

Dose-Dependent Effects of Okra Seed Oil on Functional, Biochemical and Histological Changes in Monosodium Iodoacetate-Induced Knee Osteoarthritis in Sprague–Dawley Rats

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Abstract

Background: Osteoarthritis (OA) involves progressive cartilage degeneration, inflammatory activity and impaired joint function. Okra (*Abelmoschus esculentus* L.) seed oil contains unsaturated fatty acids, phenolics and tocopherols and exhibits antioxidant activity. This study evaluated whether oral okra seed oil attenuates MIA-induced knee OA in a dose-dependent manner. **Methods:** Thirty male Sprague–Dawley rats were observed as five groups (n=6): normal control, MIA control, MIA+diclofenac sodium (5 mg/kg/day), MIA+okra seed oil 400 mg/kg/day, and MIA+okra seed oil 800 mg/kg/day. Hindlimb grip strength was assessed at baseline and weekly for four weeks. Serum COMP, IL-1 β , TNF- α and MMP-13 and Safranin O/Fast Green histology with modified Mankin scoring were included. **Results:** MIA produced progressive loss of grip strength and increased biochemical and histological indices of joint damage. Both okra seed oil doses improved outcomes relative to MIA control, with the 800 mg/kg/day group showing the larger effect. Diclofenac produced a strong functional and anti-inflammatory response, while high-dose okra seed oil showed a comparatively strong structural pattern. **Conclusion:** The observed pattern supports a dose-responsive effect of okra seed oil in experimental OA.

Keywords

Abelmoschus esculentus; okra seed oil; osteoarthritis; monosodium iodoacetate; grip strength; MMP-13; COMP.

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INTRODUCTION

Osteoarthritis (OA) is a chronic whole-joint disorder involving progressive articular cartilage degradation, synovial inflammation, subchondral bone remodelling, pain and functional impairment. Contemporary reviews emphasise that OA biology reflects interactions between cartilage, synovium, bone, inflammatory mediators and extracellular-matrix turnover rather than simple mechanical wear [3,4,16,17].

Inflammatory cytokines and matrix-degrading enzymes participate in the catabolic joint environment. IL-1 β and TNF- α contribute to inflammatory signalling, while

MMP-13 is a major collagenase involved in type II collagen breakdown and cartilage matrix loss [3-5]. Cartilage oligomeric matrix protein (COMP) is increasingly used as a cartilage-related biomarker; recent clinical work showed higher serum and synovial-fluid COMP in knee OA and associations with disease severity, pain and physical disability [6,7,19].

Preclinical animal models remain important for evaluating candidate OA interventions. The monosodium iodoacetate (MIA) rat model is widely used because it produces reproducible pain-related behaviour and progressive cartilage pathology, although its rapid chemically induced course differs from

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naturally developing human OA [8-10,13]. Grip-strength impairment is a recognised functional feature of MIA-induced OA and has been quantified using hindlimb grip-strength testing in several rat studies [11-13,18].

Okra seed oil has a plausible biochemical basis for investigation in OA. Guo et al. reported that okra seed oil contains a high proportion of unsaturated fatty acids, particularly linoleic and oleic acids, together with phenolic compounds and tocopherols, and demonstrated antioxidant activity [1]. Talebi et al. independently characterised the fatty-acid profile and antioxidant activity of okra seed oil from different okra populations [2]. These compositional data support exploratory testing in an oxidative and inflammatory disease setting, but they do not establish efficacy in OA.

The present experimental concept therefore tests oral okra seed oil at two dose levels in MIA-induced knee OA. The central hypothesis is that okra seed oil will improve functional performance and reduce biochemical and histological evidence of joint damage, with a larger effect at the higher dose. Diclofenac is included as a positive control because recent validation work in the rat MIA model demonstrated an early-phase analgesic response to oral diclofenac [10].

MATERIALS AND METHODS

Study design

Thirty adult male Sprague–Dawley rats were randomly allocated to five groups (n=6/group). All experimental procedures involving animals were conducted at the Animal House of Al-Iraqia University, Baghdad, Iraq.

Group	OA induction	Treatment
Normal control	Saline	Vehicle
MIA control	MIA	Vehicle
Positive control	MIA	Diclofenac sodium 5 mg/kg/day, oral
OSO-400	MIA	Okra seed oil 400 mg/kg/day, oral
OSO-800	MIA	Okra seed oil 800 mg/kg/day, oral

OA induction and treatment

A single intra-articular MIA injection into the left knee was used to induce OA. MIA is a widely used chemical model of rat knee OA, with pathological changes that evolve over time [8-10]. Treatment began 24 h after MIA administration and continued once daily for 28 days. Diclofenac sodium (5 mg/kg/day, orally) served as the positive control. Okra seed oil was administered orally at 400 or 800 mg/kg/day.

Hindlimb grip strength

Hindlimb grip strength was assessed at baseline and at weeks 1, 2, 3 and 4 using a calibrated grip-strength meter. Multiple technically valid trials were obtained for each rat and averaged. Hindlimb grip strength is an established pain- and function-related outcome in the MIA rat model [11-13,18].

Biochemical analysis

At day 28, serum COMP, IL-1 β , TNF- α and MMP-13 were measured using rat-specific ELISA assays under standardised conditions. COMP was used as a cartilage-related

biomarker, whereas IL-1 β , TNF- α and MMP-13 were included as indicators of inflammatory and catabolic activity relevant to OA [3-7,19].

Histological analysis

Knee joints were fixed, decalcified, paraffin embedded and sectioned. Safranin O/Fast Green staining was used to evaluate cartilage morphology and proteoglycan loss. Cartilage damage was graded using a modified Mankin scoring system. OARSI recommendations for rat OA histopathology provide an established framework for reproducible assessment [14].

Statistical analysis

Data are presented as mean $\pm$ standard deviation (SD). Because animal-level raw data and verified inferential outputs were not available in the manuscript dataset used for this draft, the present Results section reports descriptive group differences only and does not assign unverified p-values. In the final submission, repeated grip-strength measurements should be analysed with an appropriate repeated-measures or mixed-effects model, while endpoint biochemical and histological outcomes should be analysed using tests appropriate to their distribution and scale. Reporting should follow ARRIVE 2.0 principles [15].

RESULTS

Hindlimb grip strength

Group	Baseline	Week 1	Week 2	Week 3	Week 4
Normal control	1.62 $\pm$ 0.12	1.61 $\pm$ 0.11	1.63 $\pm$ 0.10	1.64 $\pm$ 0.11	1.65 $\pm$ 0.10
MIA control	1.61 $\pm$ 0.11	1.17 $\pm$ 0.12	1.04 $\pm$ 0.11	0.96 $\pm$ 0.10	0.91 $\pm$ 0.09
Diclofenac	1.60 $\pm$ 0.13	1.25 $\pm$ 0.11	1.35 $\pm$ 0.12	1.45 $\pm$ 0.11	1.51 $\pm$ 0.10
OSO-400	1.61 $\pm$ 0.12	1.20 $\pm$ 0.12	1.27 $\pm$ 0.11	1.35 $\pm$ 0.12	1.41 $\pm$ 0.11
OSO-800	1.60 $\pm$ 0.11	1.23 $\pm$ 0.10	1.34 $\pm$ 0.11	1.45 $\pm$ 0.10	1.50 $\pm$ 0.09

MIA produced a progressive reduction in hindlimb grip strength across the four-week period. At week 4, mean grip strength was 0.91 $\pm$ 0.09 in the MIA control group, compared with 1.41 $\pm$ 0.11 in the OSO-400

group and 1.50 $\pm$ 0.09 in the OSO-800 group. The diclofenac group reached 1.51 $\pm$ 0.10, while the normal control remained stable at 1.65 $\pm$ 0.10.

Serum biomarkers at week 4

Group	COMP (ng/mL)	IL-1 β (pg/mL)	TNF- α (pg/mL)	MMP-13 (ng/mL)
Normal control	42 $\pm$ 6	18 $\pm$ 4	22 $\pm$ 5	34 $\pm$ 6
MIA control	86 $\pm$ 10	46 $\pm$ 7	51 $\pm$ 8	78 $\pm$ 9
Diclofenac	57 $\pm$ 7	25 $\pm$ 5	29 $\pm$ 6	52 $\pm$ 7
OSO-400	68 $\pm$ 8	34 $\pm$ 6	38 $\pm$ 6	61 $\pm$ 8
OSO-800	56 $\pm$ 7	27 $\pm$ 5	31 $\pm$ 5	49 $\pm$ 7

The MIA group showed the highest mean concentrations of COMP, IL-1 β , TNF- α and MMP-13. The OSO-400 group showed

intermediate values, whereas the OSO-800 group showed lower concentrations across all four biomarkers. The descriptive values in the

OSO-800 group were close to those observed with diclofenac.

Histological cartilage damage

Group	Modified Mankin score
Normal control	0.8 ± 0.7
MIA control	9.8 ± 1.5
Diclofenac	5.2 ± 1.2
OSO-400	6.4 ± 1.3
OSO-800	4.6 ± 1.1

MIA produced marked cartilage damage, with a mean modified Mankin score of 9.8 ± 1.5 compared with 0.8 ± 0.7 in the normal control group. The score was lower in both okra seed oil groups (6.4 ± 1.3 at 400 mg/kg/day and 4.6 ± 1.1 at 800 mg/kg/day). The diclofenac group had a mean score of 5.2 ± 1.2 .

DISCUSSION

The apparent dose-response pattern is one of the most notable features of the study. Across grip strength, serum biomarkers and histological scoring, the 800 mg/kg/day group generally showed a larger descriptive improvement than the 400 mg/kg/day group. Reproduction of this pattern in a larger, adequately powered experiment would strengthen the case for dose-related activity and would help define the lowest effective dose. Future work should also characterise the oil batch chemically, evaluate local joint mediators and oxidative-stress markers, and include rigorous animal-level statistical analysis to clarify the relationship between functional improvement and cartilage preservation.

A plausible basis for the observed pattern is provided by the composition of okra seed oil. Guo et al. reported that okra seed oil contains a high proportion of unsaturated fatty acids, particularly linoleic and oleic acids, together with phenolic compounds and tocopherols, and demonstrated antioxidant activity [1].

Talebi et al. also characterised the fatty-acid constituents and antioxidant activity of okra seed oil [2]. Oxidative and inflammatory processes contribute to OA progression, so these constituents provide a reasonable biological rationale for further mechanistic investigation. Importantly, the composition studies do not themselves demonstrate anti-osteoarthritic efficacy; the present findings should therefore be viewed as experimental evidence requiring confirmation rather than as a mechanism already established in the literature.

The histological findings strengthen the interpretation of the biochemical results. The untreated MIA group had the highest modified Mankin score, whereas both okra seed oil groups had lower scores. The 800 mg/kg/day group showed a lower mean score than the 400 mg/kg/day group and was also descriptively lower than the diclofenac group. Because histological scoring directly reflects cartilage structural damage, this pattern raises the possibility that the higher dose exerted a cartilage-preserving effect. Nevertheless, a claim of superiority to diclofenac would require prespecified statistical comparison and adequate power; the present descriptive data do not establish such superiority.

The biochemical findings followed the same dose-related pattern. MIA produced the

highest mean levels of IL-1 β , TNF- α , MMP-13 and COMP, whereas treatment with okra seed oil was associated with lower values, particularly at 800 mg/kg/day. IL-1 β and TNF- α are central inflammatory mediators in OA, and MMP-13 contributes to extracellular-matrix degradation, particularly type II collagen breakdown [3-5]. COMP is a cartilage-related biomarker that has been associated with knee OA severity and functional impairment [6,7,19,21]. Taken together, the lower values observed in the treated groups are compatible with attenuation of inflammatory and cartilage-catabolic activity, although circulating biomarkers alone cannot establish a local mechanism within the joint.

The functional findings are particularly relevant. Grip strength fell steadily in untreated MIA animals, whereas both okra seed oil groups showed recovery over time. By week 4, the mean value in the 800 mg/kg/day group was close to that observed in the diclofenac group. Hindlimb grip strength has been used as a pain- and function-related measure in MIA-induced OA [11-13,18], so the direction of change observed here is compatible with reduced functional impairment. However, because the current manuscript does not include verified inferential statistics from animal-level data, these comparisons should be interpreted descriptively rather than as proof of statistical equivalence or superiority.

The present study showed a consistent descriptive pattern across functional, biochemical and histological outcomes. MIA administration was followed by progressive loss of hindlimb grip strength, higher concentrations of cartilage- and inflammation-related biomarkers, and marked histological cartilage damage. In contrast, rats treated with okra seed oil showed better functional performance and

lower biomarker and Mankin values, with the 800 mg/kg/day group showing the strongest overall response. This concordance across outcome domains is important because the MIA model is characterised by both pain-related functional impairment and progressive cartilage pathology [8-13,18,22,23].

Limitations

- The study used a small pilot sample (n=6/group), which limits precision and the strength of between-group inference.
- The pilot sample size may be insufficient to detect modest between-group effects and limits generalisability.
- The study evaluated only two okra seed oil doses; additional dose-ranging and safety work would be needed to define an optimal therapeutic window.
- Biochemical markers were assessed at the study endpoint; transient changes earlier in the disease course may therefore have been missed.
- Oil composition can vary by cultivar, extraction method and storage; batch characterisation is important.

CONCLUSION

Oral okra seed oil was associated with a dose-related descriptive improvement in hindlimb grip strength, lower serum IL-1 β , TNF- α , MMP-13 and COMP values, and reduced histological cartilage damage in rats with MIA-induced knee OA. The 800 mg/kg/day group showed the strongest overall response across the measured outcomes and approached the diclofenac group for several functional and biochemical measures. These findings support further investigation of okra seed oil as a potential chondroprotective and function-preserving intervention. Confirmation with animal-level inferential statistics, larger sample sizes and mechanistic

analyses is required before definitive efficacy or comparative claims are made.

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