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RESEARCH ARTICLE

Extracted alginate from the marine algae *Cystoseira indica* alleviates toxicity and inflammation pathways in a rat model of osteoarthritis

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ABSTRACT

Background: Naturally derived compounds are gaining attention as promising therapeutic agents for managing chronic inflammatory conditions. Brown algae are rich sources of bioactive substances with potent anti-inflammatory properties. Among these conditions, osteoarthritis (OA) is a common degenerative joint disease marked by persistent inflammation, cartilage degradation, and reduced quality of life. Exploring marine-derived compounds such as alginate from brown algae offers a potential avenue for developing safer, more sustainable therapies for OA and other inflammation-related disorders. Aim: This study aimed to evaluate the therapeutic potential of alginate extracted from the brown algae Cystoseira indica in mitigating OA using a rat model. **Aim:** This study aimed to evaluate the therapeutic potential of alginate extracted from the brown algae *Cystoseira indica* in mitigating OA using a rat model. **Materials and Methods:** OA was induced in rats by intra-articular administration of monosodium iodoacetate (MIA). The experimental design included a control group, an MIA-induced OA group, and an alginate-treated group (MIA + alginate). Samples were collected at intervals of 2, 4, 6, and 10 weeks post-induction to assess the levels of pro-inflammatory cytokines, biochemical markers, and histopathological changes in the knee cartilage. **Results**: OA induction led to a significant increase in the pro-inflammatory and oxidative stress markers, including malondialdehyde (MDA), IL1β, TNF-α, beta-glucuronidase (β-G), acid phosphatase (ACP), and aggrecans 1. Moreover, these OA-induced effects were associated with a marked reduction in glutathione (GSH) levels. Alginate treatment significantly mitigated OA progression, as evidenced by reduced levels of MDA, IL1β, TNF-α, β-G, ADAMTS4, and matrix metalloproteinase-3 (MMP-3), coupled with increased GSH levels and improved cartilage recovery and regeneration. **Conclusion**: Alginate from *C. indica* demonstrates significant potential in mitigating OA through its anti-inflammatory effects, highlighting its promise as a therapeutic agent for inflammation-related joint disorders.

Keywords: Algae, Alginate, Antioxidant, Anti-inflammatory, *Cystoseira indica*, Osteoarthritis, Proinflammatory cytokines

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INTRODUCTION

Osteoarthritis (OA) represents a multifaceted and gradually progressing joint disorder characterized by the perturbation of chondrocyte-matrix interactions and the alteration of metabolic responses within chondrocytes (Zheng et al., 2021). Prominent risk factors encompass age, gender, genetic predisposition, micronutrient uptake, oxidative stress, and mechanical joint traumas (Xia et al., 2014). Traditionally categorized as a non-inflammatory form of arthritis primarily associated with mechanical cartilage injury, the current understanding recognizes the significant involvement of inflammatory mechanisms in OA pathogenesis and progression. This is attributed, in part, to the excessive release of cytokines by chondrocytes and inflammatory immune cells, notably interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α), which contribute to articular cartilage degradation and the advancement of OA (Liao et al., 2020). Furthermore, OA patients manifest elevated levels of lysosomal enzymes, such as glycosidase, β-glucuronidase, and phosphatase, in their knee synovial fluids, with their activity directly linked to synovial fluid pleocytosis in those patients (Naz et al., 2020). Key protagonists in the regulation of chondrocyte metabolism and inflammation encompass interleukin-1 (IL-1), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and aggrecans (ADAMTS-4), with their expression serving as indicators of chondrocyte inflammation and degeneration (Haneda et al., 2018). Elevated

expression of chondrocytic IL-1β stimulates the production of reactive oxygen species (ROS) and lipid peroxidation, while NF-κB expression can instigate the production of TNFα and IL-1β, thereby amplifying nitric oxide (NO) production and heightening oxidative stress, ultimately contributing to cartilage degeneration (Lin and Karin, 2007).

Moreover, the extracellular matrix (ECM) of articular cartilage is comprised of water, collagen fibrils type II secreted by chondrocytes, and aggrecan molecules (Jaabar et al., 2022). Preservation of cartilage ECM integrity relies on the delicate balance between chondrocyte catabolism and anabolism (Jaabar et al., 2022). Perturbations in this equilibrium occur during the progression of osteoarthritis (OA), leading to alterations in ECM composition, notably through heightened collagenase activity, particularly matrix metalloproteinases MMP1 and MMP3. MMP-3 (Roughley and Mort, 2014), in particular, orchestrates the degradation of multiple ECM components by activating various proMMPs, thereby initiating cartilage degradation pathways (Wan et al., 2021). Additionally, the disruption of articular cartilage in severe OA triggers the influx and release of specific lysosomal enzymes within the joints. Notably, the expression of aryl sulfatases (AS) and acid phosphatase (ACP) is observed to increase in the advanced stages of the disease (Olszewska-Slonina et al., 2015).

Currently, the chemical induction of OA via the injection of monosodium iodoacetate (MIA) stands as the most frequently employed technique in rat models. MIA induction not only exhibits a short latency period, typically manifesting within one to two weeks in a dose-dependent manner (Schuelert and McDougall, 2009) but also disrupts chondrocyte metabolism and facilitates disease monitoring via various arrays of biochemical and cellular tests. Additionally, it has been substantiated that the inflammatory pathways involved in osteoarthritis (OA) can be effectively targeted through the application of anti-inflammatory pharmaceutical agents and naturally derived polymers in rigorously validated animal model investigations (Paglia et al., 2021). Among the diverse array of natural polymers, alginate, a ubiquitous natural polysaccharide prominently featured in the pharmaceutical industry (Mollah et al., 2021), has exhibited considerable promise in the realms of tissue engineering and wound healing. Prior research has posited that alginate could serve as a surrogate for cartilage glycosaminoglycans and has demonstrated its capacity to augment chondrogenic differentiation in both stem cells and progenitor cells (Ewa-Choy et al., 2017). Consequently, based on the chondrogenic properties exhibited by alginate the principal objective of the present study is to investigate the potential therapeutic benefits of alginate in the context of OA utilizing a rat model of MIA-induced osteoarthritis.

MATERIAL AND METHODS

Chemicals

MIA was acquired from Sigma Aldrich (Merck KGaA, Darmstadt, Germany). 3% Na_2CO_3 and NaOH were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Reduced glutathione, acid phosphatase, matrix metalloproteinases-3, and malondialdehyde kits were procured from Biodiagnostic Co., Egypt. ELISA kits for aggrecans, β-glucuronidase, Interleukin 1 β, and Tumor Necrosis Factor-α were purchased from BIOMATIK, Canada.

Sample collection

Brown alga *Cystoseira indica* (*C. indica*) was gathered from the Red Sea, air-dried, incubated at 30°C overnight, and subsequently ground using an electric grinder. Alginate extraction was then promptly conducted.

Alginate extraction

Alginate extraction was performed following the method of (Haug and Larsen, 1971). Ground *C. indica* algae were formaldehyde-treated overnight. Alginate extraction occurred using 0.2 M hydrochloric acid under agitation overnight. After filtration and rinsing, the residue was pH-adjusted to 8 and filtered. Sodium chloride was added to reduce solution viscosity and ethanol-induced alginate precipitation. Collected alginate subjected to 60% aqueous ethanol, pure ethanol, and ether washes. Alginate fibers were dried overnight at 30-40°C, dissolved in distilled water, and dialyzed for mineral removal. The extracted alginate was freeze-dried and subjected to Fourier transform infrared FT-IR and 1H nuclear magnetic resonance (1H-NMR) spectroscopy for analysis of chemical structure validation.

Chemical analysis by NMR spectroscopy

The chemical composition of sodium alginates extracted and purified from *C. indica* was analyzed via NMR spectroscopy. The sodium alginates, after freeze-drying, were dissolved in D2O and subjected to multiple drying cycles prior to NMR spectrum acquisition. 1H-NMR experiments were conducted using a JEOL JNM-ECA500II (500 MHz) spectrometer at a temperature of 70°C, with an acquisition time of 1.74 seconds (JEOL, Tokyo, Japan).

Experimental design

Seventy-seven male albino rats (200-230 g) were obtained from the Medical Technology Center of the

Medical Research Institute (MRI), University of Alexandria. They were housed and acclimatized according to MRI's ethical guidelines (AU 0122421812). The rats were divided into a control group of twenty-eight rats. Seven rats were sacrificed after two weeks as a baseline value for comparison with other groups. Group I: Twenty-eight rats received a single intra-articular injection of 2 mg/Kg b.w. of MIA in 25 µl sterile saline, with seven rats collected at two, four, six, and ten-week intervals. Group II: Twenty-one rats were initially injected with 2 mg/Kg b.w. of MIA in 25 µl sterile saline, followed by weekly intra-articular injections of 0.1 ml/kg b.w. of a 1% sterile water solution of alginate starting two weeks later. Seven rats were collected at two, four, and eight-week intervals after treatment.

Biochemical analysis of rat sera

Upon completion of the study experiments, blood samples were extracted from anesthetized rats via the caudal vena cava. These samples underwent ELISA analysis for IL1β (Kricka LJ, 2012), TNF-α (Kricka LJ, 2012), Aggrecans (Yamanishi et al., 2002), β-glucuronidase (Fishman et al., 1967), ACP (Seligman et al., 1951), MMP-3 (Chen et al., 2006), MDA (Halliwell & Chirico, 1993), and GSH (Beutler et al., 1963) adhering to the manufacturer's guidelines.

Histopathological preparation and examinations

At the end of the experiment, rats were humanely euthanized under isoflurane anaesthesia. The knee joints that were injected on the right side were then extracted. From each experimental group, knee joints injected on the right side were obtained from five rats and processed to create paraffin blocks. To prepare the paraffin blocks, the injected knee joints were first removed and fixed in 10% buffered formalin for 24 hours. Decalcification was subsequently carried out using 5% nitric acid, followed by dehydration, clearing, and embedding in paraffin wax. The knee joints were sectioned at a thickness of 5µm, with frontal sections focusing on the medial aspect of the knee joints. These sections were stained with hematoxylin and eosin (H&E) stain (Glasson et al., 2010). Histopathological features of the articular cartilage were assessed using a light microscope and a digital camera (BX-51 and DP-50; Olympus Corporation, Tokyo, Japan) for image capture and evaluation.

Statistical analysis

The obtained data of the present study were statistically analyzed using the IBM SPSS software package version 20.0. (Kirkpatrick LA, 2013). A one-way ANOVA test was used for comparison between groups with GSH, MDA, TNF-α, aggrecans, and β-glucuronidase. A two-way ANOVA test was used for comparison between groups with IL-1β. A difference was considered significant at $P < 0.05$.

RESULTS

Structural characterization of alginate extracted from brown algae

The isolated alginate from *Cystoseria indica,* which exhibited a higher proportion of guluronic acid, characterized by an M/G ratio of 0.33. The FT–IR spectrum of the extracted alginate is displayed in Figure 1A. Key alginate peaks are observed at 3451 cm^{-1} (representing hydroxyl -OH groups), 1625 cm^{-1} (indicating carbonyl C=O groups), and 1418 cm^{-1} (associated with carboxyl COOH groups). Notably, the extracted alginate exhibits bands resembling those of commercial sodium alginate within the 3500–1300 cm^{-1} range. In the fingerprint region, four distinct bands emerge at 946, 900, 818, and 780 cm^{-1}. The C–O stretching vibration of the carboxylate group has been red-shifted to 948 cm−1. The band at 888 cm^{-1} corresponds to the b-D-mannuronic C1–H deformation vibration. Furthermore, the presence of a-L-guluronic acid residues in the polymannuronic acid-enriched fraction is suggested by the 780 cm^{-1} band. The chemical composition of the extracted sodium alginate was determined through 1H-NMR spectroscopy (Figure 1B). As reported by Haug and Larsen in 1971, the first fraction resulting from partial hydrolysis with 0.3 M HCl of sodium alginate primarily consists of hetero-polymeric blocks (MG). The second fraction, soluble at pH 2.85, is notably enriched in poly-mannuronic acid (MM), while the third fraction, which remains insoluble at pH 2.85, exhibits a heightened content of poly-guluronic acid (GG). In summary, the FT-IR spectra and NMR of seaweed-derived alginate fractions exhibit distinctive bands in the fingerprint region, offering the potential for the identification of MG, MM, and GG fractions within extracted alginates.

Effect of alginate on inflammatory, oxidative stress, and antioxidant markers

As depicted in Figure 2A and Table S1, the injection of MIA in male rats resulted in a notable elevation of inflammatory markers, specifically IL1β and TNF-α, in their sera. This increase was evident at two weeks post-treatment and persisted up to ten weeks when compared to the control group. Conversely, the commencement of alginate treatment in the fourth week after MIA injection induced a marked and substantial reduction in the expression of these markers. This reduction persisted throughout the six- and ten-week observation periods, as illustrated in Figure 2A and Table S1. Similar trends were observed in the case of aggrecans, β-glucuronidase, MMP-3, ACP, and MDA levels. Alginate treatment led

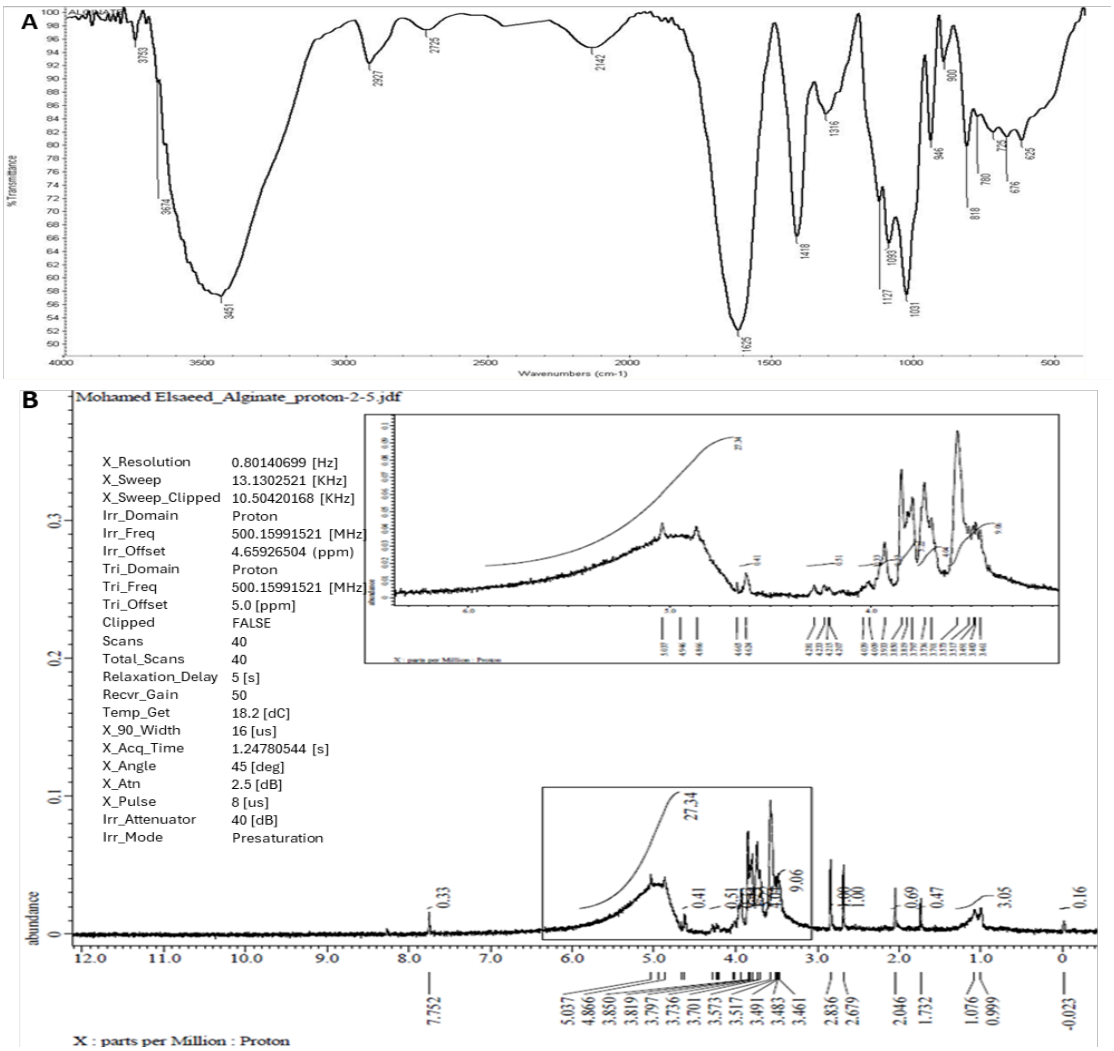

Figure 1. Chemical characterization of extracted alginate using FTIR (A), H-NMR (B) and Expansion of Low field of the 1H-NMR at 500 MHZ. Briefly, lyophilized alginate was formulated with Kbr to generate a small disk for spectral functional group analysis on FTIR. For H-NMR, powdered alginate was dissolved in D2O and properly dried before NMR spectrum acquisition.

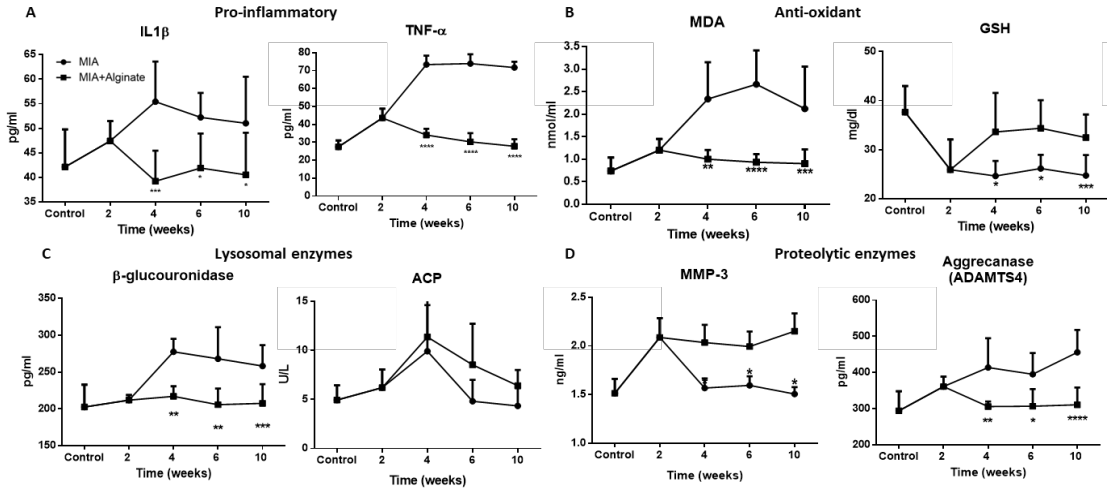

Figure 2. Effect of MIA injection and alginate treatment on pro-inflammatory markers IL-1β and TNF-α expression (A), antioxidant MDA and GSH (B), lysosomal enzyme β-glucuronidase and ACP ©, and proteolytic enzyme MMP-3, and aggrecans (D) expression in different rat groups. Briefly, rats were injected with MIA alone for ten consecutive weeks or supplemented with alginate from the second week (MIA for ten weeks and alginate for eight weeks). Rats were anesthetized, and blood samples were collected on a biweekly basis (two, four, six, and ten-week post-injection) and subjected to ELISA analysis of respective factors according to the manufacturer's protocol. N = 28, 28, and 21 for the control, MIA induction, and MIA+alginate groups, respectively. Statistical significance: *P<0.05.

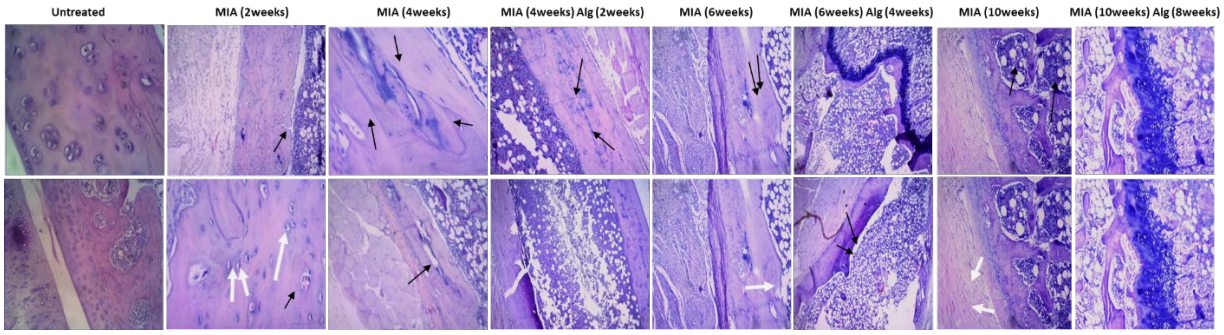

Figure 3. Histological alternation in rat knee cartilage in different rat groups (control, MIA induction, and MIA+alginate injection) during different time points (two, four, six, and 10 weeks). Briefly, at different points, five rats were anesthetized using diethyl ether and sacrificed, and knee cartilage tissue was collected for H&E histological evaluation. Knee tissues were fixed in 10% buffered formalin followed by decalcification in 5% nitric acid. Then, specimens were subjected to serial dehydration, paraffin embedding, sectioning, and H&E staining. Black and white arrows indicate different histological findings that were reported in the result section. n = 5, magnification = x100 for control group and x40 for all other groups.

Table 1. The coefficient correlation between the different parameters studied.

		GSH	MDA	β-gluco	Aggreac	IL1β	TNF-α
GSH	Pearson Correlation	1	-0.427**	-0.325**	-0.387**	-0.288**	-0.487**
	Sig.		0.000	0.004	0.000	0.008	0.000
MDA	Pearson Correlation		1	0.532**	0.448**	0.544**	0.550**
	Sig.			0.000	0.000	0.000	0.000
β-gluco	Pearson Correlation			1	0.360**	0.441**	0.728**
	Sig.				0.002	0.000	0.000
Aggreac	Pearson Correlation				1	0.225	0.503**
	Sig.					0.051	0.000
IL1β	Pearson Correlation					1	0.555**
	Sig.						0.000
TNF-α	Pearson Correlation						1
	Sig.						

** Correlation is significant at the 0.01 level (2-tailed).

to a significant reduction in the concentration of these markers compared to the group subjected only to MIA induction, as depicted in Figures 2B, C, and D & Table S2. Notably, after ten weeks of MIA injection and eight weeks of concurrent alginate treatment, the levels of aggrecans (Figure 2D), β-glucuronidase (Figure 2C & Table S2), and MDA (Figure 2B and Table S2) were significantly lower. Additionally, the introduction of alginate supplementation led to a substantial increase in the antioxidant glutathione (GSH) levels in the sera of male rats compared to the MIA-induced group, as depicted in Figure 2B & Table S2. Based on the reported results and subsequent statistical correlation analyses, a significant negative correlation was evident between GSH and MDA, aggrecans, β-glucuronidase, IL1β, and TNF-α, as detailed in Table 1. Conversely, there was a significant positive correlation between MDA, aggrecans, β-glucuronidase, IL1β, and TNF-α, as outlined in Table 1.

Effect of alginate on knee joint cartilage of rats

Figure 3 illustrates the histological features of the negative control group and the concurrent tissue alterations following MIA and alginate injections. The control group displays characteristics indicative of healthy knee joints, including an intact cartilaginous plate, absence of denudation, no cartilaginous cracks or fissures, homogenous distribution of the cartilaginous matrix, and a normal arrangement of chondrocytes. Injecting MIA into the rat knee for two weeks induced mild focal cartilaginous fissuring, focal chondroid hyperplasia, neovascularization within the cartilaginous matrix, and disorganization of chondrocyte-arrangement.

After four weeks of MIA induction, changes in chondroid matrix density, cartilaginous cracking and fissuring (particularly in the superficial cartilage, indicated by the white arrow), necrotic bony trabeculae, and focal chondroid atrophy were observed. Conversely, rats subjected to four weeks of MIA induction followed by two weeks of knee alginate injections displayed mild chondroid regeneration, an absence of fissures or cracks, and reduced chondrocyte disorientation. At the six-week mark of MIA induction, features included edema (indicated by the black arrow), denudation (white

arrow), decreased chondroid matrix, chondrocyte displacement, and atrophy, as well as chondral inflammation. Finally, ten weeks of MIA induction resulted in prominent fibrocartilaginous changes in the subchondral bone (black arrow), chondrocyte inflammation, proliferation (white arrow) with lobular disarrangements, and focal cracking of the chondroid matrix. In contrast, the eight-week treated group with alginate exhibited an increase in chondroid matrix intensity, an arranged and preserved chondrocyte lobular array, no indications of chondral fissures or cracks, and no denudation of the articular surface.

DISCUSSION

Osteoarthritis (OA) is the most prevalent joint disorder globally, impacting various body joints. Currently, pharmacological approaches to treat OA include non-steroidal anti-inflammatory drugs (NSAIDs), steroids, and disease-modifying OA drugs DMOAD (Lou and Bu, 2025), which is realized to be the only strategy for the treatment of pain and restoring the function of the diseased joints. Commonly used drugs are dexamethasone, etoricoxib, indomethacin, and diacerein, their mechanisms inhibiting MMPs expression like MMP1, MMP2, MMP3, MMP9, and MMP13 (Li et al., 2015). OA involves an accumulation of inflammatory mediators in synovial fluid, including prostaglandins, TNF, IL-1β, IL-6, ROS, and complement components (Sanchez-Lopez et al., 2022). These agents collectively trigger collagen degradation and cartilage breakdown (Sellam and Berenbaum, 2010). In our study, MIA induction in rats increased lipid peroxidation marker (MDA) and decreased glutathione levels, signifying an elevation in lipid peroxidation in osteoarthritic rats, and this aligns with previously reported findings (Surapaneni & Venkataramana, 2007).

In osteoarthritic joints, inflammation-associated tissue degeneration disrupts articular cartilage homeostasis. A hallmark of OA is synovial membrane inflammation, which recruits neutrophils and macrophages, releasing inflammatory cytokines IL-1β and TNF-α (Haraden et al., 2019). These cytokines induce IL-6, IL-8, and metalloproteinases, accelerating cartilage matrix breakdown (Hsueh et al., 2021). Chondrocyte-generated reactive oxygen species (ROS) due to synovial inflammation contribute to oxidative stress in OA (Vyas et al., 2015). Granulocytes and macrophages in arthritic areas produce superoxide and hydrogen peroxide radicals, depleting antioxidant enzymes, causing cell damage, and increasing lipid peroxidation (Zhao et al., 2023). Superoxide dismutase (SOD) and catalase detoxify radicals, while glutathione (GSH) is oxidized to GSSG, leading to GSH depletion during oxidative stress. Alginate may preserve SOD levels by scavenging free radicals and mitigate GSH decline, as observed in arthritis-induced groups, through its antioxidant properties (de la Coba et al., 2009).

Lipid peroxidation is a crucial mechanism in arthritis-induced injury (Yeom et al., 2006). Alginate treatment significantly enhances antioxidant activity compared to MIA-induced rats, protecting against free radical formation and mitigating inflammation. Alginate's antiarthritic effects include retarding lipid peroxidation and modulating the cellular antioxidant defence system. Histopathological examination of joint tissue reinforces alginate's protective potential, with reduced inflammation and oedema observed upon treatment (Yeom et al., 2006).

Prominent proinflammatory cytokines, such as TNF-α, IL-1β, and IL-6, primarily secreted by macrophages, feature prominently in the chronic state of OA (Zhao et al., 2023). These cytokines exacerbate pathological processes, fostering synovial tissue proliferation, joint destruction, and programmed cell death (Guan et al., 2024). TNF-α assumes a pivotal role in OA progression, inciting the production of other proinflammatory cytokines and collagenase enzymes (Ulfgren et al., 1995). In our study, the arthritic group displayed elevated levels of IL-1β, IL-6, and TNF-α, whereas alginate treatment significantly attenuated these cytokine levels. Synovial macrophages in arthritis secrete IL-1β, IL-6, and TNF-α, instigating collagenase and PGE2 synthesis, culminating in collagen degradation (Ulfgren et al., 1995). Furthermore, alginate exhibited antioxidative properties, as evident in the significant reduction of MDA levels and a corresponding increase in GSH levels. Alginate treatment also notably reduced lysosomal enzyme activity (Figure 2C & Table S2), indicative of diminished connective tissue degradation (Figure 3). These findings were substantiated by correlation coefficients, illustrating a significant negative correlation between GSH and other parameters and a highly significant positive correlation between MDA and the remaining parameters, encompassing β-glucuronidase, aggrecans, IL-1β, and TNF-α. Additionally, a significant positive correlation between IL-1β and TNF-α was observed, reinforcing the potential anti-inflammatory effects of alginate (Liao et al., 2020).

Following MIA induction, there was a notable increase in the concentration of MMP-3 in rat sera within the induction group compared to the control group, a result that is consistent with prior finding (El Beialy et al., 2019). This enzyme is known to activate several pro-MMPs, thereby initiating the synthesis of

MMP-3, which marks the initial stage in MMP-mediated degradation processes (Wan et al., 2021). MMP-3 has been implicated in promoting the growth of cartilage blood vessels, thereby facilitating the infiltration of inflammatory cells (Behl et al., 2021), and impeding the differentiation of mesenchymal stem cells into chondrocytes, leading to cartilage deterioration (Wan et al., 2021). In addition, cartilage exhibits low levels of protease inhibitors (Fransès et al., 2010) that counterbalance elevated MMP levels. It has been reported that the expression of MMP-3 in synoviocytes surpasses that in cartilage, which coincides with the observed angiogenesis in the knees of individuals with osteoarthritis (Koyama et al., 2021).

The progression of arthritis triggers lysosomal membrane fusion with the surrounding cellular membranes, resulting in the release of B-glucuronidase, B-hexosaminidase, cathepsin D, and ACP enzymes. This release leads to the solubilization of collagen and other structural macromolecules within connective tissue, altering the metabolism of glycoproteins and glycosaminoglycans (GAGs), and ultimately disrupting cellular activities (Giuliani et al., 2002). Our study demonstrated a significant increase in serum ACP and β-glucuronidase (β-G) levels in the MIA induction group compared to the control group, consistent with prior research (Primakoff and Myles, 2000).

Despite the stage or affected joint (hip or knee), ACP and β-G activity remains elevated throughout osteoarthritis (OA) due to cartilage destruction (Olszewska-Slonina et al., 2015). Notably, lysosomal rupture, the liberation of lysosomal enzymes, and the amplification of chondrocyte apoptosis were observed during OA progression (Takahashi et al., 2004). Lysosomal enzymes exhibit higher activity in synovial fluid, possessing proteolytic properties that likely contribute to joint aseptic loosening (Niissalo et al., 2002). The lack of nerves in the articular cartilage area results in OA patients not sensing significant pain during the early stages. However, as OA develops in the cartilage, damage occurs to the subchondral fat pad, subchondral bone, and other surrounding structures, exposing nerve endings and leading to intense pain (Hu et al., 2021). MMP-3 has been widely implicated as one of the key cytokines contributing to the progression of OA (Belluzzi et al., 2019).

In addressing the multifactorial nature of cartilage degradation in osteoarthritis (OA), the quest for an effective natural product targeting various components of this complex mechanism is imperative (Hermann et al., 2018). Current therapeutic options for OA patients involve physical therapy re-habilitation or pharmacological symptom management, which, while offering relief, come with long-term side effects. Therefore, a pressing need exists for a treatment that addresses the root causes of OA. Marine algae are a prolific source of natural bioactive compounds with diverse biomedical applications, notably rich in the alginate polymer (Cox et al., 2010). Alginate, a biocompatible natural polymer, serves as a substitute for cartilage glycosaminoglycans, demonstrating promise in cartilage tissue engineering (Mierisch et al., 2003).

In vitro studies underscore alginate's ability to expedite chondrogenesis and enhance progenitor cell proliferation (Igarashi et al., 2010; Igarashi et al., 2012).

In our study, a two-week MIA injection in male rat knees induced cartilaginous fissuring, disorganization of chondrocytes, and focal chondroid hyperplasia, elevated IL-1β, TNF-α, aggrecans, β-glucuronidase, and MDA levels, and decreased GSH levels compared to controls. This pattern persisted at 2, 4, and 8 weeks in MIA-induced rat sera. Alginate treatment mitigated MIA-induced oxidative stress, significantly reducing MDA levels and increasing GSH levels at these time points. Biochemical results demonstrated the ameliorative effects of intra-articular alginate injections, notably reducing chondroid matrix damage, fissuring, and chondrocyte disarrangement. Alginate treatment further improved chondroid regeneration and chondrocyte arrangement and reduced cartilage edema and denudation after two weeks. At six weeks, alginate-preserved chondroid matrix prevented cartilage denudation and edema. After ten weeks, alginate prevented fibrocartilaginous changes in subchondral bone, chondrocyte proliferation, and matrix cracking (Figure 4). Alginate treatment enhances chondroid matrix production, preserves cartilage integrity, and reduces edema and denudation (Figure 4). Histopathological analysis revealed that alginate treatment increased chondroid matrix intensity, maintained chondrocyte organization, prevented fissures and cracks, and inhibited articular surface denudation in the OA-induced rat model. These findings align with the biochemical data, indicating alginate's anti-inflammatory effects and its potential to ameliorate OA-related knee joint lesions. Collectively, these results underscore alginate's promise as an OA treatment, calling for further research to validate these findings and elucidate alginate's mechanism of action in OA.

CONCLUSION

In summary, our study shows that alginate from *C. indica* effectively alleviates inflammation and

oxidative stress in a rat OA model. Alginate significantly decreased pro-inflammatory cytokines IL-1β and TNF-α and decreased the activities of enzymes like beta-glucuronidase and aggrecans. It also exhibited antioxidant effects by decreasing MDA and raising GSH levels, supporting cartilage regeneration. These findings underline alginate's potential as an OA therapy, either alone or combined with other treatments. Further studies are needed to validate these results and explore the optimal alginate dosage for humans.

STATEMENTS AND DECLARATIONS

The authors have no competing interests to declare that they are relevant to the content of this article.

ETHICAL APPROVAL

The study was approved by the ethical guidelines of MRI, University of Alexandria (Appendix 1, Guiding Principles for Biomedical Research Involving Animals, 2011). The ethical approval number is AU 0122421812. Across the experiments, we compared non-treated animals and osteoarthritic animals at different time points after treatment.

FUNDING

The authors have no relevant financial or non-financial interests to disclose.

COMPETING INTERESTS

The authors have no relevant competence of interest.

AVAILABILITY OF DATA AND MATERIALS

The data sets that support this manuscript are available from the corresponding author upon a reasonable request.

AUTHORS CONTRIBUTIONS

Mohamed Helal & Amany El-Sikaily: Authors contributed equally to the manuscript.

ABBREVIATIONS

ACP: Acid phosphatase
ADAMTS-4: Aggrecans
ANOVA: Analysis of Variance
FT-IR: Fourier-transform infrared spectroscopy
GSH : Glutathionne
IL-1 : Interleukin-1
IL-1β : Interleukin-1 beta
MDA: Malondialdehyde
MIA: Monosodium iodoacetate
MRI: Medical Research Institute
MMP-3: Matrix metaloprotienases-3
NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells
NMR: Nuclear magnetic resonance
NO: Nitric oxide
OA: Osteoarthritis
TNF-α: Tumor necrosis factor-alpha

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