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Original Article

Evaluation of the Effect of Solcoseryl on Promotion of Bone Regeneration in Calvarial Bony Defect – An Experimental Pilot study

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Abstract

Aim: To evaluate the effectiveness of Solcoseryl on bone regeneration in rabbit calvarial bony defect through histological examination and histomorphometric analysis.
Materials and Methods: Twenty New Zealand rabbits were divided into 4 groups. Two cavities were drilled in the calvarial bone of each rabbit. Defects were left empty in group A as a control group or filled with either Solcoseryl in group B, DM Bone in group C or (Solcoseryl & DM Bone) mixture in group D. Rabbits were sacrificed on day 14; the defects were removed and prepared for histological and histomorphometric analysis. The area percent of newly formed bone was estimated. Comparison of the results of all groups were statistically analyzed by ANOVA and Post-hoc tests.
Results: Histological evaluation revealed presence of granulation tissues in control group while woven bone formation was evident in all experimental groups with evident osteoid tissue in group C & D. Histomorphometric analysis revealed that the quantity of newly formed bone was the greatest in mixture group. The greatest area percent of uncalcified bone was recorded in control.
Conclusion: Combination of Solcoseryl and DM Bone has a synergistic effect on bone formation quantitatively and qualitatively.

Keywords: Bone regeneration; β-TCP; HA; Scaffold; Solcoseryl

Introduction:

Alveolar ridge defects are developed as a result of surgery, trauma, infection or congenital malformations. The goals of osseous replacement are contours maintenance, dead space elimination and reducing postoperative infection; and thus

enhance bony and soft tissue healing. **(Kumar et al., 2013)**

Bone grafting is the process of bone transferring from a donor to recipient sites **(Joshi et al., 2010).** Autogeneous bone is the gold standard and the most preferred because there is less risk of graft rejection as the graft is originated from the patient′s body. Disadvantage of autologous grafts is that additional surgical sites is required, often resulting donor site morbidity and limited availability **(Kumar et al., 2013; Nandi et al., 2010).** This encouraged the introduction to a variety of bone substitutes to aid in bone grafting. This included allografts, xenografts and alloplasts. **(Nkenke and Stelzle, 2009)**

Alloplastic grafts are synthetic bone graft which can be created from ceramics such as calcium phosphates (e.g. Hydroxyapatite and tricalcium phosphate), bioglass, and calcium sulphate. The combination of hydroxyapatite with tricalcium phosphate used to give the advantage of both osteoconduction and resorbability. **(Kumar et al., 2013).** This combination with a percentage of (60% HA/40% TCP) provides a good microenvironment for bone ingrowth with its interconnected porous structure. **(Balçik et al., 2007)**

Bone is a highly vascularized tissue and angiogenesis is crucial for bone regeneration. Neovascularization helps to support the mesenchymal stem cells and osteoblasts necessary for bone repair. Several studies have shown that osteogenesis is preceded by angiogenesis in a bone fracture model. Thus, controlled delivery of both angiogenic and osteogenic growth factors can promote bone healing. **(Patel et al., 2008)**

Solcoseryl is a deproteinated hydrolysate of blood extract from calves. It stimulates cell proliferation and collagen formation. It is also widely used in medical practice, primarily for stimulation of regeneration processes in post-burn therapy of the skin. **(Magakian et al., 2009).** Solcoseryl stimulates ATP synthesis and promotes angiogenesis. It has growth factor-like activity and cytoprotective effects that accelerate returning of reversibly damaged cells to their normal state. **(Eissa et al., 2013; Hartung et al., 1991)**

The study of healing capacity of surgically produced cranial defects in rabbits with different healing periods were shown that the healing period of 2 and 4 weeks could be recommended for evaluating the early phase of the bone healing. **(Sohn et al., 2010).** The hypothesis of the current study was that the use of Solcoseryl would enhance the process of bone formation in New Zealand rabbits.

Materials and methods:

Materials:

SOLCOSERYL® Paste (Legacy Pharmaceuticals Co., Switzerland) contains protein-free dialysate of calves' blood which is chemically and biologically standardized.

DM Bone® (META-BIOMED Co., LTD., Korea) is a fully synthetic, biocompatible and bioactive bone grafting material which is resorbable and able to be replaced by new bone. It consists of 60% HA, 40% β-TCP.

Experimental and control groups:

Twenty adult New Zealand rabbits approximately 6 months old were obtained from the Animal House, Faculty of Medicine, Cairo University. The experiment was conducted according to the recommendations of the Ethics Committee on animal's experimentation of the Faculty of Dentistry, Cairo University. Two cavities were drilled in the calvarial bone of each rabbit. Animals were divided randomly into 4 groups; 5 rabbits with 10 defects in each group. **(Table 1)**

Table (1): Number of Groups, Rabbits and Defects.

Groups	No. of Rabbits	No. of Defects
Group A: negative control	5	10
Group B: Solcoseryl	5	10
Group C: DM Bone (Si-HA/β-TCP)	5	10
Group D: (Solcoseryl & DM Bone) mixture	5	10

Surgical procedure:

Rabbits were anaesthetized by intramuscular injection of Ketamine/Xylazine, then local anesthetic solution was injected.

Two separated rectangular 10x5 mm full-thicknesses cranial defects were made in the parietal bones using fisher stainless steel bur on a low-speed electric handpiece. **(Wong & Rabie, 2007; Wong & Rabie, 2010; Wong & Rabie, 2003)**

Initially, the outlines of the defects were made by making holes of full thickness at the parietal bones using a stainless steel ruler as a guide. Then the holes were connected to complete the outline of the defect. During bone cutting, irrigation with copious amount of sterile saline was used to eliminate thermal damage of the tissues. The central bone segment was then carefully dissected off the dura and removed. The defects were packed with the study materials or left empty according to the group in which the rabbit belonged. **(Fig. 1)**

Post-operative care:

Periosteum approximation was performed and sutured with interrupted 4/0 Vicryl absorbable suture then the skin was sutured with interrupted 3/0 black silk sutures. Postoperatively the rabbits were given I.M injection of Cefotaxime as an antibiotic and Diclofenac sodium as analgesic once daily for 3 days.

The rabbits were sacrificed 2 weeks after surgery and the defects with the surrounding tissue were removed for histological preparation.

Sample analysis:

I- Histological examination:

Immediately after sacrifice of the experimental animals, the parietal bones were removed and fixed in 10% neutral buffered formalin for 48 hours, then washed and soaked in EDTA for decalcification for 4 weeks. Following decalcifications, the specimens were dehydrated in ascending grades of alcohol, cleared in xylol and then embedded in paraffin blocks. Paraffin cross sections of 5µ thickness were cut and mounted on clean glass slides, then stained with Hematoxylin and Eosin (H&E) stain, for histopathological examination and histomorphometric analysis under a light microscope. Moreover, the slides were stained histochemically by Masson trichrome stain to detect bone trabeculae and collagen fibers formation.

The plane of sections was parallel to the parietal bone and the number of sections was 4 from each defect (8 from each rabbit); 2 sections were stained by H&E stain and another 2 sections were stained by Masson trichrome stain.

II- Histomorphometric analysis:

The area percent of the newly formed bone, in the region of bone repair previously identified in the histopathological observation for each specimen, was measured. The stained sections were assessed by ordinary light microscope and image analyzer computer system using the software Leica Application Suite version 3.7.0 [build: 681]

Statistical analysis:

The data obtained from computer image analysis were tabulated and statistically analyzed. Analysis of variance (ANOVA) test was used for statistical analysis of the difference between groups and between different regions within each group. Tukey's post hoc test was performed as ANOVA test revealed significant difference. P value ≤ 0.05 was considered statistically significant and p-value ≤ 0.01 was extremely significant.

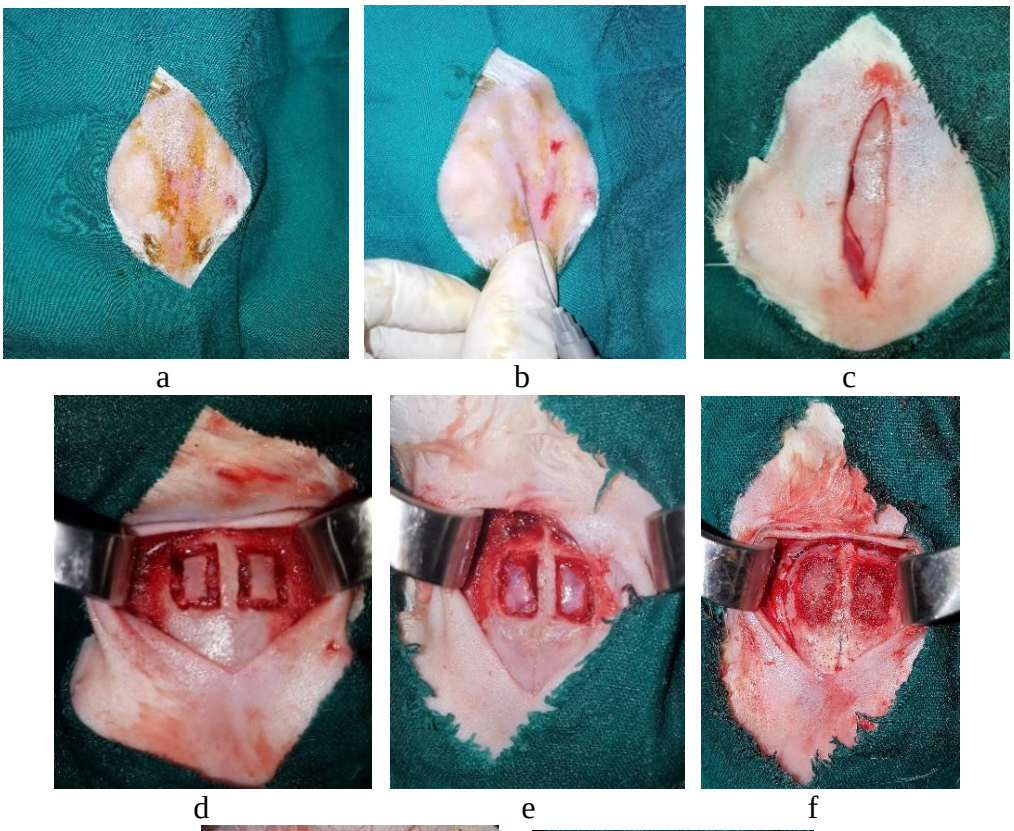

Fig.1: A photograph showing: a) Surgical site disinfection, b) Injection of local anaesthesia, c) Mid-sagittal incision, d) creation of two rectangular 10x5 mm full-thickness cranial defects, e) Group A: Defects without grafting material, f) Group B: Filling defects with Solcoseryl paste, g) Group C: Filling defects with DM Bone granules, h) Group D: Filling defects with (Solcoseryl and DM Bone) mixture, i) Suturing of periosteum and j) Suturing of skin.

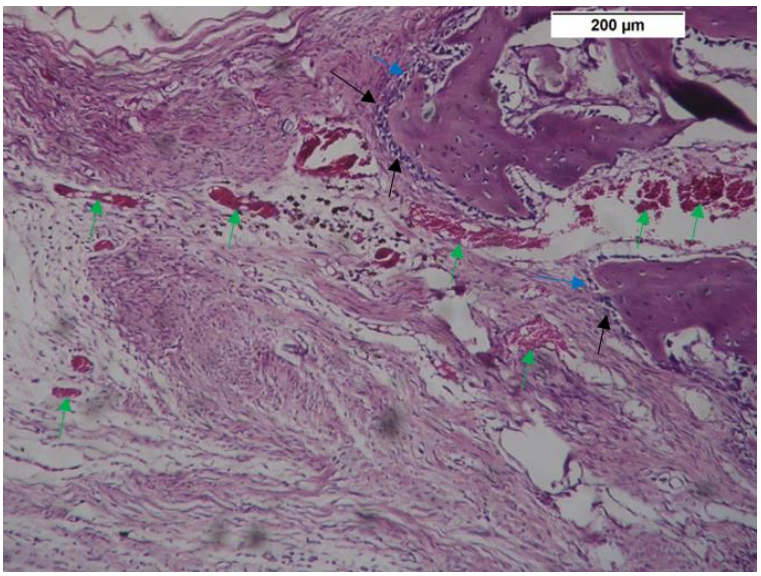

Fig.2: A photomicrograph of Group A showing: dense fibrous collagen bundles, few and small pieces of woven bone surrounded by well-arranged osteoblasts (black arrows), many blood vessels engorged by red blood cells (green arrows), few scattered osteoclasts (blue arrows) and thick granulation tissue. **(H&E Mag. x200).**

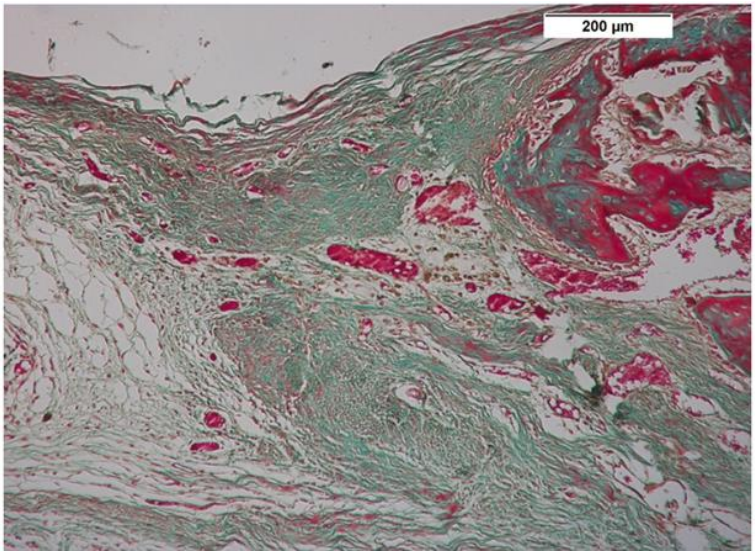

Fig.3: A photomicrograph of Group A showing: immature collagen fibers (green color), intermingled with mature collagen fibers (red color), granulation tissues at the center with blood vessels engorged by red blood cells, areas of woven bone; unmineralized (green region) and mineralized (red region). **(Masson trichrome Mag. x200).**

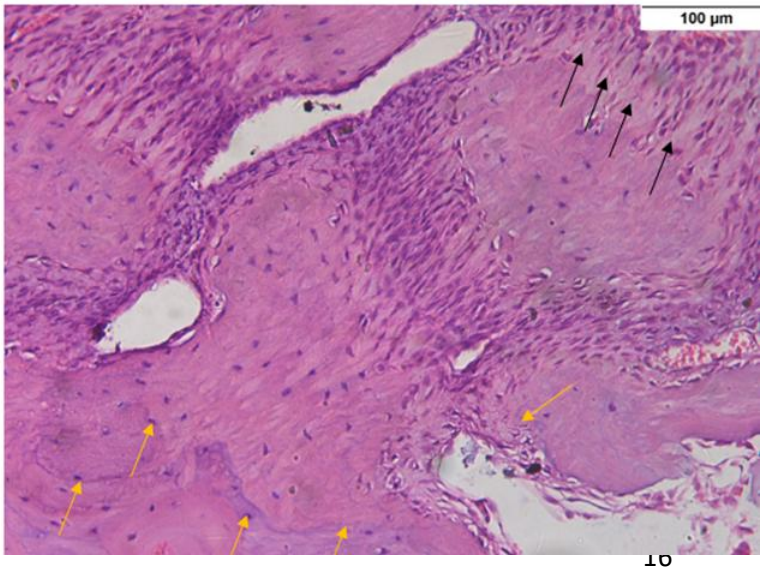

Fig.4: A photomicrograph of Group B showing: osteoid bone intermingled by osteoblasts (black arrows), reversal lines (orange arrows) and granulation tissue. **(H&E. Mag. x100).**

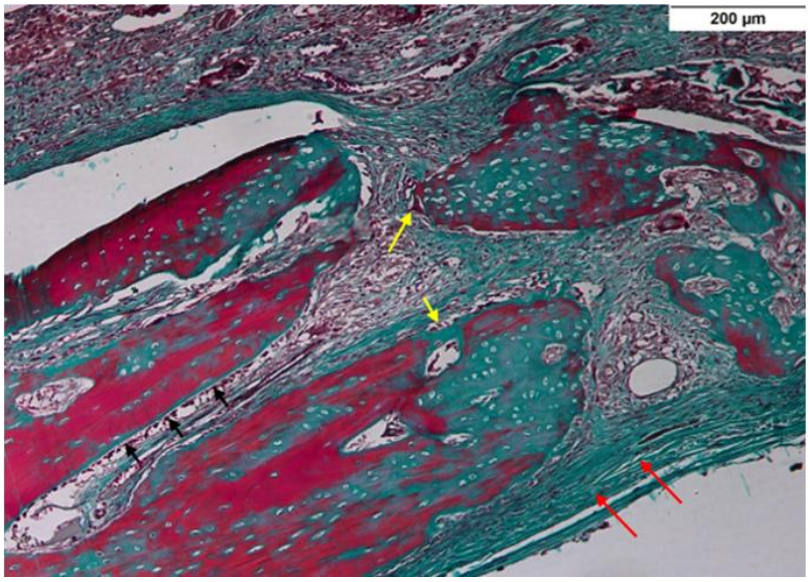

Fig.5: A photomicrograph of Group B showing: arrangement of collagen fibers into dense bundles (red arrows), areas of unmineralized woven bone, palisaded osteoblasts secreted thin layer of osteoid tissue (black arrows) and osteoclasts (yellow arrows). **(Masson trichrome Mag. x200).**

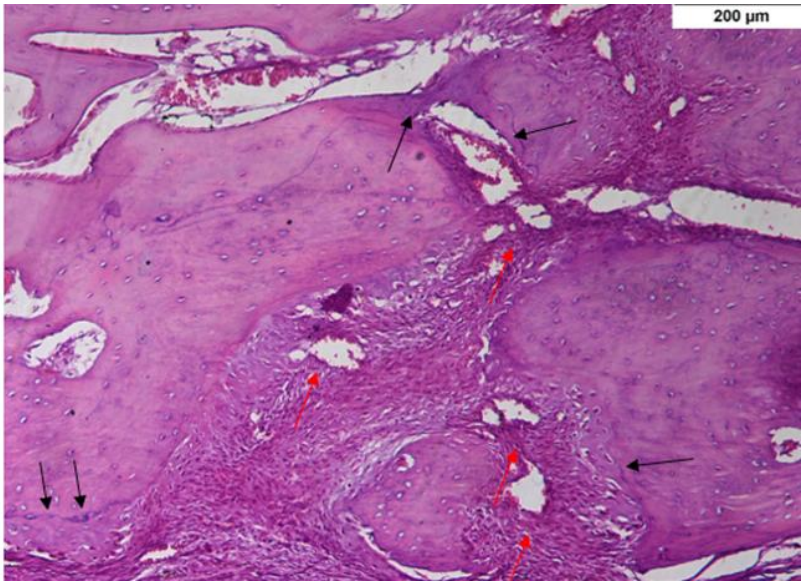

Fig.6: A photomicrograph of Group C showing: large amount of granulation tissue in the center of defect with formation of woven bone. Note reversal lines (black arrows) and many blood vessels (red arrows). **(H&E. Mag. x200).**

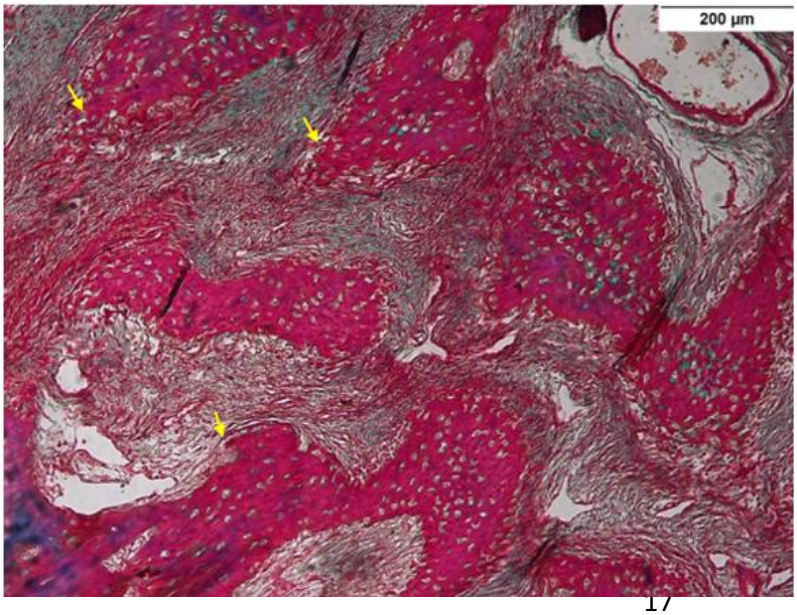

Fig.7: A photomicrograph of Group C showing: formation of mineralized woven bone with many osteoclasts at the periphery (yellow arrows) and arranged granulation tissue in between. **(Masson trichrome Mag. x200).**

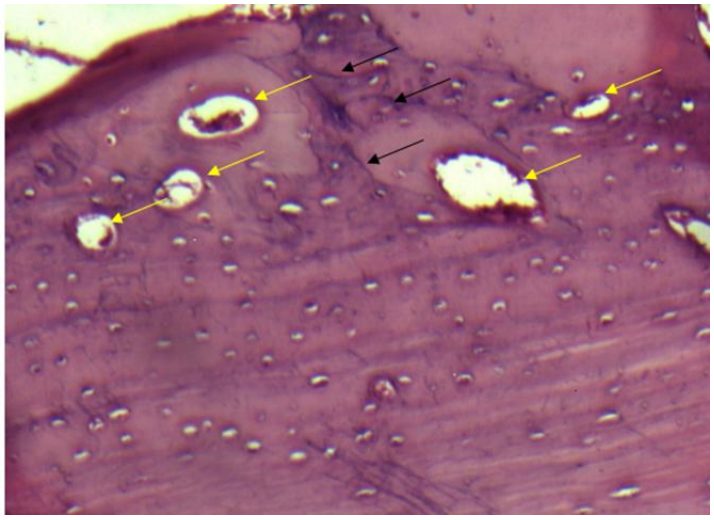

Fig.8: A photomicrograph of Group D showing: compact bone with defect in the center filled with woven bone, many reversal lines (black arrows) and many nutrient canals (yellow arrows). **(H&E. Mag. x200).**

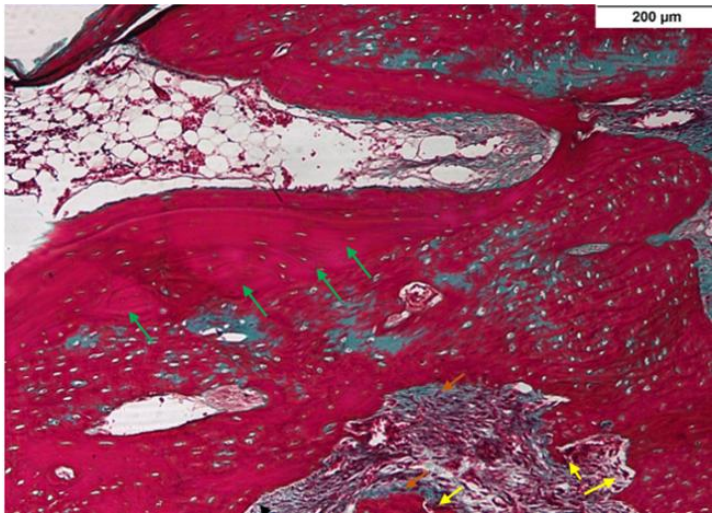

Fig.9: A photomicrograph of Group D showing: lamellar bone (green arrows), osteoblasts arranged in palisading manner at the surface (black arrows), osteoclasts (yellow arrows) and new bone formation (orange arrows). **(Masson trichrome Mag. x200).**

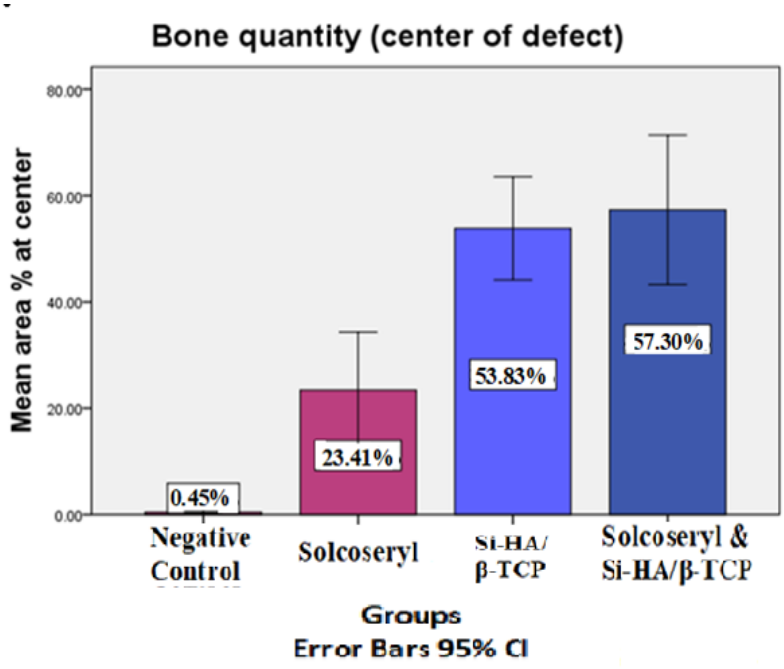

Fig. (10): Column chart showing: mean area percent of bone at center of the defect.

Table (2): Area percent of bone at the center of the defect (ANOVA test)

Groups	Mean	Std. Dev	Std. Error	95% Confidence Interval for Mean		Min	Max	F	P
				Lower Bound	Upper Bound				
Negative Control group	0.45[c]	0.13	0.06	0.24	0.66	0.30	0.60	71.42	<0.0001*
Solcoseryl	23.41[b]	6.86	3.43	12.50	34.31	13.52	28.92		
Si-HA/β-TCP	53.83[a]	6.10	3.05	44.12	63.54	45.26	59.68		
(Solcoseryl & Si-HA/β-TCP)	57.30[a]	8.84	4.42	43.24	71.36	45.72	66.95		

Significance level p<0.05, * significant
Tukey's post hoc test: means sharing the same superscript letter are not significantly different

Table (3): Area percent of uncalcified bone at the center of the defect (ANOVA test)

Groups	Mean	Std. Dev	Std. Error	95% Confidence Interval for Mean		Min	Max	F	P
				Lower Bound	Upper Bound				
Negative Control group	47.82[a]	0.89	0.45	46.39	49.24	46.90	48.60	1023.0	<0.0001*
Solcoseryl	17.90[b]	1.46	0.73	15.57	20.23	16.41	19.58		
Si-HA/β-TCP	9.61[c]	0.96	0.48	8.08	11.14	8.36	10.70		
Solcoseryl & Si-HA/β-TCP	8.24[c]	1.21	0.60	6.32	10.16	6.62	9.53		

Significance level p<0.05, * significant
Tukey's post hoc test: means sharing the same superscript letter are not significantly different

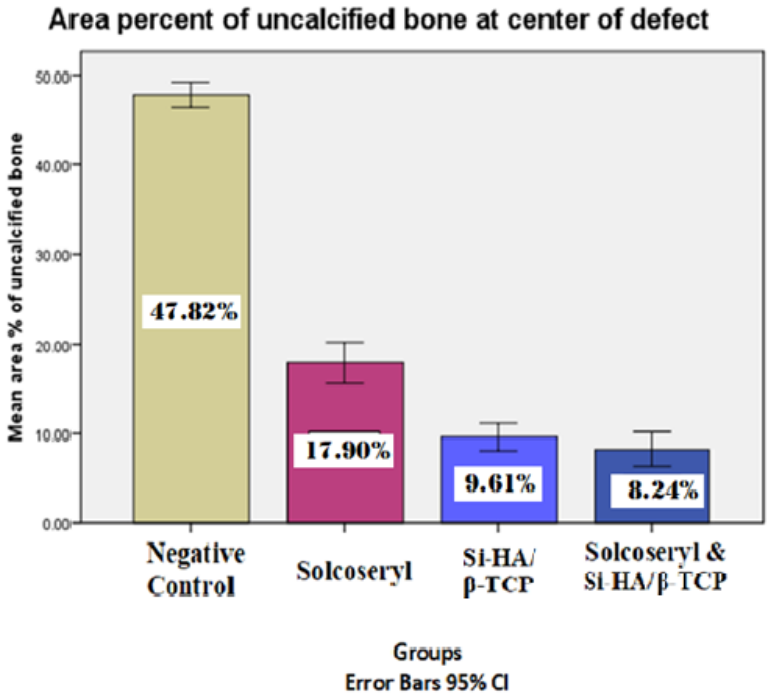

Fig. (11): Column chart showing: mean area percent of uncalcified bone at center of the defect

Results:

Clinical evaluation:

All rabbits remained healthy and recovered rapidly after operation during the course of the study. There were no signs of infections in any of the rabbits.

Histological evaluation:

Group A: Negative Control: The center of defect filled with well-formed granulation tissues formed of collagen fibers, fibroblasts and many blood vessels which were engorged by red blood cells. At the periphery of the defect, pieces of woven bone with mineralized and unmineralized areas were noticed. At the periphery of woven bone, osteoblasts were well arranged and a layer of osteoid tissue was noticed. **(Fig.2&3)**

Group B: Solcoseryl: There were areas of granulation tissue which were reduced in size. Higher cellular activity was observed indicating progress in the healing process. In the center of defect area, there were large pieces of woven bone. There were also reversal lines at the lateral walls of defect which separated between newly formed bone and old bone. **(Fig.4&5)**

Group C: DM Bone (Si-HA/β-TCP): The area of defect was almost filled with woven bone which was consisted of large sized osteocytes and granulation tissue. Many reversal lines were seen and lateral to it osteoid tissues and new bones were formed. **(Fig.6&7)**

Group D: (Solcoseryl & DM Bone) mixture: Absence of granulation tissue completely which were replaced by woven bone **(Fig.8)**. Haversian system and lamellar bone were replaced the woven bone which denoting advance in the healing process. The periphery of the woven bone was showing osteoid tissue formation and arrangement of osteoblasts **(Fig.9)**.

Histomorphometric analysis:

I- Quantitative bone area percent (H&E stain):

The quantity of bone at the center of the surgically-created defect was evaluated as area percent using H&E stained sections. The greatest mean area percent of bone was recorded in (Solcoseryl & Si-HA/β-TCP) mixture group, followed by DM Bone group, then Solcoseryl group; with the least value recorded in the negative control group. ANOVA test revealed that the difference between groups was extremely statistically significant ($P<0.0001$). Tukey's post hoc test revealed no significant difference between (Solcoseryl & Si-HA/β-TCP) mixture group and DM Bone group. **(Table 2, Fig. 10)**

II- Qualitative (uncalcified) bone area percent (Masson trichrome stain):

The quality of bone at the center of the surgically-created defect was evaluated as area percent of uncalcified bone using Masson Trichrome stained sections. The greatest mean area percent of uncalcified bone was recorded in the negative control group, followed by Solcoseryl group, then DM Bone, with the least value recorded at (Solcoseryl & Si-HA/β-TCP) mixture group. ANOVA test revealed that the difference between groups was extremely statistically significant ($P<0.0001$). Tukey's post hoc test revealed no significant difference between (Solcoseryl & Si-HA/β-TCP) mixture group and DM Bone group. (**Table 3, Fig. 11)**

Discussion:

New Zealand rabbits are commonly used for medical research. Some of their advantages are that ease of handling, their appropriate size and similarities with humans bone. (Pearce et al., 2007; Sohn et al., 2010)

Moreover, New Zealand rabbits are characterized by short developmental period and faster skeletal changes and bone turnover when compared with other species such as rodents. These rabbits achieve skeletal maturity shortly after reaching complete sexual development at approximately 6 months of age. Therefore, these rabbits were selected as experimental animals in the present study. **(Newman et al., 1995)**

The present study aimed to compare between the healing of defects in rabbit's parietal bone grafted with Solcoseryl, to those grafted with DM Bone, to those grafted with mixture of (Solcoseryl & Si-HA/β-TCP) and to negative control defects. The results were assessed using histological and histomorphometric analysis.

In this study, defects were created as rectangular defects of 10x5 mm full thicknesses in the rabbit's parietal bone based on well-established protocol done in previous studies. **(Wong & Rabie, 2007; Wong & Rabie, 2010; Wong & Rabie, 2003).** This is in agreement with **Abdel-Ghany et al., 2017** who studied the comparative effectiveness of two different forms of phytoestrogens as a graft material in rectangular 10 x 5 mm full thickness cranial bony defects in rabbits.

Moreover, the combined effect of nano-hydroxyapatite (n-HAp) silica gel bone substitute with Solcoseryl paste was studied in rectangular 10×5 mm full-thickness cranial bony defect in rabbits to detect new bone formation. It was noticed to have a synergistic osteoinductive effect on bone quantitatively and qualitatively. **(Abdel Hamid et al., 2018)**

The result of the current study showed that the negative control defects were filled with well-formed granulation tissues which is in accordance with **Landry et al., 1996** and **Calixto et al., 2007** who described the early phases of bone healing. They showed that the blood clot gradually was absorbed and replaced by granulation tissue.

Solcoseryl has growth factor-like activity and promotes angiogenesis which precedes osteogenesis **(Eissa et al., 2013; Hartung et al., 1991)**, so consequently Solcoseryl enhance the proliferation of osteoblasts and bone formation.

Abdel Hamid et al., 2018 noticed that, in the Solcoseryl group, the bony defects were totally filled with osteoid tissue surrounded by plump cells in connective tissue stroma. This represented the initial scaffold of new bone formation with calcification in the centers of osteoid areas. This has been supported by the histopathological findings of the current study among Solcoseryl group where we noticed areas of granulation tissue, large pieces of woven bone and also reversal lines at the lateral walls of defect which separate between the newly formed bone and old bone.

Balçik et al., 2007 oberved that the radiological grade of healing and bonding to the native bone was slightly better in HA/TCP (60/40) composite ceramics than pure HA ceramics at 18 weeks. Therefore in this study DM Bone [Si-HA/β-TCP (60/40)] was used. In DM Bone group, the area of defect was almost filled with woven bone and granulation tissue. Many reversal lines were seen and lateral to it osteoid tissues and new bones were formed.

It was reported that scaffolds should act as delivery vehicles for bone growth factors such as bone morphogenic proteins and transforming growth factors to induce bone tissue growth through the scaffolds (Matsushita et al., 2006)**.** This is supported by results were seen in the mixture group, in which there was absence of granulation tissues completely and were replaced by woven bone. Then haversian system and lamellar bone had replaced the woven bone which denoting advance in the healing process.

According to the obtained results, the bone healing quality and quantity had significantly higher bone area percentage with (Solcoseryl & Si-HA/β-TCP) mixture as compared to the control and the other experimental groups.

From the clinical point of view, this animal study could introduce a new combined treatment of (Solcoseryl and Si-HA/β-TCP) that could accelerate postoperative physiological ingrowth of bone into dental implants sites, cystic bony defects or even extraction sockets.

Conclusion:

Combination of Solcoseryl and DM Bone (Si-HA/β-TCP) has a synergistic effect on bone formation quantitatively and qualitatively.

Conflicts of interest and source of funding:

The authors declare no conflicts of interest. The authors are responsible for the content of the paper and did not receive any grant from funding agencies.

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