Official Dental Journal of Suez Canal University
DENTAL SCIENCE Updates
Print ISSN 2636-3836
Online ISSN 2636-3844
www.dsujournals.ekb.eg
DSU — Vol. 6, No. 2, September (2025) — PP. 329:339

INTRA-ARTICULAR INJECTION OF HUMAN PLACENTAL EXTRACT FOR MANAGEMENT OF TEMPOROMANDIBULAR JOINT OSTEOARTHRITIS IN MALE WISTAR RATS: AN EXPERIMENTAL STUDY

Ahmed Sayed Eltaweel [1], Ahmed Abdelmohsen Younis [2], Mona K Tawfik [3], Mohamed Kamal El-Kherbetawy [4], Mohamed Said Hamed [5]

DOI: 10.21608/dsu.2025.355045.1283
Manuscript ID: DSU-2501-1283

KEYWORDS

Histopathological, Human Placental Extract, Temporomandibular Joint Osteoarthritis

- E-mail address: ahmed.eltaweel.egy@gmail.com

1. Postgraduate Student at Oral and Maxillofacial Surgery Department, Faculty of Dentistry, Suez Canal University, Ismailia, Egypt
2. Lecturer in Oral and Maxillofacial Surgery Department, Faculty of Dentistry, Suez Canal University, Ismailia, Egypt
3. Professor of Pharmacology, Faculty of Medicine, Suez Canal University, Ismailia, Egypt
4. Lecturer in Pathology Department, Faculty of Medicine, Suez Canal University, Ismailia, Egypt
5. Professor of Oral and Maxillofacial Surgery, Faculty of Dentistry, Suez Canal University, Ismailia, Egypt

ABSTRACT

Introduction: Temporomandibular Joint Osteoarthritis (TMJ OA) is a type of osteoarthritis that specifically affects the temporomandibular joint (TMJ). Human placental extract (HPE) has shown potential as a therapeutic option for treating temporomandibular joint osteoarthritis (TMJ OA). **Aim**: To evaluate the regenerative effect of HPE in the management of degenerative TMJ OA in male rats. **Methods**: This investigation conducted on 32 male rats, divided into four groups of eight animals each; Group I, served as a control, receiving only saline injections and thus representing a baseline, untreated state. Group II, the temporomandibular joint (TMJ OA) control group, was subjected to a surgical procedure that created a full-thickness osteochondral defect via power drills, and these animals were subsequently terminated two weeks post-procedure. Group III was subjected to the same surgical procedure as group II then administered HPE via intra-articular injections into the TMJ at a dosage of 10 mg/kg daily for a duration of two weeks; these rats were then euthanized in the fourth week. Group IV was subjected to the same surgical procedure as group II then administered HPE via intra-articular injections into the TMJ at a dosage of 10 mg/kg daily for a duration of two weeks; these rats were then euthanized in the fifth week. **Results**: Histological evaluation of samples of all rats which injected intra-articular injection of HPE on TMJ, proved that HPE facilitated healing of temporal bone and articular disc in comparison with other groups, group IV HPE treated group with sacrificing at 6th week was the nearest to normal group I. Blood results revealed that all rats which injected intra-articular of HPE on TMJ showed a reduction in levels of inflammatory markers IL-1β, IL-6, and CRP. **Conclusion**: HPE has anti-inflammatory and regenerative properties on TMJ OA according to histological evaluations of rats and blood sample evaluations by reducing inflammatory markers such as IL-1β, IL-6, and CRP.

INTRODUCTION

The TMJ, commonly referred to as the jaw joint, is a synovial articulation that enables the complex motions necessary for essential life functions. It forms the connection between the mandibular condyle and the temporal bone's mandibular fossa. This anatomical structure, encompassing the TMJ, dentition, and associated soft tissues, plays a critical role in activities like respiration, mastication, and vocalization. Classified as a ginglymoarthrodial joint, the TMJ exhibits both rotational capability in the sagittal plane and translational movement

along its axis. This dual functionality permits a broad spectrum of movement, which is passively controlled by factors such as ligamentous tension and the activity of surrounding musculature [1].

TMJ OA is a degenerative condition that uniquely affects the TMJ, the synovial joint connecting the lower jawbone, or mandible, to the temporal bone at the base of the skull. This condition involves the degeneration of the cartilage within the joint and the supporting bone, leading to pain, inflammation, and impaired jaw movement. Symptoms may include joint pain, stiffness, clicking or popping sounds during jaw movement, and difficulty opening or closing the mouth. TMJ OA can result from wear, tear, trauma, and other factors affecting joint health[2].

While clinical manifestations of TMJ OA osteoarthritis are documented in roughly 8-16% of the general population, the condition can present on one or both sides of the jaw and displays a notable prevalence in women compared to men [3, 4].

The American Academy of Orofacial Pain categorizes TMJ OA into primary and secondary types. Primary TMJ OA is characterized by the absence of any discernible local or systemic causative elements. Conversely, secondary TMJ OA is associated with a preceding traumatic event or a pre-existing underlying disease [5].

HPE is a water-based solution produced from the human placenta. Following collection from full-term deliveries, placentas are immediately frozen and subsequently subjected to comprehensive testing for infectious pathogens, such as the human immunodeficiency virus, hepatitis B virus, and hepatitis C virus. After passing these screenings, the placentas are fragmented, and their bioactive constituents are isolated using water through techniques including enzymatic molecular separation and chemical hydrolysis. Both the extraction and sterilization phases are performed under meticulously regulated conditions to guarantee the product's safety and effectiveness [6].

The HPE preparation is supplied in 2-milliliter vials, each comprising a complex mixture of biologically active substances. This mixture encompasses collagen, elastin, laminin, vitamins, trace minerals, nucleic acids, peptides, cytokines, growth factors, and a wide spectrum of amino acids. The specific amino acids contained within the extract are arginine at 0.08%, lysine at 0.1%, phenylalanine at 0.08%, tyrosine at 0.03%, leucine at 0.12%, methionine at 0.03%, valine at 0.04%, alanine at 0.08%, serine at 0.07%, and threonine at 0.06%. These amino acids are essential for bolstering protein synthesis and providing metabolic building blocks crucial for fostering healing and enabling tissue regeneration [7].

The application of HPE is gaining traction as a viable treatment modality for osteoarthritis affecting the TMJ OA. Derived from the placenta of humans, HPE is characterized by its significant anti-inflammatory and antioxidant effects, coupled with its ability to promote growth. Consequently, these attributes suggest that HPE could be effective in reducing inflammation, preventing cellular apoptosis, and promoting the regeneration of cartilage within osteoarthritic joints [8].

This study aimed to evaluate the regeneration effect of HPE for management of degenerative TMJ OA on male rats.

MATERIALS AND METHODS

The utilized materials included HPE in ampoule form (Laennec, Japan Bio Products Co., Ltd., Korea), normal saline (500 mL; Otsuka Pharmaceutical Company, Egypt), and a micromotor device (SAESHIN Strong 204 Micromotor and Handpiece,

Korea). Additional materials comprised 10% povidone iodine (Betadine, Nile Company, Egypt), suture material (Truglyde, India), and anesthetics, including xylazine HCl and ketamine HCl (Xyl-aject 5%; Adwia Company, Egypt). Diagnostic assessments were conducted using IL-1β, IL-6, and CRP ELISA kits (Egyptian Trade Company, Egypt). Imaging was performed with a calibrated standard digital microscope camera (TuopCam® XCAM-1080PHA, Hangzhou, Zhejiang, China) mounted on an Olympus® CX23 microscope (Japan), with a resolution of 10 MP (3656 x 2740 pixels). Histological slides stained with hematoxylin and eosin (H&E) were captured at original magnifications of 100x and 400x using objectives of 10x and 40x, supported by the UIS optical system (Universal Infinity System, Olympus, Japan).

Animals

This investigation employed 32 adults male Wistar rats, each with a weight of 200 ± 30 grams, procured from the Agricultural College of Suez Canal University. During the experiment, these rats were kept in sanitary, well-aerated stainless-steel enclosures, with a consistent temperature maintained at 25±3°C. A one-week acclimation period was provided for the animals before the experimental procedures commenced. Adherence to ethical guidelines was strictly observed throughout the study, with all protocols receiving prior approval from the Ethical Committee of the Faculty of Dentistry at Suez Canal University, Egypt (approval number: 520/2022).

Methods

Sample size calculation:

To determine the appropriate sample size, G*Power software (version 3.1.9.2), created by **Faul *et al***. [9] at the University of Kiel, Germany (Copyright (c) 1992-2014), was utilized. Analysis based on prior research, employing a large effect size (f) of 0.80, an alpha (α) level of 0.05, and a beta (β) level of 0.05, yielding 95% statistical power, indicated a necessary sample size of 32. This sample was then evenly divided into four groups for the purposes of this study.

Induction of osteoarthritis

Induction of general anesthesia was achieved with an intramuscular injection of ketamine HCl at a dose of 50 mg/kg, subsequently sustained by a combination of xylazine HCl (5 mg/kg) and ketamine HCl (0.3 mg/kg) also administered intramuscularly. The unilateral temple region of each rat was prepared for surgery by shaving the fur, cleansing with a 10% povidone-iodine solution, and sterile draping. Following a surgical incision in the area of the left TMJ, osteoarthritis was experimentally induced by creating a full-thickness osteochondral defect. This defect, created with a power drill, penetrated into the subchondral bone, measuring 3-4 mm in diameter and 4 mm deep, thereby simulating osteoarthritic changes in the left TMJ condyle of group II, III, and IV. Furthermore, three triangularly arranged subchondral drill holes, each extending a minimum of 3 mm into the subchondral bone, were created within the defect area using a 1.6 mm Kirschner wire.

Therapeutic intervention involved the intra-articular injection of HPE into the TMJ of groups III and IV at a dosage of 10 mg/kg/day for a duration of two weeks. Groups I and II, serving as controls, received intra-articular injections of saline solution instead.

Grouping of rats:

This investigation involved 32 male rats, partitioned into four groups of eight animals each:

Group I (Saline Control): This group served as a negative control, with rats receiving intra-articular injections of normal saline into the TMJ to mimic a no-treatment scenario.

Group II (TMJ Lesion Control): In this group, a full-thickness osteochondral defect was surgically induced using power drills. Subsequently, rats received intra-articular injections of normal saline into the TMJ for two weeks prior to euthanasia.

Group III (HPE-Treated, 4th Week Sacrifice): following the surgical creation of a full-thickness osteochondral defect, rats in this group received intra-articular injections of HPE into the TMJ at a dose of 10mg/kg/day for two weeks. Euthanasia was performed at the conclusion of the fourth week.

Group IV (HPE-Treated, 6th Week Sacrifice): similar to Group III, these rats underwent surgical creation of a full-thickness osteochondral defect and received intra-articular injections of HPE (10mg/kg/day) for two weeks. They were euthanized at the end of the sixth week.

Upon completion of the experimental period, all rats were humanely euthanized under ketamine anesthesia (50 mg/kg, administered intraperitoneally). TMJ tissue samples were promptly harvested from each group for the assessment of pathological alterations. These tissues were fixed in a 10% phosphate-buffered paraformaldehyde solution, dehydrated, and decalcified in 8% trichloroacetic acid for a period of two weeks before being embedded in paraffin. Serial sagittal sections of the paraffin-embedded specimens were prepared, stained with hematoxylin and eosin (H&E), and subjected to blinded microscopic examination. Histopathological analysis was employed to evaluate tissue regeneration and pathological changes across all groups.

Furthermore, blood samples were collected immediately post-euthanasia to quantify inflammatory markers, including interleukin-6 (IL-6), interleukin-1β (IL-1β), and serum CRP, using an enzyme-linked immunosorbent assay (ELISA) diagnostic kit.

Statistical analysis

Data organization, tabulation, and subsequent statistical analyses were performed in a systematic manner. The Kolmogorov-Smirnov test was initially used to verify the normality of the data distribution. For each experimental group, descriptive statistics were computed, encompassing the mean ± standard deviation (SD) and the range (maximum-minimum values). Comparisons among the four groups were conducted using a one-way analysis of variance (ANOVA), followed by Duncan's post hoc test for pairwise comparisons when significant differences were detected. Statistical significance was established at a p-value of ≤ 0.05. Histological data [10], due to their non-parametric nature, were analyzed using the Kruskal-Wallis's test to compare the four groups.

All statistical computations were carried out using SPSS software (Statistical Package for Social Science), version 25.0 for Windows (IBM Corp, Armonk, NY), with the significance threshold set at $p < 0.05$.

RESULTS

Histopathological results:

Group I (Saline control group) normal bone plate showing compact bone formed of osteocytes within lacunae surrounded by lamellar bone, signs of the normal histological bone plate in this group of rats **(Figure 1, A&B)**.

Sagittal sections taken from the defect site in Group II (TMJ lesion control group) displayed discontinuous bone margins at the fracture location,

alongside central fibrous tissue heavily infiltrated by inflammatory cells and a significant number of proliferating fibroblasts. Histological evaluation of the bone plate within this group exhibited distinct inflammatory characteristics, underscoring a substantial inflammatory reaction in the region of the induced lesion **(Figure 1, C&D)**.

Group III (HPE treated group with sacrificing at 4th week) sections in defect site showed connected edges with obliteration of defect by callus mainly cartilaginous type with ossification surrounded by fibroblastic proliferation and inflammatory cells. There is early woven bone formation surrounding active osteoblastic cells, and signs of healing of bone plates appear histologically in this group of rats, this means that human placenta extract intra-articular injection has a significant regeneration effect according to histological evaluation. **(Figure 2, A&B).**

Group IV (HPE treated group with sacrificing at 6th week) sections in defect site showed totally connected and obliterated defect site edges with obliteration of defect by callus formed of maturing woven bone trabeculae with ossification surrounded by minimal fibroblastic proliferation and inflammatory cells. There are osteoblastic cells, noted minimal cartilaginous tissue, and signs of healing of bone plate nearly normal appear histologically in this group of rats. This means that (HPE) intra-articular injection has significant regeneration effect, according to histological evaluation **(Figure 2, C&D).**

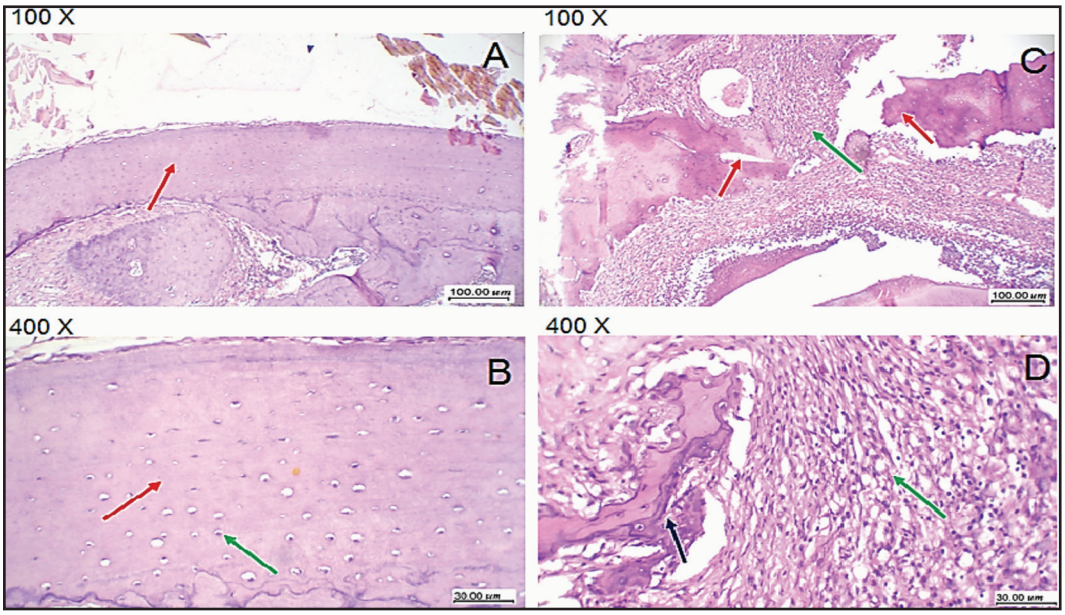

Fig. (1) Photomicrographs of sagittal sections of the TMJ from rats in group I (normal saline control) and group II (TMJ lesion control). For group I, image (A) illustrates a normal bone plate with compact bone containing osteocytes (green arrow) within lacunae, surrounded by lamellar bone (red arrow) at 100x magnification, while image (B) provides a closer view at 400x, detailing osteocyte and lamellar bone structures. For group II, image (C) highlights the defect site with discontinuous bone edges (red arrow), central fibrous tissue (green arrow) infiltrated by inflammatory cells and fibroblasts, and peripheral osteoblastic activity with early chondroid callus formation (black arrow) at 100x magnification. Image (D) magnifies these features at 400x, offering a detailed view of inflammatory cell infiltration, fibrous tissue, and developing chondroid callus.

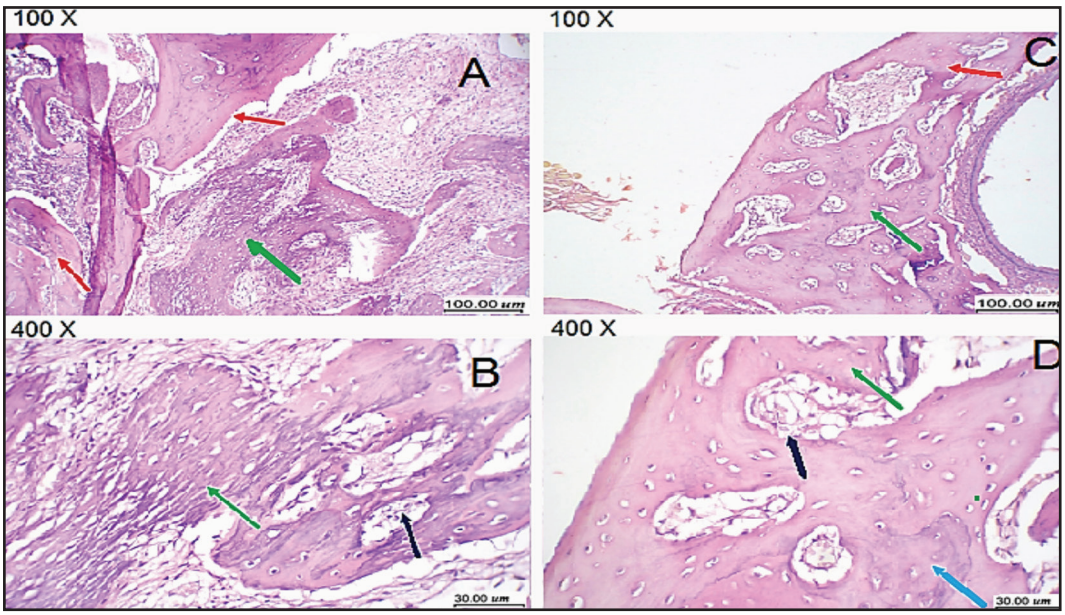

Fig. (2) Photomicrographs of a sagittal section in rats TMJ treated groups group III and group IV, for group III (HPE treated group with sacrificing at 4th week), (A, B) showed sections in defect site showed connected edges (red arrow) with obliteration of defect by callus (green arrow) with ossification surrounded by fibroblastic proliferation and inflammatory cells. There is early woven bone formation surrounded by active osteoblastic cells (black arrow). (A) (H&E. orig. mag. 100). (B) with higher magnification of (A) (H&E. orig. mag. 400). For group IV (HPE treated group with sacrificing at 6th), (C, D) showed sections in defect site showed totally connected and obliterated defect site edges (red arrow) with obliteration of defect by callus (green arrow) formed of maturing woven bone trabeculae (green arrow) with ossification surrounded by minimal fibroblastic proliferation and inflammatory cells. There are osteoblastic cells (black arrow). There is still noted minimal cartilaginous tissue (blue arrow). (C) (H&E. orig. mag. 100). (D) with higher magnification of (C) (H&E. orig. mag. 400)

Blood sample Statistical results

Analysis of interleukin-1β (IL-1β) levels, as detailed in Table 1, revealed statistically significant differences between the experimental groups, confirmed by a one-way ANOVA ($F = 1451.28$, $p<0.001$). Subsequent pairwise comparisons demonstrated significant distinctions between each individual group and all other groups, highlighting substantial variations in IL-1β expression under the different experimental conditions. The high mean values were recorded in group II (TMJ lesion control group) followed by group III (HPE treated group with sacrificing at 4th week) and group IV (HPE treated group with sacrificing at 6th week) while group I (Saline control group) was the lowest one. **Figure (3) and Table (1).**

Table (1) *Comparison between studied groups as regards IL-1β*

Groups	Mean	Std. Deviation	Minimum	Maximum	F test	P value
Group I	42.32[d]	1.88	39.0	46.0		
Group II	101.22[a]	8.17	84.0	116.0		
Group III	71.78[b]	4.40	64.0	81.0	1451.28	**<0.001****
Group IV	51.44[c]	2.07	48.0	56.0		

***(a, b, c, d) different superscript letters mean significant difference between groups (a)mean highest value followed by (b)and (c) while (d) was the lowest one.*

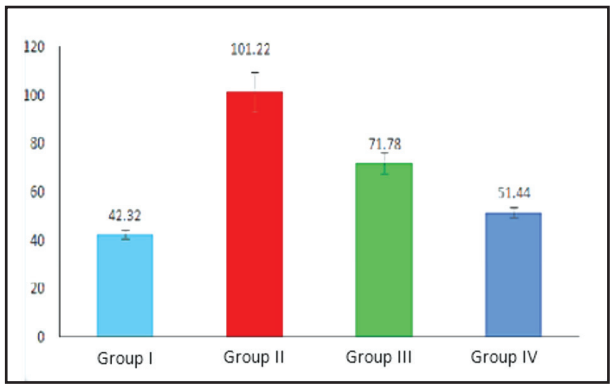

Fig. (3) Comparison between four groups showed significant differences between groups as regard to IL-1β.

The analysis of interleukin-6 (IL-6) levels, as shown in Table 2, indicated statistically significant differences between the experimental groups, substantiated by a one-way ANOVA ($F = 2973.631$, $p < 0.001$). Further pairwise comparisons revealed significant distinctions between each group and all others, emphasizing marked variations in IL-6 expression across the different experimental conditions. The high mean values were recorded in group II (TMJ lesion control group) followed by group III and group IV (HPE treated group with sacrificing at 6th week) while group I (Saline control group) was the lowest one. **Figure (4) and Table (2).**

Table (2) *Comparison between studied groups as regards IL-6*

Groups	Mean	Std. Deviation	Minimum	Maximum	F test	P value
Group I	75.30[d]	4.08	69	83		
Group II	148.88[a]	3.95	143	156		
Group III	128.24[b]	4.86	119	137	2973.631	<0.001**
Group IV	97.82[c]	4.53	90	108		

***(a, b, c, d) different superscript letters means significant difference between groups (a) mean highest value followed by (b) and (c) while (d) was the lowest one.*

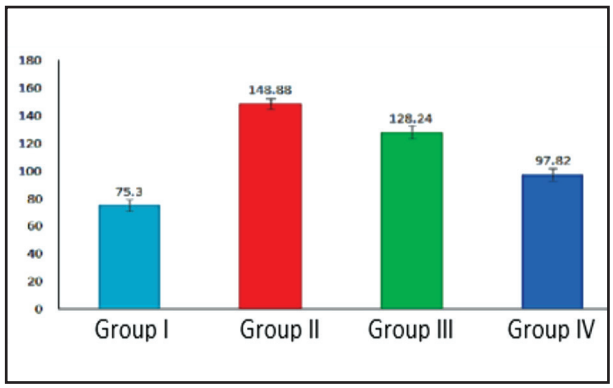

Fig. (4) Comparison between four groups showed a significant difference between groups as regard to IL.

Statistical analysis of CRP levels using one-way ANOVA indicated a significant difference among the groups ($F = 1066.24$, $p<0.001$). Subsequent pairwise comparisons identified significant differences between most groups, with the exception of group I (Saline control) and group IV (HPE-treated, 6th week sacrifice), which did not differ significantly from each other. The highest mean CRP values were observed in group II (TMJ lesion control), followed by group III (HPE-treated, 4th week sacrifice) then group I. Notably, group IV displayed the lowest mean CRP values, suggesting a potential therapeutic benefit associated with the prolonged administration of HPE.

DISCUSSION

Variations in diagnostic standards influence the reported frequency of TMJ OA; however, its incidence and associated symptoms are comparable to osteoarthritis found elsewhere in the body. Research findings suggest that TMJ OA is present in an estimated 8–16% of individuals within the general population [11].

The use of HPE has shown potential as an effective treatment for TMJ OA. These properties make HPE a promising candidate for reducing inflammation, preventing cell death, and promoting cartilage regeneration in OA-affected joints [12].

This research aims to evaluate the efficacy of HPE in promoting regeneration and mitigating the degenerative effects of TMJ OA in a male rat model.

HPE is placenta from human babies that are frozen as soon as they are removed from their mothers once they have reached full term. After being examined for the human immune deficiency virus, hepatitis B, and hepatitis C viruses [13]. The HPE formulation is provided in 2-milliliter ampules, each encompassing a diverse array of bioactive constituents. This composition includes vitamins, antioxidants, minerals, growth factors, collagen, elastin, laminin, vitamins, trace elements, nucleic acids, peptides, cytokines, and various amino acids, among which are arginine at a concentration of 0.08%, lysine at 0.1%, and threonine at 0.06%, These amino acids are known to support protein synthesis and provide metabolic substrates essential for healing and tissue repair [14].

Elevated vascular permeability constitutes a defining feature of acute inflammation. Upon inflammatory stimulation, the release of mediators like histamine, prostaglandins, and leukotrienes induces dilation of arterioles and venules, concurrently increasing vascular permeability. Consequently, fluid and plasma proteins leak into the abdominal cavity, culminating in edema formation. Research indicates that HPE mitigates the intensity of acetic acid-induced peritoneal inflammation, implying a potential role for HPE in suppressing small blood vessel permeability during the acute inflammatory phase [15].

In our experimental study, we selected male rats as in other study carried out by selim et al. [16] to evaluate the healing of TMJ OA in male rats.

In the present experimental study, rats were divided into four groups as in another study conducted by **Hu *et al.*** [17] to evaluate inflammation and healing of TMJ OA in each group.

In our study, mechanical induction was performed on rats TMJ to induce inflammation in various TMJ tissues. The technique involved creating full-thickness osteochondral defects using power drills sizes 3-4 mm in the subchondral bone of the TMJ condyles in the following groups, group II (TMJ lesion control group), group III (HPE treated group with sacrificing at 4th week) and group IV (HPE treated group with sacrificing at 6th week). As previously described, this method caused substantial morphological changes in the TMJ in these groups. The condylar cartilage and articular disc showed signs of erosion and reduction in thickness. Additionally, the fracture site displayed bone edges characterized by irregularity and discontinuity, coupled with central fibrous tissue containing a high density of inflammatory cells. The periphery was marked by the presence of proliferating fibroblasts. Substantial disruption of the temporal bone's normal architecture was evident, signifying pronounced pathological alterations.

Our results matched with a study of **Nagai *et al.*** [18] which reported that creating a full-thickness osteochondral defect using power drills

in the subchondral bone of TMJ condyles induced inflammation leading to destruction and alternation of different TMJ tissues, osteochondral damage seen in these conditions.

Our results disagree with other studies [19] which reported that drill-induced models may not accurately reflect the typical progression of (TMJ OA) and limit their translational value.

In our experimental study on rats, histological evaluation revealed that all rats, in group III (HPE treated group with sacrificing at 4th week) and in group IV (HPE treated group with sacrificing at 6th week), which received intra-articular injections of HPE into the TMJ, showed significant regeneration effect in morphological changes compared to those of induction group II (TMJ lesion control group). Furthermore, intra-articular injection of HPE into the TMJ of the treated groups (Groups III and IV) resulted in a significant decrease in inflammatory cell infiltration, a reduction in articular cartilage degeneration, and a diminution of bone abnormalities within the TMJ. According to histological evaluation, effect of HPE on group III (HPE treated group with sacrificing at 4th week) showed there is early woven bone formation surrounded active osteoblastic cells with obliteration of defect by callus mainly cartilaginous type with ossification surrounded by fibroblastic proliferation and inflammatory cells. Additionally, according to histological evaluation, effect of HPE on group IV (HPE treated group with sacrificing at 6th week), TMJ tissues showed better healing, the condylar cartilage, temporal bone and articular disc showed almost normal morphology. In addition, the defect site in group IV showed totally connected and obliterated defect site edges formed of maturing woven bone trabeculae with ossification surrounded by minimal fibroblastic proliferation and inflammatory cells in this group.

The results of this study corroborate the findings of prior investigations by Kim et al. [20], **Gwam *et al.*** [21], and **Maly *et al*.** [22] that examined the therapeutic effects of HPE on TMJOA. Their research demonstrated that HPE effectively mitigated joint deformities and cartilage damage, as evidenced by histopathological analysis. These studies indicated that HPE significantly impacted the management of TMJ OA in rats, with histopathological assessments revealing evidence of articular disc repair. Furthermore, other research has highlighted the significant advantages of HPE, including its ability to decrease inflammatory cell infiltration, reduce joint deformities, promote histological improvements, and address conditions such as osteoarthritis and rheumatoid arthritis. Histopathological examinations have also shown that intra-articular administration of HPE exerts a protective effect on existing cartilage. Collectively, these findings emphasize the anti-inflammatory and regenerative capacity of HPE.

Our results disagree with a study by **Lee *et al.***[23], which suggested that HPE treatment did not significantly affect tissue morphology changes or reduction of osteoarthritic changes in histopathological assessments with TMJ OA. However, their study suggested that HPE might have some biological activity.

To analyze inflammatory markers, specifically IL-1β, IL-6, and CRP, blood samples were collected from all rats in each experimental group. The results indicated a significant elevation of IL-1β, IL-6, and CRP in group II (the TMJ Lesion control group). Conversely, a substantial reduction in these inflammatory markers was observed in the treated groups, group III (HPE treated group, sacrificed at the 4th week) and group IV (HPE treated group, sacrificed at the 6th week).

These findings are consistent with prior research by **Naraoka *et al.*** [24], which emphasized the crucial role of IL-1β, IL-6, and CRP in the

degenerative processes associated with TMJ OA. Moreover, their study reported a marked decrease in inflammatory mediators across all treated groups, further substantiating the anti-inflammatory effects observed in our current investigation.

Our conclusions are matching previous research by **Yoon *et al.*** [25] which informed that IL-1β is a pro-inflammatory cytokine that plays a role in the development of osteoarthritis by promoting inflammation and cartilage degradation.

Our outcomes matching **Lee *et al.*** [26], which informed that IL-6 is a key cytokine involved in inflammation and joint degradation. HPE may help to reduce IL-6 levels by downregulating the inflammatory response and promoting healing processes in the TMJ.

Our outcomes matching **Kim *et al.*** [27], who found that HPE may help to lower CRP levels through its anti-inflammatory properties by reducing inflammation in the TMJ, HPE can potentially decrease the production of CRP as part of the body's inflammatory response.

The findings of this study differ from those reported by **Chen *et al.*** [28], **Zhang *et al.*** [29], and Yang et al. [30], whose research indicated that HPE did not produce a significant decrease in IL-1β, IL-6, and CRP levels in their respective TMJ OA models.

CONCLUSION

From the results of the current study, HPE intra-articular injection in the temporomandibular joint has anti-inflammatory and regenerative properties on TMJ OA according to histological samples evaluations and blood samples evaluations by reducing inflammatory markers such as IL- 1β, IL-6, and CRP.

REFERENCES

1. Whyte S, Brooks RC, Chan HF, Torgler B. Sex differences in sexual attraction for aesthetics, resources and personality across age. PLoS One. 2021;16:250-251.
2. Li H, Wu, X., Li, C., Chen, Z., & Zhu, J. Advances in the pathogenesis, diagnosis, and treatment of temporomandibular joint osteoarthritis. J Oral Rehabil. 2023;50:657-669.
3. Mejersjo C. Therapeutic and prognostic considerations in TMJ osteoarthrosis: a literature review and a long-term study in 11 subjects. Cranio. 1987;5:69-78.
4. Boyan BD, Tosi LL, Coutts RD, Enoka RM, Hart DA, Nicolella DP, et al. Addressing the gaps: sex differences in osteoarthritis of the knee. Biol Sex Differ. 2013;4:4-10.
5. De Leeuw R KG. Orofacial pain: guidelines for assessment, diagnosis, and management. . Am J Orthod Dentofacial Orthop. 2008;134:171-200.
6. Fernández-Barat L, López-Aladid R, Torres A. Reconsidering ventilator-associated pneumonia from a new dimension of the lung microbiome. EBioMedicine. 2020;60:102-995.
7. Kwon D, Kim H, Kim J, Suh SC, Kim I, Kim KJ. A survey of deep learning-based network anomaly detection. Cluster Computing. 2019;22:949-961.
8. Gwam C, Ohanele C, Hamby J, Chughtai N, Mufti Z, Ma X. Human placental extract: a potential therapeutic in treating osteoarthritis. Ann Transl Med. 2023;11:322-500.
9. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods. 2007;39:175-191.
10. Allen HL, Wase A, Bear WT. Indomethacin and aspirin: effect of nonsteroidal anti-inflammatory agents on the rate of fracture repair in the rat. Acta Orthop Scand. 1980;51:595-600.
11. Gonzalez-Perez LM, Infante-Cossio, P., Granados-Nunez, M., & Urresti-Lopez, F. J. Temporomandibular Joint Osteoarthritis: A Review of Current Clinical and Diagnostic Approaches. Int J Oral Maxillofac Surg. 2016;45:221-226.
12. Kim SJ, & Shin, H. J. . The effect of human placental extract on osteoarthritis of the temporomandibular joint in an experimental rat model. J Maxillofac Surg. 2010;38:372-377.

13. Park MS, Lee, K. S., & Han, S. H. Safety and efficacy of human placental extract in various therapeutic applications. . Reprod biol endocrinol. 2005;3:1-7.

14. Choi YA, Seol, D. W., & Kim, H. J. Composition and Biological Effects of Human Placental Extracts in Regenerative Medicine. . Journal of Biomolecular Research & Therapeutics. 2018;7:1-10.

15. Burton MJ, Ramke J, Marques AP, Bourne RRA, Congdon N, Jones I, et al. The Lancet global health commission on global eye health: Vision beyond 2020. Lancet Glob Health. 2021;9:489-551.

16. Selim M, Hamed G, Dahab M, Hendawy H, younis A. Effect of apitoxin versus hyaluronic acid in treatment of induced temporomandibular joint osteoarthritis in albino rats: An experimental study. Egyptian Journal of Oral and Maxillofacial Surgery. 2024;15:142-148.

17. Hu Z, Xiao M, Cai H, Li W, Fang W, Long X. Glycyrrhizin regulates rat TMJOA progression by inhibiting the HMGB1-RAGE/TLR4-NF-κB/AKT pathway. J Cell Mol Med. 2022;26:925-936.

18. Nagai M, & Ono, S., & Matsumoto, H., and Kitamura, Y. Animal Models for Bone and Cartilage Tissue Engineering. Tissue Engineering Part B. 2014;20:468-484.

19. Lee SH, & Kim, H. J., and Park, J. W. The limitations of mechanical induction methods in replicating TMJ osteoarthritis in animal models. . Journal of Experimental Orthopaedics. 2020;7:27-40.

20. Kim J-k, Kim T-h, Park S-w, Kim H-y, hoon Kim S, youl Lee S, et al. Protective effects of human placenta extract on cartilage degradation in experimental osteoarthritis. Biol Pharm Bull. 2010;33:1004-1010.

21. Gwam C O, C., Hamby, J. Human placental extract: a potential therapeutic in treating osteoarthritis. . Annals of Translational Medicine 2021;11:322-500.

22. Maly K, Andres Sastre E, Farrell E, Meurer A, Zaucke F. COMP and TSP-4: Functional roles in articular cartilage and relevance in osteoarthritis. Int J Mol Sci. 2021; 22:22-42.

23. Lee JH, Park, K. J., & Kim, S. H. The impact of human placental extract on histopathological changes in temporomandibular joint osteoarthritis. Arch Oral Biol. 2022;139:19-25.

24. Naraoka T, Ishibashi Y, Tsuda E, Yamamoto Y, Kusumi T, Toh S. Periodic knee injections of collagen tripeptide delay cartilage degeneration in rabbit experimental osteoarthritis. Arthritis Res Ther. 2013;15:32-100.

25. Yoon JH. Human placental extract and its effects on pro-inflammatory cytokines in osteoarthritis. J Orthop Sci. 2018;23:604-611.

26. Lee JY. Intra-articular injection of human placental extract for temporomandibular joint osteoarthritis: A randomized controlled trial. Clinical Oral Investigations. 2021;25:4217-4224.

27. Kim JS. Anti-inflammatory effects of human placental extract in vitro and in vivo. . J Inflamm Res. 2017; 10:137-146.

28. Chen Y, & Wang, R., and Zhao, Q Human placental extract and its influence on inflammatory cytokine expression in joint disorders: An experimental study. CEI. 2022;208:89-98.

29. Zhang L, & Liu, J., and Wang, X. Evaluation of human placental extract in modulating inflammatory cytokines in a rat model of temporomandibular joint osteoarthritis. J Inflamm Res. 2021;14:345-357.

30. Yang Y, & Zhang, H., and Wang, Z. Investigating the effects of human placental extract on inflammatory markers in a rat model of temporomandibular joint osteoarthritis. . Inflammation Research. 2021;70:177-185.