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The Role of Magnesium Oxide Nanoparticles In The Healing Process of Cervical Esophageal Anastomosis in Dogs :An Experimental study

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THE current designed to investigate the role of Magnesium Oxide Nanoparticles in healing process of cervical esophageal anastomosis in dogs. An Adults eighteen healthy stray dogs of both sexes were allocated equally in to two groups 9 of each, each main group was Moreover ,divided into 3 subgroups of 3 dogs in each according to the period post-surgery at 7,15,30 days. All animals underwent to end - end cervical esophageal anastomosis and the mucosal layer was sealed 2/0 silk with a simple interrupted pattern and the knot tied within the lumen, Whereas, the muscular layer was closed with 2/0 polygalactin (Vicryl) in an interrupted horizontal mattress, finally the neck muscles were closed routinely. In group one G1 (control group) the mucosa and muscularis layers at anastomotic site were sutured only without treatment, while, in group two G2 (MgO NPs) were treated by spreading of MgO NPs. The result of the study showed that the healing process were superior with the MgO NPs group compared to control group significantly at $P < 0.05$, based up on both histological and gross pathological observations. this study conclude that local spreading of MgO NPs was accelerate healing of esophageal anastomosis and promote return the tissue to their Furniture and function pre surgery.

Keywords: MgO NPs, Esophageal anastomosis, Dogs.

Introduction

Many abnormal disorders and affections affect the esophagus as esophageal foreign bodies like bones, fishhooks, needles, and sticks, esophageal neoplasia [1] ,esophageal fistula (Broncho-esophageal fistula) [2]. these esophageal disorders need esophagectomy (esophageal anastomosis) [3-5] , esophageal operation commonly associated with high rate incidences of various complications [6, 4] such as leakages [7] , wound dehiscence [8] and Stricture or stenosis [9] , specially might be developed after anastomosis of esophagus [10] , unfortunately normal esophageal anastomosis healing is multifactorial , healing fails when any phase of healing process is disrupted [11] and the esophagus, unlike other digestive organs, lack of serosa (which promotes a rapid seal due to exudation of fibrin) and omentum, longitudinal tension weakening of the esophagus tissue, segmental blood supply, and continuous motion of the suture site, consider a predisposing factors demonstrate poor healing and leads to scar tissue formation around anastomotic site and causing narrowing the esophagus [12, 5] as an inelastic anastomosis and a fixed-size scar around the anastomosis and causing stenosis [13, 4].

Nanoparticles possess a wide range of biological properties, including anti-inflammatory,

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anti-bacterial action and accelerate and enhance wound healing with minimal scar formation [14] . MgO NPs have ability to accelerate wound healing by spreading locally on the tissues [15]. Due to prescience a high chance of major complications related to healing process of esophageal tissues and effect of nanoparticles on the healing process, Thus in this study we introduce using MgO NPs for investigate its roles on healing

Material and Methods

Eighteen adults' healthy stray dogs in both sexes weighted 20 to 30 kg with an average 25 kg ± 1.3 and aged 18-36 months an average 27 months ± 1.5 were used in present study; all animals underwent same condition of housing and feeding in animal's field of college of veterinary medicine, university of Mosul. Animals were randomly divided into two equal main groups with nine dogs, each main group was then divided into three subgroups of three dogs in each to the period post-surgery at 7,15,30 days. The protocol of anesthesia including atropine sulphate (Vapco, Jorden) at dose 0.05mg/kg BW/SC as a pre-anesthetic medication, five mint later a mixture of ketamine HCl at 15 mg/kg, (Rotexmedica , Germany) with xylazine at 5mg/kg B.w (Interchemie, Holand) intramuscularly (16) . All the animals underwent to end - end cervical esophageal anastomosis was applied and the mucosa was closed with 2/0 silk in a simple interrupted pattern and the knot tied within the lumen. , while , the muscular layer was closed with 2/0 polygalactin (Vicryl) in an interrupted horizontal mattress, finally the neck muscles were closed with polygalactin size 1 using simple continuous and the skin was sutured with silk size 1 using simple interrupted pattern.

In group one G1 (control group) the mucosal and muscularis layers at anastomotic site were sutured only without treated by any materials while in group two G2 (MgO NPs) were treated by speeding MgO NPs manufactured in Iran size 20 nm at dose 100 µg / animal , MgO NP powder was dissolved at the time of operation in 1 ml of distal water.

The post-operative care includes systemic antibiotic penicillin- streptomycin, in a dose of 10.000 I.U /Kg / B. W. Penicillin and 10mg/ kg /B. W. streptomycin for 3 days by I.M route injection and all animals were given intravenous fluid therapy for two days after surgical operation and oral feeding starts in third day after operation with milk as a liquid food to the 5th day , then gradually returned to the normal solid food. Skin sutures were removed 7 days after the operation.

Appraisal of the esophageal healing in this study was based on gross pathology, histological study and scoring, the autopsy collected after esophagatomy in alive animals in two groups at 7th, 15th , 30th P.ODs.

The gross pathological study was depended on clarity and binding line of anastomotic site and the histological study was performed staining of haemetoxyline-eosin and Masson - trichrome stains to compare the healing process between the two groups along the duration of 7, 15 , and 30. Scoring the histological study to convert non parametric healing process into numerical data that can be easily processed statistically to clarify the differences between different groups .Using SPSS version 22.0 to measures the significant differences at $P<0.05$ to compare between different mean for different treatments, in which the One Way ANOVA with Duncan's test were used to measures the significances between different groups at $P<0.05$.

Results

The macroscopic investigation indicated that, the line of anastomosis in G1 was clearly at 7th, 15th, and 30th P.O.Ds, while in G2 the clarity of anastomotic line was decreasing with the time after operation (7th , 15th, and 30th) Fig. (1, 2).

The histological study In G1 (control group) at 7th P.O.Ds , showed necrosis and sloughing to the endothelial layer and deposition of collagen fibers with infiltration of mononuclear inflammatory cells Fig.3. , and the Masson's trichrome stain showed deposition of collagen fibers that take a bright green color Fig.4. At 15th P.O.Ds showed few infiltration of mononuclear inflammatory cells with deposition of immature collagen fibers , with few fibroblast showed hyperplasia Fig.5 and Masson's trichrome section study revealed deposition of immature collagen fibers that take a bright green color in layer of granulation tissue Fig. 6.

At 30th P.O.Ds the histological study showed a weak re-epithelization process and immature collagen fibers with hyperplasia of fibroblast and infiltration of few inflammatory cells Fig. 7. The Masson's trichrome showed immature deposition collagen fibers that take a bright green color within granulation tissue Fig.8.

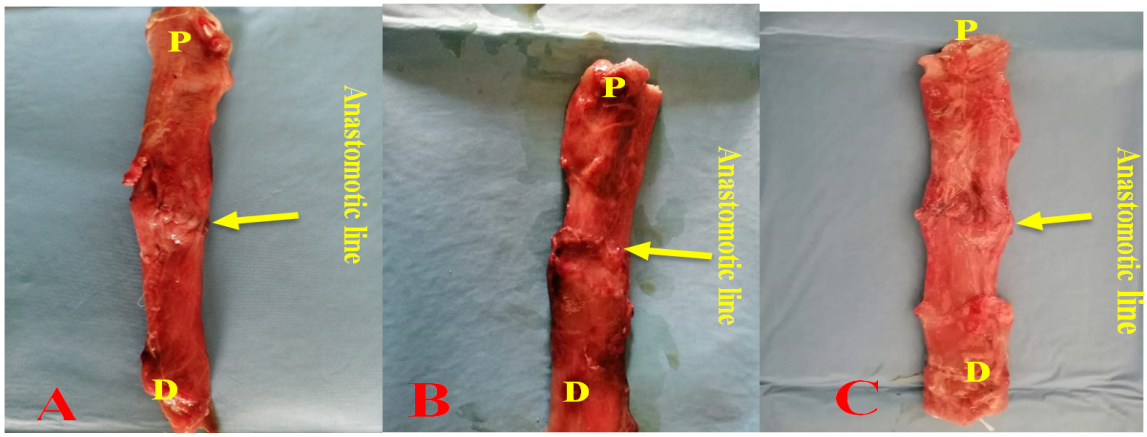

Fig. 1. Macroscopic investigation indicated that the anastomosis more clear in group 1 (Control group) A: At 7th , B : At 15th , C : At 30th P.O.Ds ,D meaning distal portion and P proximal portion.

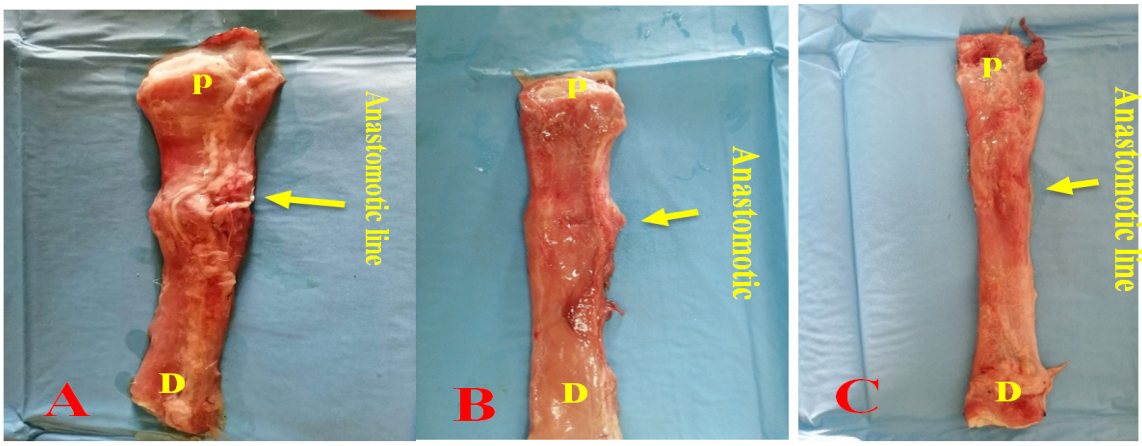

Fig. 2. Macroscopic investigation indicated that the anastomosis less clear in the in group G2 (MgO NPs group) A: At 7th , B : At 15th , C : At 30th P.O.Ds. D meaning distal portion and P proximal portion.

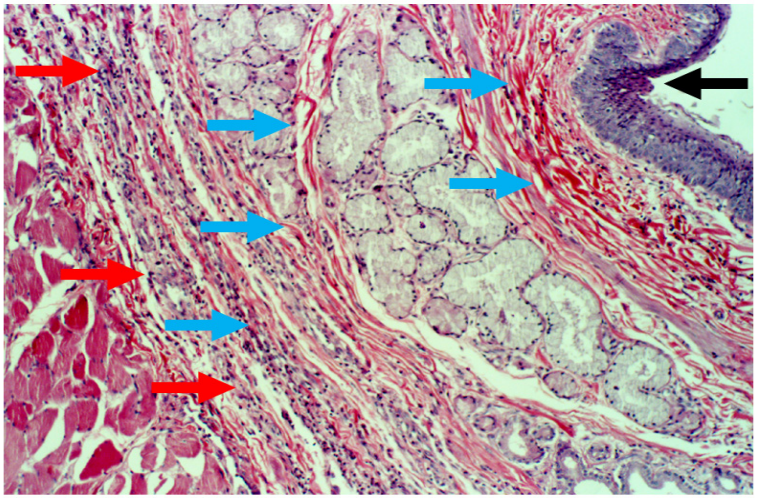

Fig. 3. Micrograph at 7th P.O.Ds in control group showed necrosis and sloughing to the endothelial layer (arrow) deposition of collagen fibers (arrow), infiltration of mononuclear inflammatory cells (arrow). H&E, 100x.

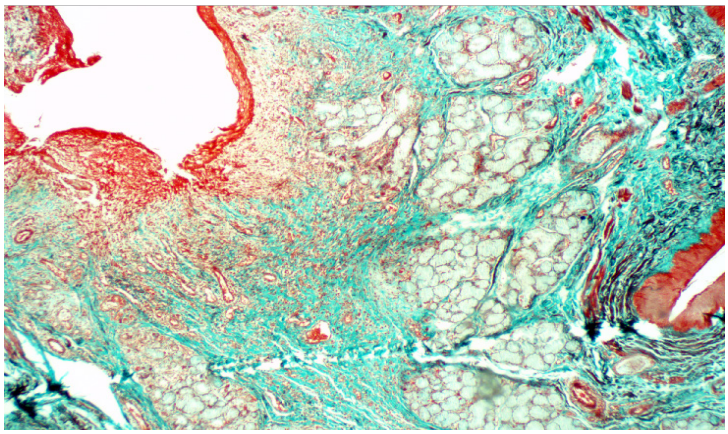

Fig. 4. Micrograph at 7th P.O.Ds in control group showed deposition of collagen fibers that take a bright green color. Masson's trichrome, 100x.

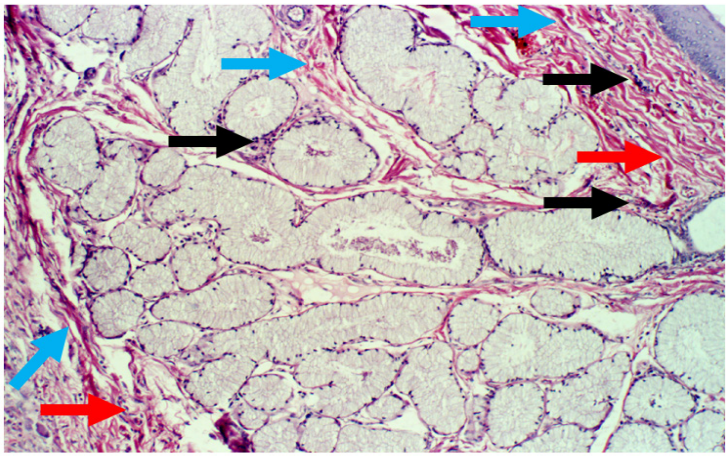

Fig. 5. Micrograph at 15th P.O.Ds in control group showed few infiltration of mononuclear inflammatory cells (arrow) with deposition of immature collagen fibers (arrow), with few fibroblast showed hyperplasia (arrow). H&E, 100x

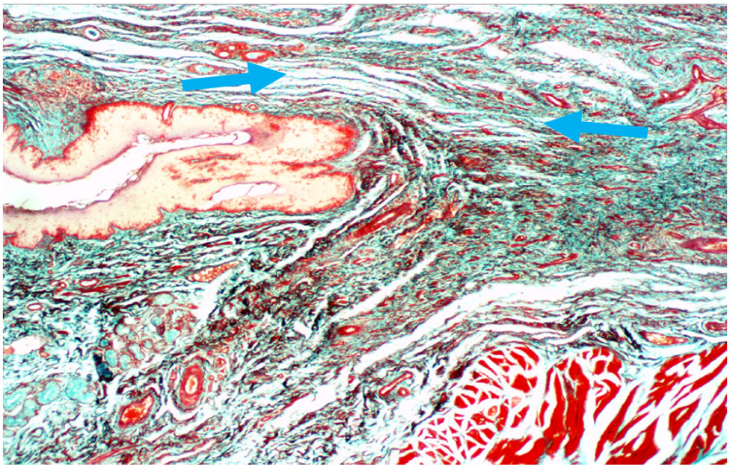

Fig. 6. Micrograph at 15th P.O.Ds in control group showed deposition of immature collagen fibers that take a bright green color in layer of granulation tissue. Masson's trichrome, 100x.

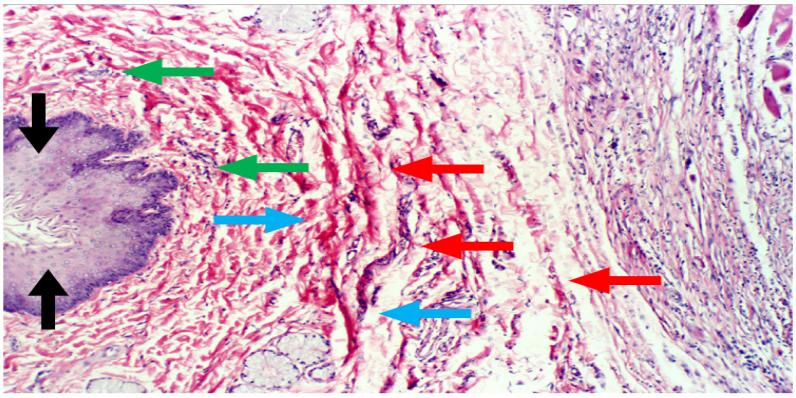

Fig. 7. Micrograph at 30th P.O.Ds in control group showed weak re-epithelization process (arrow) immature collagen fibers (arrow) hyperplasia of fibroblast (arrow) with few infiltration of inflammatory cells (arrow). H&E, 100x.

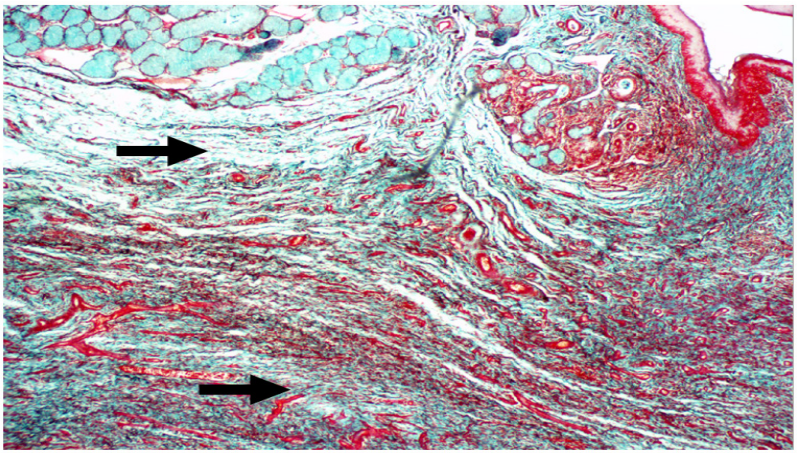

Fig. 8. Micrograph at 30th P.O.Ds in control group showed immature deposition collagen fibers that take a bright green color within granulation tissue. Masson's trichrome, 100x.

In G2 (MgO NPs), at 7th P.O.Ds., showing inflammatory infiltration of mononuclear inflammatory cells with deposition of immature collagen fibers and hyperplasia of fibroblasts and edema Fig.9., and the Masson's trichrome study was showed few deposition of collagen fibers that take a bright green colour Fig.10.

At 15th P.O.Ds Site of anastomosis showed inflammatory infiltration of mononuclear inflammatory cells with increase in deposition of collagen fibers and hyperplasia of fibrocytes Fig.11. Masson's trichrome staining showed increase in the amount of collagen fibers that appear as bright green colour. Fig.12.

At 30th P.O.Ds, at anastomotic site showed re-epithelization process immature collagen fibers with congestion of blood vessels Fig.13. ,the Masson's trichrome stain showed deposition of collagen fibers that take a bright green color Fig.14.

Scoring of histological study current at the 7 days post surgery reflected infiltration of inflammatory cells as a results of the inflammatory respond were the highest in MgO NPs group, and it low significant differences was recorded in control group at $P<0.05$. A similar result was showed in the next 15 and 30 days after operation, these results indicate that the best inflammatory response associated with infiltration of inflammatory cells were recorded in MgO NPs group in compare with control group in 7, 15 and 30 days post operation Table (1).

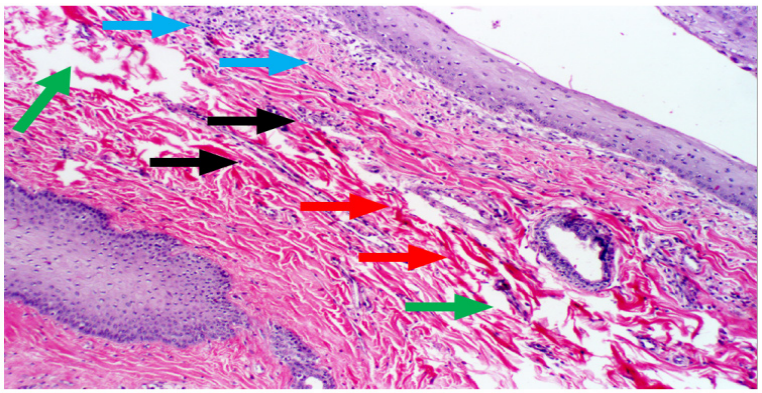

Fig. 9. Micrograph at 7th P.O.Ds in MgO NPs group showed inflammatory infiltration of mononuclear inflammatory cells (arrow) with deposition of immature collagen fibers (arrow) and hyperplasia of fibroblast (arrow) and edema (arrow). H&E, 100x.

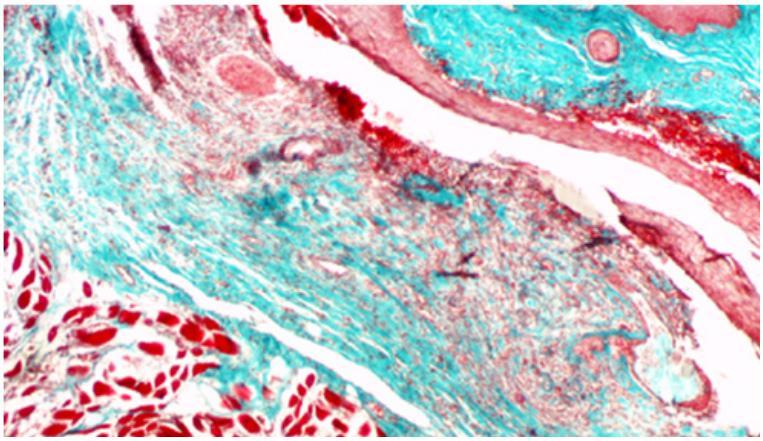

Fig.10. Micrograph at 7th P.O.Ds in MgO NPs group showed few deposition of collagen fibers that take a bright green color. Masson's trichrome, 400x.

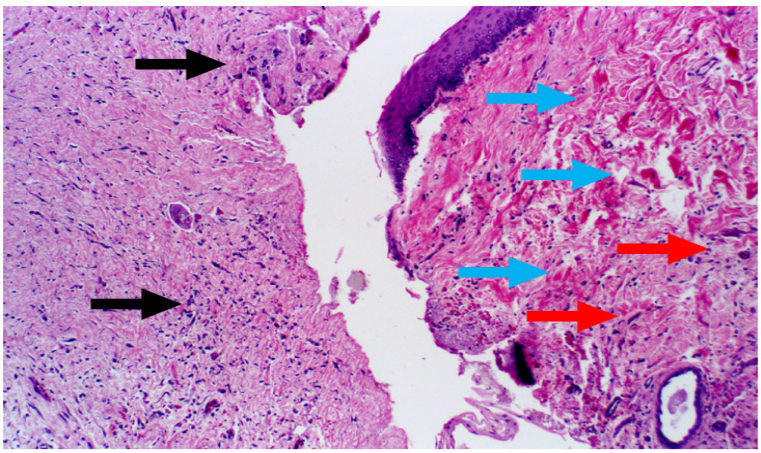

Fig.11. Micrograph at 15th P.O.Ds in MgO NPs group showed inflammatory infiltration of mononuclear inflammatory cells (arrow) increase in deposition of collagen fibers (arrow), and hyperplasia of fibrocytes (arrow). H&E, 100x.

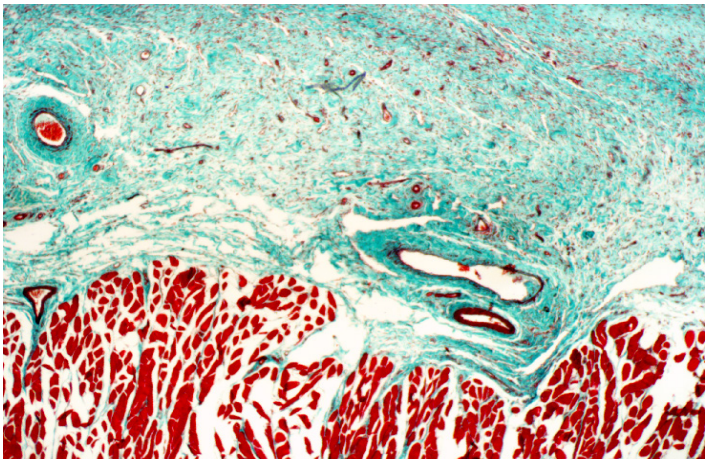

Fig.12. Micrograph at 15th P.O.Ds in MgO NPs group showed increase in the amount of collagen fibers that appear as bright green color. Masson's trichrome, 100x.

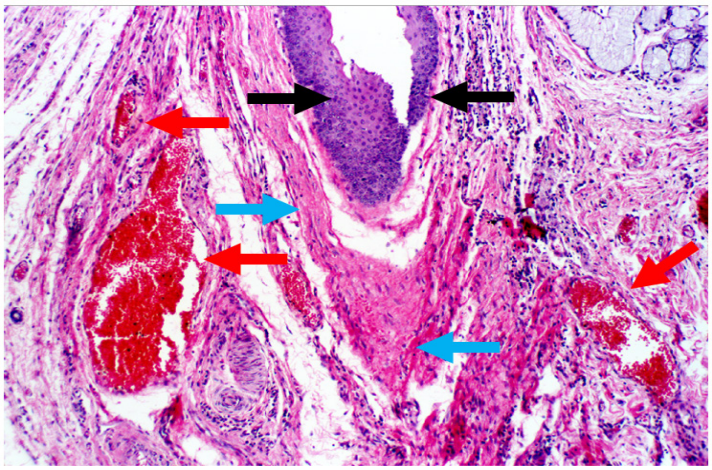

Fig. 13. Micrograph at 30th P.O.Ds in MgO NPs group View to site of anastomosis, showed re-epithelization process (arrow) immature collagen fibers (arrow) with congestion of blood vessels (arrow). H&E, 100x.

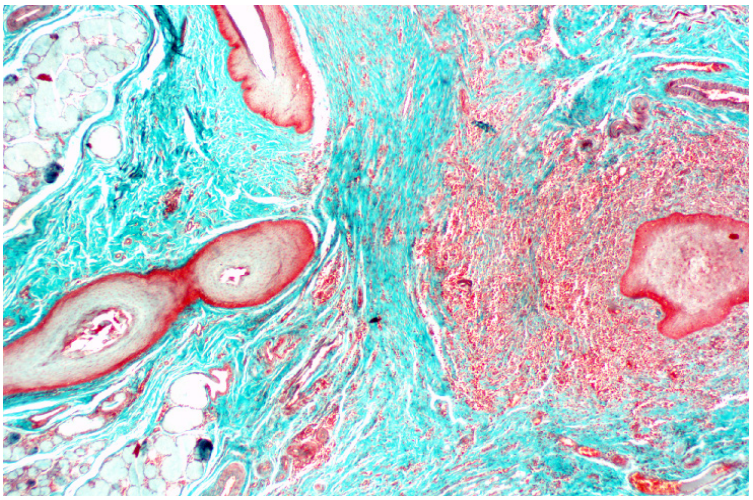

Fig.14. Micrograph at 30th P.O.Ds in MgO NPs group showed deposition of collagen fibers that take a bright green color. Masson's trichrome, 100x.

The result of Granulation tissue formation and maturation current in the 7 days post operation showed that the formation of granulation tissue was the highest in control group, while , lowest significant differences was recorded in MgO NPs group at P<0.05. A similar result was showed in the next 15 and 30 days after operation, these results indicate that the lowest amount of granulation tissue formation were recorded in MgO NPs group in compare with control group in 7, 15 and 30 days post operation Table (2).

The result of angiogenesis and newly blood vessels formation current in the 7 and 15 days post operation showed that the formation of newly blood vessels were the highest in MgO NPs group, and lowest significant differences was recorded in control group at P<0.05. These results indicate that the highest angiogenesis process was recorded in MgO NPs group in compare with control group in 7 and 15 days post operation Table (3). In contrast and in coordination with fast and perfect healing process the newly blood vessels formation were diminished in lowest pattern in MgO NPs group then in control group, this indicate the healing process was finished rapidly in MgO PNs group in compare with control and other groups at P<0.05.

The result of Re-epithelization of esophageal mucosa current in the 15 and 30 days post operation showed that re-epithelization process were the highest process in MgO NPs group, and lowest significant differences was recorded in control group at P<0.05. in contrast and in compare with the normal healing process this stage was not presence in the 7 days post operation in all groups. These results indicate that the best and fast re-epithelization process was recorded in MgO NPs group as acompare with control group in 15 and 30 days post operation Table (4).

TABLE 1. Inflammatory respond and infiltration of inflammatory cells

Group	7 days	15 days	30 days
Control	0.45 ± 0.04^{D}	1.77 ± 0.07^{D}	2.46 ± 0.09^{D}
MgONPs	2.21 ± 0.80^{A}	3.27 ± 0.84^{A}	4.91 ± 0.94^{A}

Different vertical letters mean presence of significant differences at P<0.05.

TABLE 2. Granulation tissue formation and maturation

Group	7 days	15 days	30 days
Control	1.61 ± 0.10^{A}	2.61 ± 0.21^{A}	3.75 ± 0.20^{A}
MgO NPs	0.94 ± 0.09^{D}	1.57 ± 0.10^{D}	2.71 ± 0.11^{D}

- Different vertical letters mean presence of significant differences at P<0.05.

TABLE 3. Angiogenesis and newly blood vessels formation

Group	7 days	15 days	30 days
Control	2.04 ± 0.12^{D}	2.94 ± 0.11^{D}	1.81 ± 0.14^{A}
MgO NPs	3.54 ± 0.19^{A}	4.18 ± 0.13^{A}	0.91 ± 0.09^{D}

- Different vertical letters mean presence of significant differences at P<0.05.

TABLE 4. Re-epithelization of esophageal mucosa

Group	7 days	15 days	30 days
Control	0.00 ± 0.00^{A}	1.24 ± 0.11^{D}	2.91 ± 0.51^{D}
MgO NPs	0.00 ± 0.00^{A}	2.87 ± 0.12^{A}	4.87 ± 0.54^{A}

- Different vertical letters mean presence of significant differences at P<0.05.

The result of fiberplasia and collagen fiber deposition current in the 15 and 30 days post operation showed that collagen fiber deposition were the lowest in MgO NPs group and highest significant differences was recorded in control group at P<0.05. In contrast and in compare with the normal healing process this stage was not presence in the 7 days post operation in all groups. These results indicate that the healing without fibrosis was recorded in MgO NPs group in compare with control group in 15- and 30-days post operation Table (5).

Discussion

Dog was used as a model for several surgical intervention as Repairing Achilles Tendon Defect [22] and esophageal anastomosis [23]. In the present study the decreasing of clarity of anastomotic line in G2 there was possibly related to good binding or healing between the two edges of anastomotic site that , enhanced and accelerated by the effect of MgO NPs , this result agrees with [15, 17] who that suggest MgO NPs have ability to accelerate wound healing when spread locally.

The result of histopathological investigation indicated that the using MgO NPs had beneficial value and was superior in progressing healing process in deferent periods as a compare to control . There are many factors play an important role in progress or inhibition healing process as infection during surgical operation [18], the MgO NPs play as important role in elimination of these infective factors, a wide range of nanoparticle showed to have or exeret anitbacteirla effect such as sliver, zinc, copper and magnesium, in which the MgO NPs showed to have antibacterial effect against 36 species of pathogenic bacteria included *Staphylococcus* and other pyogenic bacteria [19] The pro-angiogenic activity of MgO NPs help in the wound healing, in which MgO NPs will increase the expression of VEGF in the wound site which considered as the primary cytokine that will increase and promote the angiogenesis process and increase in the formation of newly blood vessels which considered the transporting vessels to bring more nutrients and cells that promote the healing process and lead by the end to finalized the healing process as soon as possible in compare with the normal healing events [20]. the MgO NPs showed that they have a great ability of stimulate the endotheial cells to be differentiated and proliferated and help in reepithelization faster than normal healing process, in which MgO NPs will exert direct effect on the phagocytic cells to increase secretion of IL-12 rapidly [21] .The antibacterial effect, angiogenic activity and endothelial cell stimulation properties of MgO NPs play an important role in decrease the healing process duration in addition to promote return the tissue to their normal status before surgical intervention[14, 24] , which is obtained in current study in compare with normal healing process as figured in control group.

Conclusion

We conclude the MgO NPs applied locally are accelerate healing of esophageal anastomosis and promote return the tissue to their normal status before surgical intervention

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Conflicts of Interest

There are no conflicts of interest declared by the authors.

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TABLE 5. Fibrosis and collagen fiber deposition

Group	7 days	15 days	30 days
Control	0.00±0.00^{A}	1.09±0.12^{A}	2.12±0.12^{A}
MgO NPs	0.00±0.00^{A}	0.55±0.11^{C}	0.67±0.10^{C}

- Different vertical letters mean presence of significant differences at P<0.05.

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دور جزيئات أوكسيد المغنيسيوم النانوية على استشفاء تفاغر المرئ في الكلاب : دراسة تجريبية

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هدفت الدراسة الحالية الى استقصاء دور جسيمات اوكسيد المغنيسيوم النانوية في تاثيرها على عملية التئام تفاغر المرئ في الكلاب . تم اجراء البحث على ١٨ كلبا ناضجا وسليما سريريا ومن كلا الجنسين .تم تقسيم الحيوانات عشوائيا الى مجموعتين رئيسيتين ضمت كلا منها ٩ كلاب , وكل مجموعة رئيسية تم تقسيمها وحسب الوقت بعد العملية الجراحية ب٧ و ١٥و ٣٠ يوم الى ثلاث مجاميع فرعية ضمت كل منها ٣ كلاب . خضعت جميع الحيوانات الى عملية تفاغر المرئ في الجزء الرقبي وبطريقة النهاية الى النهاية , حيث تم غلق الطبقة المخاطية للمرئ بخيط الحرير الجراحي بحجم ٠/٢ وبطريقة الخياطة البسيطة المتقطعة مع وضع العقدة الجراحية داخل تجويف المرئ , بينما , الطبقة العضلية للمرئ تم غلقها بالخيط الجراحي البوليكالاكتين حجم ٠/٢ وبطريقة المرتبة الافقية المتقطعة , واخيرا تم غلق عضلات الرقبة والجلد بالطريقة التقليدية . في المجموعة الاولى(مجموعة السيطرة) تم فقط غلق الطبقة المخاطية والطبقة العضلية للمرئ دون اضافة اي مادة على مكان التفاغر , بينما في المجموعة الثانية (مجموعة جسيمات اوكسيد المغنيسيوم النانوية) تم نشرها على موضع التفاغر وبعد الخياطة مباشرة .اظهرت نتائج الدراسة الحالية ان عملية استشفاء تفاغر المرئ كانت افضل في مجموعة جسيمات اوكسيد المغنيسيوم النانوية عند مقارنتها مع مجموعة السيطرة معنويا. اعتمادا على الدراسة المرضية العيانية و النسيجية . نستنتج من هذه الدراسة ان جسيمات اوكسيد المغنيسيوم النانوية عند اضافتها موضعيا على خط التفاغر تسرع استشفاء المرئ بالاضافة الى

تحفيز نسيج المرئ بالرجوع الى حالته الطبيعية قبل العملية الجراحية.

الكلمات المفتاحية: جسيمات اوكسيد المغنيسيوم النانوية , تفاغر المرئ , الكلاب.