GEORGIAN MEDICAL NEWS

ISSN 1512-0112

NO 11 (344) ноябрь 2023

ТБИЛИСИ - NEW YORK

ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

Monthly Georgia-US joint scientific journal published both in electronic and paper formats of the Agency of Medical Information of the Georgian Association of Business Press. Published since 1994. Distributed in NIS, EU and USA.

GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman** (**Кириллица**), для текста на грузинском языке следует использовать **AcadNusx.** Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - http://www.spinesurgery.ru/files/publish.pdf и http://www.nlm.nih.gov/bsd/uniform_requirements.html
В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректура авторам не высылается, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or compu-ter-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman** (**Cyrillic**), print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf
In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.

ავტორთა საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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Содержание:

GEORGIAN MEDICAL NEWS
No 11 (344) 2023

COMPARISON BETWEEN PRE- AND POST-OPERATIVELY BOTOX INJECTION IN SECONDARY WOUNDS HEALING

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Abstract.

Tension impacts wound healing and scarring. Tissue stress reduces blood flow and promotes fibroblastic response in wound treatment. Botox injections reduce tension. A total of 18 male albino rats weighing 250–350 grams each got 1 IU of Botox and saline injected into a subcutaneous muscle in the center of each 1.5 CM two circles with 4.5 CM a gap between them. Group A seven days pre-operatively; group B, after wound incidence. The study compared Botox-treated wounds to those treated immediately after surgery. Group A animals were surgery-ready after 7 days, removed full-thickness skin. Group B got Botox and saline in separate cages following full-thickness skin excision. Each group had three equal subgroups. Each group had immunohistochemistry tests on days 3, 7, and 14. and histological test on day 14 only, Skin biopsies following euthanasia showed significant variations between A and B groups. On day three, the group A showed increase significantly MMP-9 expression than B group. On day 7, the group A displayed a significant increase CD31 expression, suggesting significant new blood vessel development than group B. On day 14, both groups showed strong MMP-9 and CD31 expression, demonstrated greater endothelial cell, and keratinocyte proliferation resulted in very well re-epithelialization. Botox injection before surgery improved wound healing and reduced fibrosis and scarring.

Key words. Botox, Wound, CD31, MMP-9, Surgery.

Introduction.

The primary objective of local wound management is to accelerate skin physiological and anatomical continuity, which keeps wounds moist, avoids external infections, and maintains tissue homeostasis. It decreases edema and increases circulation, saving time and money while also improving the quality of life [1,2].

Botulinum toxin-induced muscle paralysis may persist 2–6 months. A to F Clostridium botulinum serotypes generate this toxin called BTX-A which has effectively treated eyelid spasms, speech stuttering, and hyperactive facial muscles for almost two decades [3,4]. Understanding these effects has made this toxin popular lately. BTX-A affected skin grafting and wound contraction in 2014 research. BTX-A injection reduces wound contraction, impacts inflammatory cells, increases collagen fibers, and reduces fat cells and hair follicles [5,6].

Park et al. examined how BTX-A affected abdominal-rectal muscle graft survival in rats with midline vertical scars in 2014. BTX-A injection improved abdominis rectus flap circulation [7]. BTX-A prevented peripheral vasoconstriction and prolonged mouse dermal flap longevity in another investigation. The BTX-A subgroup exhibited higher survival rates than the control group. The BTX-A group had good blood flow and unconstructed arteries throughout the week. BTX-A pretreatment raised flap retention and blood flow [8,9].

Since BOTOX® is commonly used for therapeutic and cosmetic purposes, our study examined how car accident wounds and other injuries heal in areas previously received Botox injections and in the regions that receive injections immediately following surgery.

Materials and Methods.

Animals: Eighteen healthy-looking male albino rats, four to six months old and 250-350 gm in weight, were divided into two main groups (n=9) according to the method and subdivided into three subgroups according to the healing periods (3rd, 7th, and 14th) days after surgery. They were isolated in clean cages at 18–22°C. They received equal quantities of grain, fruit, and vegetables and water. Researchers monitored health. The rats were separated into two groups A (pre-surgery Botox injection), with its control AC and B (post-surgery Botox injection) with its control BC. and given two wounds on their dorsum, one near the head for Botox and one near the tail for saline.

Group A include two steps procedure:

Step one of the research: we injected group A with Botox in the targeted location seven days before surgery. Animals preparation include the rats received general anesthesia (intra-peritoneal ketamine and xylazine) and each rat's dorsum was marked, shaved, and sterilize the two identical circular wounds, 1.5 cm in diameter and 4.5 cm between them [10]. BTX-A (1U) (1U=0.2ml) was injected into the subcutaneous muscle of all group A animals through the center of a circle [11] to relax the muscles (The maximum of the paralytic effect 1-4 weeks after injection) [12]. Another circle of 0.2ml normal saline was injected similarly.

Step two of the research: On the seventh day following injection, group A wounds had full-thickness skin removal (Figure 1).

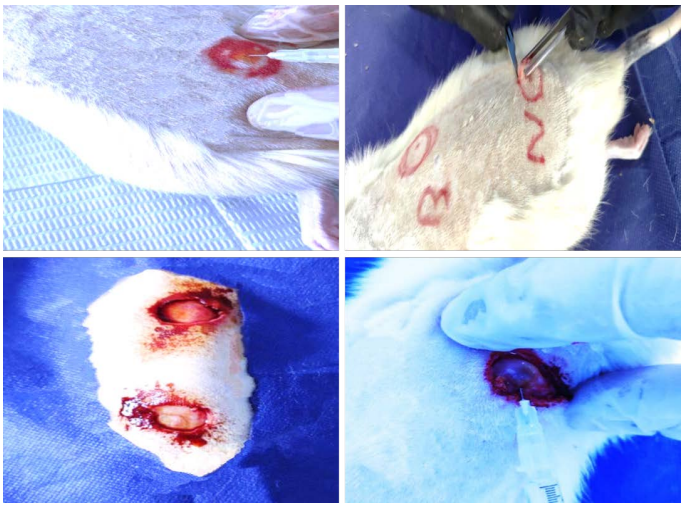

Figure 1. *Surgical procedure steps.*

In group B the same process applied here except injection of BTX-A and saline were done (post-operatively) immediately after surgery. To prevent wound infection, compression bandages were used to compress the surgical site and washed with normal saline and wiped with 10% povidone-iodine. All experimental animals were kept in separate cages. Immunohistochemical evaluation of wounds on the 3rd, 7th, and 14th and histological analysis on day 14 only of healing.

Immunohistochemical and histopathological examination: Tissue samples of the resulted wounds were taken from animals that were killed on days 3, 7, and 14. The tissue was fixed, processed, and stained with (hematoxylin and eosin), MMP-9, and CD31 [13,14].

Matrix metalloproteinase 9 (MMP-9): Matrix metalloproteinase-9 is the primary enzyme responsible for extracellular matrix breakdown in chronic wounds, inflammation, wound healing, and the release of tissue-bound cytokines and growth factors may all be monitored by measuring the levels of (MMP-9). Its expression is linked to the presence of inappropriate collagen deposition during the healing of wounds [15].

Cluster of differentiation31 (CD31): CD31 is a molecule that connects platelets to vascular endothelium. CD31's primary function in immunohistochemistry is to identify endothelial cells in tissue sections. Cell-cell adhesion is facilitated by CD31, which aids in the development of new blood vessels, it may be used as a biomarker for the degree of angiogenesis [16,17].

Results.

In immunohistochemical finding (MMP-9), on the day three group A had moderate (++) expression in the cells underneath the epidermis of wounds, both groups B and AC wounds expressed weak MMP-9 (+), two wounds of group BC had negative expression (-), and one had weak expression (Tables 1-4, Figures 2-9).

Table 1. *MMP9 and CD31 means expression intensity scores in the skin specimens from control and treatment groups of the pre-and post-surgery groups.*

Time period	Group	**MMP9** (Median)	**CD31** (Median)
3rd day	AC	1	0
	A	2	2
	BC	0	1
	B	1	2
7th day	AC	1	1
	A	2	3
	BC	1	1
	B	2	2
14th day	AC	2	1
	A	3	3
	BC	2	1
	B	3	3

Data expressed as Median of the scores, A= pre-surgery treatment group, AC= pre-surgery control group, B= post-surgery treatment group, and BC= post-surgery control group.
The scores represent: 0 (-) negative expression, 1 (+) mild positive expression, 2 (++) moderate positive expression, and 3 (+++) strong positive expression

Table 2. *Comparisons of the intensity means scores of MMP9 and CD31 immunohistochemistry expression between the control group and the treatment group within pre-surgery at the same day.*

Time period	**MMP9 (P-value)**	**CD31 (P-value)**
3rd Day	0.025*	0.068
7th Day	0.025*	0.034*
14th Day	0.025*	0.034*
Mann-Whitney U test was used for the comparisons between groups at p≤0.05. (*): Significant difference.		

Table 3. *Comparisons of the intensity means scores of MMP9 and CD31 immunohistochemistry expression between the* ***control*** *group and the* ***treatment*** *group within* ***post-surgery*** *at the same day.*

Time period	**MMP9 (P-value)**	**CD31 (P-value)**
3rd Day	0.099	0.114
7th Day	0.114	0.025*
14th Day	0.025*	0.025*
Mann-Whitney U test was used for the comparisons between groups at p≤0.05. (*): Significant difference.		

Table 4. *Comparisons of the intensity means scores of MMP9 and CD31 immunohistochemistry expression between the treatment groups within pre and post-surgery at the same day.*

Time period	**MMP9 (P-value)**	**CD31 (P-value)**
3rd Day	0.025*	0.197
7th Day	0.197	0.025*
14th Day	1	0.114
Mann-Whitney U test was used for the comparisons between groups at p≤0.05. (*): Significant difference.		

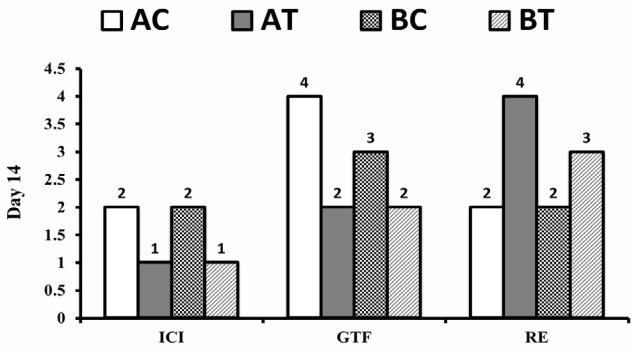

Figure 2. *The histopathological descriptive median scores of the inflammatory cell's infiltration ICI, granulation tissue formation GTF, and re-epithelialization RE of thcontrol and treatment groups of the pre- and post-surgery groups at day 14 of the study period.*

On the day seven, groups A and B wounds showed moderate expression of MMP-9, one specimen of group A showed strong expression while one specimen of group B showed weak expression, both group AC and BC showed weak expression of MMP-9 in the cells below the epidermis (Table 1-4, Figure 2-9).

On the day fourteen treatment groups had similar strong MMP-9 expression while both control groups showed moderate expression (Table 1-4, Figure 2-9).

While in immunohistochemical finding (CD31), on the day three both treatment groups displayed moderate CD31expression in two wounds, one specimen of group A had intense (+++) CD31expression and one of group B expressed light (+) (Table 1-4, Figure 2-9).

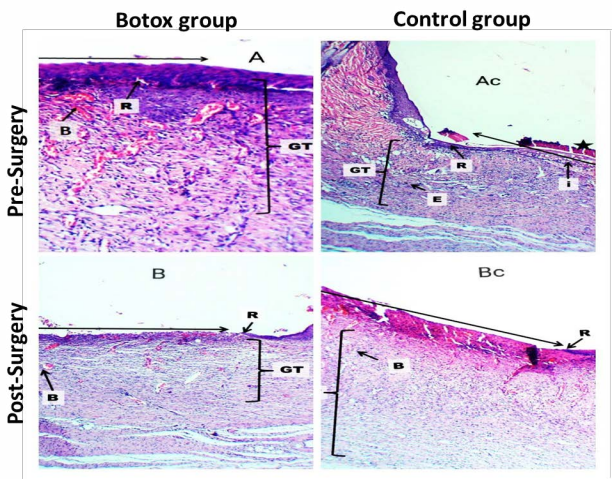

Figure 3. *Histological sections of rat skin (after 14 days) showed inflammatory cells infiltration (i), destruction of epithelium layer of skin with re-epithelialization (R) granulation tissue (GT) and newly formed blood vessels (angiogenesis) (B) fibroblast cells (E). H&E stain, 40X magnification.*

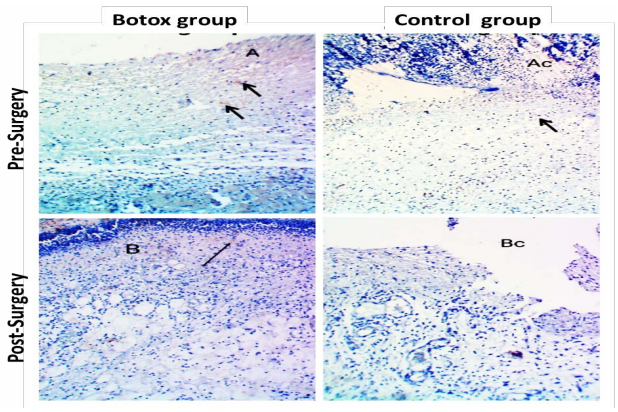

Figure 4. *Microphotograph of the wounds site of pre-surgery treatment A, pre-surgery control AC, post-surgery treatment B, and post-surgery control BC groups after three days immunohistochemistry for MMP-9 showed expression of MMP-9 of all groups in the cells below the epidermis. 100x magnification.*

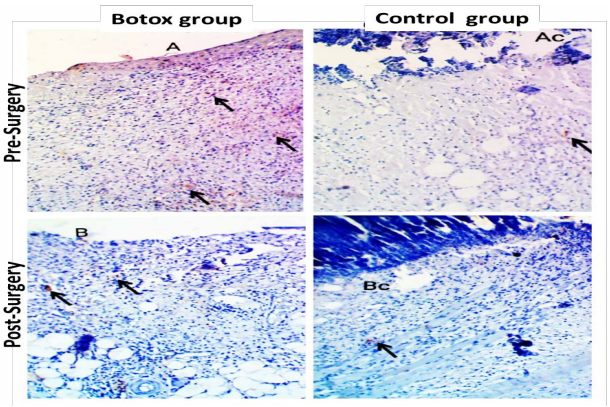

Figure 5. *Microphotograph of the wounds site of pre-surgery treatment A, pre-surgery control AC, post-surgery treatment B, and post-surgery control BC groups after seven days immunohistochemistry for MMP-9 showed expression of MMP-9 of all groups in the cells below the epidermis. 100x magnification.*

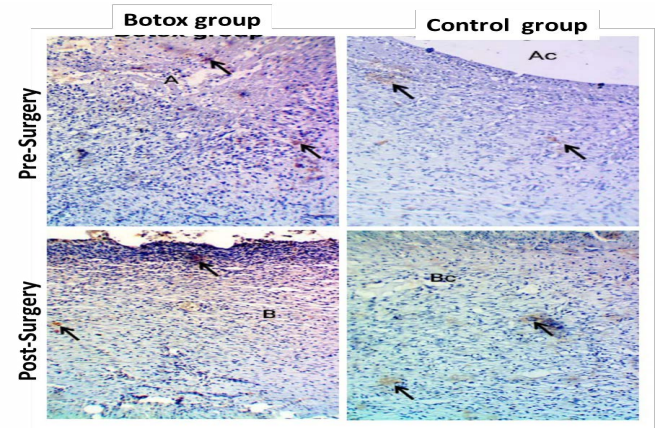

Figure 6. *Microphotograph of the wounds site of pre-surgery treatment A, pre-surgery control AC, post-surgery treatment B, and post-surgery control BC groups after fourteen days immunohistochemistry for MMP-9 showed expression of MMP-9 of all groups in the cells below the epidermis. 100x magnification.*

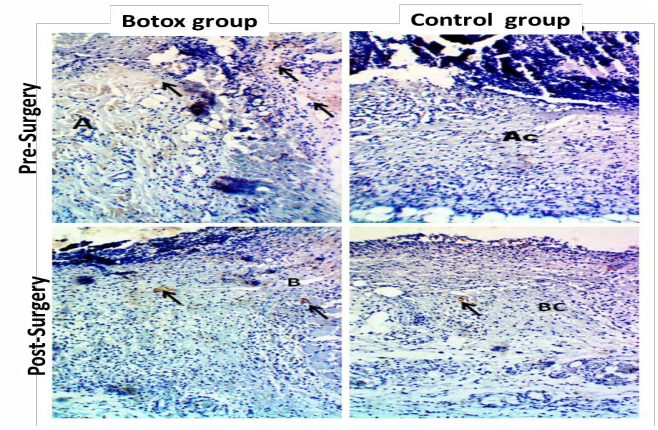

Figure 7. *Microphotograph of the wounds site of pre-surgery treatment A, pre-surgery control AC, post-surgery treatment B, and post-surgery control BC groups after three days immunohistochemistry for showed CD31 expression of angiogenesis for all groups in the cells below the epidermis. 100x magnification.*

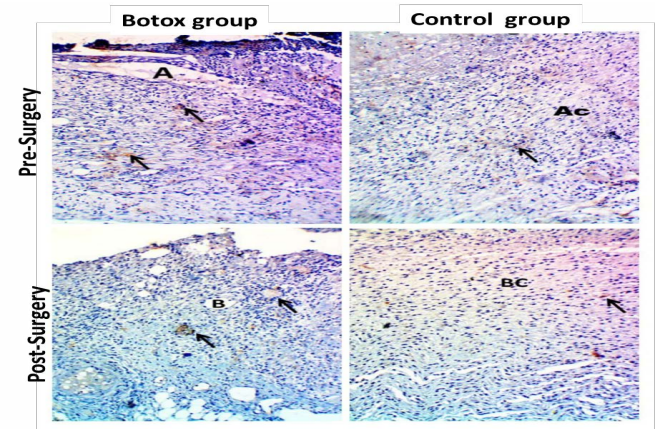

Figure 8. *Microphotograph of the wounds site of pre-surgery treatment A, pre-surgery control AC, post-surgery treatment B, and post-surgery control BC groups after seven days immunohistochemistry for showed CD31 expression of angiogenesis for all groups in the cells below the epidermis. 100x magnification.*

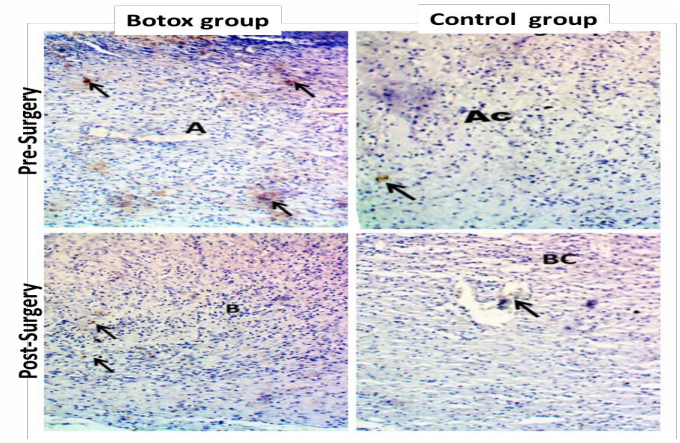

Figure 9. *Microphotograph of the wounds site of pre-surgery treatment A, pre-surgery control AC, post-surgery treatment B, and post-surgery control BC groups after fourteen days immunohistochemistry showed CD31expression of angiogenesis for all groups in the cells below the epidermis. 100x magnification.*

The BC group showed light CD31 expression in all specimens while explained group AC absent (-) expression in two wounds and light expression in one of them (Table 1-4, Figure 2-9).

On the day seven, AC group wounds expressed light CD31protein. One species lacked wound expression. Group A wounds showed strong CD31 protein expression. BC group wounds showed light protein expression in all wounds. B group wounds showed moderate CD31 protein expression in cells below the epidermis (Table 1-4, Figure 2-9).

After 14 days, AC group wounds revealed light expression of CD31 protein while one specimen displayed moderate expression in the wound field. Group A wounds, cells below the epidermis expressed intense cd31 protein. BC group wounds displayed light cd31 protein expression. B group wounds revealed moderate production of cd31 protein in cells below the epidermis, whereas one specimen displayed intense expression (Table 1-4, Figure 2-9).

The histological finding on the day fourteen groups A and B had no inflammatory cell infiltration. Only one specimen demonstrated low wounds field inflammatory cell infiltration. Groups AC and BC showed minimal wounds field inflammatory cell infiltration (Table 1-4, Figure 2-9).

Angiogenesis and fibroblast cells in granulation tissues were limited in all groups A and B wounds. Group AC specimens have extensive wounds granulation tissue development. Group BC wounds had moderate granulation tissue development (Table 1-4, Figure 2-9).

Group A specimens demonstrated full-field re-epithelialization. Group B specimens demonstrated uneven re-epithelialization over the wounds field. AC and BC specimens demonstrated re-epithelialization over half of the wounds field (Table 1-4, Figure 2-9).

Discussion.

Reduce tension in large wounds that are too large for suturing is very important [18]. Tension lowers blood flow and stimulates fibroblastic response, affecting wound healing and scarring [19,20]. Muscular contracture elimination can get better skin wounds. Botulinum toxin type A paralyzes striated muscle for 2–6 months. For almost 20 years, people have safely utilized it in therapeutic setting [21]. On day 14, H.&E. stain showed that all groups had less inflammatory cells due to normal healing process [18].

Group A had better re-epithelialization and skin regeneration than group B because BTX-A injection before skin surgery reduces sympathetic nor-epinephrine production, improving circulation and wound healing [22].

A and B wound healing and re-epithelialization were better than AC and BC the reason was keratinocyte and endothelial cell migration and proliferation increased blood vessel sprouting, re-epithelialization, and remodelling after one IU Botox injection agreed with [22,23].

The 3rd day immunohistochemical study with cluster of deffentiotion (CD31) showed no significant difference between groups because angiogenesis occurs mostly during wound healing's proliferation phase [24]. Group A had a significant increase in cd31 expression on day 7 because BTX-A releases proangiogenic cytokines, such as VEGF, that stimulate endothelial cell proliferation, migration, and differentiation (angiogenesis) from the wound site's preexisting vessel bed [18,25]. The two groups reached the peak of BTX-A impact on the 14th day, therefore cd31 increased but was insignificant [12]. After 3 days, group A had a significant increase in MMP-9 gene expression compared to groups B, BC, and AC due to the impact of BTX-A on wound healing through elevated MMP-9 levels, which have anti-inflammatory properties. MMP-9 gene expression was similar in groups B and BC due to near BTX-A administration time. On day 7, the median scores of groups A and B are not significantly different due to BTX-A effectiveness convergence [12].

MMP-9 modulates ECM breakdown and deposition, fibroblast activity, and collagen networks to prepare wounds for re-epithelization, hence treatment groups had more MMP-9 gene expression than controls [26,27]. On day 14, both treatment groups had similar MMP-9 expression and a significant increase compared to control groups because BTX-A affects wound MMP-9 levels, the MMP-9 secreted by keratinocyte cells at this stage, which alter keratinocyte migration and proliferation from the stratum Basale layer of the epidermis in wound edges, resulting in re-epithelization and skin regeneration. BTX-A reduces wound border tension by denervating underlying muscles, improving wound healing [22,28-30]. In addition to these aforementioned variation in the response could be also associated with the variation in the status of localized milieu of oxygen supply, immunomodulatory cytokine, cellular structure and behviours [31-33].

Conclusion.

The results revealed that both the procedures showed significant improvement of secondary intention wounds and gave a satisfactory result of healing acceleration in terms of re-epithelization and skin regeneration. We can recommend that the Botox injection may be considered as an alternative modality of therapy to enhance wound healing. The best method is Botox injection prior to the wound incidence.

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