International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG(USA) Volume 6, Issue 2 - 2019

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2019.06.02.012

Condylar cartilage degradation as response to single dose Botulinum toxin - A injection in masticatory muscles of young rabbits

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Abstract

Botulinum toxin is a neurotoxin derived from anaerobic fermentation of various strains of spore-forming bacillus Clostridium botulinum. Injection of the toxin in masticatory muscles induces muscle weakness and paralysis. Reviews of clinical studies have shown improvement in the symptoms of temporomandibular joint disorders, myofascial pain, tension headache, and chronic migraines. The present work aimed to evaluate the effect of injection of single dose of botulinum toxin A in masseter and temporalis muscles on condylar cartilage of rabbits. Thirty two young adult rabbits were divided into two main groups. Control group (the saline rabbits) contained eight rabbits and received 0.2 ml saline injections divided equally on masseter and temporalis muscles. Experimental group (botulinum toxin A injected rabbits) contained twenty four rabbits and allocated equally into three subgroups (A, B & C). Rabbits in experimental subgroups received single dose of BTX-A injection in masseter and temporalis muscles. The work was terminated after two six and twelve weeks for subgroups A, B and C respectively. Results showed dark chondrocytes with condensed chromatin and large number of empty lacunae in experimental subgroups A & B. Thickness of condylar cartilage was found to be significantly decreased in time dependant manner from experimental subgroup A to C.

Keywords: Botulinum toxin A, masseter muscle, temporlais muscle, condylar cartilage

Introduction

Botulinum toxin (BTX) is a large complex protein having eight distinct neurotoxin serotypes (A,B,C,D,E,F,G,H). The toxin is produced by obligate anaerobic bacteria Clostridium botulinum **(Scaglione, 2016)[35].** Among the group of its serotypes, botulinum toxin type A (BTX-A) is the most potent one **(Miller & Clarkson E, 2016)[27].** It has the most sustained action in laboratory animals and human beings **(Keller & Neale, 2001[22] ; Foran et al., 2003[16])**. BTX-A consists of 150 kDa neurotoxin itself and a number of neurotoxin associated complexing proteins (NAPs) **(Inoue et al., 1996[20] ; Sharma, 2003[36]).**

Currently BTX-A in purified and complex forms are both available in therapeutic and cosmetic applications to treat neuromuscular disorders. Xeomin (incobotulinumtoxin A, Merz Pharmaceuticals, Germany) is free from complexing proteins while Botox (onabotulinum toxin A, Allergan, USA) contains NAPs.

On cholinergic nerve terminals, BTX-A neurotoxin binds to specific glycoprotein structures. Thus the toxin has high selectivity docking action for cholinergic synapses. After internalisation, the neurotoxin binds to the N-ethylmaleimide-sensitive

factor attachment protein receptor (SNARE). BTX- A cleaves synaptosomal-associated proteins of 25 kDa (SNAP-25). Proteolytic cleavage of the SNARE protein complex prevents the docking of the acetylcholine vesicle on the inner surface of the cellular membrane resulting in blockade of vesicle fusion. Chemical denervation occurs together with reversible paralysis of the target muscle that lasts for 4 – 6 months in humans **(Dressler et al., 2005)[12].** The complexing proteins NAPs by themselves have no role in the intracellular biochemical process involved in the blockade of neurotransmitter release **(Wang et al., 2014)[39].**

Commercially available forms of BTX-A are increasingly used by clinicians. Local injections of the toxin were used for the treatment of strabismus, muscle spasm or hyperactivity such as cerebral palsy **(Koman et al., 2003)[23],** several pain syndromes such as myofacial pain **(Porta, 2000)[31]** and treatment of dynamic facial wrinkles for cosmetic demands **(Carruthers et al., 2004)[6].**

Conditions that affect masticatory muscles such as benign masseter hypertrophy are treated with intramuscular injections of BTX-A **(Miller & Clarkson, 2016[27] ; Chen et al., 2015[8]).** For temporomandibular joint disorders (TMDs), BTX injection targets the muscles of mastication. The masseter and temporalis muscles are the most common muscles injected **(Dutt et al., 2015)[14].**

BTX- A was used to manage myofacial pain syndrome (MPS) symptoms in ten patients. They were injected with BTX-A in masseter and temporalis muscles extra orally. Results were evaluated 1, 2, 3 and 6 months after the last injection. The study showed that BTX-A decreased the severity of symptoms and improved functional abilities of TMJ because of its action as muscle relaxing agent **(Zayed et al., 2015)[41].**

In cartilage degenerative process cytokines produced by both synovial cells and chondrocytes, are key mediators of cartilage destruction. Interleukins induce catabolic process involving many proinflammatory enzymes, including the cyclooxygenases (COX). There are two isoforms of COX, COX-1 and COX-2, in mammals which catalyze the conversion of arachidonic acid to prostaglandins (PGs). COX enzymes catalyze the formation of prostaglandin H2 (PGH2) from arachidonic acid, followed by the isomeration of PGH2 to prostaglandin E2 (PGE2) **(Hardy et al., 2002)[18].** PGs are considered one of the most potent inflammatory mediators. Elevated PGs levels in the synovial membrane and fluid play critical role in the development of vasodilatation, fluid extravasation, and pain in synovial tissues. Moreover, there is increasing evidence that PGs (especially PGE2) are mediators involved in articular cartilage erosion and degradation **(Fattahi & Mirshafiey, 2012)[15].**

The effects of BTX-A in masticatory muscles include paralysis, weakness, and atrophy. Much less studied are the side effects that could affect condylar cartilage. The present study aimed to evaluate the effect of injection of single dose of BTX-A in masseter and temporalis muscles on condylar cartilage.

Materials and Methods

A total number of thirty two young adult healthy male New Zealand White (NZW) rabbits weighing 2-2.5 kg were used in this study. Animals were housed in separate cages at the Faculty of Agriculture, Minia University under the optimal experimental conditions. They had free access to food and ad-libitum water for the whole experimental period. All experiments were carried out in accordance with research protocols established by the animal care committee of the National Research Center, Egypt, which follows the recommendations of the National Institutes of Health Guide for Care and use of Laboratory Animals (Publication No.85-23, revised 1985).

Botox

Botox (Botox®; Onabotulinumtoxin A; Allergan, 100 U) was purchased from Allergan Scientific Office 53 El Sheikh Mohamed, El Nadi Street, Nasr City, 6th Zone, Cairo, Egypt.

For the injections, Botox was reconstituted using 2 ml of 0.9% saline per 100U, which means that the concentration of Botox solution was 5U per 0.1 ml **(Park et al., 2015)[30]**. Injection was made using 28-gauge, 0.5 inch needles on a 1 ml insulin syringe.

Experimental design:-

Rabbits were randomly divided into two main groups as following:-

Control group:

The control group included 8 rabbits received single unilateral masseter and temporalis injection of 0.1 ml

of 0.9% saline in each muscle. Injection was done in the middle of the superficial part of masseter muscle (selected among three separate injection sites) and in the middle of temporalis muscle **(Rafferty et al., 2012[33]; Park et al., 2015[30]).**

Experimental group:

The experimental group included twenty four rabbits divided equally and randomly into three subgroups (A, B and C). Each subgroup contained 8 rabbits. Animals in experimental subgroups received the same volume of BTX-A (0.1 ml saline containing 5 units of BTX-A) injected in the same locations as controls. Rabbits were sacrificed after two weeks in subgroup A, six weeks in subgroup B and twelve weeks in subgroup C.

Histological procedures:

The mandibular condyle specimens were cut through the condylar neck 1cm below the head and parallel to the occlusal plane. The specimens were fixed in 10% neutral buffered formalin then demineralized using 10% ethylene diaminetetracetic acid (EDTA) solution at pH 7.8 in a microwave oven **(Wallington, 1972[38]; Page, 1996[28]).**

The demineralized condylar specimens were washed by tap water and prepared for histological investigation by dehydration using ascending grades of alcohol. Specimens were then cleared from alcohol with xylene followed by infiltration with paraffin in constant temperature oven (60 ℃) for 2-3 hours. Condyle specimens were then removed and placed in the center of the box of melted paraffin, the bottom of which was the surface of cutting. Condyle specimens were placed to enable taking coronal sections through the widest portion of each condyle. The box containing the paraffin embedded condyle specimen was then immersed in cool water to harden. By the use of microtome, serial coronal sections of condyle (4-6 microns) were done from the paraffin blocks. Paraffin ribbons were mounted on the prepared microscope slides. The slides were placed on a constant temperature drying table at about 37-42 ℃ **(Luna, 1968)**[25].

For **immunohistochemical staining** with COX-2, the slides were deparaffinized in xylene and hydrated in descending grades of alcohol. For antigen exposure, the tissues sections were heat treated for 3.5 minutes with citrate buffer (10mM, pH 6.0) in a pressure cooker. Using Hydrogen peroxide in water solution 6% for 30 minutes in the dark; inhibited endogenous peroxidase activity. After that, the tissue sections were washed with phosphate-buffered saline (PBS) (10 mM, pH 7.2) followed by overnight incubation, in a moist chamber, at 4°C, with polyclonal anti-rabbit (COX-2) antibody (Cell Signaling #4842, dilution 1:50). The slides were incubated with Super Picture Poly HRP conjugate (Zymed, USA, nº. 87-8963) for 30 minutes at 37ºC. The slides were incubated with diaminobenzidine (DAB, Sigma, USA, D-5637) for 5 minutes at 37ºC for detection to be counterstained with Harris Hematoxilina, dehydrated with ethanol and cover slipped with mounting medium (Knittel, StarFrost, Germany).

Measuring area fraction of COX-2 immunopositivity:

Image J 22 soft ware was used for area fraction measurement of COX2 immunonopositivity. Area fraction was measured in a standard measuring frame per 6 photomicrographs in each group using a magnification x 400 by light microscopy transferred to the monitored screen. Areas containing positively immunostained tissues were used for evaluation regardless the intensity of staining. These areas were masked by a red binary color that could be measured by the computer system as follows:

1. Soft ware converted the image type to 8-bit gray scale.
2. The image was then color threshold to select only the brown color of COX-2 immunopositivity.
3. The brown color was then masked by a red binary color to measure area fraction which is the percentage of the pixels in the brown color that have been highlighted in red.

Measuring thickness of condylar cartilage:

Thickness of the condylar cartilage was measured at the most apical portion of the condyle. Measuring included all condylar cartilge zones (fibrous, proliferative, maturational and hypertrophic) **(Matthys et al., 2015)[26].**

Image analysis for condylar cartilage thickness was done using Image J 22 soft ware. Standard measuring frame per 5 photomicrographs for each group using a magnification x 400 by light microscopy were transferred to the monitored screen.

Steps of measuring condylar cartilage thickness:

1. Straight line tool was selected to make a line that corresponds to condylar cartilage thickness.
2. Centimeter was the unit of length measurement.
3. The distance in pixels field was automatically filled in based on the length of the line selection.

Statistical analysis:

The results of measuring condylar cartilage thickness and area fraction immunoreactivity for COX-2 were presented as mean and standard deviation. Mann-Whitney test was used to study the significant difference between groups. P value of less than 0.04 was considered as cutoff for significance. Data entry and analysis was done using SPSS versions 20, graphics were made by excel.

Results

1) Histological study of condylar cartilage using hematoxylin and eosin:

The control group:-

Longitudinal coronal sections of mandibular condyle in saline treated animals revealed the articular cartilage following the contour of the condylar head. Condylar cartilage appeared to include all zones (fibrous, proliferative, maturative, hypertrophic and calcified) (Fig.1).

The most superficial layer of the condylar cartilage showed dense fibrous connective tissue with scattered cells. The cells of the fibrous zone appeared to be fibroblast-like cells. The underlying proliferative zone is mainly cellular with mesenchymal chondrocyte precursors for the underlying zones. In the maturative zone, chondrocytes at various stages of maturation were clearly detected. Cellular morphology was found to be changed from flattened to spherical in shape in this zone. In the hypertrophic zone, chodrocytes became larger and died. Deeper to this zone, bone and marrow spaces appeared to replace the cartilage (Fig.1).

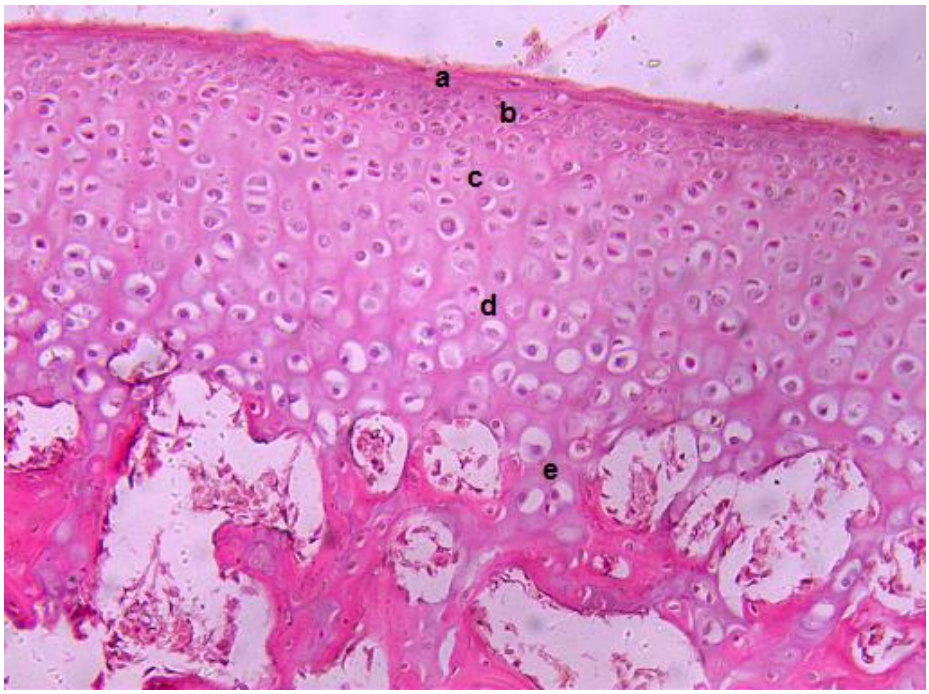

Fig.(1): Photomicrograph of longitudinal coronal section in the condyle of control group showing: a- fibrous zone b- proliferative zone c- maturative zone d-hypertrophic zone e- calcified cartilage (H& E, X 200).

The experimental group

a). Experimental subgroup A (sub Gp A):

Longitudinal coronal sections of mandibular condyle in BTX-A treated animals of this subgroup revealed intensively stained fibrous layer (Fig.2). Dark chondrocytes with condensed chromatin inside the nuclei are clearly visible in the maturative cell layer. Most of chondrocytes lacunae appeared to be empty in the hypertrophic zone zone (Fig. 3).

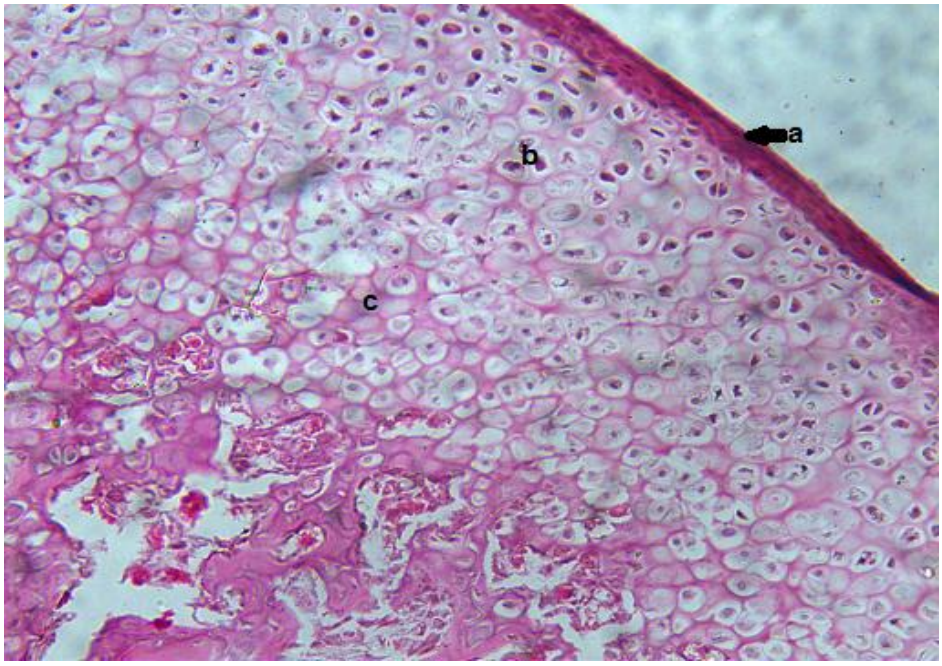

Fig.(2): Photomicrograph of longitudinal coronal section in the condyle of experimental subgroup A showing: a- densely stained fibrous layer b- dark stained chodrocytes in maturative layer c- empty lacunae in hypertrophic zone (H& E, X 200).

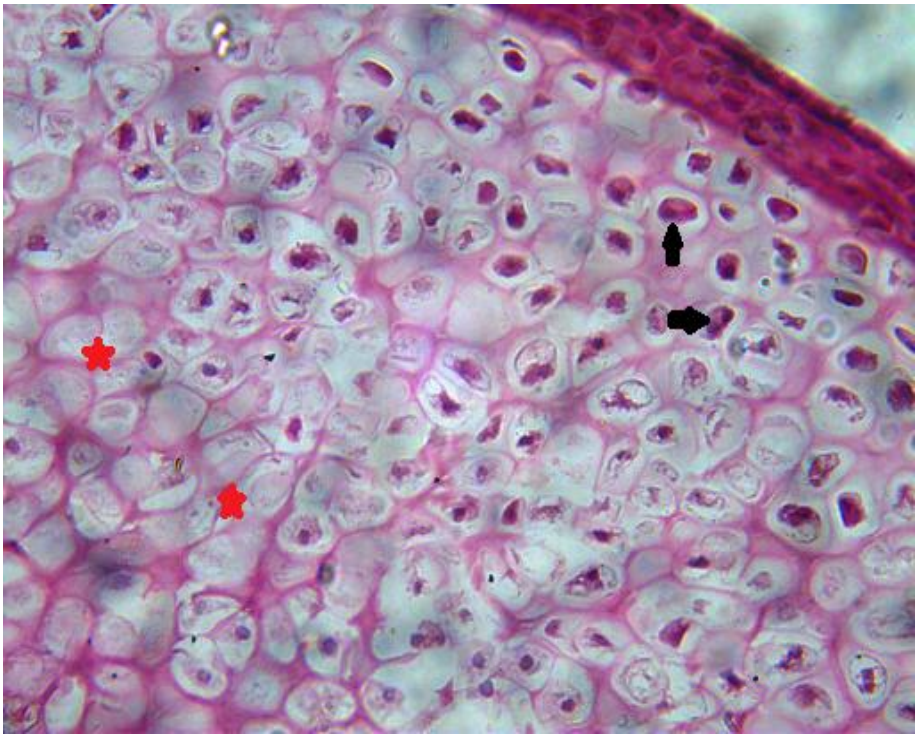

Fig. (3): Photomicrograph of longitudinal coronal section in the condyle of experimental subgroup A showing: empty lacunae (red stars), dark stained nuclei (black arrows) (H & E, X 400).

b) Experimental subgroup B (Sub Gp B):

Longitudinal coronal sections of mandibular condyle in BTX-A treated animals of this subgroup showed obvious decrease in the thickness of condylar cartilage (Fig.4).

Higher magnification of the same field revealed shrunk shaped chondrocytes in the proliferative zone with small condensed nuclei at the periphery of the lacunae (Fig.5).

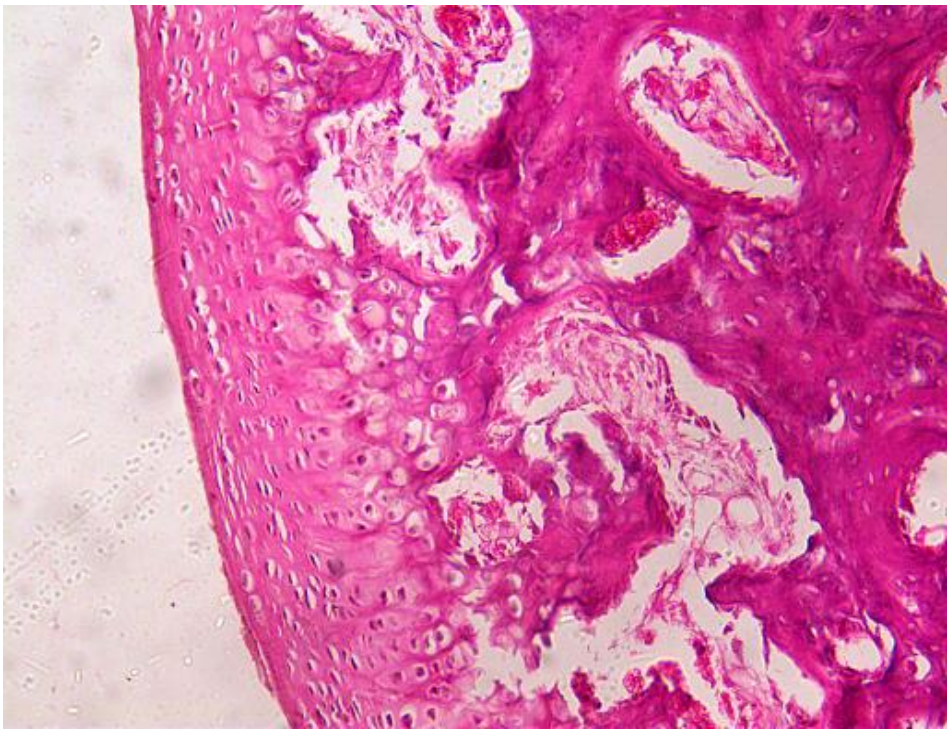

Fig. (4): Photomicrograph of longitudinal coronal section in the condyle of experimental subgroup B showing: Obvious decrease in condylar cartilage thickness (H & E, X 200).

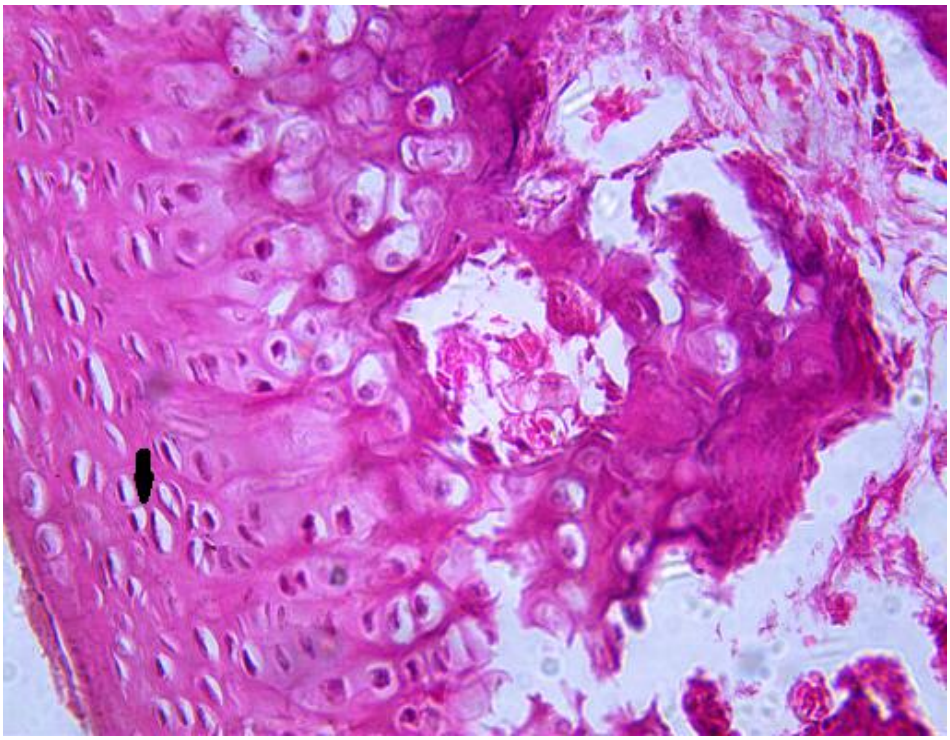

Fig. (5). Photomicrograph of longitudinal coronal section in the condyle of experimental subgroup B showing: shrunk chodrocytes with peripherally located condensed nuclei (black arrow) (H & E, X 400).

c) Experimental subgroup C (Sub Gp C):

Longitudinal coronal sections of mandibular condyle in BTX-A treated animals of this subgroup clarified that condylar cartilage thickness became thin and deteriorated (Fig.6).

Chondrocytes in the proliferative and maturative zones appeared to lose their normal architecture and shape. Chodrocytes were shrunk with complete loss of their nuclei. Some bone marrow spaces appeared congested with blood (Fig.7)

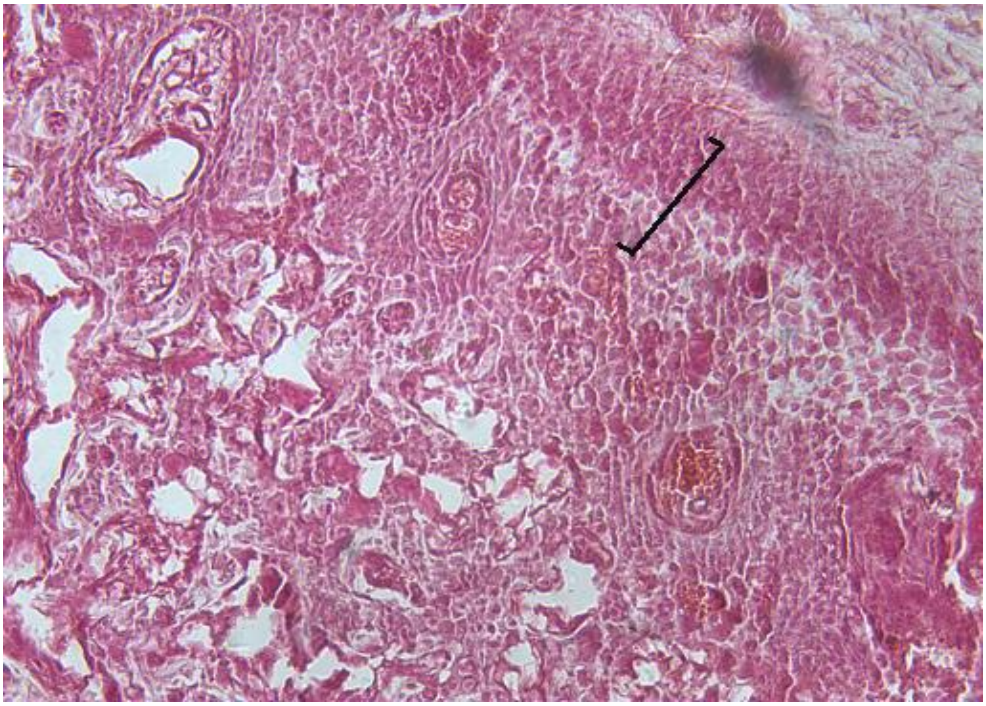

Fig. (6). Photomicrograph of longitudinal coronal section in the condyle of experimental subgroup C showing: Massive decrease in condylar cartilage thickness (H & E, X 200).

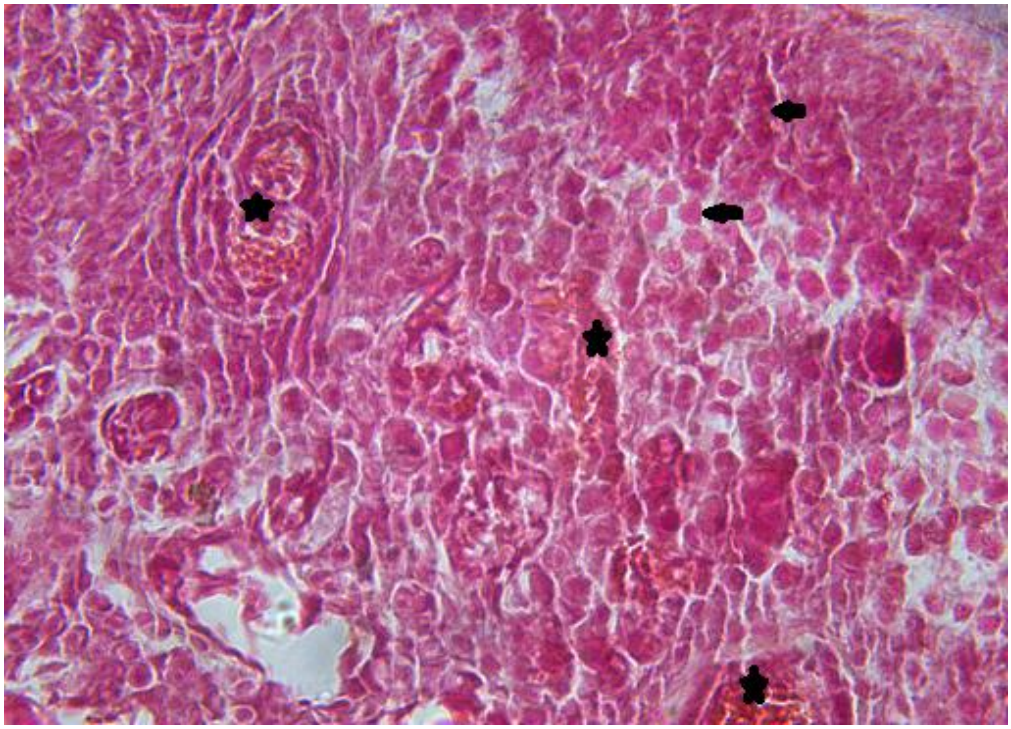

Fig. (7). Photomicrograph of longitudinal coronal section in the condyle of experimental subgroup C showing: complete loss of chondrocytes architecture and nuclei (black arrows), congested bone marrow spaces (black stars) (H & E, X 400).

2) Immunohistochemical results using COX- 2:

The positive immunoreactivity for COX-2 appeared in the form of brown coloration of chondrocytes and fibroblasts' nuclei.

The control group:

Longitudinal coronal sections of mandibular condyle specimens of control group appeared with few and mild detectable immunostained chondrocytes' nuclei (Fig. 8).

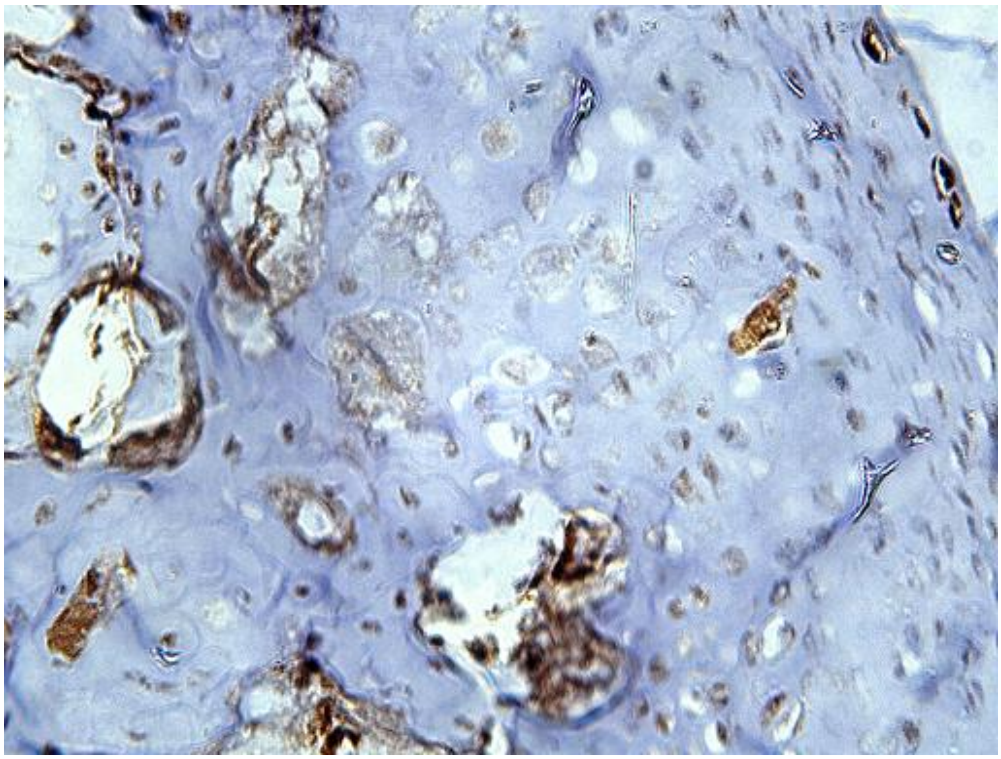

Fig. (8): A photomicrograph of longitudinal coronal section in condyle of control group showing few and mild detectable immunostained chondrocytes and fibroblats' nuclei (COX-2, X 400).

The experimental group:

a) Experimental subgroup A (sub Gp A):

Longitudinal coronal sections of mandibular condyle specimens in this subgroup appeared with moderate detectable immunostained fibroblasts and chondrocytes' nuclei (Fig. 9).

b) Experimental group B (Sub Gp B):

Longitudinal coronal sections of mandibular condyle specimens in this subgroup appeared with intense detectable immunostained chondrocytes and fibroblasts' nuclei (Fig. 10).

c) Experimental group C (Sub Gp C):

Longitudinal coronal sections of mandibular condyle specimens in this subgroup appeared with intense detectable immunostained fibrous and condylar cartilage layers (Fig. 11).

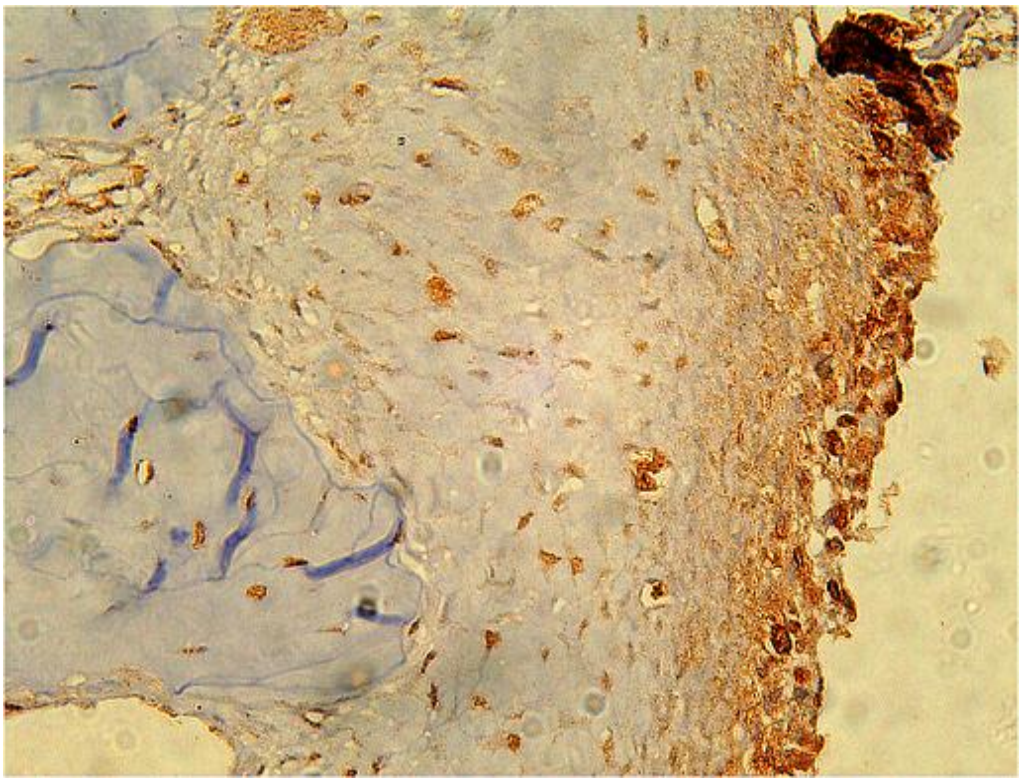

Fig. (9): A photomicrograph of longitudinal coronal section in condyle of experimental subgroup A showing moderate detectable immunostained fibroblasts and chondrocytes' nuclei (COX-2, X 400).

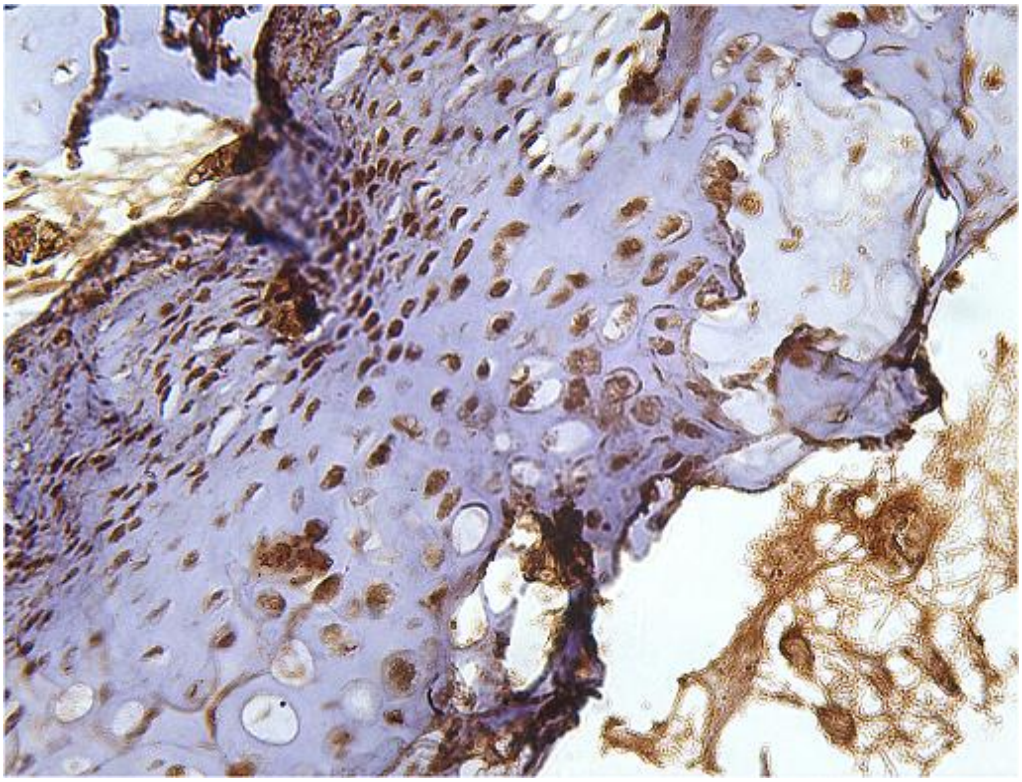

Fig. (10): A photomicrograph of longitudinal coronal section in condyle of experimental subgroup B showing intense detectable immunostained chondrocytes and fibroblasts' nuclei (COX-2, X 400).

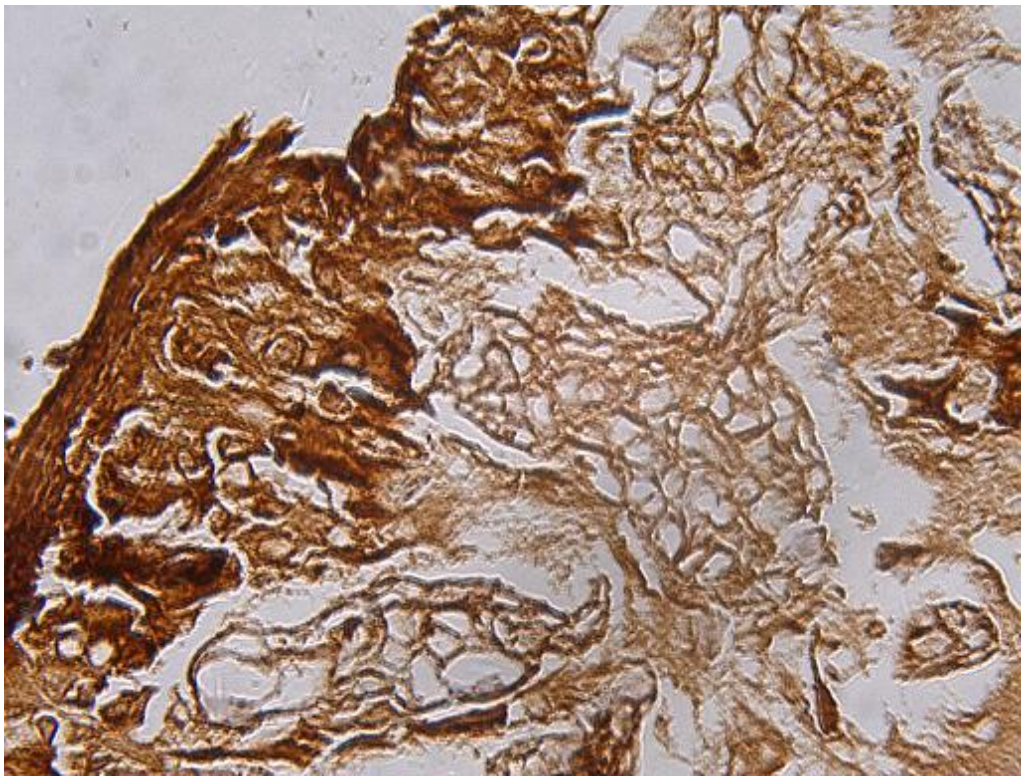

Fig. (11): A photomicrograph of longitudinal coronal section in condyle of experimental subgroup C showing intense detectable immunostained fibrous and condylar cartilage layers (COX-2, X 400).

3) Image analysis results

COX-2 area fraction

On measuring the area fraction of COX-2 immunopositivity, disappearance of the red binary color was observed in most nuclei of chondrocytes and fibroblast cells of control group while significant increase in the binary color was measured in the experimental subgroups in comparison to control group [table 1, 2; fig. 12].

Table 1:

Area fraction	Control	Sub Gp A	Sub Gp B	Sub Gp C	p1	p2	p3
Range	8.1-12.7	16.6-20.08	23.6-34.4	45.5-53.2	0.001*	0.001*	0.001*
Mean ±SD	10.8±1.7	18.5±1.3	28.7±4.2	49.01±3.2			

P1 (control vs Sub Gp A), p2 (Control vs sub Gp B), p3 (Control vs Sub Gp C). *significant.

Table 2:

Area fraction	Sub Gp A	Sub Gp B	Sub Gp C	p1	p2	p3
Range	16.6-20.08	23.6-34.4	45.5-53.2	0.001*	0.001*	0.001*
Mean ±SD	18.5±1.3	28.7±4.2	49.01±3.2			

P1 (Sub Gp A vs B), p2 (Sub Gp A vs C), p3 (Sub Gp B vs C). * significant.

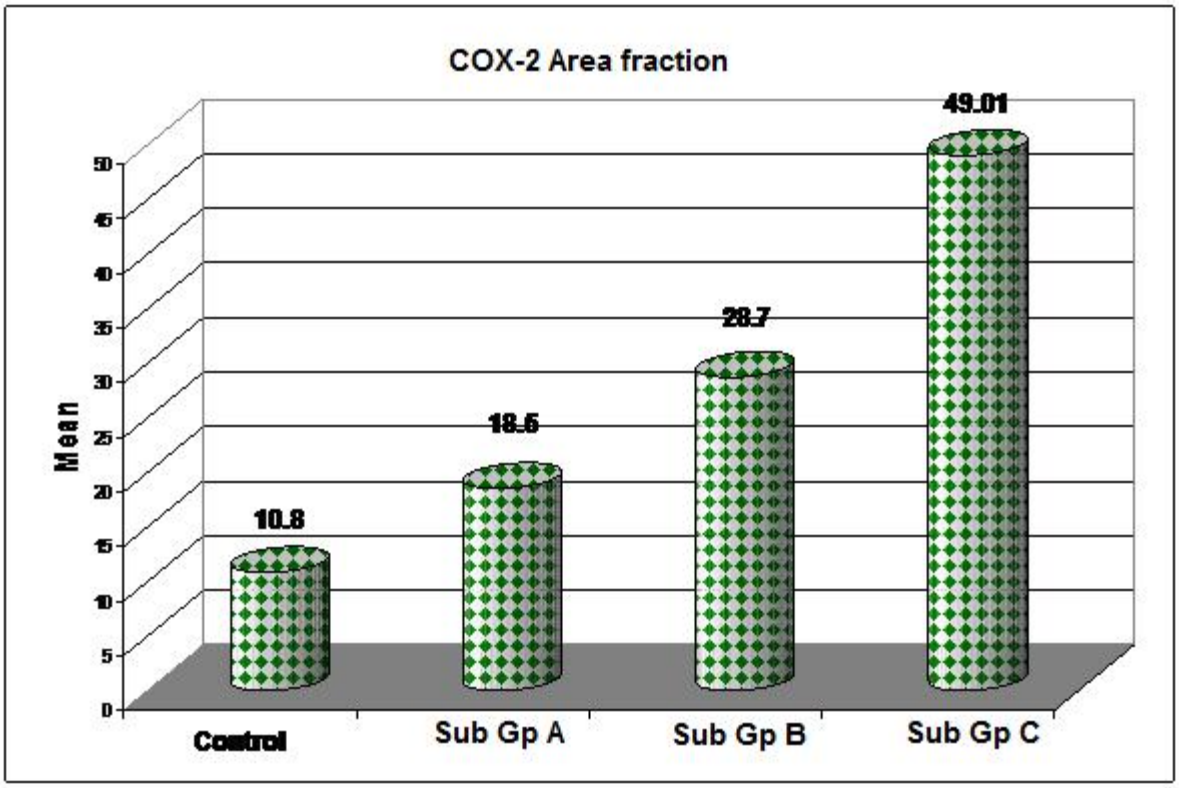

Fig. (12): A histogram showing comparison between the control and experimental subgroups regarding COX-2 area fraction

Condylar cartilage thickness

Statistical analysis results in tables 3 & 4 strongly support the significant BTX-A condylar cartilage loss in all experimental subgroups compared to control group (fig. 13).

Table 3

Thickness	Control	Sub Gp A	Sub Gp B	Sub Gp C	p1	p2	p3
Range	3.6-4.6	3.1-4.08	1.1-2.2	1.09-1.6	0.01*	0.001*	0.001*
Mean ±SD	4.1±0.4	3.4±0.3	1.9±0.4	1.3±0.2			

P1 (control vs Sub Gp A), p2 (Control vs sub Gp B), p3 (Control vs Sub Gp C). *significant.

Table 4:

Thickness	Sub Gp A	Sub Gp B	Sub Gp C	p1	p2	p3
Range	3.1-4.08	1.1-2.2	1.09-1.6	0.008*	0.001*	0.05*
Mean ±SD	3.4±0.3	1.9±0.4	1.3±0.2			

P1 (Sub Gp A vs B), p2 (Sub Gp A vs C), p3 (Sub Gp B vs C). * significant.

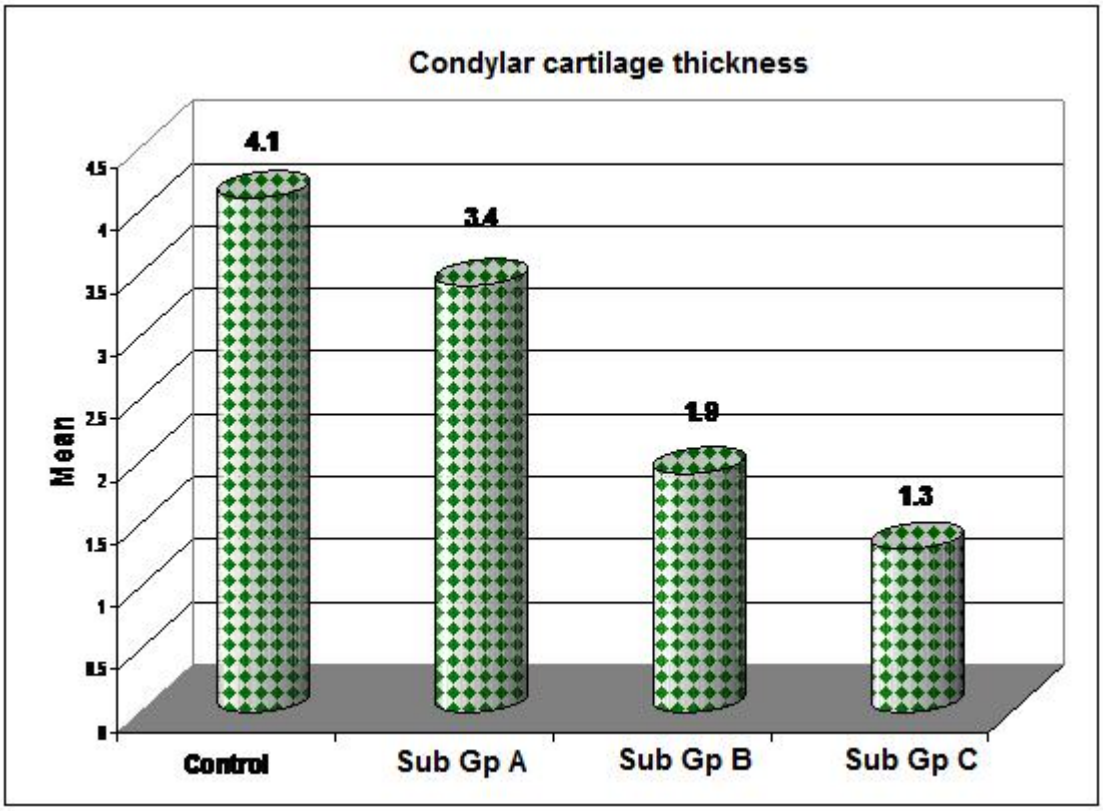

Fig. (13): A histogram showing comparison between the control and experimental subgroups regarding condylar cartilage thickness

Discussion

Injection of BTX-A into the muscles of mastication has been recently used to improve the symptoms of TMDs, myofascial pain syndrome, tension headache, and chronic migraines. The main targeted muscles are the masseter and the temporalis, where several study reports have shown effective relieve of orofacial pain **(Connelly et al., 2017[10]; Sidebottom et al., 2013[37]; Ivask et al., 2016[21]).** Consequently masseter and temporalis muscles were chosen to be injected in the present work.

Several clinical studies have investigated the effects of BTX-A injection on tempromandibualr joint and its associated skeletal muscles. In particular, the histopathological findings about the effect of injection of BTX-A in masticatory muscles on the condylar cartilage are little. In the present study, the effect of single dose injection of BTX-A into the masseter and temporalis muscles on the condylar cartilage of rabbits was evaluated histologically and immunohistochemicaly.

Light microscopic results revealed several structural changes in condylar cartilage specimens stained with hematoxylin and eosin. Dark chondrocytes in maturative zone and empty lacunae in hypertrophic zone were clearly detected in experimental subgroup A.

In dying chondrocytes of articular cartilage, condensed or dark chondrocytes appeared to be a type of cell death occurring in vivo in hypertrophic chondrocytes and termed chondroptosis. This form of apoptosis is free from phagocytosis **(Roach et al., 2004)[34].** In osteoarthirites, IL-1β was described as an apoptogen in chondrocytes of rats **(Charlier et al., 2016)[7].**

This result could be explained on the biases of releasing inflammatory cytokines from synovial cells after exposure to NAPs which are associated with BTX-A. It was proved that exposure to NAPs only and BTX-A complex significantly increased release of inflammatory cytokines such as IL-6, MCP-1, IL-8, TNF-α from neuronal SH-SY5Y cells in vitro **(Wang et al., 2014)[39].**

Proinflammatory cytokines were found to induce mitochondrial stress, cytochrome c release, activation of caspase-9 and -3, and DNA fragmentation in β cells of rat and human islets resulting in intrinsic proapoptotic signaling **(Grunnet et al., 2009)[17].**

In the present study BTX-A containing NAPs (onabotulinum toxin A, Allergan, USA) was used for intramuscular injections. Author in this study agrees with the previous postulation regarding the mechanism of chondroptosis.

The reason for the presence of large number of empty lacune is presumed to be elimination of apoptotic chondrocytes by phagocytosis-independent process. This process was reported to be mediated by digestion of cytoplasmic components and organelles, the formation of multiple autophagic vacuoles and the extrusion of cellular components into the lacunae space, leaving remnants and vesicular debris, distinct from the apoptotic bodies formed during apoptosis. The final stage of chondroptosis leaves the lacunae empty **(Charlier et al., 2016)**[7]. It was manifested that Botox injection in masseter muscle of transgenic mice (Col10a1-RFPcherry) led to decrease in chondrocyte proliferation and differentiation and increased chondrocyte apoptosis **(Dutra et al., 2016)[13].**

In experimental subgroups B & C, obvious decrease in condylar cartilage thickness was detected. This result was time dependent as proved by statsistical analysis of condylar cartilage thickness in experimental subgroups in comparison to control one.

This result is supported by findings reported after injection of 0.3 unit of Botox (Botox®; Onabotulinumtoxin A; Allergan, USA) into masseter muscle of 5-week-old transgenic mice (Col10a1-RFPcherry). Authors recorded significant decrease in condylar cartilage thickness together with decrease in chondrocytes proliferation and differentiation and increase in cell apoptosis one month after treatment **(Dutra et al., 2016)[13].**

In contrast to this finding, it was found that injection of 10U BTX-A (Botox™, Allergan Inc., Irvine, CA) in the inferior portion of superficial masseter muscle of young rabbits revealed thickening of condylar cartilage as age effect rather than thinning as treatment result **(Matthys et al., 2015)[26].**

COX is an enzyme that converts arachidonic acid to prostaglandins which cause pain, bone and cartilage resorption in TMJ. PGE2 is considered one of the major mediators of inflammation **(Alstergren & Kopp, 2000)[1].** It was reported that osteoarthritis cartilage releases PGE2 50-fold higher than normal cartilage **(Amin et al., 1997)[2]**. The therapeutic effect of non-steroidal anti-inflammatory drugs (NSAIDs) is assigned to their ability to inhibit COX activity **(Zweers et al., 2011)[42].**

Regarding immunohistohemical results, the present work demonstrated significant increase in the area fraction of COX-2 immunoreactivity from subgroup A to subgroup C. This finding might be attributed to the production of inflammatory cytokines from synovial cells and chondrocytes after injection of BTX-A. It was reported that IL-6 can stimulate chondrocytes and synovial cells to produce prostaglandins resulting in cartilage degradation **(Qu et al., 2015)[32].**

It was proved that pure BTX-A and BTX-A complex bind to different cells developed from human neuronal and non-neuronal tissues, and induce different cytokine release from the neuronal cell line SH-SY5Y **(Wang et al., 2014)[39].**

In addition, it was reported that intra-articular injection of BTX- A in canine joints is not related to inhibition of release of PGE2 **(Heikkilä et al., 2017)[19].**

In contrast to this result, it was reported that injection of capsaicin into the prostate of an adult male rat resulted in inflammation. Expression of COX-2 was found to be reduced in rats' prostate pretreated with 20 U BTX-A one week before injection of capsaicin **(Park & Park, 2017)[29].** Moreover, it was proved that intravesical BTX-A administration inhibited COX-2 expression and reduced rat bladder hyperactivity and inflammation **(Chuang et al., 2009)[9].** It was manifested that injection of BTX-A in induced arthritic rat ankle joint reduced cartilage degradation and infiltration of inflammatory cells **(Wang et al., 2017)[40].**

Data obtained from immunohistochemical results of this work suggest evocation of inflammatory response resulting in cartilage degradation. It was documented that pure BTX-A binds specifically to nerve cells, whereas NAPs bind to nerve cells and to other non-neuronal cell types. Thus NAPs are not considered passive group of associated proteins of BTX-A complex **(Arndt et al., 2005)[3].** Furthermore, NAPs were found to have stronger immunogenicity than that of purified BTX-A, thus showing a higher potential to induce host immune response **(Kukreja et al., 2009)[24].**

Pure BTX-A was proved to result in no cytokine response after 48 h of incubation with human neuronal and non neuronal cell lines. However, BTX-A complex and NAPs were found to exert selective cytokine response, suggesting that NAPs binding has significant host response **(Wang et al., 2014)[39].**

On the other hand, some pre-clinical studies presented that atrophy of masticatory muscles following BTX-A injection impaired craniofacial bone development by reducing the size of mandibular condyle and changing its morphology **(Balanta-Melo et al., 2019)[4].** In the present work it is suggested that BTX-A might spread from injection site to underlying structures including TMJ. In accordance to this proposal, a study used muscle biopsy to identify BTX-A spread, reported a diffusion gradient of over a distance of 30–45 mm from the point of injection into latissimus dorsi muscle of rabbits **(Brodsky et al., 2012[5])**. Most clinicians endorse that diffusion of BTX-A happens. They documented that diffusion is dose dependent and its extent and clinical importance is controversial **(Brodsky et al., 2012[5]; Dover et al., 2018[11])**. Further studies using radio-labeled BTX-A are needed to confirm spread of the toxin into TMJ capsule and synovial membrane from masticatory muscles' injection site.

Conclusion

It was concluded that BTX-A injection in masticatory muscles of young rabbits resulted in time dependant deteriorated histological changes in articular cartilage. These changes were proved to be evocated by elaboration of the proinflammatory enzyme (COX-2).

References

1. Alstergren, P., & Kopp, S. (2000): Prostaglandin E2 in temporomandibular joint synovial fluid and its relation to pain and inflammatory disorders. J Oral Maxillofac Surg., 58: 180-6.
2. Amin, A.R., Attur, M., Patel, R.N., Thakker, G.D., Marshall, P.J., Rediske, J., Stuchin, S.A., Patel, I.R. & Abramson, S.B. (1997): Super-induction of cyclooxygenase-2 activity in human osteoarthritis-affected cartilage. Influence of nitric oxide. J Clin Invest ., 99: 1231-7.
3. Arndt, J.W., Gu, J., Jaroszewski, L., Schwarzenbacher, R., Hanson, M.A., Lebeda, F.J. & Stevens, R.C. (2005): The structure of the neurotoxin-associated protein HA33/ A from Clostridium botulinum suggests a reoccurring beta-trefoil fold in the progenitor toxin complex. J Mol Biol., 346: 1083-1093.
4. Balanta-Melo, J., Toro-Ibacache, V., Kupczik K., & Buvinic, S. (2019): Mandibular Bone Loss after asticatory Muscles Intervention with Botulinum Toxin: An Approach from Basic Research to Clinical Findings. Toxins., 11(2): 84.
5. Brodsky, M. A., Swope, D. M., & Grimes, D. (2012): Diffusion of botulinum toxins. Tremor and other hyperkinetic movements (New York, N.Y.)., 2: tre-02-85-417-1..
6. Carruthers, J., Fagien, S., Matarasso, S.L. Consensus recommendations on the use of botulinum toxin type A in facial aesthetics. (2004): Plastic Reconstr Surg., 114 (6 suppl): 1S-22S.
7. Charlier, E., Relic, B., Deroyer, C., Malaise, O., Neuville, S., Collée, J., Malaise, M.G. & De Seny, D. (2016): Insights on Molecular Mechanisms of Chondrocytes Death in Osteoarthritis. Int J Mol Sci., 17(12): 2146.
8. Chen, Y.W., Chiu, Y.W., Chen, C.Y., Chuang, S.K. (2015): Botulinum toxin therapy for tempromandibular joint disordrs: a systematic review of randomized controlled trials. Int J Oral Maxillofac Surg., 44(8): 1018-26.
9. Chuang, Y-C., Yoshimura, N., Huang, C-C., Wu, M., Chiang, P-H., Chancellor, MB. (2009): Intravesical Botulinum Toxin A Administration Inhibits COX-2 and EP4 Expression and Suppresses Bladder Hyperactivity in Cyclophosphamide-Induced Cystitis in Rats. European Urology ., 56(1): 159-167.
10. Connelly, S.T., Myung, J., Gupta, R., Tartaglia, G.M., Gizdulich, A., Yang, J., Silva, R. (2017): Clinical outcomes of Botox injections for chronic temporomandibular disorders: do we understand how Botox works on muscle, pain, and the brain? Int J Oral Maxillofac Surg ., 46: 322-327.
11. Dover, J.S., Monheit, G., Greener, M. & Pickett, A. (2018): Botulinum Toxin in Aesthetic Medicine: Myths and Realities. Dermatol Surg., 44(2): 249-260.
12. Dressler, D., Saberi, F.A. & Barbosa, E.R. (2005): Botulinum toxin mechanisms of action. Arq Neuropsiquiatr., 63(1): 180-185.
13. Dutra, E.H., O' Brien, M.H., Lima, A., Kalajzic, Z., Tadinada, A., Nanda, R. & Yadav S. (2016): Cellular and Matrix Response of the Mandibular Condylar Cartilage to Botulinum Toxin. PLoS ONE., 11(10): 15.

14. Dutt, C.S., Ramnani, P., Thakur, D. & Pandit, M. (2015): Botulinum Toxin in the Treatment of Muscle Specific Oro-Facial Pain: A Literature Review. J Maxillofac Oral Surg., 14(2): 171-175.
15. Fattahi, M.J. & Mirshafiey, A. (2012): Prostaglandins and rheumatoid arthritis. Arthritis. Arthritis ., 2012: 7.
16. Foran, P.G., Mohammed, N., Lisk, G.O., Nagwaney, S., Lawrence, G.W., Johnson, E., Smith, L., Aoki, K.R. & Dolly, J.O. (2003): Evaluation of the therapeutic usefulness of botulinum neurotoxin B, C1, E, and F compared with the long lasting type A. Basis for distinct durations of inhibition of exocytosis in central neurons. J. Biol. Chem., 278: 1363-1371.
17. Grunnet, L.G., Aikin, R., Tonnesen, M.F., Paraskevas, S., Blaabjerg, L., Størling, J., Rosenberg, L., Billestrup, N., Maysinger, D. & Mandrup-Poulsen, T. (2009): Proinflammatory Cytokines Activate the Intrinsic Apoptotic Pathway in β-Cells. Diabetes., 58: 1807–1815.
18. Hardy, M.H., Seibert, K., Manning, P.T., Currie, M.G., Woerner, B.M., Edwards, D., Koki, A. & Tripp, C.S. (2002): Cyclooxygenase 2–Dependent Prostaglandin E2 Modulates Cartilage Proteoglycan Degradation in Human Osteoarthritis Explants. ARTHRITIS & RHEUMATISM., 46(7): 1789-1803.
19. Heikkilä, H.M., Hielm-Björkman, A.K., Innes, J.F.& Laitinen-Vapaavuori, O.M. (2017): The effect of intra-articular botulinum toxin A on substance P, prostaglandin E2, and tumor necrosis factor alpha in the canine osteoarthritic joint. BMC Vet Res., 13: 74.
20. Inoue, K., Fujinaga, Y., Watanabe, T., Ohyama, T., Takeshi, K., Moriishi, K., Nakajima, H., Inoue, K. & Oguma, K. (1996): Molecular composition of Clostridium botulinum type A progenitor toxins. Infect. Immun., 64: 1589-1594.
21. Ivask, O., Leibur, E., Akermann, S., Tamme, T. & Voog-Oras, U. (2016). Intramuscular botulinum toxin injection additional to arthrocentesis in the management of temporomandibular joint pain. Oral Surg Oral Med Oral Pathol Oral Radiol ., 122: e99-e106.
22. Keller, J.E. & Neale, E.A. (2001): The role of the synaptic protein snap-25 in the potency of botulinum neurotoxin type A. J. Biol. Chem., 276: 13476-13482.
23. Koman, L.A., Smith, B. & Balkrishnan, R. (2003): Spasticity associated with cerebral palsy in children: guidelines for the use of botulinum A toxin. Paediatr Drugs., 5: 11-23.
24. Kukreja, R., Chang, T.W., Cai, S., Lindo, P., Riding, S., Zhou, Y., Ravichandran, E. & Singh, B.R. (2009): Immunological characterization of the subunits of type A botulinum neurotoxin and different components of its associated proteins. Toxicon., 53: 616-624.
25. Luna, H.L. (1968): Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. [Book] / ed. LG Luna. - New York : Mc Graw-Hill,. - 3rd: 70.
26. Matthys, T., Ho Dang, H.A., Rafferty, K.L. & Herring, S.W. (2015): Bone and cartilage changes in rabbit mandibular condyles after a single injection of botulinum toxin. Am J Orthod Dentofacial Orthop., 148(6): 999-1009.
27. Miller, J. & Clarkson, E. (2016): Botulinum Toxin Type A: Review and Its Role in the Dental Office. Dent Clin North Am., 60(2): 509-21.
28. Page, K.M. Bone. (1996). Theory and Practice of Histological Techniques [Book Section] / ed. eds. In Bancroft JD and Stevens A. - New York: Churchill Livingstone: [s.n.].
29. Park, J. & Park, H.J. (2017): Botulinum Toxin for the Treatment of Neuropathic Pain. Toxins. 9(9): 260.
30. Park, S., Park, Y., Ji, Y., Park, S. & Kim, S. (2015): Effects of a botulinum toxin type A injection on the masseter muscle: An animal model study. Maxillofacial Plastic & Rec. Surg., 37: 10.
31. Porta, M.A. (2000): Comparative trial of botulinum toxin type A and methylprednisolone for the treatment of myofacial pain syndrome and pain from chronic muscle spasm. Pain., 85: 101-105.
32. Qu, X.Q., Wang, W.J., Tang, S.S., Liu, Y. & Wang, J.L. (2015): Correlation between interleukin-6 expression in articular cartilage bone and osteoarthritis. Genetics and Molecular Research., 14 (4): 14189-14195.
33. Rafferty, K.L., Liu, Z.J., Ye, W., Navarrete, A.L., Nguyen, T.T., Salamati, A. & Herring, S.W. (2012): Botulinum toxin in masticatory muscles: Short- and long-term effects on muscle, bone, and craniofacial function in adult rabbits. Bone., 50: 651-662.
34. Roach, H.I., Aigner, T. & Kouri JB. (2004): Chondroptosis: A variant of apoptotic cell death in chondrocytes? Apoptosis., 9: 265-277.
35. Scaglione, F. (2016): Conversion Ratio between Botox, Dysport, and Xoemin in Clinical Practice. Toxins., 8: 65.

36. Sharma, S.K., Ramzan, M.A. & Singh, B.R. (2003): Separation of the components of type A botulinum neurotoxin complex by electrophoresis. Toxicon., 41: 321-331.
37. Sidebottom, A.J., Patel, A.A. & Amin, J. (2013): Botulinum injection for the management of myofascial pain in the masticatory muscles. A prospective outcome study. Br J Oral Maxillofac Surg., 51: 199-205.
38. Wallington, E.A. Histological Methods for Bone [Book Section]. London: Butterworths, 1972.
39. Wang, L., Sun, Y., Yang, W., Lindo, P. & Singh, B.R. (2014): Type A botulinum neurotoxin complex proteins differentially modulate host response of neuronal cells. Toxicon., 82: 52-60.
40. Wang, L., Wang, K., Chu, X., Li, T., Shen, N., Fan, C., Niu, Z., Zhang, X. & Hu, L. (2017): Intra-articular injection of Botulinum toxin A reduces neurogenic inflammation in CFA-induced arthritic rat model. Toxicon., 126: 70-78.
41. Zayed, O.M., Khedr, M.M.S., Sadakah, A.A.M. & El-deeb, A.E. (2015): Evaluation of using botulinum toxin (A) in the treatment of myofacial pain syndrome. Tanta Dental Journal., 12: 156-162.
42. Zweers, M.C., de Boer, T.N., van Roon, J., Bijlsma, J.W., Lafeber, F.P. & Mastbergen, S.C. (2011): Celecoxib: considerations regarding its potential disease-modifying properties in osteoarthritis. Arthritis Res Ther., 13: 239.

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DOI:10.22192/ijarbs.2019.06.02.012	

How to cite this article:
Saher Sayed Mohammed. (2019). Condylar cartilage degradation as response to single dose Botulinum toxin - A injection in masticatory muscles of young rabbits. Int. J. Adv. Res. Biol. Sci. 6(2): 94-109.
DOI: http://dx.doi.org/10.22192/ijarbs.2019.06.02.012