Original Article

Role of Botulinum Toxin A (Botox) as a New Modality in Induced Rhematoid Arthritis Versus Corticosteroids in Adult Male Albino Rat

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ABSTRACT

Introduction: Rheumatoid arthritis (RA) is defined as a chronic immune-mediated disease and the Botox is tried to be a new treatment. The aim of our work to compare the ameliorating actions of the peri-articular Botox as a new modality and the systematic corticosteroids on the rheumatoid arthritis in the adult albino rats.

Materials and Methods: Rats were randomly allocated into 5 weight-matched groups, each of 5 rats; Group 1: ten rats were divided into the negative control were left without any intervention and the positive control were injected intra-articularly with saline, Group 2 induced RA by injecting rats with Freund's Complete Adjuvant (CFA), Group 3 were injected with Botox together with CFA and Group 4 were injected with dexamethasone (DEX) with CFA.

Results: The RA induced rats showed significant improvement histologically, biochemically and radiologically when given corticosteroids with mild amelioration when given Botox.

Conclusions: We have concluded that the use of Botox and dexamethasone were effective therapies of the RA with the priority to dexamethasone in the management of the RA.

Key Words: Arthritis, Botox, Dexamethasone, Knee and Rheumatoid.

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INTRODUCTION

Rheumatoid arthritis (RA) is defined as a chronic immune-mediated disease characterized by progressive symmetric inflammation of the joints[1]. Treatment of Rheumatoid arthritis (RA) involved new researches that used managements targeting specific cytokines including tumor-necrosis factor and others[2].

Leuchtweis *et al*.[3] induced rheumatoid arthritis in the rats by injecting bovine serum albumin intra-articular and they observed the persistent hypersensitivity and the inflammations as possible etiologies for rheumatoid arthritis.

Moreover, De Sousa *et al*.[4] described a delayed-type hypersensitivity (viz., type IV hypersensitivity) as a possible way for RA resulting in central sensitization due to immune and neuronal cells activation, leading to the local release of a variety of cytokines.

In addition, Firestein and McInnes[5] assumed that the explanation of RA is not clear yet, but many mechanisms were suggested such as gene–environment interactions, immune disorders and stromal tissue disorders.

Comella[6] mentioned seven types of Botulinum toxins including subtype A produced by fermentation of Clostridium bolutinum bacteria and they elucidated that only subtype A toxin (Botox Cosmetic) is the exclusively one approved for cosmetic use.

As mentioned by Wijbrandts *et al*.[7], the activated macrophages of the synovium derived from circulating monocytes that secrete various mediators and participate in arthritis induction and tissue injury. Moreover, studies done by Haringman *et al*.[8] had recognized the sublining CD68+ macrophages as a biomarker to evaluate clinical response to various anti-arthritis therapies with an association with clinical improvement.

Therefore, LaBranche *et al*.[9] suggested the CD68+ sublining macrophages as a synovial biomarker, with a high sensitivity in discriminating between effective and ineffective therapies or placebo, useful in an early stage of drug development.

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Corticosteroids and hyaluronic acid preparations were used as anti-arthritis therapies and they had been obviously demonstrated to be effective as illustrated by Hua *et al.*[10].

Cui *et al.*[11] described the clostridium botulinum neurotoxins as zinc dependent endopeptidases causing membrane proteolysis and inhibition of synaptic vesicle release of neurotransmitters at the nerve terminals and thus reversibly block neurotransmission. They added that release of substance-P from primary afferent neurons and release of acetylcholine at neuromuscular junctions are also inhibited reducing the refractory joint pains.

So, Stevens *et al.*[12] suggested new therapies for the treatment of arthritis and the reduction of the pain resulting. These include systemic therapy with monoclonal antibodies directed against nerve growth factor (NGF), intra-articular vanilloids, such as highly purified synthetic trans-capsaicin (CNTX-4975), and peri-articular onabotulinumtoxin A.

Accordingly, the aim of our study to compare the ameliorating actions of the peri-articular injections of botulinum toxin A (Botox) as a new modality and the systematic injection of the corticosteroids on the artificially induced rheumatoid arthritis in the adult albino rats.

MATERIALS AND METHODS

A- Drugs and Chemicals:

Complete Freund's adjuvant (CFA) and dexamesathone were purchased from Sigma-Aldrich Chemical Company (St. Louis, Mo., USA). Botulinum toxin A is available in Egypt in the form of Botox 100 IU from el Dawaya Company.

B- Experimental design:

This study was conducted on 40 adult male albino rats; with average weight 150 - 200 gm. The animals were kept in standard housing conditions and were freely supplied with food and water for 1 week before the experiment. The experiment was done in the animal house of Ain Shams University in the duration from 1 April to 28 April, 2022. The rats were bred under standard laboratory and environmental conditions approved by the Animal Ethics Committee, Ain Shams University.

Rats were randomly allocated into 4 weight-matched groups, each of 10 rats:

Group 1: Ten rats were divided into two subgroups (5 rats each):

Group I-a (negative control): Five rats were left without any intervention.

Group I-b (positive control): Five rats were injected intra-articularly once in the right knee joint with 0.1 ml of saline.

Group 2: We induced RA by injecting rats with 3 doses in the knee joints of the hind limbs with 3 days interval between every 2 doses via intra-articular injection with 0.1 ml. of 0.1 % Complete Freund's Adjuvant (CFA)[13].

Group 3: The rats were injected peri-articularly with botulinum toxin A (Botox) at a dose of 14 U/ Kg together with CFA[14].

Group 4: The rats were injected subcutanously with dexamethasone (DEX) at a dose of 1.5 mg/Kg together with CFA[15].

C- Methods:

At day 28, we collected blood samples from the retro orbital plexus of veins at the inner canthus of rat's eyes after approval by the Animal Ethics Committee, Ain Shams University for laboratory investigations to detect (RF, interleukin-6 and CRP) to confirm diagnosis of RA in which these investigations increase more than normal and subjected to the following studies[16, 17, 18].

Animals in each treatment group were euthanized, their hind limbs were dissected. MR and Toshiba CT in vivo imaging of rat knee joints employed a Esaote O.scan Elite for limbs (Esaote Elite, Genoa Florence, Italy) using the standard DPA coil and a Tosiba-CT scanner (Aquilion One Vision 640 slice, Minato, Tokyo, Japan).

1. The radiological protocol:

The MRI protocol began with a standard low-resolution localization sequence. These were subsequently used to localize the correct plane for the two dimensional or thinner slab three-dimensional images as well as for quantitative analysis. The protocol took approximately 20 minutes and the parameters of the sequences used were as follows: 1- Sagittal T2 FSP (fast spin echo sequences) is to detect the joint effusion and 2- Sagittal T1* gradient to detect the cartilage thickness. The parameters were: reception time/echo time (TR/TE)

14.3 / 5.9 ms, flip angle 12° and field of view (FOV) 40 mm, acquisition time 2 minutes 54 seconds[19].

In the CT scan analysis, the embedded specimens were examined with an in-plane pixel size and slice thickness of 5mm, was performed. To cover the entire thickness of the dissected limb. The CT scanner's had built-in software were used to make a 3-D, MIP and VR reconstruction from the set of scans[20].

2. Light microscopic study:

Thereafter, the knee joints were extracted; the knee joints of all groups were fixed in10 % neutral buffered formalin. The specimens were then washed properly under running water, dehydrated by transferring through ascending grades of alcohol, then transferred to xylene to clear the specimens from alcohol. The specimens were infiltrated with paraffin wax and embedded in the center of the paraffin wax blocks. The embedded specimens were sectioned 6 microns thick. The sections were mounted on clean glass slides and stained with Hematoxylin and eosin and toluidine blue stains for histological examination and detection of any structural changes in the different components of the knee joints[21].

3. Immunohistochemical study for CD68:

For immunohistochemical examination, the tissue sections were blocked for 10 min in phosphate-buffered saline containing 20 % rabbit serum and then incubated overnight at 4_C with anti-human CD68 monoclonal antibody (1: 1000; DAKO). After treating with this antibody at room temperature for 10 min, sections were then incubated for 10 min with appropriate Vectastain ABC reagent (Vector Laboratories, Burlinghame, CA, US), using 3,3¢-diaminobenzidine- 4HCL (DAB) (Sigma, ST Louis, MI, US) for the color reaction for 5 min, which resulted in brown staining of antigen-expressing cells. After treatment with the anti-human CD68 monoclonal antibody, we compared the expression pattern of CD68 with that of the control group[22].

4. Image analysis and morphometric study:

For quantitative analysis, morphometric image J program image analyzer was used. The image analyzer was initially calibrated automatically to translate the image analyzer program's measurement units (pixels) into actual measurement units (µm). At magnification of 400, ten non-overlapping microscopic fields were randomly chosen in each articular image for the assessment of the area percentage of toluidine blue reaction[23].

5. Statistical analysis:

The mean values of all measurements were subjected to statistical analysis. Data were coded and entered using the GraphPad Priism Version 7. Data was summarized using mean and standard deviation. Comparisons between groups were done using one way analysis of variance (ANOVA) with Tukey's multiple comparisons test when comparing more one variable in more than two groups[24].

RESULTS

Biochemical findings:

With regard to inflammatory markers including RF and CRP, treatment of rats with botox or DEX significantly improved serum levels of RF and CRP. Moreover, the inflammatory markers showed significantly ($P > 0.05$) better amelioration of the serum levels of RF and CRP in DEX treated group as compared with Botox (Table 1 and Histograms 1, 3).

For cytokines in the present research namely IL6, treatment of rats with DEX significantly ($P > 0.05$) improved the serum level of IL6 compared with arthritis induced rats. In addition, our study reported that treatment with botox showed non-significantly ($P \geq 0.05$) better amelioration of the serum levels of IL6 as compared with dexamethasone (Table 1 and Histogram 2).

Radiological analysis:

The knees of the rats of the control group exhibited average thickened cartilage (2.2 mm) and smoothening of its outer surface and the joint cavity is preserved (1.9 mm) (Figure 1). Induction of RA in the rats of group 2 led to marked radiological damage in the form of marked reduction of the size of the joint spaces (0.9 mm) with bright signals seen subcortical suggesting bone marrow edema. Also, there was calcifications seen in the vicinity of the joint capsule and the cartilage thickness was markedly reduced (1.4 mm) (Figure 2). Administration of Botox to rats of group 3 was disappointing as the joint spaces were seen markedly reduced in distance (0.9 mm) with bright signals seen subcortical suggesting bone marrow edema and there were fine calcifications seen in the vicinity of the joint capsule. Moreover, the cartilage thickness was mildly increased (1.6 mm) (Figure 3). In contrary, giving dexamethasone to group 4 produced some improvement in the radiological appearance of the knee joints as the articular cartilage and the joint cavity were partially regained (1.4 mm) and (1.1 mm) respectively (Figure 4).

Histological findings:

Examination of different stained sections of the positive control revealed similar findings as compared to the negative control group. Hx&E stained sections of the knee joint showed the articulated lower end of femur and upper end of tibia with both menisci in between. The joint space appeared clear (Figure 5a). The articular cartilage appeared as a typical hyaline cartilage with regular surface. The tidemark line was seen as a well-defined boundary separating uncalcified from calcified cartilage (Figure 1a). The arthritic knees showed destroyed articular space, extensively degenerated cartilage. Moreover, decreased chondrocytes from the femoral and tibial condyles, increased fibrotic tissue and bone destruction were observed (Figure 5b). In the botox-treated group, the joint spaces were mildly restored, and there was moderate degeneration. In addition, a number of chondrocytes and inflammatory infiltrates were noted (Figure 5c). This improvement was more obvious in the dexamethasone-treated rats than the botox- treated group (Figure 5d).

In toluidine blue stained sections of the control groups, the matrix of the articular cartilage appeared to have homogenous affinity to toluidine blue staining reflecting its good proteoglycan content. The calcified cartilage appeared to have less affinity to stain than the non-calcified cartilage (Figure 2a). The degree of cartilage damage was markedly higher in the arthritic group. Botox and DEX treatments significantly ($P > 0.05$) reduced the cartilage damage with more ameliorating effect of DEX than Botox (Figure 6, Histogram 4 and Table 1).

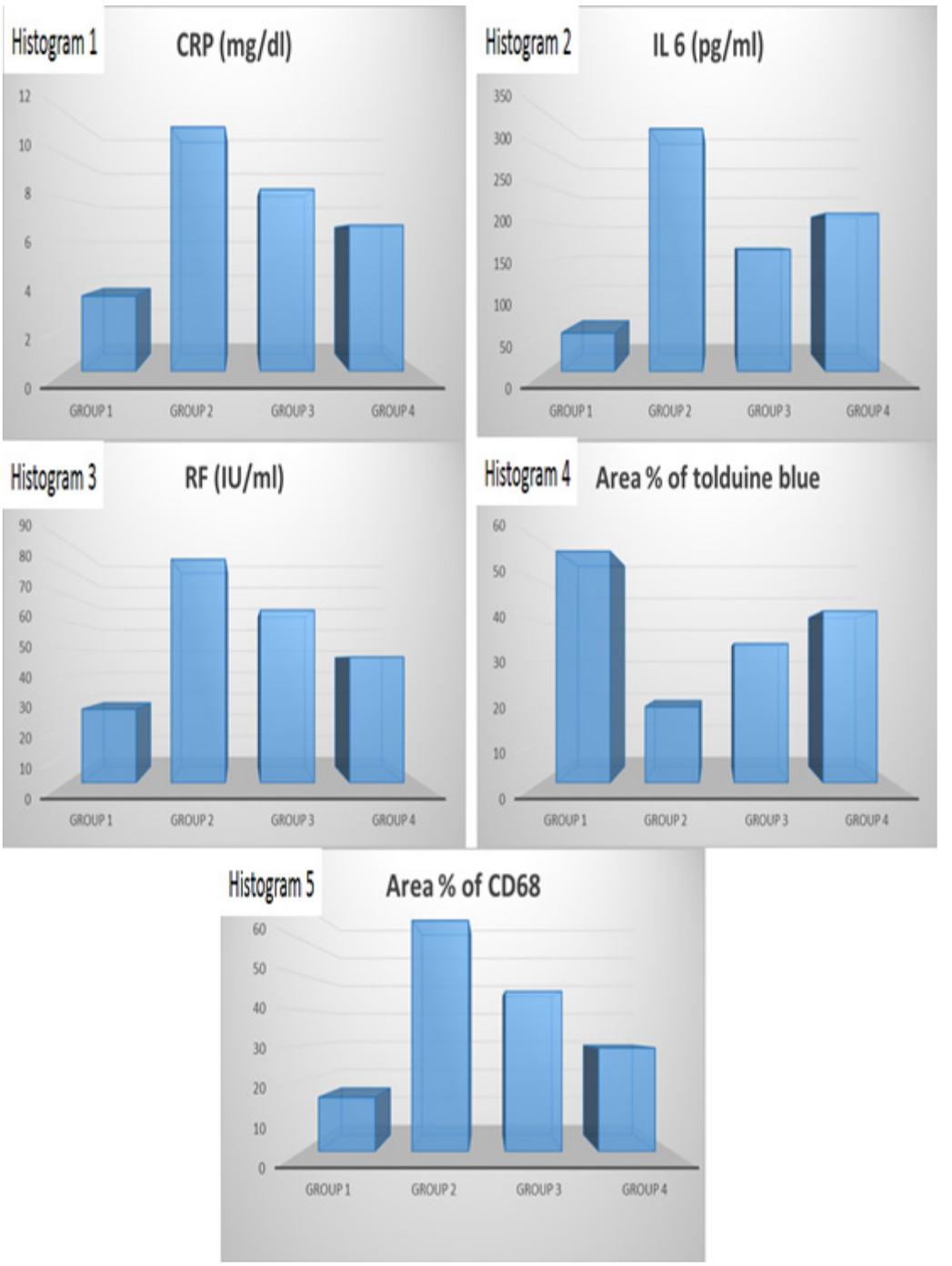
Immunohistochemical study:

Our trial had characterized CD68+ macrophages as a synovial biomarker after treatment with botox and DEX and showed that arthritic rats had an increase in the number of CD68+ synovial macrophages. In addition, botox and DEX administrations significantly ($P > 0.05$) decreased the levels of CD68+ macrophages ($p < 0.05$) with significant ($P > 0.05$) amelioration of DEX treatment than Botox therapy (Figure 7, Tble 1 and Histogram 5).

Table 1: Comparison between different groups:

The Groups / The Markers	Group 1 (mean ± SD)	Group 2 (mean ± SD)	Group 3 (mean ± SD)	Group 4 (mean ± SD)
Serum CRP (mg/dl)	3.49 ± 0.37	11.31 ± 0.36& $	8.43 ± 0.40@ $	6,77 ± 0.54@ &
Serum IL 6 (pg/ml)	51.79 ± 4.02	327.33 ± 18.76$	164.54 ± 8.67	213.16 ± 7.75@
RF (IU/ml)	30.16 ± 5.07	80.89 ± 3.31& $	61.33 ± 2.36@	49.67 ± 2.13@
Area % of toluidine blue	57.36 ± 2.75@&	18.87 ± 1.72* $	34.49 ± 1.81* $	42.55 ± 1.95@ &
Area % of CD68	15.29 ± 1.18	65.34 ± 1.31@ & $	45.29 ± 1.54@ $	29.28 ± 2.18@ &

P-values > 0.05 were considered significant.
*Statistically significant as compared to control group (group 1).
@ Statistically significant as compared to group 2.
& statistically significant as compared to group 3.
$ Statistically significant as compared to group 4.



Comparison between different groups

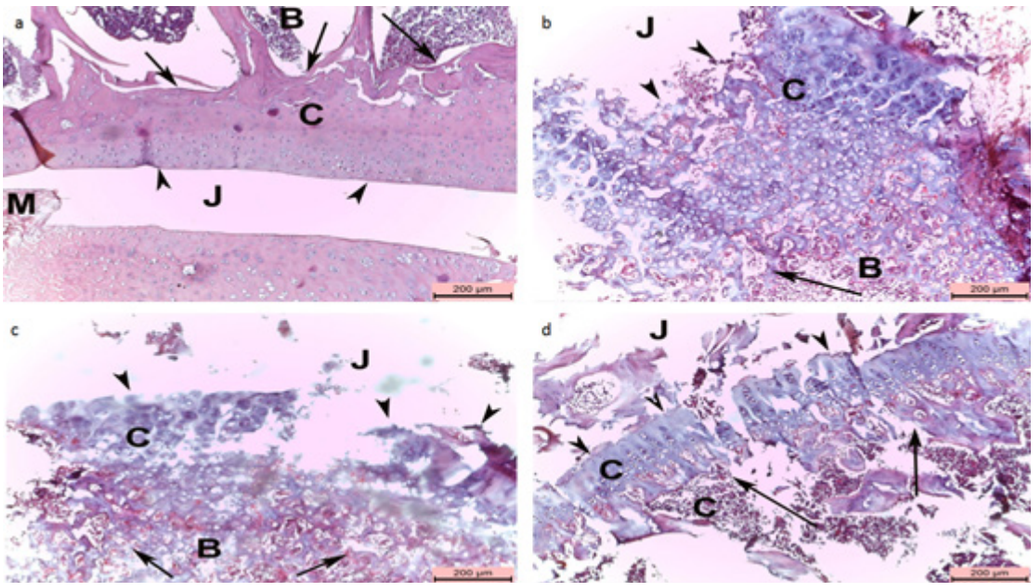


Figure 1: Photomicrographs of the knee joints of different groups including a) the control group showing a smooth articular surface (arrow heads) and a regular tide mark (arrows) separating the articular cartilage (C) from the underlying subchondral bone (B) and regular joint space (J).the meniscus (M) was noticed. b) The arthritis group 2 showing disrupted articular surface (arrow heads) with chondrocytes showing degeneration with pyknotic and vesicular nuclei (C) and the subchondral bone (B) was destructed. c) The Botox arthritic group 3 showing unnoticed improvement of the histological structure of the affected joints. d) Dexamethasone-treated arthritic group 4 showing partially smooth articular surface (arrow heads), thickened articular cartilage (C) and the subchondral bone (B) was observed. Moreover, the cartilage (C) showed hypercellularity and cloning. Regular tide mark can be seen (arrows). (H&E ×400).

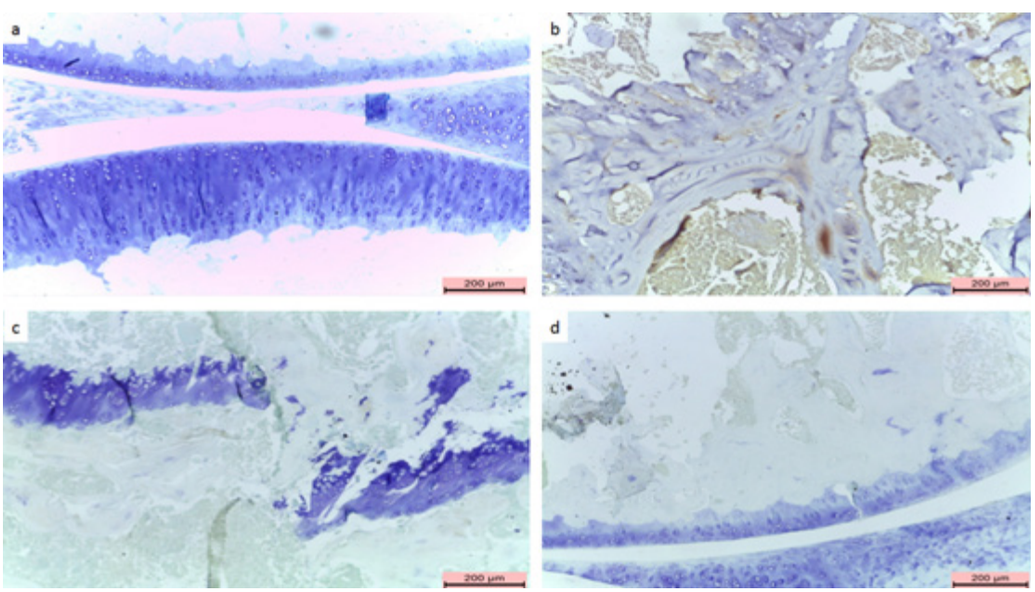


Figure 2: Photomicrographs of the knee joints of different groups stained by tolduine blue showing the cartilage of a) the control group. b) The arthritis group 2. c) The Botox arthritic group 3. d) Dexamethasone-treated arthritic group 4. (tolduine blue ×400).

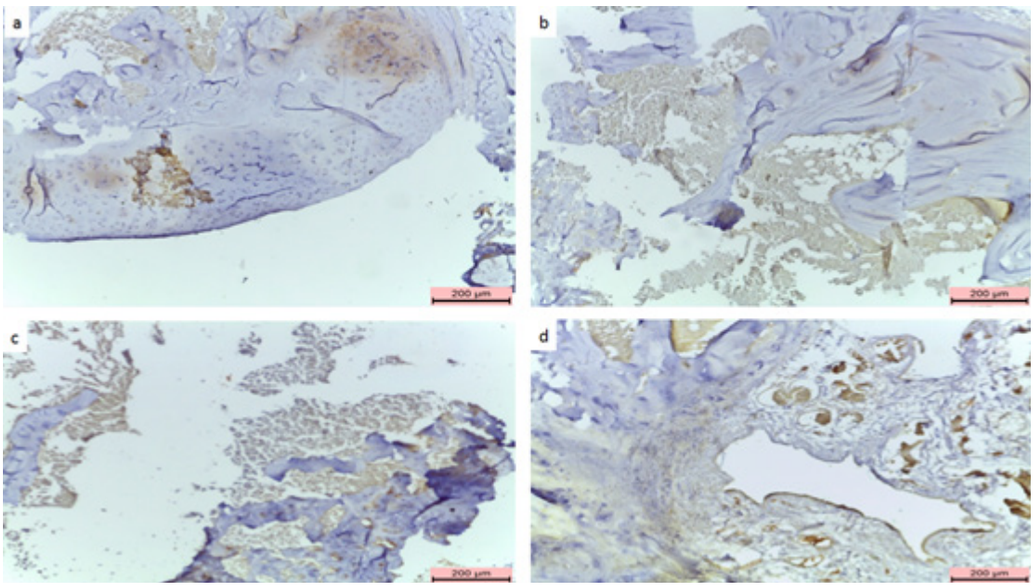


Figure 3: Representation of the immunohistochemical evaluation showing the numberof CD68 cells in the knee joints of different groups: a) the control group. b) The arthritis group 2. c) The Botox arthritic group 3. d) Dexamethasone-treated arthritic group 4. (CD68 ×400).

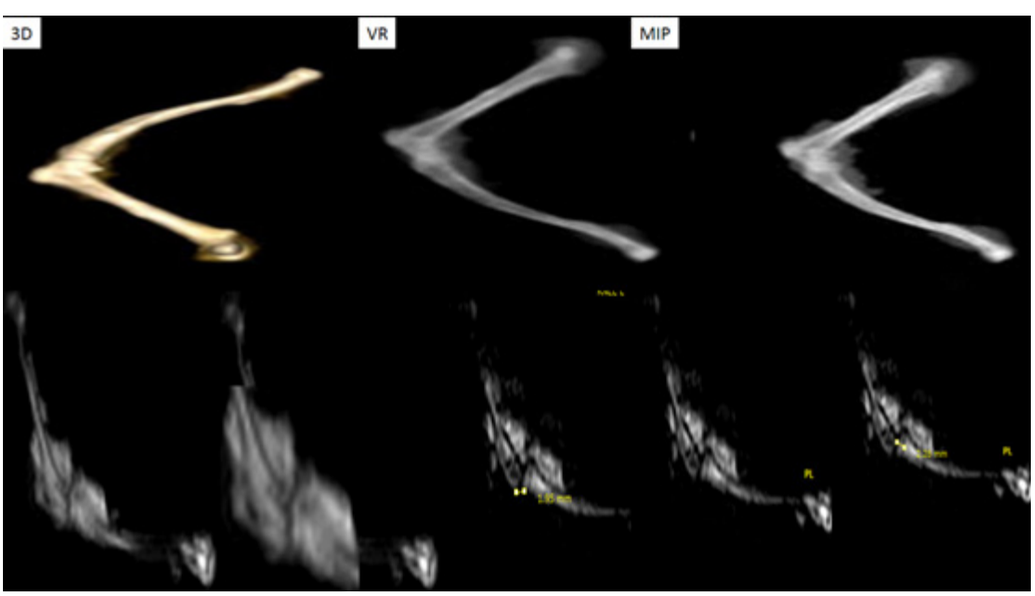


Figure 4: The limbs of the control group showed that the cartilage was evident with increase in its thickness (2.2mm) and smoothening of its outer surface, also the joint cavity is obvious (1.9mm).

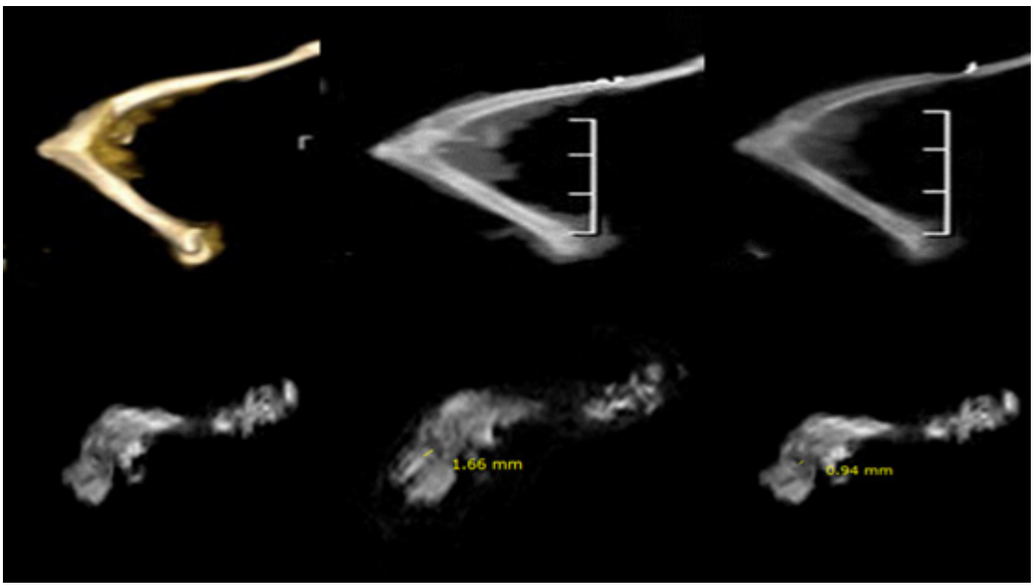


Figure 5: The limbs of the group 2 showed that the joint space was markedly reduced in size (0.9mm) with bright signals seen subcortical suggesting bone marrow edema consistent with acute injury, also there was fine calcifications seen in the vicinity of the joint capsule suggesting traumatic injury. The cartilage thickness was mildly increased (1.6mm, the effect of buttox in the acute injury is minimal.

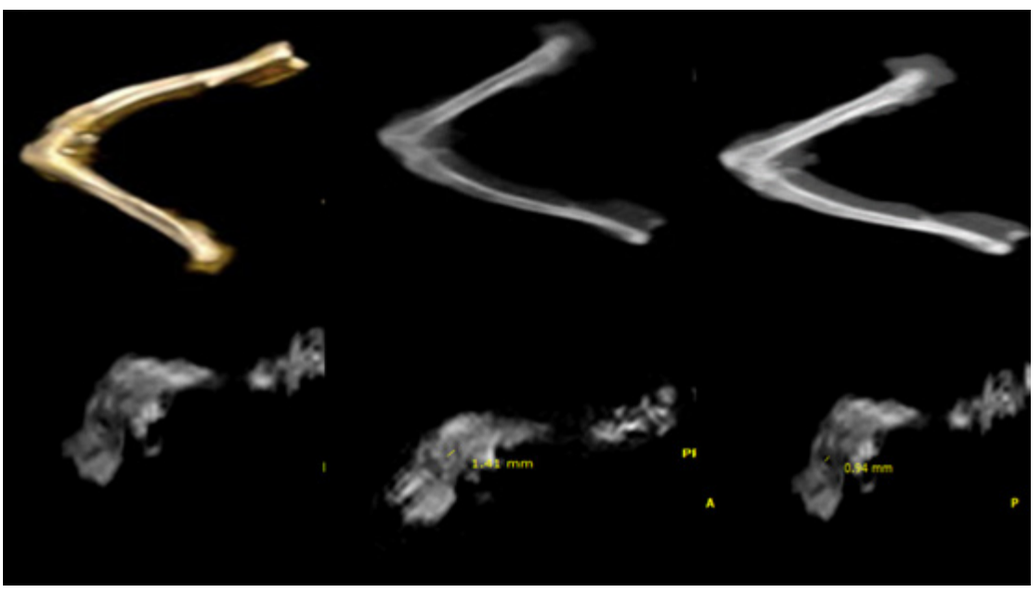


Figure 6: The limbs of the group 3 showed that the joint space was markedly reduced in size (0.9mm) with bright signals seen subcortical suggesting bone marrow edema consistent with acute injury, also there was calcifications seen in the vicinity of the joint capsule suggesting traumatic injury. The cartilage thickness was not affected (1.4mm).

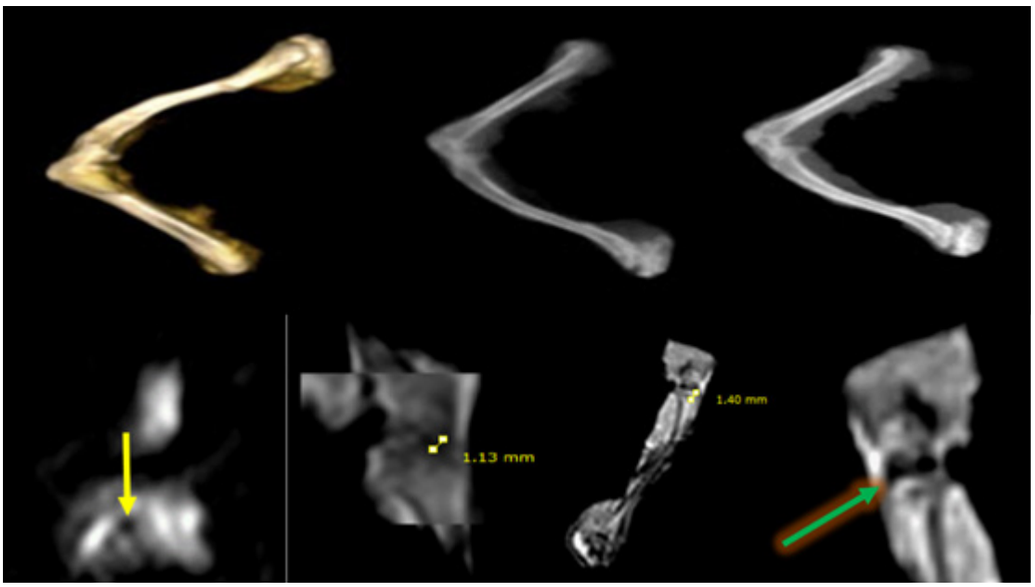


Figure 7: The limbs of the group 4 showed that the epiphyseal cartilage was mildly reduced in thickness(1.4mm) with irregular outline and narrowing of the joint cavity(1.1mm), also there was an osteochondral defect seen in the in intercondylar region (yellow arrow). There was relative bright signal in the meniscus possibly representing meniscal tear (green arrow).

DISCUSSION

Neurotoxins are being extensively investigated for use to manage chronic joint pain. Clinical evidence suggests clostridium botulinum A as a pain killer especially for individuals with nociceptive pain. Few clinical trials have been studied on the inflammatory joint diseases including RA and accordingly, the comparisons are hardly to be understood and cannot effectively be made between the studies[25].

Synovial histopathology in RA was characterized by formation of new capillaries and infiltration of inflammatory cells; predominantly mononuclear cells (T lymphocytes, B lymphocytes and plasma cells, and macrophages), although PMCs were also detected. In our trial, we found that knees of the RA-induced group exhibited destroyed articular space, extensively degenerated cartilage with decreased chondrocytes from the femoral and tibial condyles. Also, increased fibrotic tissue bone destruction was observed. In the botox-treated group, the joint spaces were mildly restored and there were moderate degeneration. In addition, a number of chondrocytes and inflammatory infiltrates were noted. These observations were more obvious in the dexamethasone-treated rats than the botox- treated group.

In agreement with our study, Saxler *et al.*[26] reported the analgesic effect of the Botox with mild amelioration on the histopathology of the joints as they documented the sensory neuropeptides including substance P (SP), calcitonin gene-related protein (CGRP) as Botox associated nociceptive chemicals. Moreover, they added that SP and CGRP were centrally enhanced in the dorsal root ganglion cells in a model of acute and chronic unilateral arthritis.

Another rat model of inflammatory arthritis in the temporomandibular (TMJ) joint done by Lora *et al.*[27] treated with Botox approved reduction of the pain behaviors of the rats after the injection of low dose formalin into the inflamed joints without significant effect on the joint histopathology. However, they explained these results in this model by the less release of SP and CGRP in the trigeminal ganglion of Botulinum clostridium A (BoNT/A)-treated animals than saline-treated animals.

In another research done by Chuang *et al.*[28], the rats exhibited persistent immunogenic hypersensitivity (PIH) of the joints after being induced by CFA with a remarkable reduction with a concomitant administration of BoNT-A together with a reduction of spontaneous and evoked nocifensive behaviors.

Moreover, in the same arthritis model, they explored the reduction of the nocifensive behaviors, as well as the expression of proinflammatory mediators A and peptides in the joints of BoNT-A treated rats in accordance with other study.

The advantage of DEX is much more pronounced in the histological evaluation as the histological damage of DEX group is much lower than the Botox group and appears similar to the healthy controls. In agreement with our study, Wang *et al.*[29] mentioned that DEX could be used in the treatment of RA patients through its anti-inflammatory action improving the histological damage resulting from the collagen disease reactions. Moreover, Burmester *et al.*[30] delineated the protective role of the corticosteroids by describing the significant differences in disease activity outcomes between the glucocorticoid groups and the placebo group at the end of the 12-week intervention period and the restoration of the normal histological architecture of the RA- synovial membranes associated with glucocorticoids therapy.

The inflammatory markers in our study including RF and CRP revealed that treatment of rats with botox or DEX improved serum levels of RF and CRP with much significantly better amelioration of the serum levels of RF and CRP in the rats treated by DEX as compared with Botox. Regarding the cytokines; namely IL6 in our research, treatment of rats with botox and DEX improved the serum level of IL6 compared with arthritis induced rats with significantly better amelioration of the serum levels of IL6 in the Botox treated rats as compared with dexamethasone.

In contrary to our trial, Mahowald *et al.*[31] used the Botox for refractory joint pain with no anti-inflammatory actions in a case series review of 12-month clinical experience. They reported a clinically and statistically significant improvement in the joint pain especially in the lower extremities after Botox injections. This report was confirmed by a recent RCT done by Xiao *et al.*[32] that established the intra-articular injection of Botox as an effective and safe treatment for chronic joint pain disorders without affecting the inflammatory state of the patients and they explained the analgesic action of BoNT/A by its ability to interact with the nociceptive neurons and/or act centrally through retrograde axonal transport.

In another theory in agreement with our study, Mika *et al.*[33] assumed that the proinflammatory factors plays a role in both the development and maintenance of pain as they detected the release of proinflammatory (IL-1β, IL-6, and IL-18) and anti-inflammatory factors (IL-1RA, IL-10 and IL-18BP). Accordingly, Pavone and Luvisetto[34] described the anti-inflammatory, immunomodulatory and neuroprotective effects of BoNT/A injections. Moreover, in an arthritis model done by Rapp *et al.*[35], they demonstrated that BoNT-A injections reduced the release of proinflammatory mediators and peptides in accordance with our study.

Regarding our experiment, Botox was less potent than Dex in reducing clinical inflammation as DEX provided bone and cartilage protection to the knee joints when compared to that of Botox.

In contrast to our research, Svensson *et al.*[36] observed that the effects of DEX particularly of low doses were more on the bone than the joints as the bone damage was associated with increased the cytokines. However, Da Silva *et al.*[37] documented the anti-inflammatory effects of higher doses of CS corticosteroids and they explicated the reason by proofing the reduction of cytokines IL-1β and IL-6 overpowering its anti-inflammatory action in arthritic patients similar to our experiment results.

In an advanced study done by Hazra *et al.*[38], they elucidated the inhibition of cytokine mRNA by the corticosteroids resulting in decreasing the IL6 suppressing the rheumatoid inflammatory activity.

LaBranche *et al.*[9] had identified the sublining CD68+ macrophages in the synovium as an important marker to assess the RA activity depending on the activated macrophages derived from circulating monocytes and secrete various mediators that participate in arthritis induction and tissue injury.

We had used the CD68+ macrophages as a synovial biomarker after treatment with botox and DEX and showed that arthritic rats had an increase in the number of CD68+ synovial macrophages. In addition, botox and DEX administrations significantly decreased the levels of CD68+ macrophages ($p < 0.05$) with significant amelioration of DEX treatment than Botox therapy.

In agreement with our study, Chou *et al.*[39] had observed the earlier anti-nflammatory of BoNT-A with less cell infiltration before executing its anti-nociceptive and they explained this phenomenon by the ability of BoNT to control the release of neurotransmitters that have nociceptive and inflammatory effects. Accordingly, Anderson *et al.*[40] suggested the BoNT-A to treat refractory arthritic pain, dermatologic pain, and postherpetic neuralgia by the inhibition of the release of neurotransmitters such as substance P, glutamate, and CGRP.

On the other hand, Favre *et al.*[41] denied the anti-inflammatory effect of BoNT-A and its ability to decrease inflammatory cell infiltrates in the affected joints and they added that BoNT-A had only an analgesic effect with no anti-inflammatory effect in the inflammatory and peripheral neuropathy diseases.

On contrary to the Botox, Gerlag *et al.*[42] described the ameliorating effects of the corticosteroids on the inflammatory nature of the RA by the measurement of the number of CD68-positive cells in the intimal and sublining regions of the synovium, using knee arthroscopy before and two weeks after treatment administration and they observed the decreased number of sub-lining CD68-positive cells in response to the treatment. Furthermore, Haringman *et al.*[43] enumerated that the number of sub-lining CD68-positive cells was directly correlated to the 28-joint count disease activity score and standardized-response mean score used to assess the disease activity in RA patients.

The radiological examination of the knees of the rats of our experiment showed that the control group exhibited average thickened cartilage (2.2 mm) and smoothening of its outer surface and the joint cavity is preserved (1.9 mm). Induction of RA in the rats of group 2 led to marked radiological damage in the form of marked reduction of the size of the joint spaces (0.9 mm) with bright signals seen subcortical suggesting bone marrow edema consistent with acute injury. Also, the cartilage thickness was mildly reduced (1.4 mm). Administration of Botox to rats of group 3 was disappointing as the joint spaces were markedly reduced in size (0.9 mm) with bright signals seen subcortical suggesting bone marrow edema and the cartilage thickness was mildly increased (1.6 mm, the effect of Botox in the acute injury was minimal). In contrary, giving dexamethasone to group 4 produced some improvement in the radiological appearance of the knee joints as the articular cartilage was mildly increased in thickness (1.4 mm) yet with irregular outline and partial regaining of the joint cavity (1.1 mm).

In contrast to our research, Sherry *et al.*[44] assumed the analgesic effect as the beneficial action of the intra-articular corticosteroid injection (IACI) but the duration of this effect is variable. In agreement with our study, the use of MRI examination by Creamer *et al.*[45] reported that the IACI at the knee joint in JRA resulted in remission for > 6 months in > 80 % of the patients with a mean duration of approximately 1.2 years and they added that the IACI could also induce remission in patients with oligo-/polyarthritis and in patients with extra-articular manifestations. Moreover, Eberhard *et al.* [46] observed that IACI influenced the gait pattern in joints that had been injected and additionally, they documented the clinical trials by utilizing the MRI technique suggested that early and continued use of IACI might be associated with less leg length discrepancy in young children with pauciarticular JRA. This may indicate decreased duration of synovitis. These results were confirmed by Hepper *et al.*[46] who noted that quantitative magnetic resonance imaging (MRI) could differentiate between therapeutically induced changes in inflammation and synovial proliferation in RA of the knee and is sensitive enough to detect change after 1 week.

The peri-articular injections of botulinum toxin type A (BoNT/A) to the rats of our study showed mild MRI changes. These results were also reported by Mahowald *et al.*[48] who utilized the Botox for refractory joint pain without significant radiological alterations. However, Singh *et al.*[49] examined eleven patients (15 joints) with chronic arthritis by the MRI who were not surgical candidates and all patients were on analgesic and/or anti-inflammatory medications and they reported a clinically and statistically significant improvement after BoNT/A injections and they added that the mean maximum decrease in lower extremity joint pain was 55 and 36 % at 4 and 10 weeks after injection, respectively. In contrary, Xiao *et al.*[32] elucidated that BoNT/A might mechanistically interact with the nociceptive neurons, as they had shown and/or acted centrally through retrograde axonal transport to produce analgesic effects that are independent of muscle relaxation with no structural effects on the joints.

CONCLUSION

In our study, we have supported the robustness of the use of Botox as a possible effective therapy of the RA in addition to the corticosteroids as treatment regimens that can enhance the drug development of the management of the RA.

CONFLICT OF INTEREST

There is no potential conflict of interest among the authors

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