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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

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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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INCUOLATION THE BOTULINUM TOXIN-B IN THE ZYGOMITICUS OF THE RAT, FOLLOWED BY EVALUATION IT'S EFFECT HISTOLOGICALLY ON THE ZYGOMATIC BONE

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Abstract.

Background: Botulinum toxin is an attenuated neurotoxin of Clostridium Botulinum gram positive bacterial, which is used in medication sialorrhea, cervical dystonia, hyperhidrosis and non-surgical cosmetic operation (aesthetic) such as facial wrinkles and reduced the bulky appearance hypertrophied of masseter muscle. This study was designed to revealed the effect of zygomiticus inoculation of botulinum toxin B in zygomatic muscle of rats on zygomatic bone. **Methods:** A total of 25 male albino rats (200-260 gm) were injected facial intramuscular by a single dose of 2.5 U/ botulinum toxin B. All experimental groups (4 groups, 5 each) were kept survived to the end period and sacrificed by time period [group 1 (G1)=14; group 2 (G2)=30; group 3 (G3)=45, and group 4 (G4)=60 days] post injection, moreover, 5 animals were administered nothing and kept as a control group till 60 days elapsed. Animals were killed by intense dose of chloroform and rats facial zygomatic bone removed. **Results:** Zygomatic bone of G1 showed irregular boney border, degenerated osteocytes, woven collagen bundles within collar bone, reddish bone matrix and detachment of fibrous layer of periosteum in G2 results showed disappearance of osteogenic cells, lacunae devoid osteocytes, and aggregated of necrotic elements of bone tissue. In G3 groups showed great crack, disorganization of collagen bundles atrophied osteocytes the matrix of bone had necrotic areas of osteocytes, fragments of bone also demonstrated. G4 group showed massive crack underneath degenerated periosteum of bone, tunnel like furrow, filled up with cellular debris and osteogenic debris within Howship's lacunae. **Conclusion:** In this present study we summarized the effect of paralyzed facial muscle by botulinum toxin B on facial bone of rats induced, secondary osteoporosis represented as irregular bone border degeneration of osteocytes, crack appeared in bony matrix, decreases in amounts of collagen bundles and separation of periosteum.

Key words. Botulinum toxin B, Rats, zygomatic bone, periosteum.

Introduction.

Botulinum neurotoxins (BoNT) are regarded to be the most toxic materials are responsible for human botulism [1]. Aesthetic treatment with BoNT for cosmetic purposes was fast increasingly [2]. The BoNT/B is used for cervical dystonia, hyperhidrosis and facial wrinkles [3]. Botulinum neurotoxin inhibits released of neurotransmitter acetylcholine production from motor neuron cells, this inhibition mechanism of neurotransmitter released was effect on neurochemical synapsis in between axon knop and skeletal muscles sarcoplasm which finally led to humans' muscle flaccid paralysis persist about 4-6 months [4].

Long term treatment of wrinkles caused by muscular contraction with using of BoNT given worse results [5]. Adequate dose of BoNT/A causes rapid muscle atrophy [6]. when using Botulinum toxin B in medication sialorrhea treatment, showed an increase in the incidence of xerostomia and dysphagia as side effects when compared with botulinum toxin A [7]. The atrophied skeletal muscle induced with spinal cord nerve injury effect on bone mineralization [8]. Multi-injection of BoNT within the masseter muscle of rabbit, leads to muscle atrophy and temporary changes in mastication force [9]. Bone was a highly vascularized, mineralized and homeostasis tissue [10]. The muscular skeletal system retains a tight regulation in between muscles contraction which released a biochemical molecules that participate in bone homeostasis [11]. The goal of current experiment to elucidate histological alteration in zygomatic bone induced by inoculation of botulinum toxin-B in zygomiticus of albino rats.

Materials and Methods.

The whole experimental study was done in the surgical lab of veterinary medicine college of Tikrit university from March 2024 until August 2024.

Animals: Twenty-five adult male albino rats weighing 200-260 gram were used in the experiment, were kept under appropriate environmental conditions of 21-25ºC and photoperiod, were allowed free access to food and water. Before the experiment animals were randomly distributed to (5) groups and housed in steel cages (1.250 × 0.5 ×0.5) meter. The 25 animals were classified into 5 groups:

Control Group: 5 animals were administered nothing and kept as a control group till 60 days elapsed, then sacrificed.

Group 1 (G1): 5 rats were injected facial intramuscular by a single dose of 2.5 U/ botulinum toxin B were kept survived until day 14 then sacrificed.

Group 2 (G2): 5 rats were injected facial intramuscular by a single dose of 2.5 U/ botulinum toxin B were kept survived until day 30 then sacrificed.

Group 3 (G3): 5 rats were injected facial intramuscular by a single dose of 2.5 U/ botulinum toxin B were kept survived until day 45 then sacrificed.

Group 4 (G4): 5 rats were injected facial intramuscular by a single dose of 2.5 U/ botulinum toxin B were kept survived until day 60 then sacrificed.

Animals were killed by intense dose of chloroform and rats facial zygomatic bone removed. The animals were kept for at least 14 days for adapt before the study start. The design of study was done to assessment intramuscular inoculation of botulinum toxin-B in zygomiticus muscle of rats on zygomatic bone. Rats were administrated by a single dose of BTXB 2.5 U. by using 25-gauge 1.0 cm needle. The animals were left for

life at different periods as followed five rats in each group, G1 – scarified after 14 days of inoculation, the G2. scarified after 30 days post injection, the G3. scarified after 45 days post injection, the G4 - scarified after 60 days post injection.

All animals were survived for their end periods and then scarified by using sufficient of inhalation inhaled chloroform inside sealed glass box. The animals were dissected, and specimens' zygomatic bone for both experimental and control groups were taken and immersed in 10% neutral buffer formalin for 48h for fixation followed de-calcification in acetic acid and nitric acid respectively for one week and cut into smallest possible size. The decalcified bone tissue was prepared for histological technique, followed by immersion in graded transfer of alcohol from (70, 80, 90 and 100) %, then clearing with xylene and embedded in paraffin wax at (60)°C. Blocking of the samples were done, and the sectioning were performed with (5µm) thickness. After staining with Hematoxylin and Eosin (HE stain) and Masson's trichrome (MT stain), ribbon was mounted on the slides by D.P.X and covered by cover slips. The slides were examined by using light microscope and photographed by manipulated camera prepared for this purpose.

Results.

Sections of control group stained with HE stain showed, bone matrix, osteocytes detected in there were lacunae and normal distributed of its around haversian canals (osteon). Bone tissue sample stained with MT stain, revealed reddish bone matrix, gray color collagen bundles (Figure 1).

The results of group1 stained with HE stain showed irregular boney border, degenerated osteocytes within lacunae, bone marrow cells inside trabeculae, woven collagen bundles within collar bone, atrophied osteocytes, lack of osteoblast cells,

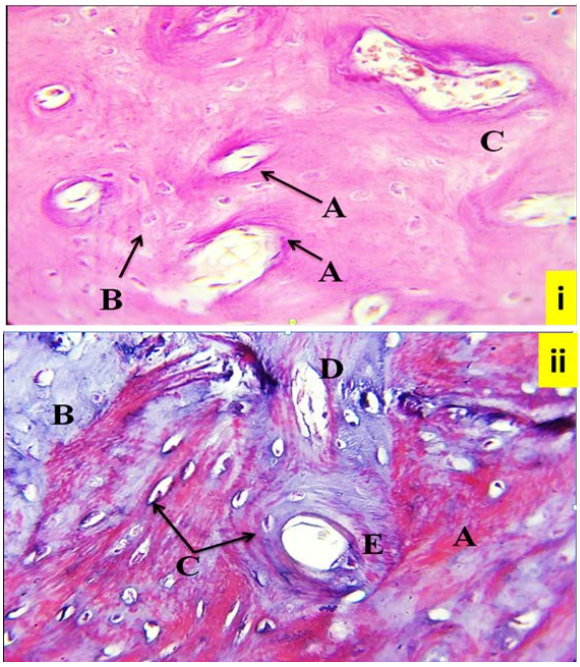

Figure 1. *Rat zygomatic bone control group. (i) Matrix of bone, haversian canals (A) osteocyte in lacunae (B) and blood vessel (C) (HE stain, 40X). (ii) Bony matrix (A), collagen bundles (B), osteocyte in lacunae (C) and blood vessel (D), great vacuoles for haversian canals (E) (MT stain, 40X).*

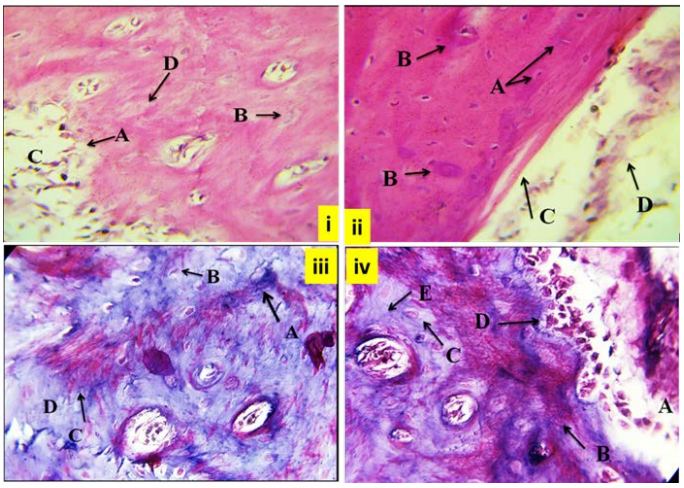

Figure 2. *G1 group, (i) showed an irregular bone border (A), degenerated osteocytes (B). bone marrow cells (C) woven collagen bundles in collar bone (D). (ii) bone tissue, atrophy of osteocytes (A), basophilic area(B) absence of osteogenic cells (C), separating of fibrotic layer of periosteum (D) (HE stain, 40X). (iii) cartilage remnant (A), degenerated osteocytes (B), reddish bone matrix (C), degeneration of collagen fibers (D). (iv) ill- defined endosteum (A), increased reddish bone matrix (C), degenerated osteocytes (C), aggregate of osteoclast cells (D), collagen fibers (E). MT stain, 40X).*

basophilic area underneath detachment of fibrotic layer of periosteum (Figure 2).

The results of group 1 samples stained with MT stain, showed cartilage remnant, degenerated osteocytes, reddish bone matrix, degeneration of collagen fibers. Other sections showed ill-defined endosteum, increased reddish bone matrix, degenerated osteocytes, aggregate of osteoclast cells within Howship's lacuna (Figure 2).

The results of G2 group stained with HE stain, revealed, separated fibrous coat of periosteum with disappearance of osteogenic cells or osteoblast, basophilic area and lacunae devoid osteocytes. Bone tissue irregular border of bone, with aggregated of necrotic elements of bone tissue, atrophied osteogenic cells, lacunae with atrophied osteocytes (Figure 3).

The results of group G2 samples stained with MT stain, reddish bone matrix, decreased of collagen bundles amount, lacunae devoid osteocytes, crack within collar bone (Figure 3). Other sections showed, remnant cartilage and chondrocytes within bone matrix, expanded lacunae, irregular border of haversian canal (Figure 3).

The results of G3 group stained with HE stain, showed, great cavities (crack) and sinuses within bone matrix, degeneration of osteocytes within lacunae has been detected, degeneration of periosteum and devoid of osteogenic cells. Other section revealed necrotic area in collar bone with atrophied osteocytes within lacunae, degenerated and detached bony mass, degenerated fibrous layer of periosteum. Disorganization of collagen bundles, necrotic of osteogenic cells layer of endosteum, expanded lacunae with degenerated osteocytes, separated of periosteal collagen bundles with atrophied osteogenic cells, degeneration in collar bone (Figure 4).

The results of group G3 samples stained with MT stain, showed increased in bone matrix, degeneration of fibrous layer

of periosteum with devoid osteogenic cell. crack in collar bone, lacunae devoid osteocytes, degeneration of osteogenic cells, necrosis in collar bone (Figure 4).

The results of group G4 stained with HE stain showed bone tissue, massive crack underneath degenerated periosteum of bone, with aggregate of cellular debris, lacunae devoid osteocytes. Necrotic area within collar bone, massive cavities with a few cellular debris aggregations within it, ill- defined nuclei of osteocytes were showed in lacunae. Other section showed irregular bony border with tunnel like furrow, filled

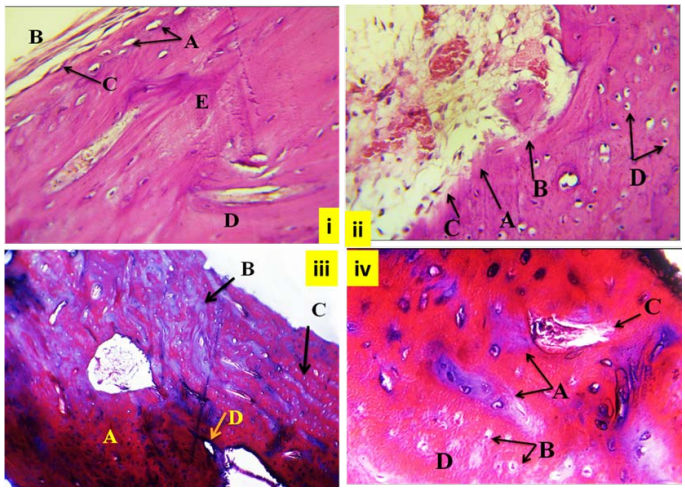

Figure 3. *Group G2, (i) Empty lacunae (A), separated fibrotic layer of periosteum (B), disappearance of osteoblast cells (C), Volkmann with micro blood vessels (D), basophilic area (E). (ii) irregular boney border (A), necrotic cells of bone tissue (B), atrophied osteogenic cells (C), lacunae contained a mini osteocytes (D) (HE stain, 40X). (iii) G2 group, reddish bone matrix (A), decreased of collagen bundles amount (B), lacunae devoid osteocytes (C), crack in collar bone (D). (iv) remnant cartilage (A), lacunae of small osteocytes (B), irregular border of haversian canal (C), bone matrix (D). (MT stain, 40 X).*

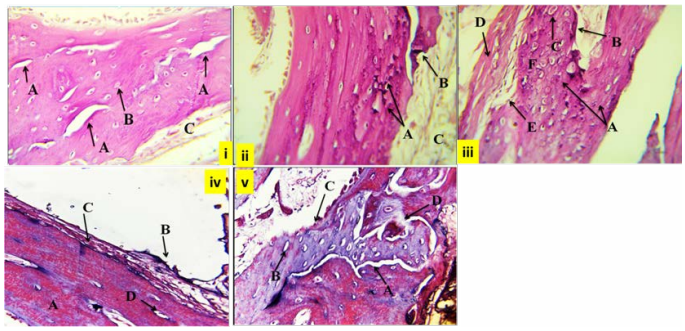

Figure 4. *G3 group, (i) bone tissue, great crack in boney matrix (A), degeneration of osteocytes (B) devoid of osteoblasts and degeneration of periosteum (C). (ii) necrotic area and atrophy of osteocyte cells within lacunae (A), degenerated and detached bony mass (B), degenerated fibrotic layer of periosteum (C). (iii) G3 group, disorganization of collagen bundles (A), necrotic cells (B), expanded lacunae with degenerated osteocyte (C), separated of periosteal collagen bundles (D), atrophied osteogenic cells (E) degeneration in collar bone (F) (HE stain, 40X). (iv) increased in boney matrix (A), degeneration of periosteum (B), devoid osteogenic cells (C), newly haversian canal (D). (v) crack in collar bone (A), lacunae devoid osteocytes (B), degeneration of osteogenic cells (C), necrosis in collar bone (D). (MT stain, 40 X).*

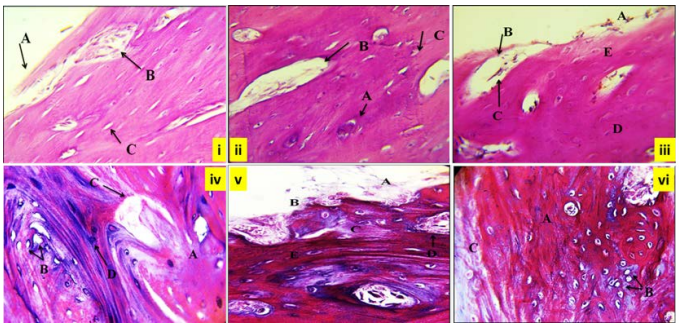

Figure 5. *G4 group, (i) partial detachment of boney matrix (A), cellular debris (B), lacunae devoid osteocytes (C). (ii) boney matrix remnant of necrotic debris (A), great cavities with necrotic material (B), lacunae with osteocytes and ill-defined nuclei (C). (iii) irregular boney border (A) tunnel likes furrow (B), necrotic cells (C), atrophied osteoblasts (D), degeneration of collagen bundles (E) (HE stain, 40X). (iv) crack in collar bone (A), lacunae devoid osteocytes (B), colloid in crack (C), disorganization with degeneration of collagen bundles (D). (v) osteogenic debris within Howship's lacunae (A), irregular bone border (B), degeneration of collagen bundles (C), haversian canal(D), and increased boney matrix (E). (vi) increased boney matrix (A), lacunae devoid osteocytes (B), degeneration of collagen bundles (C). (MT stain, 40 X).*

up with cellular debris, atrophied osteoblasts layer devoid periosteum, and degeneration of collagen bundles within bone matrix (Figure 5).

The results of group G4 samples stained with MT stain showed crack in collar bone, lacunae devoid osteocytes, colloid material in crack of collar bone, disorganization with degeneration of collagen bundles. osteogenic debris within Howship's lacunae, irregular bone border, degeneration of collagen bundles, increased of boney matrix. increased of boney matrix, degeneration of collagen bundles and lacunae devoid osteocytes (Figure 5).

Discussion.

The Botox are used as therapeutic numerous clinical condition and Aesthetic indications [12]. Reduced the bulky appearance hypertrophied of masseter muscle [3]. In current study was designed to reveal the role of botulinum toxin B on the facial zygomatic bone of rats. The bone was undergoing constant bony changes (remodelling), with frequent events changes in between osteoclasts and osteoblasts [13,14]. Osteoblasts produced osteoid [15]. This relationship maybe explained how these compensatory mechanisms to retain the bone strength in case of bone loss [16]. Gajraj (2005) showed that the period of flaccid paralysis induced with botulinum toxin-A was extend about 8 weeks linked with neural [17].

The histological examination of G1 group showed, that irregular boney border, degenerated osteocytes within lacunae, bone marrow cells inside trabeculae, woven collagen bundles within collar bone. The presence of multi- scattered irregular bone border cavities situated by a group osteoclast there is evidence of osteoporosis [18]. MT stain, of G1 group showed cartilage remnant, degenerated osteocytes, reddish bone matrix, degeneration of collagen fibers. The present results in agreed with El-Yamany et al. (2024), who showed that a few lacunae devoid osteocytes following, abnormal collagen with decreasing

in the collagen fibers deposition and distribution within bone matrix [19].

The results of G2 group revealed separation of periosteal fibrous coat of with disappearance of osteoblast. Warden revealed the number of osteoclasts increased by double the number post Botox-muscular injection 5 days these number increasing induces rapid bone degradation and giving a maximal decrease in bone mass through 2– 4 weeks [19]. The results of G3 group, showed, great crack, necrotic area in collar bone and disorganization of collagen bundles. The MT stain showed decreases in collagen bundles in bone matrix, degeneration of fibrous layer of periosteum in compared with control group. Periosteum is a connective tissue which covered the boney surfaces and is closely connected to the bone cortex. The periosteum represented into two layers: outer fibrous layer with and inner layer contain an osteogenic [20]. Periosteum highly vascularized tissue convey minerals, oxygen, and other substances needed to reconstitute bone tissue [21].

The results of group G4 stained showed bone tissue, massive crack underneath degenerated periosteum, with aggregation of cellular debris and appeared a necrotic area within collar bone. The results of MT stain showed crack in collar bone, lacunae devoid osteocytes, colloid material in crack of collar bone. Botulinum toxin significantly inhibit bone formation at both the periosteum and endosteum cortical surfaces [22].

Results obtained from the current study showed the histological alterations increased throughout the length of the experimental period. Ho Dang 2013 declare the injection of 10 U BTX in masseter muscles of rabbits reduced a cortical thickness and fewer trabeculae of condyle bone, these differences prolong post 12 weeks and announced this boney alteration due to changing in muscular loading [23]. Dutra et al. (2016) confirmed that the injection of botulinum toxin within masseter muscle effect on mandibular bone mineralization and matrix formation a decrease in the osteoblast cells activities [24]. Botulinum toxin A effect on formation calluses in fractured femur bone of rat, which convert the calluses into woven bone, the main causes of this boney defect due to atrophied quadriceps and failed to generate a sufficient loudening force on bone [25].

Type I collagen is the most abundant type of collagen in bones play a role in its toughness [26]. The main reason of osteoporosis was bone de-mineralization [27,28], that may reduce bone stiffness and may be associated with a collagen fibrillogenesis and modification of the content of collagen crosslinks [29]. The twice injection of 20 and 25 units of BTX in temporalis and superficial masseter muscle of volunteers are reduced masticatory force and may induced degradation of the cortical and trabecular bony areas of the condylar bone through 6 months [30]. Skeletal muscles in serve the intact bone, the muscular loading produced a mechanical force which sensed on the cellular membrane of osteocyte is transduced into anti-apoptotic signals mediated by integrins, focal adhesion kinases, and several cytoskeletal proteins [30].

Takata and Yasui (2001), who declare that botulinum toxin induced a flaccid paralysis (disuse muscle) was associated with an imbalanced bone remodelling process through an increasing in bone resorption and decreasing within bone composition [31]. Ashley et al. (2007) announced that contracted muscles participate in vascular pump, denervated muscles loss their ability in bumping blood, with losing vasoconstrictor of nerve fibers, which finally affecting on muscle and bone vascular blood activity [32]. The indirect effect of botulinum toxin inoculation of hind limb muscle on bone led to significant decreasing in mineralization and density of bone this effect when in compared with the direct effect of botulinum toxin such as decreases in muscle force contraction muscle atrophy which affects more on bone maintenance. The innervation of bone tissue plays a major role in bone homeostasis [33]. The sensory neuropeptide receptors in boney osteoblasts revealed the direct communication in between osteoblasts and neurons [34].

The botulinum toxin role mechanism inhibits of acetylcholine (ACH) release in between nerve ending synapsis. Therefore, acetylcholine level, can participate in bone protection, so the toxin-induced osteoporotic [35]. Botulinum neurotoxin inhibits released of neurotransmitter acetylcholine production from motor neuron cells, this inhibition mechanism of neurotransmitter released was effect on neurochemical synapsis in between axon knop and skeletal muscles sarcoplasm which finally led to humans' muscle flaccid paralysis persist about 4 – 6 months [4].

Deng et al. (2021) showed that the botulinum toxin injection and the neurectomy of hind limb induced decreases in trabecular bone formation and higher osteoporosis [36]. The immobilized limb of experimental animals showed the increasing activity of osteoclast, and the serum ALP (a marker of bone formation) is decreased, which reflect the bone osteoporosis by inhibition of osteoblastic activity [37]. Numerous factors are released from contracting muscle (VEGF, TGF-β, IL-6, TNF-α, substance P, and glutamate) may also play a major role in bone homeostasis. The botulinum toxin paralyzed skeletal muscles and block the surge of these factors [38]. Inhibiting endothelial growth factor VEGF secretion was effect on osteocyte-mediated angiogenesis [39].

Conclusion.

The histology of the control group has shown the normal bone architecture while the treated rat group revealed that continuous progressive bone and collagen degeneration, necrosis, and architectural abnormal structure.

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