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Original Article

Analgesic and anti-inflammatory activities of mangiferin gel for musculoskeletal injuries in cancer patients

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Abstract: Objective: Musculoskeletal injuries, a global public health concern, are among the most significant causes of long-lasting disability and meager performance in activities of daily living (ADLs). Methods: Mangiferin (5%) was used to formulate a gel, extracted from aqueous-methanolic (30:70) extracts of M. indica leaf. Participants (n = 200) diagnosed with musculoskeletal injuries were separated into four groups (n = 50/group). Group I and II received phonophoresis with 5% mangiferin gel and 1% diclofenac gel, respectively, while Group III and IV received superficial massage with the same gels. Color, stability test, pH, spreadability test, pain, onset of pain relief, stiffness, ADLs were evaluated through the Numeric Pain Rating Scale (NPRS), Global Pain Relief Scale (GPRS), and Western Ontario and McMaster Universities Arthritis Index (WOMAC) Scale. Results: NPRS was relieved in Group-I, while WOMAC was also reduced in Group-I, along with ADLs and stiffness measures; this improvement was greater than that in Group-II for all measures. Also, the NPRS of Group III was reduced along with WOMAC and ADLs scores and stiffness, more effectively that the same measures in Group IV. Conclusion: Mangiferin gel 5% has been proven more effective than diclofenac diethyl-ammonium gel 1% in treating human musculoskeletal injuries. Phonophoresis enhanced the effect of both gels, strongly suggesting that the topical application of mangiferin gel combined with phonophoresis could be a valuable therapeutic alternative to reduce inflammation and relieve pain significantly. The formulation of mangiferin gel is nature-based, cost-effective, eco-friendly, and prepared easily.

Keywords: Mangiferin gel, diclofenac diethyl-ammonium, phonophoresis, pain, musculoskeletal injuries

Introduction

The complex disease known as cancer is caused by multiple interactions between genes and the environment [1]. Globally, cancer is a significant public health issue. Global statistics indicate that the incidence of cancer will rise over the next few decades, with more than twenty million new cases projected annually by 2025 [2]. It was estimated that there would be 8.2 million cancer-related deaths and 14.1 million new cases in 2012 based on GLOBOCAN data [3]. In Europe, the most common malignancies diagnosed are those of the female breast, colon, prostate, and lung. Lung cancer continues to be the most prevalent form of cancer and the leading cause of death globally [4]. Significant side effects of cancer therapy and metastases to the bone, include bone loss and muscle weakness. In particular, there is a strong likelihood that malignancies of the breast, prostate, and lung may metastasize to the bone; in individuals with advanced stages of the disease, this likelihood is 73%, 68%, and 36%, respectively [1-3]. Cancer patients are more likely to fall and incur fracture because of their weakening muscles and loss of bone. It has been demonstrated that women receiving chemotherapy for recently-diagnosed breast cancer have a five-fold increased risk of frac-

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tures [3]. High levels of interleukin-6 and heightened oxidative stress are signs of an increased inflammatory response that is linked to cancer cachexia [1, 5]. Tumor growth and metastasis are also associated with these same factors. When bone metastases are present, they alter the intracellular calcium released in skeletal muscle through ryanodine receptor 1 (RyR1) channels [3]. These channels are needed for muscle contraction and excitation.

Cancer patients with bone metastases also show evidence of RyR1 Ca^{2+} channel leakage in skeletal muscle biopsies [6]. When muscular and soft tissue injuries result in inflammation, inflammatory biomarkers like IL-6, IL-1, TNF-α, PGE-2, COX-2, and MCP-1 are elevated [7]. It is possibility that increased muscle mass, function, and strength could stop cachexia from developing further or occurring altogether [1, 7]. These musculoskeletal injuries also hamper survival, quality of life, and performance status. Compared to healthy controls, chemotherapy-treated cancer patients had reduced hand-grip strength, longer chair rise times, and shorter 12-minute walking distances [8, 9]. Chemotherapy may also lessen the body's capacity to withstand or react to anti-tumor therapies, perhaps leading to an extended hospital stay, an increased likelihood of treatment disruptions, or even a decreased probability of survival. A reduction in lean body mass, which affects strength and the ability to move, also lowers quality of life, being the hallmark of sarcopenia, as it affects ADLs and causes severe pain [10, 11].

Mangifera indica, otherwise known as mango or 'Chaunsa', is a native of the Indo-Pak region and a member of the Anacardiaceae family [12]. Many countries have used mango extract from the tree's roots and pulp from the fruits, bark, leaves, stems, and kernels for medical purposes [13, 14]. Numerous chemical components, such as flavonoids, saponins, alkaloids, vitamins C and B, minerals, and phenols, are present in *M. indica* leaves [15, 16]. The biological activities of the leaf extract include immunomodulatory, cardioprotective, hepatoprotective, anti-diabetic, anti-microbial, analgesic, anti-allergic, and anti-inflammatory effects [17-19].

M. indica has a xanthone molecule named mangiferin in its various sections. Mangiferin is produced by hydrolyzing the aglycone 1,3,6,7-tetrahydroxy-xanthone with R-acetobromoglucose and forming an O-glycosidic bond (**Figure 1**) [20]. It has hepatoprotective, anti-aging, anticancer, anti-diabetic, antiviral, and immunomodulatory qualities [21, 22]. Mangiferin is used to treat a variety of eye conditions. In human white blood cells, mangiferin reduces the oxidative damage that hydrogen peroxide induces in lipids [23, 24].

In this study, we extracted mangiferin from *M. indica* leaves and created a gel to treat several forms of inflammation and pain associated with musculoskeletal injuries, including injuries to the soft tissues and muscles, which are thought to be the most frequent causes of pain, chronic disease, and impaired functioning in activities of daily life in cancer patients. Mangiferin is a miraculous medication with anti-inflammatory and anticancer properties, but sadly, its low absorption prevented it from being used therapeutically. Several researchers have recently experimented with mangiferin nanoparticles to enhance absorption; however, this is the first time we have attempted to do so via phonophoresis, which utilizes ultrasound to enhance the absorption of topicals through the skin.

Materials and methods

Drugs, chemicals, and instruments

SAMI Pharmaceuticals (Pvt). Ltd., Karachi, SD, Pakistan supplied the 1% diclofenac diethyl ammonium. Duksan Pure Chemicals Co., Ltd., South Korea, supplied carbpol-940. Merck, Germany, supplied the methanol. Sigma-Aldrich, Pakistan, supplied the sodium benzoate, triethanolamine, and glycerol. SARCO Chemicals, Multan, PU, Pakistan, supplied distilled the water. We purchased a therapeutic ultrasound, Enraf NoniusSonoplus 590. The additional reagents and substances utilized in this investigation were all pharmaceutical grade.

High-performance liquid chromatography (HPLC) analysis

When using C-18 in HPLC (Hitachi High-Tech), a binary solvent gradient technique was applied, and a column (with an internal diameter of 250 mm) appropriate for separating one polyphenol (mangiferin) and two flavonoids (quercetin and

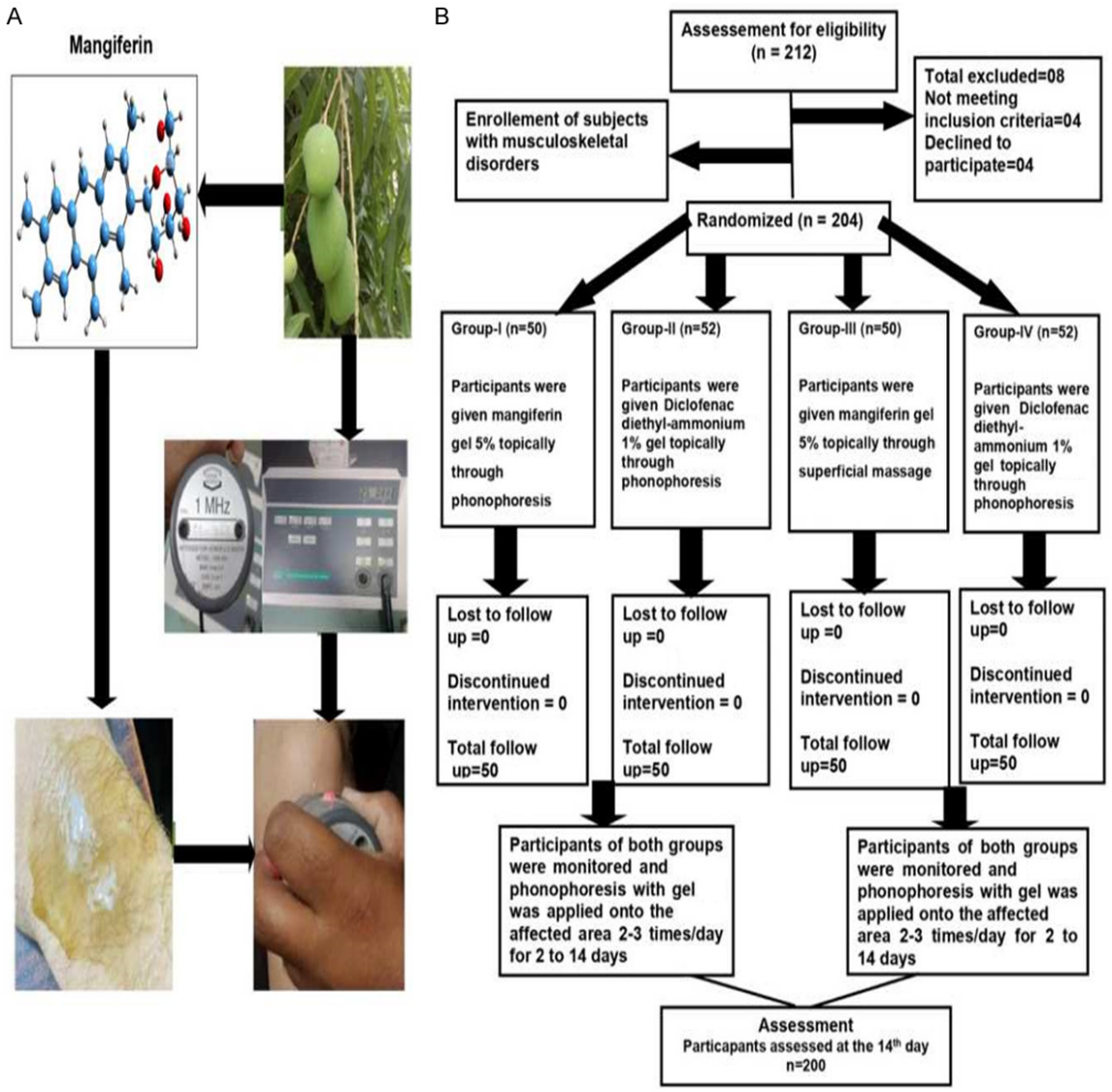

Figure 1. CONSORT diagram of methodology (A) used for anti-inflammatory activity and photographic representation of methodology used for anti-inflammatory activity (B).

isoquercetin) in thirty-six minutes was utilized. The flow rate was 0.0008 L/min, and 5 m was the film depth at a temperature of 30°C in the oven. As a reference point, mangiferin, isoquercetin and quercetin were acquired from Aldrich (St. Louis, USA). Methanol was used to make the dilutions in order to reach g/mL. Sample retention periods were contrasted as per industry standards. The resolution and separation factors were used to evaluate the effectiveness of HPLC-separated components [15, 16].

Antioxidant activity

Antioxidant activity was performed using 2,2-diphenylpicrylhydrazyl (DPPH), Nitric oxide (NO) assay, and Superoxide Dismutase Assay (SOD).

DPPH assay

The methanol-diluted sample was combined with mangiferin to get a final volume of 5 mL for the DPPH test. Subsequently, the conjunction was kept in the darkness for a duration of 40 minutes. The absorbance of the given solution at a wavelength of 517 nm was determined using a spectrophotometer (Shimadzu Corporation, Alibaba, Hangzhou, China), UV-1800 UV-Vis spectrophotometer [25-27]. The trial was conducted in triplicate, and the reduction in vitamin C (standard) equivalency was record-

ed at 35%, 36%, 37%, and 38%. A mathematical calculation was employed to calculate the percent of DPPH scavenging capacity [28-30].

1% = A (blank) - B (sample)/A (blank) × 100

Measurement of NO scavenging capacity

We gradually added distilled water to the isolated mangiferin, which allowed the concentrations to be adjusted from 100 to 1,000 µg/mL. The method used for Gallic acid (standard) was the same. The dilutions for the tests were stored at 4 degrees Celsius. The process employed a recently synthesized Griess reagent, Sigma-Aldrich (Merck Group). One milliliter of each extract concentration was mixed with 0.5 milliliters of phosphate-buffered saline containing 10 millimeters of sodium nitroprusside (ranging from 100 to 1,000 g/mL) [25-27] and left to incubate for three hours at 25 degrees Celsius. An equal amount of recently prepared Griess reagent was added. The preparation of the control samples was the same as the test samples, except for the extracts and the same amount of buffer. Similar extracts were present in the color tubes, but sodium nitroprusside was absent. The reaction mixture was dispensed onto a 96-well plate in a volume of 150 µL [28-30]. The UV-Vis spectrophotometer reader (Alibaba, Hangzhou, China) was used to measure the absorbance at 546 nm, as previously mentioned in our past communications. The mangiferin content was extracted and quantified using the following formula. The inhibition percentage of the standard was also calculated and recorded. The nitrite radical scavenging activity of Gallic acid and the extracts was considered.

Percent NO scavenging activity: A (blank) - B (sample)/A (blank) × 100

Gel formulation and quality tests

Extracted mangiferin 5% was mixed in distilled water, followed by the addition of triethanolamine, carbpol-940, sodium benzoate and glycerol. These ingredients were then thoroughly mixed and put into plastic bottles with spray caps for application. Carbopol-940 was used as a gelling agent to prepare the mangiferin gel. Sodium benzoate was used as a preservative. Triethanolamine and glycerol were used as emulsifiers [31].

Study design and ethical consideration

Using the lottery methodology, 200 participants with musculoskeletal injuries, including soft tissue and muscle injuries [32], were divided equally into four groups. The original baseline history was collected to assess efficacy and equality of distribution. Using phonophoresis by therapeutic ultrasound (continuous mode, 1 MH, 0.8 W/cm^2) [33, 34], Group I and Group II were given topical applications of 5% mangiferin gel and 1% diclofenac diethylammonium gel, respectively. In addition, using superficial massage, Group III and Group IV received topical application of mangiferin gel 5% and diclofenac diethyl-ammonium 1% gel, respectively, on the pretentious parts of participants (**Figure 1A**). If the injuries worsened, participants in both groups were monitored and instructed to stop topical application of gel onto the afflicted area two to three times a day for two to fourteen days, depending on the severity of the injuries, with distinct attention paid to using a loose bandage and to keep it out of the eyes. The gel's application quantity depends upon the site/area of injury and the number of affected areas (**Figure 1B**).

Anti-inflammatory and analgesic activities

The anti-inflammatory and analgesic effects of topically applied mangiferin and diclofenac gels combined with massage and phonophoresis on various muscle and soft tissue injuries in humans were evaluated using the following standard pain rating scales.

Numeric pain rating scale (NPRS)

NPRS is an 11-point numeric scale that ranges from 0 to 10, primarily used for scoring pain. Where 0 indicates no pain and 10 indicates extreme pain. We use NPRS in this study pretreatment and post-treatment for anti-nociceptive activity because it is simple and easy to understand [35].

Western Ontario and McMaster Universities Arthritis Index (WOMAC)

WOMAC scale is mainly used for the assessment of functional ability. It has 24 scores in three essential areas: pain, stiffness, and ADLs [35].

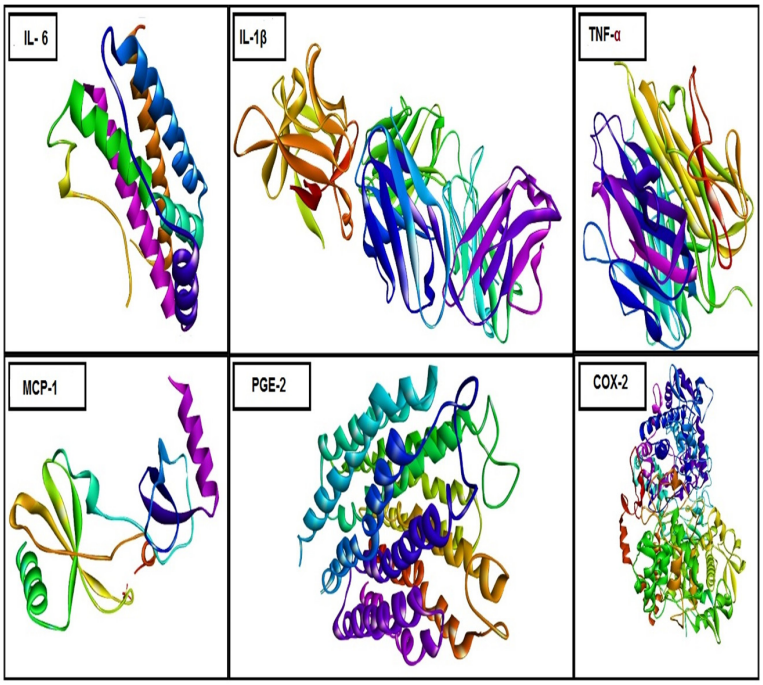

Figure 2. Crystal structures (3D) of standard docking proteins.

MMFF-94's force field, the ligands' residual energy was reduced to a minimum (**Figure 2**). Likewise, ligand atoms were assigned Gasteiger partial charges, and non-polar hydrogen atoms were combined to determine rotatable bonds. The AutoDock methods were used to add the required hydrogen atoms and pertinent properties to the protein molecule; these were then used in the docking calculations. Furthermore, Gasteiger partial charges were applied to the ligand atoms, and the non-polar hydrogen atoms were combined to identify the rotatable bonds. The program Visualizer-3.1 was utilized to watch how each protein interacted with its ligand.

Global pain relief scale

The global pain relief scale is a seven-point rating system based on the proportion of patients who reported at least moderate pain alleviation. It is used to assess the degree of pain relief that patients have received [36].

Data collection

Written consent forms were signed by participants involved in this study. The specific point of time was measured to identify the significant pain relief effect (0-12 min). An NPRS score was used to quantify the pain level before and after applying both gels. WOMAC was utilized to quantify stiffness, pain, and activities of daily living (ADLs), and the half-life of the gel was used to determine the end-point time of the pain reduction effect [35-37]. The intensity of pain was quantified both pre and post-administration of gels, by NPRS and the global pain relief scale. No type of skin allergy or other side effects of mangiferin gel were reported.

Molecular docking of mangiferin

"AutoDock 4.2" was used to dock all of the produced ligands and enzymes of the IL-1β (4g6j), IL-6 (1aLu), MCP-1 (1dok), TNF-α (1tnf), COX-2 (5kir) and PGE-2 (2dww), enzymes. Using

Statistical analysis

The software GraphPad Prism eighth edition was used to analyse the data. The quantitative data were measured as mean ± SD, the means of each group were compared pre and post administration of gels using a paired t-test, and the means of four separate groups were compared using one-way analysis of variance (ANOVA) using SPSS-23. A significance level of $P<0.005$ indicates a 95% confidence interval.

Results

HPLC analysis

Under optimized UTPP conditions (temperature of 30 ± 2°C, pH of 6, frequency of 25 kHz, duty cycle of 50%, power of 180 W, soaking time of 5 min, the saturation of ammonium sulphate of 40% (w/v), solute to solvent ratio of 1:40, and slurry to t-butanol ratio of 1:1), the maximum yield of mangiferin was obtained from *M. indica* leaves in 25 min. Mangiferin was confirmed at a retention time of 7.5 min concerning standard mangiferin (**Figure 3**).

Antioxidant assays

The DPPH assay revealed an antioxidant potential of mangiferin with reference to standard

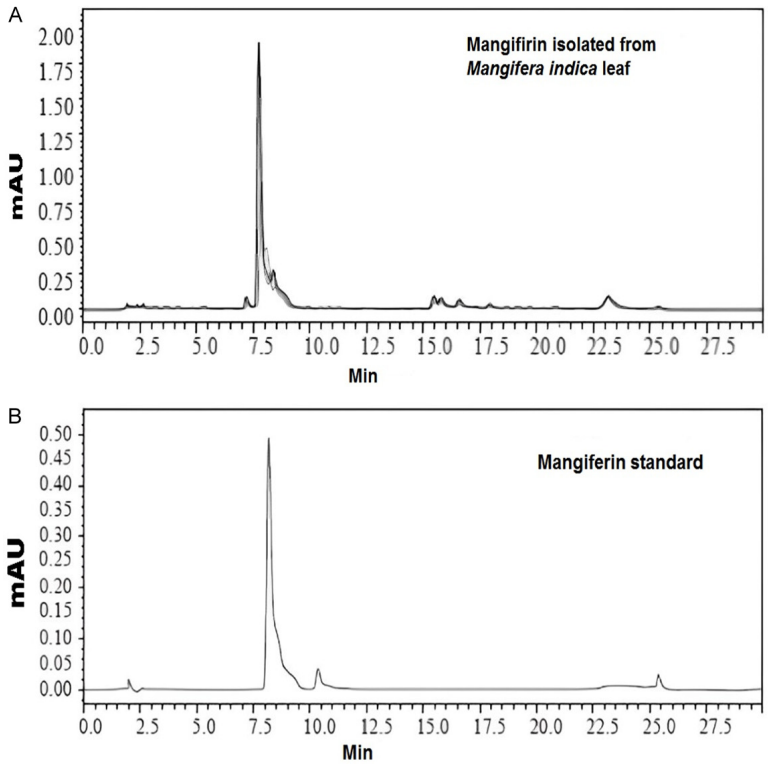

Figure 3. HPLC chromatogram of mangiferin extracted from the leaves of *M. indica* (A) and standard mangiferin (B) with respect to retention time (7.5 min).

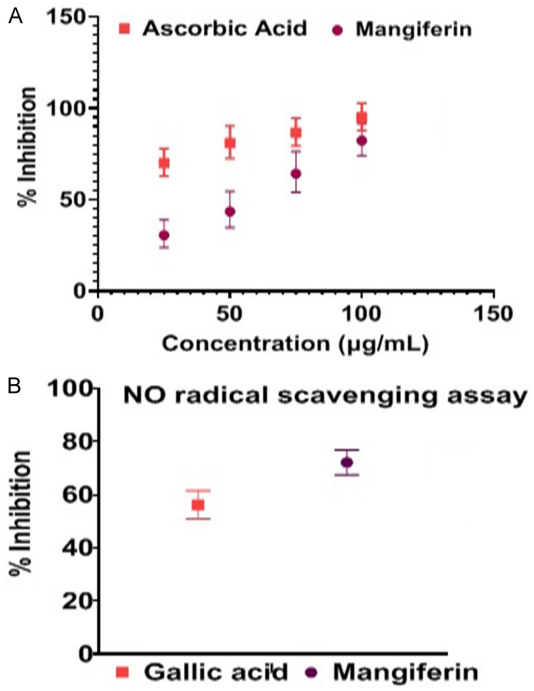

Figure 4. Antioxidant potential of mangiferin using DPPH assay concerning ascorbic acid (A), antioxidant potential of mangiferin using NO scavenging assay with reference to standard gallic acid (B).

ascorbic acid (**Figure 4A**). The antioxidant potential of mangiferin using NO scavenging assay was performed in reference to standard gallic acid (**Figure 4B**).

Stability test of mangiferin gel

Upon visual assessment, the mangiferin gel appeared to be a homogeneous substance with excellent spreadability characteristics. It had a distinct smell, was opaque, and had a pH value of 6.3 ± 0.2 (Hanna HI-98127 pH Tester). Its color was yellowish-green. Reliable results were obtained based on appearance, density, and pH, which were stable throughout 12 months.

Musculoskeletal injuries included in this study

Prevalence of musculoskeletal injuries included in the study (**Figure 5**).

Cooling effect

After administering mangiferin and diclofenac gels, cooling was evaluated, and the alteration between the two groups and the point of departure was calculated by measuring the entire amount of time. According to the results, the cooling effect produced by mangiferin gel on the applied areas is more potent than that of diclofenac gel. The study's findings demonstrated that, compared to diclofenac, mangiferin gel produced a more significant cooling impact, ranging from 80% to 90%, on areas applied within 3-4 minutes (**Figure 6**).

Anti-nociceptive activity based on global pain relief scores

The results of the global pain relief scale were highly significant in groups that received mangiferin gel with phonophoresis ($P<0.001$) and massage ($P<0.005$), compared to groups that received diclofenac diethyl-ammonium gel with phonophoresis ($P<0.002$) and massage ($P<0.005$) (**Figure 7**).

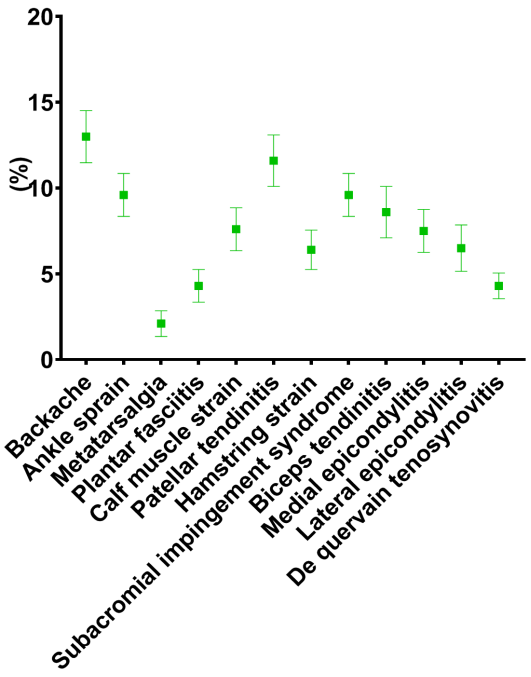

Figure 5. Incidence of muscular and soft tissue injuries in the human cohort population included in this study.

Onset of pain relief (NPRS score)

Time (0-12 min) was noted after gel application (test formulation vs. control formulation) when there was a decrease of 2 points in a total of 11 points of the NPRS. As shown in **Figure 8**, the mangiferin gel applied through phonophoresis ($P<0.000$) revealed a significantly faster anti-nociceptive response compared to mangiferin gel delivered through massage ($P<0.02$), or diclofenac gel delivered through phonophoresis ($P<0.001$) and diclofenac gel applied with massage ($P<0.05$). Phonophoresis had clearly ($P<0.05$) an adjuvant effect on pain relief when combined with test gel application ($P<0.000$) or the control gel ($P<0.001$).

Anti-nociceptive activity based on NPRS score

While diclofenac diethyl-ammonium gel demonstrated considerable anti-nociceptive action delivered through phonophoresis ($P< 0.001$) and massage ($P<0.05$), mangiferin gel demonstrated significant anti-nociceptive activity when delivered through phonophoresis ($P<0.000$) in contrast to massage ($P<0.002$) (**Figure 8**).

Anti-nociceptive and anti-inflammatory activities based on WOMAC scores

The results shown in **Figure 9** indicate that the application of mangiferin gel with phonophoresis resulted in significant increases in pain relief measures (pain $P<0.000$, stiffness $P<0.003$, and ADLs $P<0.001$). On the other hand, research on the effects of mangiferin gel applied through massage had less robust rain relief measures (pain via WOMAC $P<0.001$, stiffness $P<0.003$, ADLs $P<0.002$); while diclofenac gel delivered with phonophoresis relieved pain measures to a lesser degree (pain $P<0.001$, stiffness $P<0.003$, ADLs $P<0.002$), and diclofenac gel applied through massage gave similar relief (pain $P<0.004$, stiffness $P<0.005$, ADLs $P<0.004$) (**Figure 10**).

Molecular docking study

The 'orientation' of the mangiferin molecule (PubChem ID: 5281647) as demonstrated by autoDock, appeared to be bounded by the active pockets of the IL-1β, COX-2, IL-6, MCP-1, TNF-α, PGE-2, and IL-6 enzymes. Significant fitness ratings and hydrogen bonding against COX-2 were seen in the association of mangiferin with the active pockets of IL-1β, MCP-1, IL-6, TNF-α, COX-2 and PGE-2. Mangiferin showed that non-covalent interactions with enzymes could result in minimal binding energy (**Figure 11**).

Proposed mechanism of action

Without altering COX-2 transcription, mangiferin reduces the amount of PGE-2 and COX-2 protein generated through LPS, exhibiting anti-inflammatory and analgesic properties. Furthermore, it can lower the plasma concentrations of MCP-1 (monocyte chemoattractant protein-1), IL-6, TNF-α, and IL-1β (**Figure 12**) [13-21]. Therapeutic use of ultrasound increases the absorption of mangiferin. As a result, a better effect is observed by promoting angiogenesis and muscle regeneration, modulating inflammation, and inhibiting fibrosis in injured muscle, which may be possible via the inhibition of pro-proliferative, pro-inflammatory, and pro-fibrosis genes.

Discussion

Deterioration of musculoskeletal function has a severe effect on quality of life. Both the tumor

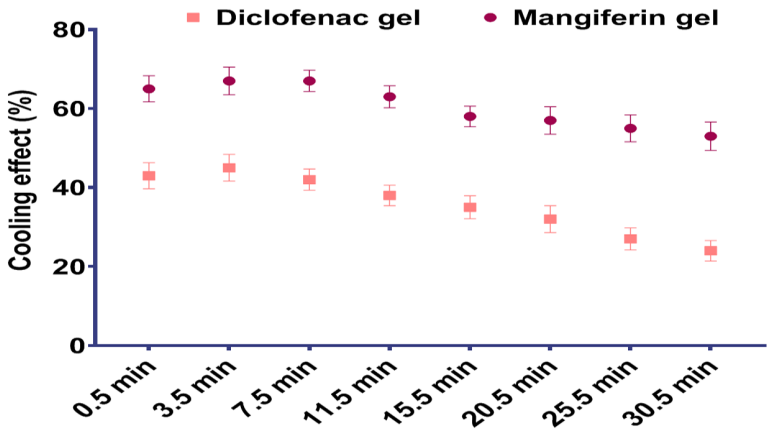

Figure 6. The % cooling effect of the mangiferin gel and diclofenac gel over time. Mangiferin gel shows the greatest cooling effect than diclofenac and the highest cooling effect at 3.5 min.

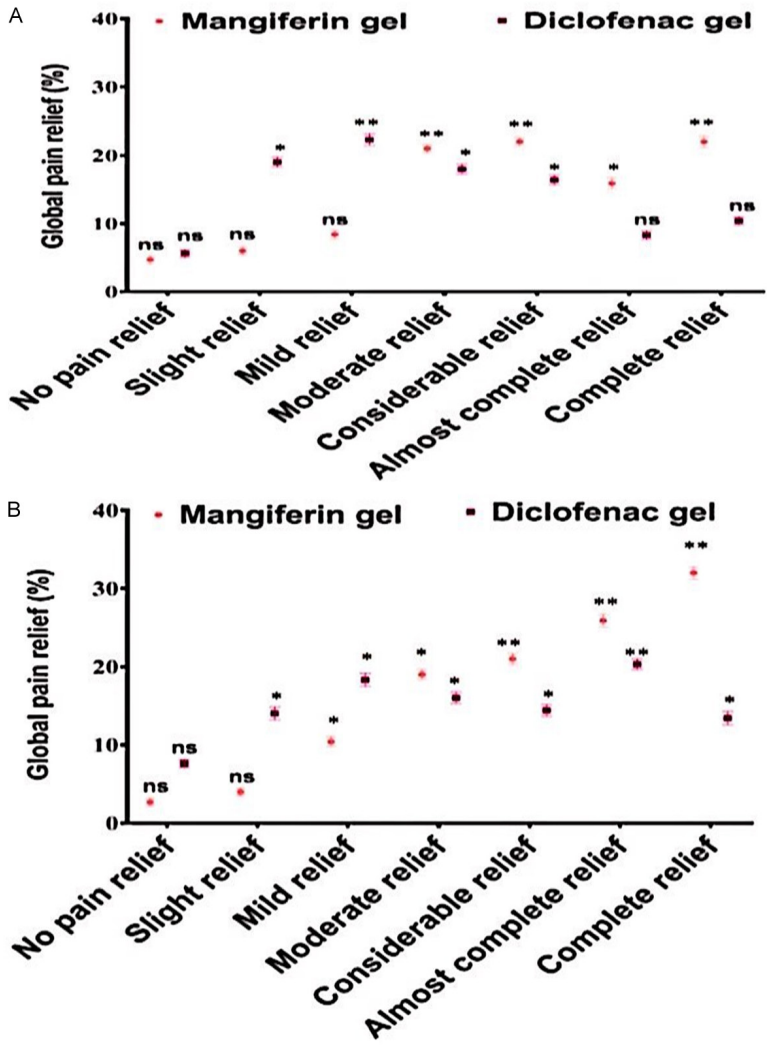

Figure 7. Comparison of pain relief negotiated by mangiferin and diclofenac gels with massage (A), and with phonophoresis (B) using Global Pain Rating Scale. ns: non-significant; *: significant; **: highly significant.

and the medicines used to minimize the tumor burden can lead to impairment of mobility and fracture risk, which are critical concerns for cancer patients. These tissues' closely linked mechanical and biological functions cause a feedback loop that connects the loss of bone mass with the loss of muscle strength and functionality (cachexia) [2, 3]. Scientists are exploring new and better therapeutic targets and therapeutic agents worldwide to increase muscle strength and avoid undesirable muscle deterioration [2, 4, 10]. The process of dealing with cancer is very unpredictable, ranging from diagnosis to treatment and survivorship. Therapeutic approaches do attempt to take musculoskeletal consequences into account for some malignancies. For example, in pancreatic cancer, where the prevalence of cachexia is high, nutrition plays a critical role in treatment. As we have already noticed, it is a matter of significance that chemotherapy can induce cachexia. Deterioration of musculoskeletal function has a severe effect on life satisfaction. Impairment of mobility and fracture risk are critical concerns for cancer patients and can be attributed to both the tumor and the medicines used to minimize the tumor burden [4, 5]. The process of dealing with cancer is very unpredictable, ranging from diagnosis to treatment and survivorship.

Mangiferin is distinctive because it does not damage healthy cells. It also does not build up in the kidneys or liver.

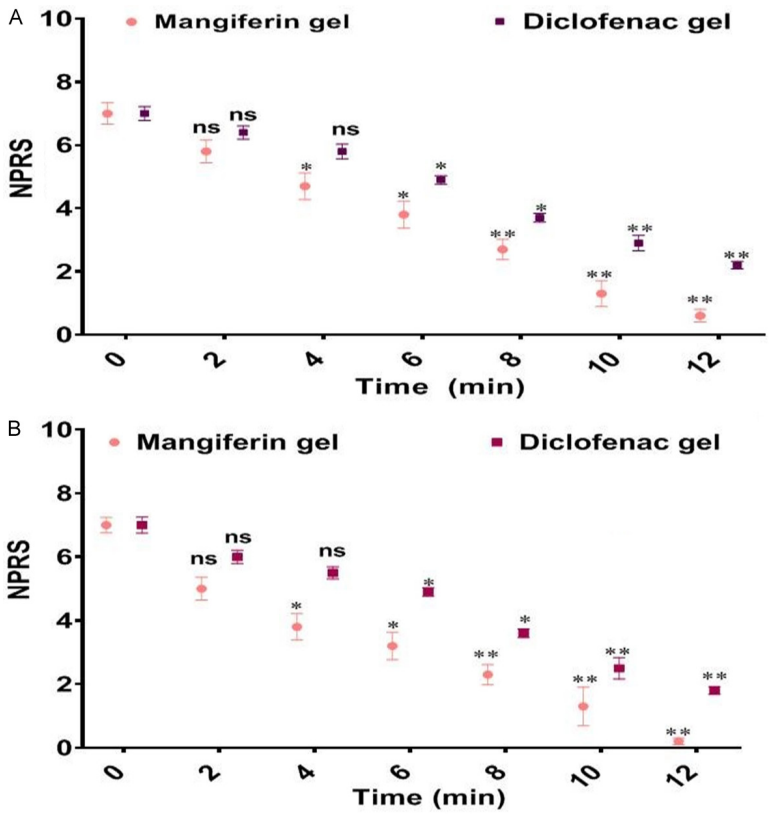

Figure 8. NPRS index for onset of pain (anti-nociceptive activity) of applied mangiferin gel vs. diclofenac with massage (A), and with phonophoresis (B). ns: non-significant; *: significant; **: highly significant.

Its oral bioavailability is about 1.2%, which is incredibly low [4]. Mangiferin's low lipophilic properties, poor intestinal permeability, and low oral absorption are the reasons for its limited bioavailability [24]. Preserving mangiferin's pharmacological properties in real-world applications requires enhancing its solubility, permeability, and organ retention duration.

Reactive oxygen species (ROS) cause damage to bone and cartilage in situations like osteoarthritis, where oxidative stress plays a significant role in the advancement of musculoskeletal illnesses [21]. The cellular antioxidant response mostly depends on the Nrf2 (nuclear factor erythroid 2-related factor 2) pathway, which is activated by mangiferin. Mangiferin (alpizarin or quinomine), is a chemical compound in the xanthone class. Its average molecular weight is 422, and its chemical formula is C19H18O11 [22].

Superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase, and other antioxidant enzymes are upregulated when Nrf2 is activated. Nrf2 translocates to the nucleus and attaches to antioxidant response elements (AREs) [19]. These enzymes lessen oxidative stress in musculoskeletal tissues by neutralizing ROS. Excessive apoptosis of myocytes and chondrocytes in musculoskeletal illnesses leads to muscle and cartilage tissue deterioration. Mangiferin spares healthy cells while inducing apoptosis in defective cells. It controls caspase activity, especially caspase-3 and caspase-9, which are essential to the apoptosis process [15-18]. It balances pro- and anti-apoptotic factors. Encouraging apoptosis to be selectively induced in damaged cells helps maintain healthy musculoskeletal tissues. Patients suffering from osteoarthritis may benefit from mangiferin's enhancement of PPARγ (Peroxisome Proliferator-Activated Receptor Gamma) expression, as it has been demonstrated to reduce inflammation and increase insulin sensitivity [21]. Mangiferin preserves cartilage and muscle integrity by inhibiting the expression of inflammatory cytokines and enzymes that break down tissue by activating PPARγ. Severe muscle atrophy, or cachexia, is a symptom that is frequently seen in individuals with cancer and chronic inflammatory diseases, including some musculoskeletal conditions [21]. Mangiferin lowers muscle atrophy by influencing the transcription factors involved in muscle breakdown, FOXO (Forkhead box O) and NF-κB. Mangaiferin reduces muscle loss and maintains muscle strength by blocking these pathways, which stops the activation of genes linked to muscle atrophy [14-17].

Mangiferin modifies the structure of many genes that control inflammation associated with NF-B [21, 22]. Mangiferin can be pro-inflammatory as it inhibits the activity of prostaglandin-endoperoxide synthase 2 (PTGS2, COX-2) and reduces PGE2 and perhaps PGD2 syn-

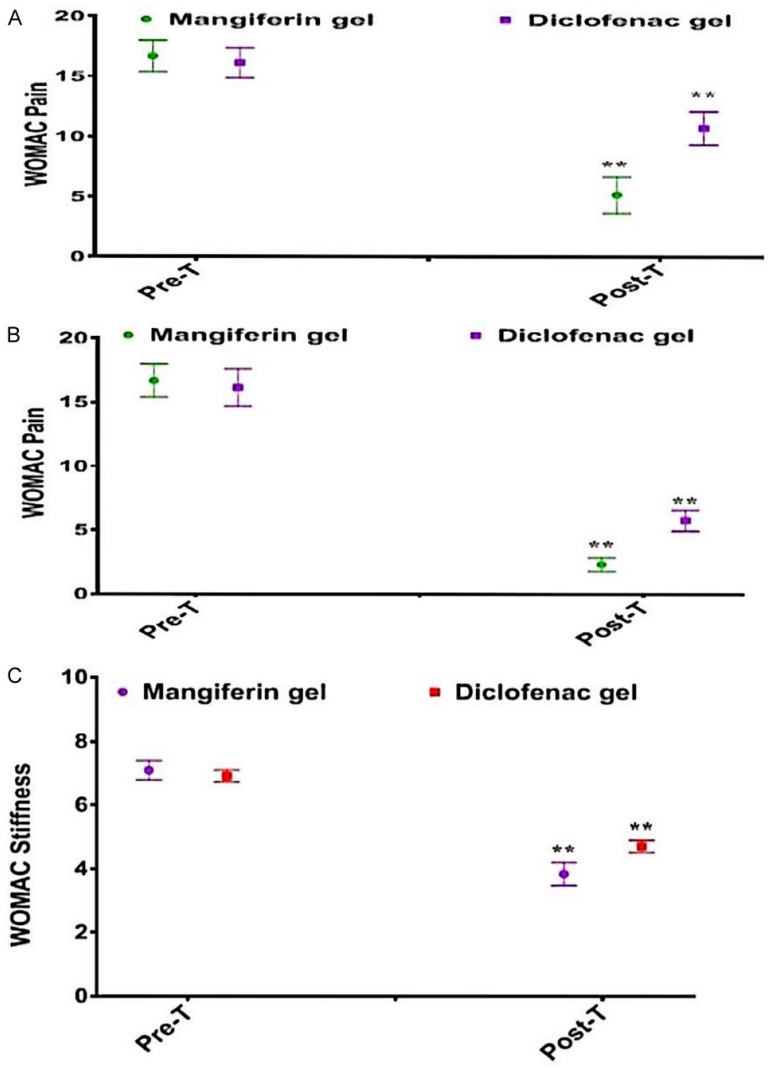

Figure 9. Relative anti-inflammatory and anti-nociceptive activities in mangiferin and diclofenac gels with massage and with phonophoresis. A. WOMAC pain (massage); B. WOMAC pain (phonophoresis); C. WOMAC stiffness (massage). WOMAC: Western Ontario and McMaster Universities Arthritis Index; ADLs: Activities of daily living; Pre-T: Pre-treatment; Post-T: Post-Treatment; *: significant; **: highly significant.

thesis [21]. By focusing on the NF-B pathway, mangiferin reduces inflammation [23]. Mangiferin inhibits various NF-B activation mechanisms, both conventional and alternative. While inhibitors of kappa B kinase and p52 govern the alternative pathway, the IB kinase complex and p50 control the traditional pathway. A substance called MGIFerin stops TNFR1 (Tumor Necrosis Factor Receptor Type-1-Associated Death Domain Protein), TNFR-Associated Factor 2 (TRAF2), NCK Interacting Kinase (NIK), and IKK factors from working. These factors cause SEAP to show up. However, it does not significantly affect p65, which is responsible for SEAP expression. Mangiferin lowers the MAPK signal [24]. It stops MAPK p38 from working, which is controlled by ERK and phosphorylated at the Jun N c terminus [15]. Mangiferin has been shown to fight tumors by working on many different signaling pathways, such as nuclear NF-B and COX-2 protein expression [24]. Mangiferin's anticancer efficacy involves apoptosis, presumably through caspase activation. Tumors begin due to abnormalities in cell growth and death. Mangiferin increases PPARgamma gene expression and decreases COX-2 transcriptional activity [21]. In vitro experiments on MDA-MB-231 cells show that mangiferin may improve PPAR gamma and COX-2 modulation.

Mangiferin gel (5%) can help muscles because it has potent anti-inflammatory and pain-relieving effects when applied through superficial massage and phonophoresis, compared to diclofenac diethyl ammonium 1% gel. This is due to the direct suppression of inflammatory biomarkers, which, in turn, regulate the inflammatory process within the skeletal muscle (**Figure 12**). Mangiferin is a miraculous compound, and its activity is profoundly increased by increasing its penetration into tissues through phonophoresis. Pain decreased significantly when evaluated through NPRS, global, and WOMAC scale readings (**Figures 9**, **10**). It was also shown that mangiferin showed profound results when mangiferin gel was given with phonophoresis.

Moreover, patients' ADLs significantly improve when mangiferin is given to them with phonophoresis. Diclofenac gel shows less significant

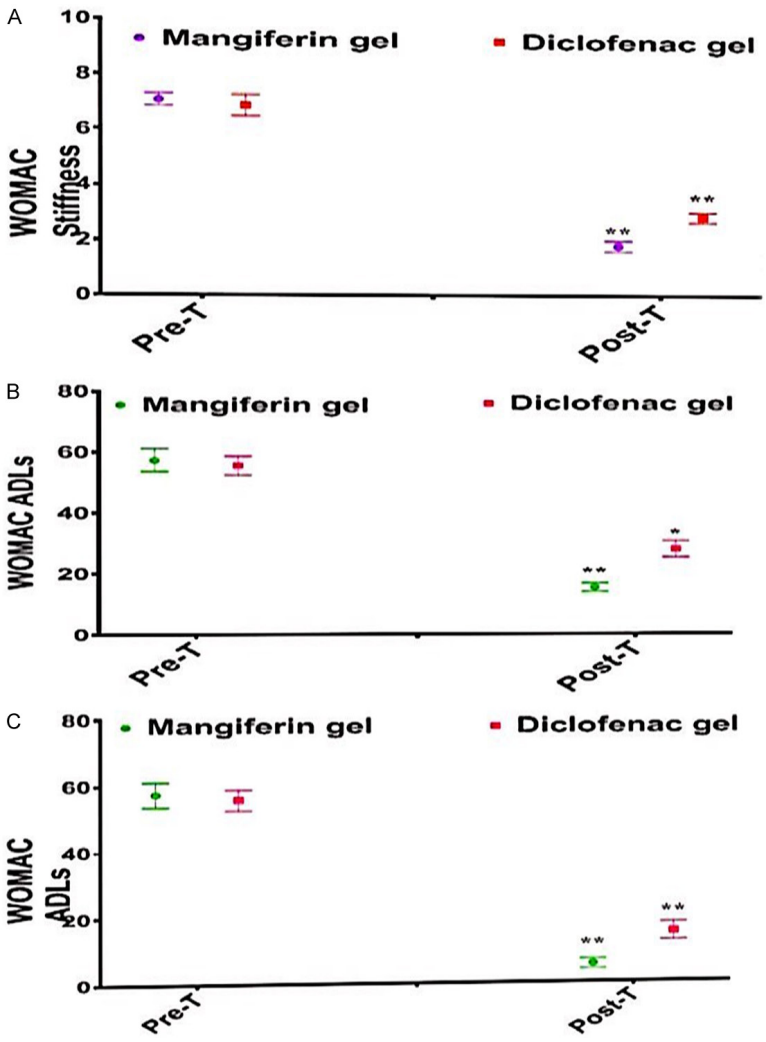

Figure 10. Relative anti-inflammatory and anti-nociceptive activities in mangiferin and diclofenac gels with massage and with phonophoresis. A. WOMAC stiffness (phonophoresis); B. WOMAC ADLs (massage); C. WOMAC ADLs (phonophoresis). WOMAC: Western Ontario and McMaster Universities Arthritis Index; ADLs: Activities of daily living; Pre-T: Pre-treatment; Post-T: Post-Treatment; *: significant; **: highly significant.

results on these parameters than mangiferin. The cooling effect of mangiferin gels is also more than that of diclofenac (**Figure 6**). Stiffness from patient injuries significantly improves when mangiferin is given to patients with phonophoresis. This comparison has been evaluated by WOMAC scale. Mangiferin anti-nociceptive activities are also depicted more powerfully when mangiferin gel is applied with phonophoresis. Mangiferin gel was easy to apply as it smelled good and showed less or no allergic reaction in patients when applied topically (**Figure 8**).

One of this study's main limitations is its comparatively small sample size. Although the study offers insightful information on treatment outcomes, the small sample size may limit how broadly the results can be applied.

Our findings imply that a functional loss of the motor unit partly causes muscle atrophy and weakening, as seen in experimental cachexia models. Mangiferin helps improve muscle loss due to chemotherapy. Future studies should employ this, which would enhance and motivate young researchers to use natural products that help ameliorate the effects of weakness and wasting of the skeletal muscles in cancer and chemotherapy-induced cachexia models.

Conclusions

Our results showed that mangiferin gel, superficial massage, and phonophoresis can reduce inflammation and pain in cancer patients. This could be due to an interruption in the production of different inflammatory biomarkers, down-regulating anti-inflammatory pathways. This report is the first of its kind. It has the advantage of acting on different pharmacological and biochemical pathways to increase mangiferin absorption with phonophoresis in cancer patients suffering from musculoskeletal ailments.

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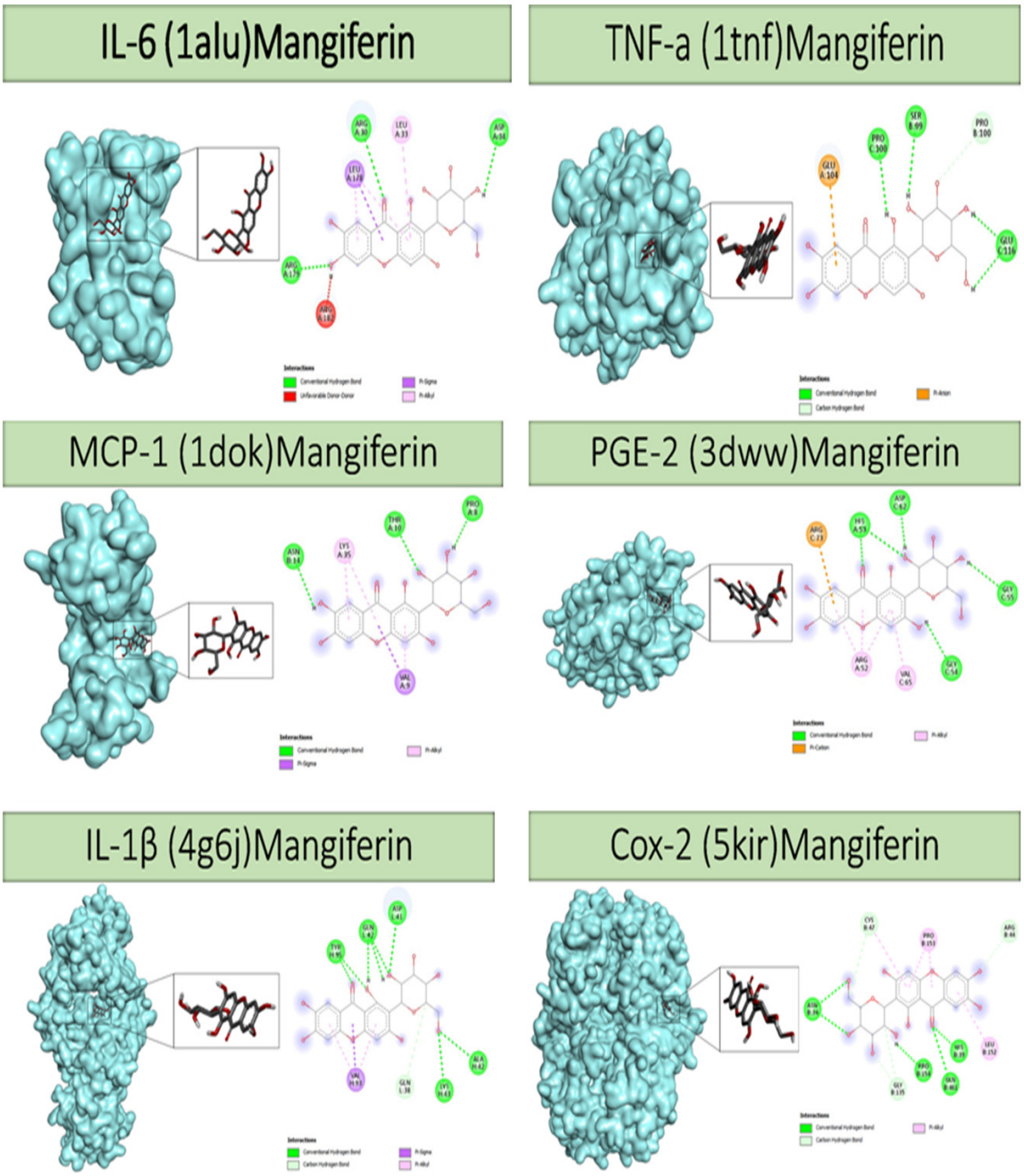

Figure 11. Direct act of mangiferin on different inflammatory biomarkers; IL-6, IL-1β, TNF-α, MCP-1, PGE-2, and COX-2.

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Disclosure of conflict of interest

None.

Abbreviations

GBD, global burden of disease; ADLs, activities of daily living; WOMAC, Western Ontario and McMaster Universities Arthritis Index; NPRS, numeric pain rating scale; NSAIDs, nonsteroidal anti-inflammatory drugs; COX-2, cyclooxygenase 2; IL, interleukin; NF-κB, nuclear factor-κB; TNF-α, tumor necrosis factor-α; DPPH, 2,2-diphenylpicrylhydrazyl; NO, nitric oxide;

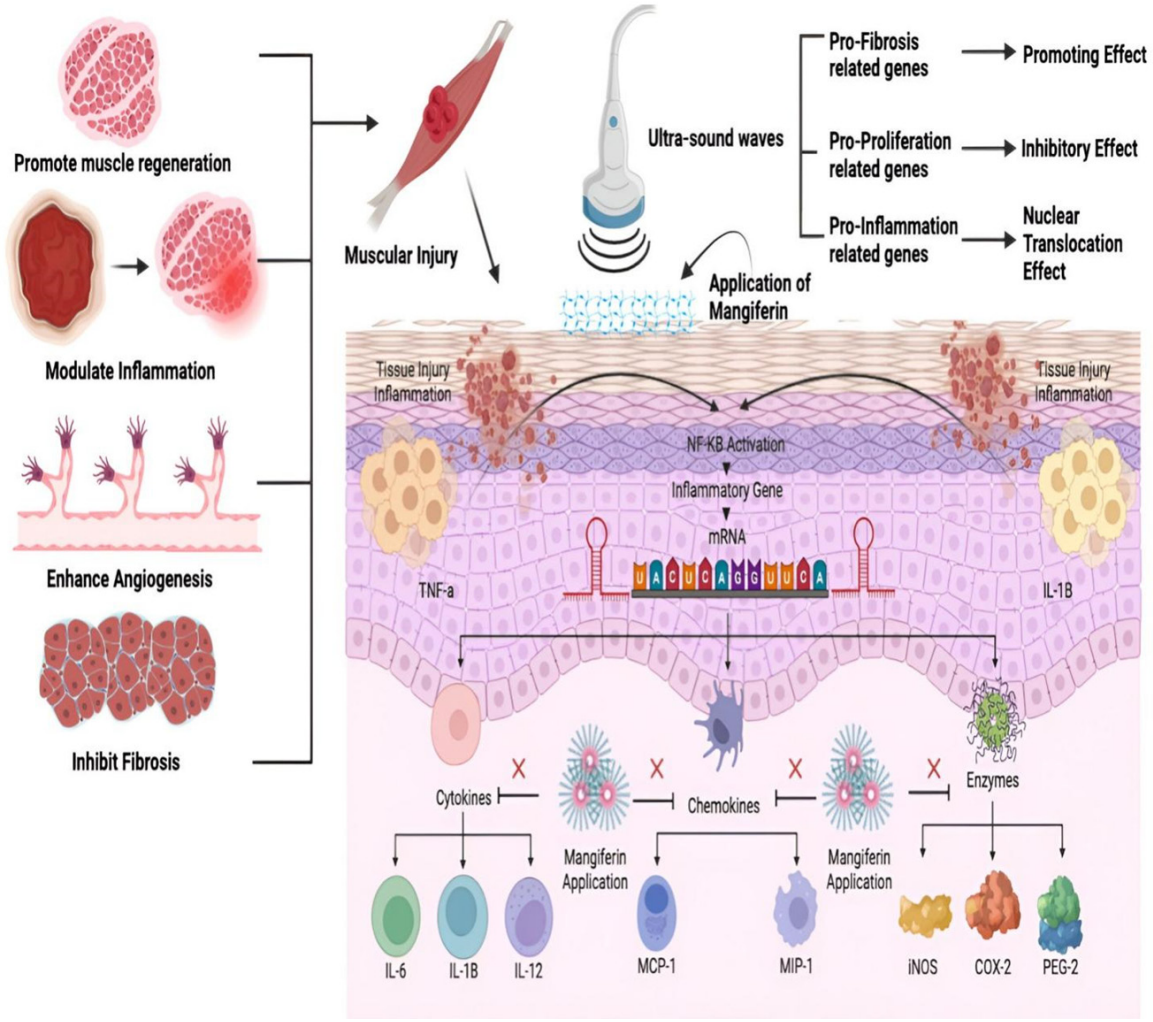

Figure 12. Proposed mechanism of action of mangiferin on various inflammatory biomarkers in skeletal muscle.

SOD, superoxide dismutase; WHO, World Health Organization; WMA, World Medical Association; ROS, reactive oxygen species; TLR-4, Toll-like receptor-4; TGF-β1, Transforming growth factor-β1; PGE-2, prostaglandin E-2; MAPK, mitogen-activated protein kinase; FTIR, fourier transform infrared spectroscopy; HPLC, high performance liquid chromatography; iNOS, inducible *nitric oxide* synthase; PGN, peptidoglycan; LPS, lipopolysaccharides.

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