant increase compared to that in N Group (89.59±1.26) (p=<0.001, <0.001 respectively) while D group (68.45±.96) showed significant decrease compared to that in N Group (89.59±1.26) (p=<0.001). D & DSR groups (68.45±.96; 95.88±1.16 respectively) showed significant decrease compared to that in NSR Group (120.51±3.03) (p=<0.001, <0.001 respectively). DSR groups (95.88±1.16) showed significant increase compared to that in D Group (68.45±.96) (p=<0.001).

	N Group	NSR Group	D Group	DSR Group	P value
5 days	89.59±1.26	120.51±3.03	68.45±.96	95.88±1.16	<0.001*
Post-hoc		P1=<0.001*	P1=<0.001* P2=<0.001*	P1=<0.001* P2=<0.001* P3=<0.001*	

Table (1): Comparison of means of number of AgNOR dots per unit area between N, NSR, D and DRS groups within 5 days interval.

Data expressed as mean±SD **SD: standard deviation**
P: Probability ***: significance <0.05**
Test used: One way ANOVA followed by post-hoc tukey's test
P1: significance vs N Group
P2: significance vs NSR Group
P3: significance vs D Group

(II) Qualitative statistical analysis for intensity of AgNOR dots:-

As shown in table (2) and figure (2), within 5 days NSR & DSR groups (161.15±2.12; 130.33±1.77 respectively) showed significant increase compared to that in N Group (113.17±2.37) (p=<0.001, <0.001 respectively) while D group (91.22±.99) showed significant decrease compared to that in N Group (113.17±2.37) (p=<0.001). D & DSR groups (91.22±.99; 130.33±1.77 respectively) showed significant decrease compared to that in NSR Group (161.15±2.12) (p=<0.001, <0.001 respectively). DSR groups (130.33±1.77) showed significant increase compared to that in D Group (91.22±.99) (p=<0.001).

	N Group	NSR Group	D Group	DSR Group	P value
5 days	113.17±2.37	161.15±2.12	91.22±.99	130.33±1.77	<0.001*
Post-hoc		P1=<0.001*	P1=<0.001* P2=<0.001*	P1=<0.001* P2=<0.001* P3=<0.001*	

Table (2): Comparison of means of intensity of AgNOR dots between N, NSR, D and DRS groups within 5 days interval.

Data expressed as mean±SD **SD: standard deviation**
P: Probability ***: significance <0.05**
Test used: One way ANOVA followed by post-hoc tukey's test
P1: significance vs 5 days
P2: significance vs 10 days **P3: significance vs 30 days**

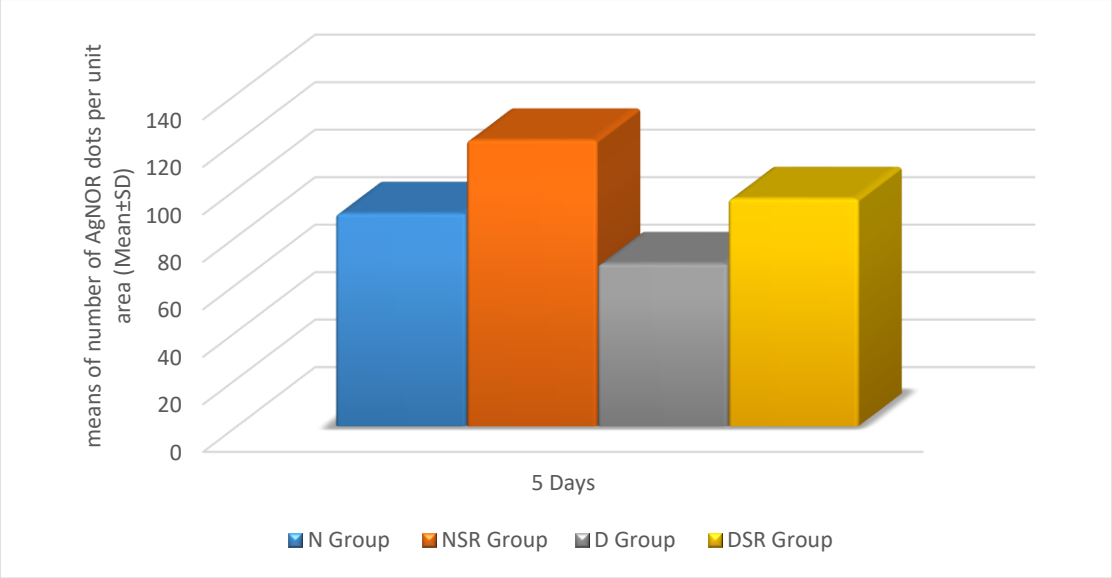


Fig. (1): Mean±SD of means of number of AgNOR dots per unit area between N, NSR, D and DRS groups within 5 days interval.

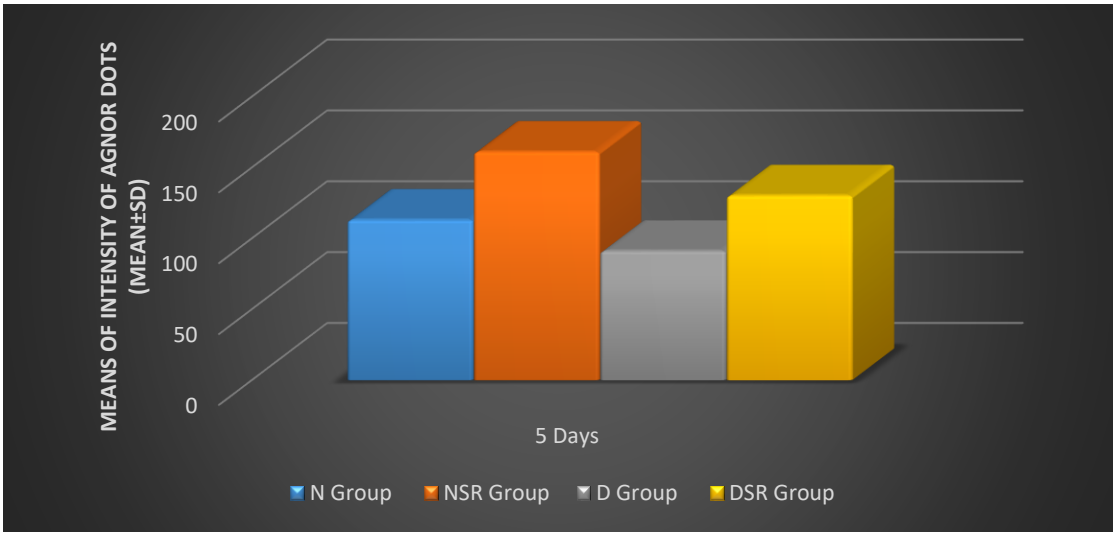


Fig. (2): Mean±SD of means of intensity of AgNOR dots between N, NSR, D and DRS groups within 5 days interval.

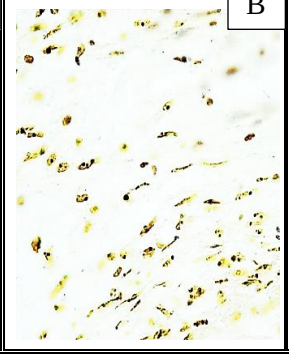
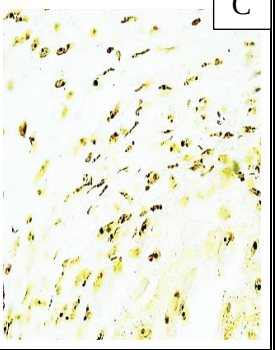
Photomicrograph of N5 group	Photomicrograph of D5 group	Photomicrograph of NSR5 group	Photomicrograph of DSR5 group
A	B	C	D
			

Table (3):- Comparison between groups at 5 days interval **(AgNOR stain X1000)**

2- Ten days interval

(I) Quantitative statistical analysis for means of number of AgNOR dots per unit area:-

As shown in table (4) and figure (3), within 10 days NSR & DSR groups (132.30±2.23; 106.37±1.88 respectively) showed significant increase compared to that in N Group (99.24±1.09) (p=<0.001, <0.001 respectively) while D group (71.77±1.01) showed significant decrease compared to that in N Group (99.24±1.09) (p=<0.001). D & DSR groups (71.77±1.01; 106.37±1.88 respectively) showed significant decrease compared to that in NSR Group (132.30±2.23) (p=<0.001, <0.001 respectively). DSR groups (106.37±1.88) showed significant increase compared to that in D Group (71.77±1.01) (p=<0.001).

	N Group	NSR Group	D Group	DSR Group	P value
10 days	**99.24±1.09**	**132.30±2.23**	**71.77±1.01**	**106.37±1.88**	**<0.001***
Post-hoc		**P1=<0.001***	**P1=<0.001*** **P2=<0.001***	**P1=<0.001*** **P2=<0.001*** **P3=<0.001***	

Table (4): Comparison of means of number of AgNOR dots per unit area between N, NSR, D and DRS groups within 10 days interval.

(II) Qualitative statistical analysis for intensity of AgNOR dots:-

As shown in table (5) and figure (4), within 10 days**:** NSR & DSR groups (176.93±3.42; 137.77±1.82 respectively) showed significant increase compared to that in N Group (128.08±1.75) (p=<0.001, <0.001 respectively) while D group (97.56±.91) showed significant decrease compared to that in N Group (128.08±1.75) (p=<0.001). D & DSR groups (97.56±.91; 137.77±1.82 respectively) showed significant decrease compared to that in NSR Group (176.93±3.42) (p=<0.001, <0.001 respectively). DSR groups 137.77±1.82) showed significant increase compared to that in D Group (97.56±.91) (p=<0.001).

	N Group	NSR Group	D Group	DSR Group	P value
10 days	128.08±1.75	176.93±3.42	97.56±.91	137.77±1.82	<0.001*
Post-hoc		P1=<0.001*	P1=<0.001* P2=<0.001*	P1=<0.001* P2=<0.001* P3=<0.001*	

Table (5): Comparison of means of intensity of AgNOR dots between N, NSR, D and DRS groups within 10 days interval.

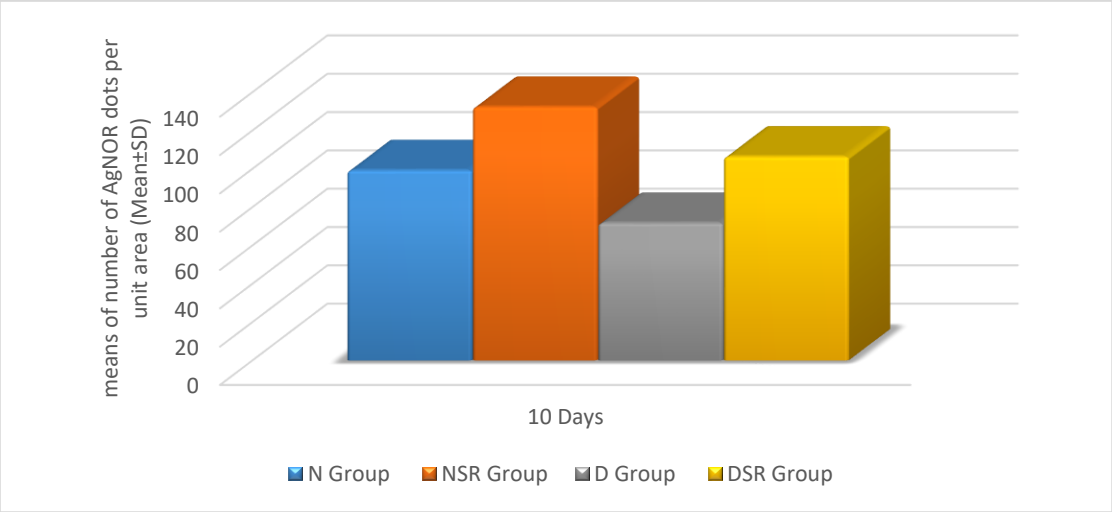


Fig. (3): Mean±SD of means of number of AgNOR dots per unit area between N, NSR, D and DRS groups within 10 days interval.

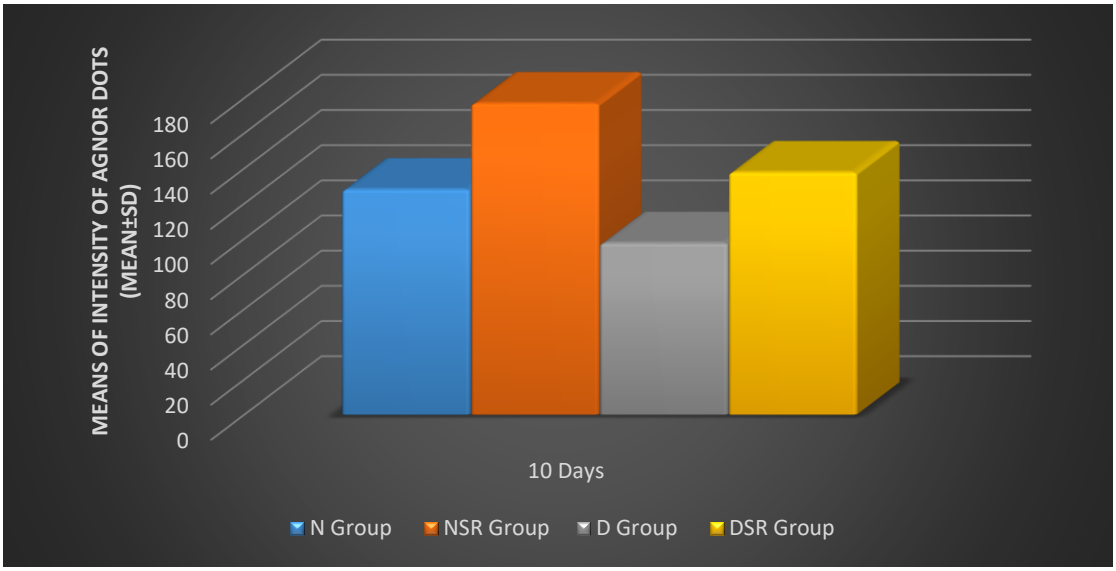


Fig. (4): Mean±SD of means of intensity of AgNOR dots between N, NSR, D and DRS groups within 10 days interval.

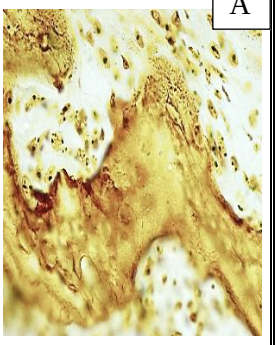
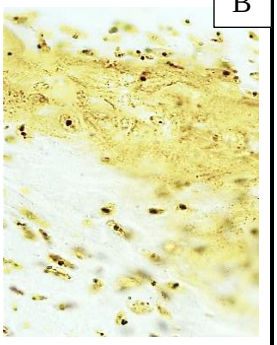
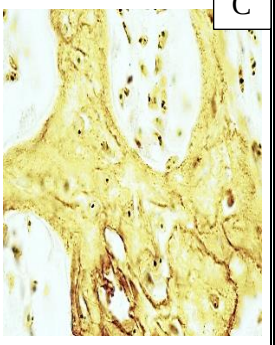
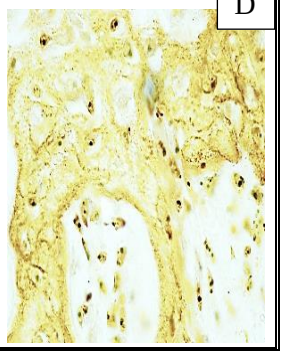
Photomicrograph of N10 group	Photomicrograph of D10 group	Photomicrograph of NSR10 group	Photomicrograph of DSR10 group
A 	B 	C 	D 

Table (6):- Comparison between groups at 10 days interval **(AgNOR stain X1000)**

3- Thirty days interval

(I) Quantitative statistical analysis for means of number of AgNOR dots per unit area:-

As shown in table (7) and figure (5), within 30 days NSR group (152.10±1.35) showed significant increase compared to that in N Group (115.17±1.27) (p=<0.001) while D & DSR groups (80.06±.60, 110.48±1.41 respectively) showed significant decrease compared to that in N Group (115.17±1.27) (p=<0.001, <0.001 respectively). D & DSR groups (80.06±.60, 110.48±1.41 respectively) showed significant decrease compared to that in NSR Group (152.10±1.35) (p=<0.001, <0.001 respectively). DSR groups (110.48±1.41) showed significant increase compared to that in D Group (80.06±.60) (p=<0.001).

N Group	NSR Group	D Group	DSR Group	P value
30 days	**115.17±1.27**	**152.10±1.35**	**80.06±.60**	**110.48±1.41**	**<0.001***
Post-hoc		**P1=<0.001***	**P1=<0.001*** **P2=<0.001***	**P1=<0.001*** **P2=<0.001*** **P3=<0.001***	

Table (7): Comparison of means of number of AgNOR dots per unit area between N, NSR, D and DRS groups within 30 days interval.

(II) Qualitative statistical analysis for intensity of AgNORs dots:-

As shown in table (8) and figure (6), within 30 days: NSR group (196.68±2.45) showed significant increase compared to that in N Group (157.71±1.97) (p=<0.001) while D & DSR groups (101.18±1.26, 150.26±2.61 respectively) showed significant decrease compared to that in N Group (157.71±1.97) (p=<0.001, <0.001 respectively). D & DSR groups (101.18±1.26, 150.26±2.61 respectively) showed significant decrease compared to that in NSR Group (196.68±2.45) (p=<0.001, <0.001 respectively). DSR groups (150.26±2.61) showed significant increase compared to that in D Group (101.18±1.26) (p=<0.001).

	N Group	NSR Group	D Group	DSR Group	P value
30 days	157.71±1.97	196.68±2.45	101.18±1.26	150.26±2.61	<0.001*
Post-hoc		P1=<0.001*	P1=<0.001* P2=<0.001*	P1=<0.001* P2=<0.001* P3=<0.001*	

Table (8): Comparison of means of intensity of AgNOR dots between N, NSR, D and DRS groups within 30 days interval.

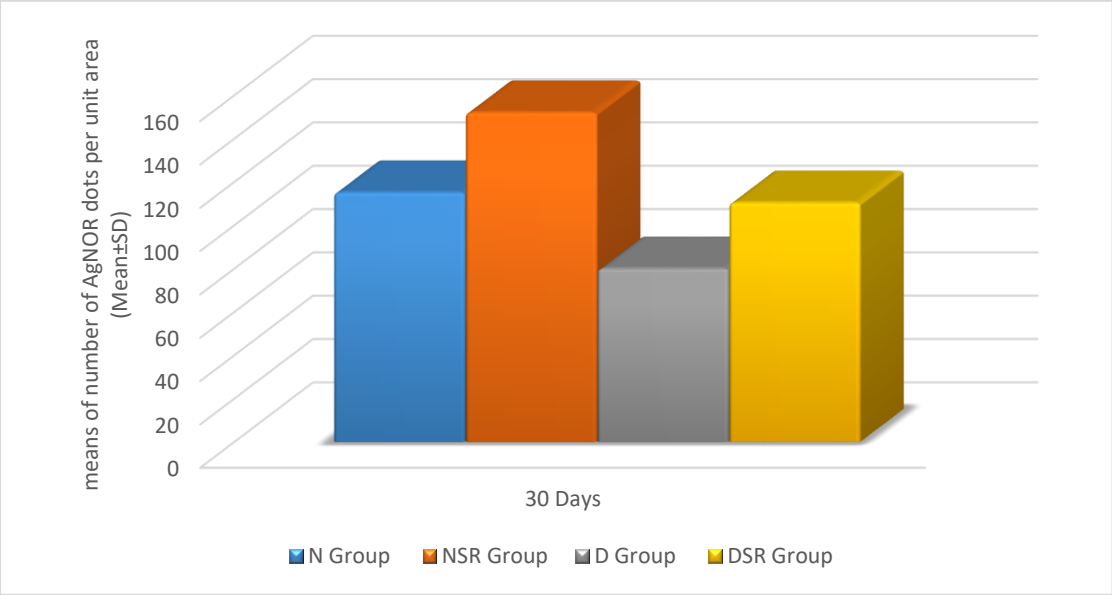


Fig. (5): Mean±SD of means of number of AgNOR dots per unit area between N, NSR, D and DRS groups within 30 days interval.

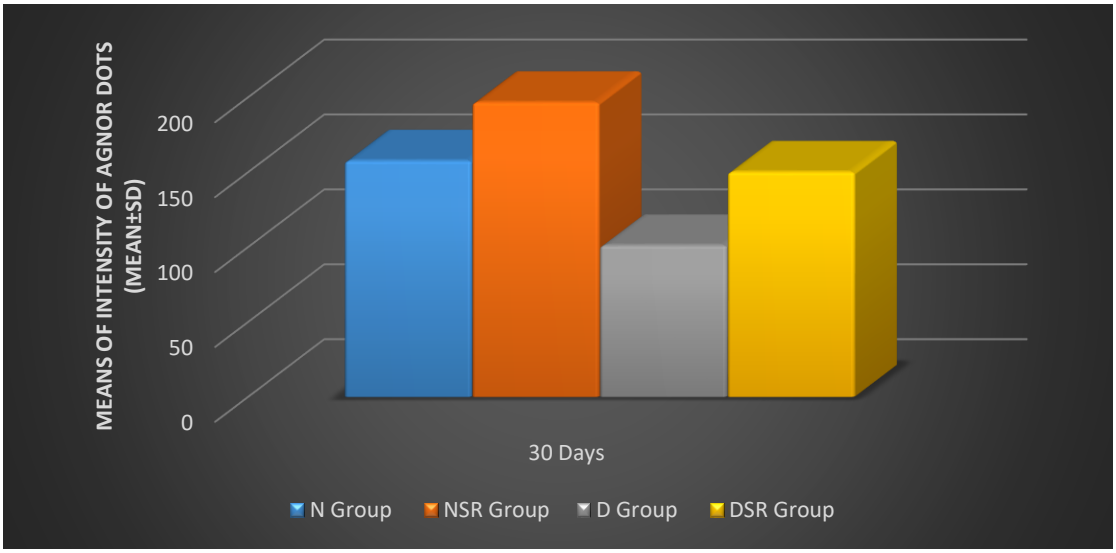


Fig. (6): Mean±SD of means of intensity of AgNOR dots between N, NSR, D and DRS groups within 30 days interval.

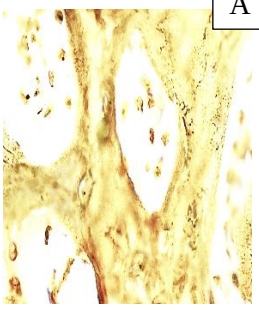
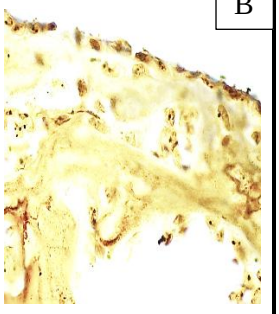
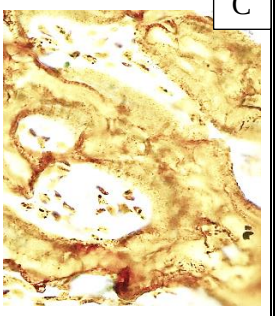
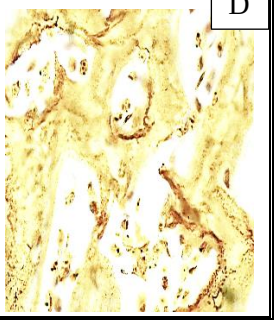
Photomicrograph of N30 group	Photomicrograph of D30 group	Photomicrograph of NSR30 group	Photomicrograph of DSR30 group
A 	B 	C 	D 

Table (9):- Comparison between groups at 30 days interval **(AgNOR stain X1000)**

III. DISCUSSION

Diabetes is one of the most important sugar metabolic disorders which have a great effect on bone healing. Diabetes is associated with bone resorption, poor osseous healing, and impaired bone regeneration [29, 30]. Diabetes impairs bone formation, weakens its mechanical strength and accelerates deterioration of bone architecture [9, 10, 11]. Moreover, Hyperglycemia produces deleterious effects on bone matrix and its components, and also affects adherence, growth and accumulation of extracellular matrix [12]. Therefore, there is need to develop new treatment plan for the bone healing in patients with diabetes mellitus.

The local application of systemic bone modulating drug that is commonly used to treat bone disease could offer a plausible alternative to growth factors. The advantage of local drug delivery is releasing the drug directly into the site of infection for a sufficient period without systemic exposure preventing both bacterial resistance and drug-related systemic side effects [13]. There are many drugs can be used systemically for the stimulation of bone metabolism. Among these drugs, the Strontium Ranelate (SrR) which has received special attention from researchers in the past few years [14, 15].

Strontium ranelate (SrR), currently used for osteoporosis treatment, appears to be a promising alternative due to its ability to promote bone formation by both stimulating osteoblast function and inhibiting osteoclast activation [16,17]. This dual effect of SrR has been demonstrated in several in vitro studies in which SrR promoted osteoblast proliferation, survival, differentiation and function, while inducing apoptosis and the reduction of osteoclast differentiation and activity [18, 19].

The quantitative and qualitative statically analysis of AgNORs revealed that, there was obvious retardation in the bone regeneration in the diabetic control group along the different intervals of the study, while in diabetic group treated with SrR gel, the bone regeneration was markedly increased along the intervals of the study. However, the bone regeneration was better in normal group treated with SrR gel than all other groups.

The quantitative and qualitative statistical analysis of AgNORs at five and ten days interval showed that, there was significant increase in the experimental normal (NSR) and diabetic groups (DSR) than the control normal (N) and diabetic (D) groups. While the quantitative and qualitative statistical analysis of AgNORs of the bone cells at thirty days interval showed that there was significant increase in the experimental normal group (NSR30) and control normal (N30) than the experimental diabetic groups (DSR30) and the control diabetic group (D30). While the experimental diabetic group (DSR30) was showed significant increase than the control diabetic group (D30).

These findings were coordinated by **Bonnelye E., et al. 2008[18]** and **Caudrillier A., et al. 2010 [19]** who stated that SrR promoted osteoblast proliferation, survival, differentiation and function, while inducing apoptosis and the reduction of osteoclast differentiation and activity. Also, these findings were parallel to **Aubin JE 1998 [31]** who

postulated that SrR treatment was sufficient to induce a significant increase in alkaline phosphatase (ALP) activity in both cell lines, indicating its effects on osteoblastic differentiation.

In addition to that, our findings were in agreement with **Zhang Y. et al. 2006** [34] who demonstrated that SrR enhance pre osteoblast replication, osteoblast differentiation, collagen type I synthesis, and bone matrix mineralization. On the other hand, because of the critical size of the defect, there was a slight retardation also in the healing of the normal control group than the diabetic group treated with SrR. These findings were in agreement with **Garcia1 P., Histing T.,** [32] who stated that the critical sized bone defect will not heal spontaneously under normal conditions during the lifetime of the individuals and have greater possibilities of clot destruction with failure of healing process or fill in from the periphery over a period of many months.

This retardation in the healing of diabetic groups was due to the harmful effect of diabetes. These results were parallel to findings of other studies which postulated that Diabetes reduces osteoblastic activity and bone mineralization. In addition, micro vascular complications and reduced blood flow also increase bone fragility [5, 6]. Additionally, these finding were in agreement with others who stated that diabetes reduce cellular proliferation in early callus and reduce collagen synthesis and content [7, 8]. Moreover, Our finding were coincidental with the results of other study which proposed that diabetes impairs bone healing processes by several factors contribute to wound healing deficiencies in diabetic patients, including decreased angiogenic responses, growth factor production, collagen accumulation, and changes in mineral metabolism [33].

IV. CONCLUSIONS

1) The local application of SrR has a positive regenerative effects on bone in case of diabetic rats with critical size bone defects.

2) The topical application of SrR is a safe method with an economic value to stimulate bone formation in diabetic rats with critical size bone defects.

3) AgNORs is one of the effective proliferative markers for qualitative and quantitative evaluation of bone regeneration.

REFERENCES

[1] Einhorn TA. The cell and molecular biology of fracture healing: Clin Orthop Relat Res. 1998; 35: 7-21.
[2] Cullinane DM, Barnes GL, Gerstenfeld LC. Fracture healing as a post-natal developmental process: molecular, spatial, and temporal aspects of its regulation. J Cell Biochem 2008; 88:873-884.
[3] Olsn J, Shernoff A, Tarlow J, Colwell J, Scheetz J, Bingham S. Dental endosseous implant assessments in a type 2 diabetic population: a prospective study. Int. J. Oral Maxillofacial. Implants 2000; 15: 811 – 818.
[4] Turkyılmaz I. one-year clinical outcome of dental implants placed in patients with type 2 diabetes mellitus: a case series. Implant Dent. 2010; 19: 323 – 329.
[5] Rakel A, Sheehy O, Rahme E, LeLorier J. Osteoporosis among patients with type 1and type2 diabetes. Diabetes & Metabolism 2008; 34:193–205.
[6] Cunha D, Gushiken M, Mardegan Issa, Iatecola J, Iatecola A, Pettian M, et al. Osteoconductive capacity of hydroxyapatite implanted into the skull of diabetics. The Journal of Craniofacial Surgery 2011; 22: 2048–2052.
[7] Beam H, Parsons J, Lin S. The effects of blood glucose control upon fracture healing in the BB Wistar rat with diabetes mellitus. J Orthop Res 2002; 20:1210–1216.
[8] Funk J, Hale J, Carmines D, Gooch H, Hurwitz S. Biomechanical evaluation of early fracture healing in normal and diabetic rats. J Orthop Res 2000; 18:126–32.
[9] Krakauer J , McKenna M , Buderer N , Rao D , Whitehouse F, Parfitt AM. Bone loss and bone turnover in diabetes. Diabetes 1995; 44: 775 – 782.
[10] Thrailkill K, Liu L, Wahl E, Bunn R, Perrien D, Cockrell G, et al. Bone formation is impaired in a model of type 1 diabetes .Diabetes 2005; 54: 2875 – 2881.
[11] Botolin S, McCabe L. Bone loss and increased bone adiposity in spontaneous and pharmacologically induced diabetic mice. Endocrinology 2007; 148: 198 – 205.
[12] Weiss R, Gora A, Nimni M. Abnormalities in the biosynthesis of cartilage and bone proteoglycans in experimental diabetes. Diabetes1981; 30: 670 – 677.

[13] Puri K, Puri N. Local drug delivery agents as adjuncts to endodontic and periodontal therapy. J Med Life. 2013; 6: 414–419.
[14] Reginster JY, Seeman E, De Vernejoul MC, Adami S, Compston J, Phenekos C, et al. Strontium ranelate reduces the risk of vertebral fractures in postmenopausal women with osteoporosis: treatment of peripheral osteoporosis (TROPOS) study. J Clin Endocrinol Metab 2005; 90: 2816-2822.
[15] lucif N Jr, Oliveira RZ, Achcar J, Lima CM, Silvah JH, Marchini JS. Effect of strontium ranelate on bone metabolism of elderly men. J Am Geriatr Soc 2015; 63: 2634-2635.
[16] Marie P. Strontium ranelate. A dual mode of action rebalancing bone turnover in favour of bone formation. Curr Opin Rheumatol. 2006; 18 (1):11-15.
[17] Saidak Z, Marie P. Strontium signaling; Molecular mechanisms and therapeutic implications in osteoporosis. Pharmacol Ther. 2012; 136 (2):216-226.
[18] Bonnelye E, Chabadel A, Saltel F, Jurdic P. Dual effect of strontium ranelate: stimulation of osteoblast differentiation and inhibition of osteoclast formation and resorption in vitro. Bone. 2008; 42(1):129-138.
[19] Caudrillier A, Hurtel-Lemaire A, Wattel A, Cournarie F, Godin C, Petit L, et al. Strontium ranelate decreases receptor activator of nuclear factor-KB ligand-induced osteoclastic differentiation in vitro: involvement of the calcium-sensing receptor. Mol Pharmacol. 2010; 78 (4):569-576.
[20] Zacchetti G, Dayer R, Rizzoli R, Ammann P. Systemic treatment with strontium ranelate accelerates the filling of a bone defect and improves the material level properties of the healing bone. Biomed Res Int. 2014; 2014: 549785.
[21] Apostu D, Lucaciu O, Lucaciu G, Crisan B, Crisan L, Baciut M, et al. Systemic drugs that influence titanium implant osseointegration. Drug Metab Rev. 2017; 49 (1):92-104.
[22] Saidak Z, Hay E, Marty C, Barbara A, Marie PJ. Strontium ranelate rebalances bone marrow adipogenesis and osteoblastogenesis in senescent osteopenic mice through NFATc/ Maf and Wnt signaling. Aging Cell 2012; 11:467–474
[23] Trere D. AgNOR staining and quantification Micron 2000;31: 127–131
[24] Cucer N, Imamoglu N, Tozak H, Demirtas H, Sarac F, Tatlýsen A, et al. Two-dimensional agnor evaluation as a prognostic variable in urinary bladder carcinoma: A different approach via total AgNOR area/nucleus area per cell Micron 2007;38: 674–679
[25] Federico L, Silvia Gatto, José Ferro, Juan O, Juan E . Preparation of platelet-rich plasma as a tissue adhesive for experimental transplantation in rabbits. Thrombosis Journal, 2006; 5: 4-18.
[26] Sirri V, Roussel P, Hernandez-Verdun D. The AgNOR proteins: qualitative and quantitative changes during the cell cycle. Micron. 2000; 31(2):121-126.
[27] Isaac F. Federiuk , Heather M, Casey, Matthew J, Michael D, et al. Induction of Type-1 Diabetes Mellitus in Laboratory Rats by Use of Alloxan: Route of Administration, Pitfalls, and Insulin Treatment. American Association for Laboratory Animal Science.2004; 54:252-257.

[28] Nahass HE, Din NNE, Nasry SA. The Effect of Strontium Ranelate Gel on Bone Formation in Calvarial Critical Size Defects. Open Access Maced J Med Sci. 2017; 5(7):994-999.
[29] Cozen L. Does diabetes delay fracture healing? Clinical Orthopaedics and Related Research 1972; 82: 134–140.
[30] Verhaeghe J, van Herck E, Visser WJ, Suiker AM, Thomasset M, Einhorn TA, et al. Bone and mineral metabolism in BB rats with long-term diabetes. Decreased bone turnover and osteoporosis. Diabetes 1990; 39:477-482.
[31] Aubin JE. Advances in the osteoblast lineage. Biochem Cell Biol 1998; 76:899–910
[32] Garcia P, Histing T, Holstein JH, Klein MW, Matthys R, Ignatius A, et al. Rodent animal model of delayed bone healing and non-union formation: A comprehensive review. European Cells and materials 2013; 26: 1-14.

[33] Erdogan O., Uçar Y., Tatlı U., Sert M., et al. clinical prospective study on alveolar bone augmentation and dental implant success in patients with type 2 diabetes. Clin Oral Implants Res. Jul 11, 2014.
[34] Zhang Y, Wei L, Chang J, Miron RJ, Shi B, Yi S, et al. Strontium incorporated mesoporous bioactive glass scaffolds stimulating in vitro proliferation and differentiation of bone marrow stromal cells and in vivo regeneration of osteoporotic bone defects. J. Mater. Chem. B, 2013; 1: 5711–5722.