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Intra-Articular Exosome and Intraarticular Laser Therapy for Osteoarthritis; Preliminary Non-Surgical Approach

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Abstract **Original Research Article**

Background**:** Stem cells and exosomes offer a non-surgical approach to treating osteoarthritis (OA). Exosomes play an essential role in intercellular communication that has been widely studied. Furthermore, multi-wavelength intra-articular laser therapy is a newly developed technique in the treatment of various diseases. Here, we investigated the therapeutic potential and mechanism of adipose tissue-derived exosomes to alleviate knee osteoarthritis compared with exosome therapy combined with intra-articular laser therapy. ***Method*****:** Adipose-derived exosomes were isolated and purified. Five volunteers received intra-articular injection of autologous exosomes in the right knee, while the left knee of these volunteers received intra-articular exosome injection and intra-articular infrared laser, 808 nm. To avoid any bias in the patient, the laser fiber was passed through the inserted needle for exosome injection, but without radiation, and on the right knee, an invisible infrared laser was applied. Visual analog scales (VAS) and Western Ontario and McMaster Universities Arthritis Index (*WOMAC*) were collected the week before the procedure, and every month until the sixth months after injection for both knees. ***Discussion*****:** Both intra-articular exosome injections improved pain and functional outcomes at 6-month follow-up in volunteers with knee osteoarthritis. The differences between exosome injection and exosomes combined with intra-articular laser therapy are in terms of clinical improvement rates and functional outcome rates. ***Conclusion*****:** Combination of stemcell or exosome therapy with intra-articular laser therapy could be considered as a feasible option in non-surgical treatment approaches. It could be also used as a preventive purpose.
Keywords: Osteoarthritis, Mesenchymal stem cells, Exosome, Extracellular Vesicles, Intraarticular laser therapy.

INTRODUCTION

Osteoarthritis (OA) is an important health problem that is common in middle-aged and elderly people worldwide and is associated with chronic pain, functional limitations, and economic burden (Wieland *et al.*, 2005). To date, physical therapy, nonsteroidal anti-inflammatory drugs (NSAIDs), and intra-articular injections have been reported to reduce the pain symptoms of osteoarthritis patients, but do not help relieve pain and degeneration of articular cartilage in a long term (Ding, 2002).

In the past, platelet-rich plasma was a popular method to treat osteoarthritis, and recently, many studies show that intra-articular injection of stem cells or exosomes plays an important role in promoting the growth factors, proliferation, differentiation and matrix synthesis of cartilage cells (Prodromos *et al.*, 2018).

It has been reported that these methods can promote re-epithelialization of chronic skin wounds, improve bone regeneration, enhance tendon and ligament repair, treat chronic femoral osteomyelitis, and it is like an explosion in the use of these methods in various fields of aesthetics and aesthetics and therapeutic medicinal purposes (Asgari *et al.*, 2024; Chen *et al.*, 2022; Tan *et al.*, 2021; M WEBER; Weber, Ghanbarzadeh, *et al.*, 2023; Weber, Mehran, *et al.*, 2023).

Many studies on the regenerative and anti-inflammatory effects of arthritis have been widely reported, and intra-articular PRP injections provide a non-surgical approach to advanced disease. Stem cell therapy and exosome therapy contain more growth factors and contains much more anti-inflammatory factors, and we can culture desired amounts of exosomes and stem cells for different purposes (Prodromos *et al*., 2019; Wong *et al.*, 2020).

Activated platelets are thought to secrete large amounts of growth factors (GFs) and cytokines, the delivery of which contributes to the main functions of PRP, including promoting proliferation and inhibiting apoptosis, and destruction of cartilage cells. However, we must reconsider that we need platelet activation, which also depends on the PRP set and the results of different harvesting and activation methods. These all, it leads us to choose stem cell and exosome instead of PRP injection, and due to the fact that exosomes are much smaller, and exosomes have unique characteristics or functions or can be separated or distinguished effectively, we did decide to inject exosomes (Iyer *et al*., 2020; Liu *et al.*, 2019; Wong *et al.*, 2020; Zhang *et al.*, 2022).

On the other hand, one of the most desirable goals of any regenerative procedure is to strengthen stem cell mitochondria and exosomes, and many studies demonstrate that low-intensity radiation (low-level laser therapy (LLLT)) with specific wavelengths known to activate cells proliferation and differentiation (Chen *et al*., 2022; Prodromos *et al*., 2018, 2019; Tan *et al*., 2021; Wong *et al*., 2020). The energy density required in LLLT is very low, typically on the order of 2 to 4 J/cm2 and with output power of less than 0.5 watts. Laser light can be absorbed by chromophores and photoreceptors identified in the mitochondrial respiratory chain. Laser irradiation in the red or near-infrared spectrum causes stimulation of mitochondria on exosomes. Photoreceptor molecules in the mitochondria absorb laser radiation as a photo initiator, then trigger a series of photochemical reactions, causing changes in cell metabolism such as protein signaling (Weber, Mehran, *et al*., 2023).

Through a series of stimuli in the mitochondrial respiratory chain, adenosine triphosphate (ATP) can be formed. This synergic effect may can cause more survival rate of exosomes and stem cells. We can bring any wavelength of laser therapy directly into the targeted area (Weber, Ghanbarzadeh, *et al*., 2023; Weber, Mehran, *et al*., 2023).

In this study, we examined the power of injection of exosome for treating OA and compare it with supply the exosomes with ATP release, increasing micro and macro blood circulation, and all effects of laser therapy.

MATERIAL AND METHODS

Autologous human adipocyte derived mesenchymal stromal cells (hADMSC) isolation.

Samples of adipose tissue were obtained from autologous healthy donors; written consent was obtained from all volunteers involved following the indications of the Helsinki Declaration. Human lipoaspirates from volunteers undergoing selective suction-assisted lipectomy will be collected; Also, the donor's blood sample was negative for viral infections including mycoplasma, cytomegalovirus (CMV), hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV) evaluated by polymerase chain reaction (PCR). The tissues were obtained using sterile techniques and stored in Hanks' Balanced Salt Solution (HBSS), without Ca & Mg with 1% (v/v) penicillin/streptomycin. In order to isolate the ASCs, approximately 5 g of adipose tissue was minced and washed with PBS buffer twice followed by centrifugation at 1200 rpm for 10 min. The supernatant was removed; the extracellular matrix will be digested with 0.2% collagenase II for 2 hours at 37°C on a shaker to release the cellular fractions. After digestion complete medium was added to stop the reaction and the digested mixture was filtered through 40-mm cell strainer and centrifuged at 1200 rpm for10 min. The supernatant was removed after centrifuging and the pellet obtained was resuspended in DMEM (DMEM) supplemented with 10% (v/v) Human serum (Human Serum is derived from plasma collected from volunteers) and 1% (v/v) penicillin/streptomycin.

Isolated cells were multiplied in a monolayer in T175 flasks using low glucose DMEM at 37°C in a humid atmosphere containing 5% CO_2

Immunophenotype analysis: For immunophenotype analysis of the expanded cells, the cells will be detached and washed with phosphatebuffered saline (PBS) containing bovine serum albumin, and incubated with primary antibodies against human (CD73, CD 105, CD90 and CD 44; and were negative for haematopoietic markers (CD45, CD 34 and HLA-DR). After washing with PBS containing bovine serum albumin, the cells will be incubated with fluorescein isothiocyanate (FITC) and phycoerythrin (PE)-conjugated secondary antibodies. After three washes, cells will be re-suspended in PBS and analyzed by flow cytometry by use of a FACSCalibur flow cytometer.

Osteogenic and adipogenic differentiation

For osteogenic differentiation, the culture expanded cells will be induced in the following osteogenic medium for 2–3 weeks: DMEM supplemented with FCS, b-glycerophosphate, dexamethasone, and ascorbic acid. Then the cells will be stained with von Kossa to reveal osteogenic differentiation. For adipogenic differentiation, the culture-expanded cells will be induced for 3 weeks in DMEM supplemented with FCS, hydrocortisone, isobutylmethylxanthine, and indomethacin. At the end of the culture, the cells will be fixed in formalin for 10 min and stained with fresh Oil red-O solution to show lipid droplets in induced cells.

hADSCs purification and characterization

In the presence of a cellular density of 80%, the human ADSCs-conditioned media will be harvested every 48 h. To sterilize the supernatants and remove larger extracellular vesicles (EVs), the conditioned media will be passed through 0.22 mm filter membranes and centrifuged at 700 g for 10 min to remove cell debris. Another round of centrifugation will be performed at 9000 g for 30 min, and the supernatant will be collected again. Exosomes will be isolated by ExoEasy Maxi kit (76064; Qiagen) 3 and re-suspended in PBS. Pellets will be washed with 0.9% NaCl and re-precipitated at 100,000 g at for 2 h. The obtained pellets will be dissolved in 0.9% NaCl to a final concentration of 1 unit EVs per 1 ml. The characterization of exosomes will be achieved by measuring expression of exosome-specific markers CD81, CD9, nex1 and CD63 by Western blot analysis and particle size by NanoSight analysis.

Autologous exosome purification and characterization

hADSCs passages 3–6 was maintained in Dulbecco's minimal essential medium (DMEM) supplemented with 10% Human AB serum (mycoplasma, CMV, HBV, HCV and HIV tests performed by PCR), 100 U/ml penicillin and 100 µg/ml streptomycin and incubated at 37°C in 5% CO2. Cells were grown to 70%–80% confluency, washed three times with PBS and incubated for 48 h in serum-free media (low-glucose DMEM/F12). In the presence of a cellular density of 80%, the hADSCs-conditioned media were harvested every 48 h. To sterilize the supernatants and remove larger EVs, the conditioned media were passed through 0.22 mm filter membranes and centrifuged at 700 g for 10 min at 4°C to remove cell debris. Another round of centrifugation was performed at 9000 g at 4°C for 30 min, and the supernatant was collected again. Exosomes were isolated by ExoEasy Maxi kit (76064; Qiagen) and re-suspended in phosphate-buffered saline (PBS). Residual polyethylene glycol and soluble proteins that might have been coprecipitated were removed, pellets were washed with 0.9% NaCl and reprecipitated at 100,000 g at 4°C for 2 h. The obtained pellets were then dissolved in 0.9% NaCl to a final concentration of 1 unit EVs per 1 ml and stored as 1 ml aliquots at −80 C until usage. The characterization of exosomes was achieved by measuring expression of exosome-specific markers CD81, CD9, nex1 and CD63 by Western blot analysis and particle size by NanoSight analysis (platforms SLM 20).

Cases

All male volunteers were 67, 68, 68, 70 and 74 years old. In their later years, they suffer a lot of pain, limited mobility, and many deformities. They all had a history of pain and loss of knee mobility and ability to walk for at least five years. Volunteers do not want surgical treatment such as total knee replacement. All were confirmed to have chronic grade 3 knee osteoarthritis.

They consulted with an orthopedic surgeon who said eventually they would need joint replacement surgery, owing to bad conditions of grade 3 to grade 4. They all requested to try any regenerate method, not operate, and it was determined they are a good candidate for this study as a volunteer.

They all asked to try any reconstructive method other than surgery, and they were determined to be good candidates for this study as volunteers. All volunteers gave written informed consent.

None of them had received any other treatments in the past year, such as PRP or steroid injections, or any other injections or physical therapy.

None of them had taken anticoagulants or corticosteroids in the past 12 months.

Inclusion criteria for our study were radiographic evidence of knee osteoarthritis (grade II-III according to the Kellgren-Lawrence classification), pain, or functional limitation in the patient's daily activities before treatment, and there are no clinical or imaging signs of disease, joint instability. Known exclusion criteria: cancer, BMI greater than 40, chronic anticoagulant use or history of opioid use, alcohol, tobacco, coagulopathy, injury neoplasia and kidney failure, any other autoimmune, neurodegenerative, or metabolic diseases. On the medical check-up list, they only had grade three of knee osteoarthritis.

Intraarticular laser therapy procedure

After injecting 40 billion exosomes in a volume of 3 ml, the flexible fiber optic cable was inserted through a hollow needle into both knee joints (BD Insyte Autoguard 22 GA × 1.00 IN intravenous catheter), and therefore they received 20 billion of exosomes in each knee. This is inserted lateral to the joint under musculoskeletal ultrasound guidance. The right knee joint received 20 minutes (min) of infrared laser intraarticular irradiation (808 nanometers), while there was no radiation at all to the left knee (this channel was turned off while the other was on and the device was on, and so that the patient does not know the details). As you know, infrared lasers are not visible and therefore the volunteers were unaware that they were not receiving any lasers on their left knee. At the end of the laser fiber, the intensity of the infrared laser is set to 100 mill watts (mW). The low-power laser machine used in this study was the Weberneedle® basic laser device (Weber Medical GmbH, Germany). The patient comes in monthly for this procedure and has not received any other treatment in between. They come for six months, monthly. Before each treatment and at the sixth follow-up periods, they filled two questionnaire forms include VAS and WOMAC.

Data Collection

The clinical picture of each case was analyzed before treatment using the Western Ontario McMaster University Osteoarthritis Index (WOMAC) to assess the patient's overall clinical condition and the Analog Scale Visualization (VAS) to specifically assess their pain.

WOMAC is a pain index measure for osteoarthritis, the most widely used parameter to evaluate knee function and a tool to evaluate disorders related to extremity osteoarthritis.

WOMAC includes a total of 24 questions and three subscales. Of these, there were 5 questions related to pain, 2 questions related to stiffness, and 17 questions related to difficulties in performing activities of daily living related to physical function.

This disease-specific tool is useful in the clinical assessment of changes in health status and clinical outcomes related to pain. WOMAC is valid and reliable for determining function in lower extremity disorders.

Results

Difficulty total parameter

The results of the Friedman test showed that the trend of changes in the total difficulty score is decreasing and significant.

Table 1: Mean and standard deviation of the difficulty total parameters

Difficulty Total							
	pre	month1	month2	month3	month4	month5	month6
Mean	57.2000	43.4000	43.4000	42.2000	36.2000	35.8000	24.8000
Std. Deviation	1.48324	1.51658	2.88097	2.38747	1.64317	3.11448	1.48324

The total difficulty parameter between before and after the fourth month (p value 0.001, and 0.027) was decreasing significant. The total difficulty parameter between before and after the fifth month (p value 0.001, and 0.021) was decreasing significant. The total difficulty parameter between before and after the sixth month (p value 0.000, and 0.000) was decreasing significant. Therefore, the first significant decreasing effect on the difficulty total appears after the fourth month. Also, the total difficulty between the first month and sixth month (p value 0.002, and 0.044) was decreasing significant.

Total stiffness parameter

The results of the Friedman test showed that the trend of changes in the total stiffness score is decreasing, but this decrease is not statistically significant.

Table 2: Mean and standard deviation of the difficulty total parameters

Stiffness total							
	pre	month1	month2	month3	month4	month5	month6
Mean	5.6000	6.0000	5.2000	5.2000	4.8000	4.8000	3.6000
Std. Deviation	.89443	1.41421	1.30384	.44721	.83666	.44721	.89443

Pain parameter

The results of Friedman's test showed that the trend of the pain score changes is decreasing and significant.

Table 3: Mean and standard deviation of the Pain parameters

Pain parameter							
	pre	MONTH1	MONTH2	MONTH3	MONTH4	MONTH5	MONTH6
Mean	15.4000	14.2000	14.4000	12.0000	11.8000	9.0000	8.8000
Std. Deviation	2.07364	1.30384	1.14018	1.00000	.44721	1.22474	1.09545

The pain parameter between before and after the fifth month (p value 0.000, and 0.009) was decreasing significant. The pain parameter between before and after the sixth month (p value 0.000 and 0.006) was decreasing significant. Therefore, the first significant decreasing effect on the pain parameter appears after the fifth month. Also, the pain parameter between the first month and sixth month (p value 0.002, and 0.035) was decreasing significant.

Walking parameter

The results of Friedman's test showed that the walking score change trend is decreasing and significant.

Table 4: Mean and standard deviation of the walking parameter

Walking Parameter								
type		pre	month1	month2	month3	month4	month5	month6
walking	Mean	3.8000	3.2000	3.2000	2.6000	2.4000	1.4000	1.4000
	Std. Deviation	.44721	.44721	.44721	.54772	.54772	.54772	.54772

The walking parameter between before and after the fifth month (p value 0.001, and 0.012) was decreasing significant. The walking parameter between before and after the sixth month (p value 0.001 and 0.012) was decreasing significant. Therefore, the first significant decreasing effect on the walking parameter appears after the fifth month.

Climbing stairs parameter

The results of the Friedman test showed that the trend of changes in the Climbing Stair score is significant.

Table 5: Mean and standard deviation of the climbing stair parameter

Climbing stair parameter								
type		pre	month1	month2	month3	month4	month5	month6
Climbing Stairs	Mean	3.2000	3.4000	3.4000	2.2000	2.8000	1.4000	1.6000
	Std. Deviation	.44721	.54772	.54772	.44721	.44721	.54772	.54772

The climbing stairs parameter between before and after the fifth month (p value 0.001, and 0.011) was decreasing significant. The climbing stairs parameter between before and after the sixth month (p value 0.001 and 0.009) was decreasing significant. Therefore, the first significant decreasing effect on the climbing stairs parameter appears after the fifth month.

Sleeping at night, Resting, and Standing parameters, Rate stiffness in the morning, and Rate stiffness in the evening

The results of the Friedman test showed that the changes in the scores of Sleeping at night, Resting, and Standing are not significant. The results of Friedman's test showed that the trend of changes in stiffness in morning and in the evening scores are not significant.

Descending stairs, and Ascending stairs

The descending stairs parameter between before and after the fourth month (p value 0.002, and 0.042) was decreasing significant. The descending stairs parameter between before and after the sixth month (p value 0.001 and 0.021) was decreasing significant. Therefore, the first significant decreasing effect on the descending stairs parameter appears after the fourth month. The ascending stairs parameter between before and after the sixth month (p value 0.000 and 0.004) was decreasing significant. Therefore, the first significant decreasing effect on the ascending stairs parameter appears after the sixth month.

Table 6: Mean and standard deviation of the descending and ascending stairs

Descending stairs, and Ascending stairs								
		PRE	MONTH1	MONTH2	MONTH3	MONTH4	MONTH5	MONTH6
Descending Stairs	Mean	3.4000	2.6000	2.4000	2.4000	1.6000	2.0000	1.6000
	Std. Deviation	.54772	.54772	.54772	.54772	.89443	.00000	.54772
Ascending Stairs	Mean	3.4000	2.6000	2.6000	2.4000	2.0000	2.2000	1.0000
	Std. Deviation	.54772	.54772	.54772	.54772	.70711	.83666	.00000

Rising from sitting and standing parameters

The rising from sitting parameter between before and after the sixth month (p value 0.001, and 0.027) was decreasing significant. Therefore, the first significant decreasing effect on the rising from sitting parameter appears after the sixth month. The standing parameter was not significant.

Table 7: Mean and standard deviation of the rising from sitting and standing parameters

Rising from sitting and standing parameters.								
TYPE		PRE	MONTH1	MONTH2	MONTH3	MONTH4	MONTH5	MONTH6
Rising from Sitting	Mean	3.4000	2.4000	3.0000	2.6000	2.0000	2.0000	1.4000
	Std. Deviation	.54772	.54772	.00000	.54772	1.00000	.70711	.54772
Standing	Mean	3.2000	2.6000	2.4000	2.4000	2.0000	2.2000	2.0000
	Std. Deviation	.44721	.54772	.54772	.54772	.00000	.44721	.00000

Bending to floor and walking on even floor parameters

The binding to floor parameter between before and after the sixth month (p value 0.002, and 0.044) was decreasing significant. Therefore, the first significant decreasing effect on the rising from sitting parameter appears after the sixth month. The walking on even floor parameter between before and after the sixth month (p value 0.000, and 0.007) was decreasing significant. Therefore, the first significant decreasing effect on the walking on even floor parameter appears after the sixth month.

Table 8: Mean and standard deviation of the bending to floor and walking on even floor parameters

Bending to floor and walking on even floor parameters								
TYPE		PRE	MONTH1	MONTH2	MONTH3	MONTH4	MONTH5	MONTH6
bending to floor	Mean	3.2000	2.8000	2.6000	2.6000	2.2000	2.2000	1.6000
	Std. Deviation	.44721	.44721	.54772	.54772	.83666	.83666	.54772
Walking on even floor	Mean	3.8000	2.4000	2.8000	2.2000	2.4000	2.0000	1.6000
	Std. Deviation	.44721	.54772	.44721	.44721	.54772	.70711	.54772

Get in/our car and going shopping parameters

The get in/out car parameter between before and after the sixth month (p value 0.000, and 0.003) was decreasing significant. Therefore, the first significant decreasing effect on the get in/out car parameter appears after the sixth month. The going shopping parameter between before and after the sixth month (p value 0.001, and 0.012) was decreasing significant. Therefore, the first significant decreasing effect on the going shopping parameter appears after the sixth month.

Table 9: Mean and standard deviation of get in/our car and going shopping parameters

Get in/our car and going shopping parameters								
TYPE		PRE	MONTH1	MONTH2	MONTH3	MONTH4	MONTH5	MONTH6
Get in/out car	Mean	3.4000	2.4000	2.8000	2.4000	2.4000	2.4000	1.0000
	Std. Deviation	.54772	.54772	.44721	.54772	.89443	.54772	.00000
going shopping	Mean	3.2000	2.4000	2.6000	2.4000	2.8000	2.4000	1.4000
	Std. Deviation	.44721	.54772	.54772	.54772	.44721	.54772	.54772

Putting on socks, rising from bed, taking off socks, lying in the bed parameters

The putting on socks parameter between before and after the sixth month (p value 0.001, and 0.016) was decreasing significant. Therefore, the first significant decreasing effect on the putting on socks parameter appears after the sixth month. The rising from bed parameter between before and after the sixth month (p value 0.000, and 0.009) was decreasing significant. Therefore, the first significant decreasing effect on the rising from bed parameter appears after the sixth month. The taking off socks parameter between before and after the sixth month (p value 0.002, and 0.035) was decreasing significant. Therefore, the first significant decreasing effect on the taking off socks parameter appears after the sixth month. The laying on bed parameter between before and after the sixth month (p value 0.001, and 0.021) was decreasing significant. Therefore, the first significant decreasing effect on the laying on bed parameter appears after the sixth month.

Table 10: Mean and standard deviation of putting on socks, rising from bed, taking off socks, lying in the bed parameters

Putting on socks, rising from bed, taking off socks, lying in the bed parameters.								
TYPE		PRE	MONTH1	MONTH2	MONTH3	MONTH4	MONTH5	MONTH6
putting on socks	Mean	3.2000	2.4000	2.6000	2.6000	2.4000	2.2000	1.2000
	Std. Deviation	.44721	.54772	.54772	.54772	.89443	.83666	.44721
Rising from bed	Mean	3.2000	2.8000	2.6000	2.6000	2.2000	2.0000	1.0000
	Std. Deviation	.44721	.44721	.54772	.54772	.44721	.70711	.00000
Taking off socks	Mean	3.8000	2.4000	2.4000	2.6000	2.0000	2.2000	1.6000
	Std. Deviation	.44721	.54772	.54772	.54772	.00000	.83666	.54772
Lying in the bed	Mean	3.0000	2.4000	2.4000	2.4000	2.0000	1.8000	1.4000
	Std. Deviation	.00000	.54772	.54772	.54772	.70711	.44721	.54772

Get in/out bath, sitting, get in/out toilet parameters

The get in/out bath parameter between before and after the sixth month (p value 0.001, and 0.027) was decreasing significant. Therefore, the first significant decreasing effect on the get in/out bath parameter appears after the sixth month. The sitting, and the get in/out toilet parameters were not significant.

Table 11: Mean and standard deviation of get in/out bath, sitting, get in/out toilet parameters

Get in/out bath, sitting, get in/out toilet parameters.								
TYPE		PRE	MONTH1	MONTH2	MONTH3	MONTH4	MONTH5	MONTH6
Get in/out bath	Mean	3.4000	2.4000	2.6000	2.2000	1.8000	1.8000	1.4000
	Std. Deviation	.54772	.54772	.54772	.44721	.83666	.83666	.54772
sitting	Mean	3.4000	2.8000	2.4000	3.0000	2.0000	2.2000	1.6000
	Std. Deviation	.54772	.44721	.54772	.00000	.70711	.83666	.54772
get in/out toilet	Mean	3.4000	2.6000	2.4000	2.6000	2.4000	2.0000	1.8000
	Std. Deviation	.54772	.54772	.54772	.54772	.54772	1.00000	.44721

Doing light duties and doing heavy duties parameters

The doing light duties parameter between before and after the sixth month (p value 0.001, and 0.012) was decreasing significant. Therefore, the first significant decreasing effect on doing light duties parameter appears after the sixth month. The heavy duties parameters were not significant.

Table 12: Mean and standard deviation of doing light duties and doing heavy duties parameters

Doing light duties and doing heavy duties parameters.								
TYPE		PRE	MONTH1	MONTH2	MONTH3	MONTH4	MONTH5	MONTH6
doing light duties	Mean	3.4000	2.6000	2.6000	2.2000	2.0000	2.2000	1.4000
	Std. Deviation	.54772	.54772	.54772	.44721	.00000	.83666	.54772
doing heavy duties	Mean	3.4000	2.8000	2.2000	2.6000	2.0000	2.0000	1.8000
	Std. Deviation	.54772	.44721	.44721	.54772	.00000	.70711	.44721

Womac Results

Table 13 shows the mean and standard deviation of the WOMAC test. The results of Friedman's test showed that the change trend of WOMAC score is significant. The WOMAC parameter between before and after the fourth month (p value 0.002, and 0.035) was decreasing significant. The WOMAC parameter between before and after the fifth month (p value 0.001 and 0.012) was decreasing significant. The WOMAC parameter between before and after the sixth month (p value 0.000 and 0.000) was decreasing significant. Therefore, the first significant decreasing effect on the WOMAC parameter appears after the fourth month. The WOMAC parameter between the first and the sixth month (p value 0.001, and 0.016) was decreasing significant. The WOMAC parameter between the second and the sixth month (p value 0.002, and 0.035) was decreasing significant.

Table 13: Mean and standard deviation of the WOMAC test

Womac Results							
	pre	month1	month2	month3	month4	month5	month6
Mean	78.2000	63.6000	63.0000	59.4000	52.8000	49.6000	37.2000
Std. Deviation	2.16795	2.30217	3.53553	2.79285	2.28035	3.20936	1.92354

Visual Analogues Scale (VAS) Parameter

Table ***14*** shows the mean and standard deviation between the right knee and the left knee.

Table 14: Mean and standard deviation of the VAS parameter

VAS for pain in the right and the left knee and mean of both knees (total)								
		pre	month1	month2	month3	month4	month5	month6
right	Mean	3.8000	3.0000	2.4000	2.4000	1.8000	1.6000	1.2000
	Std. Deviation	.44721	.00000	.54772	.54772	.44721	.54772	.44721
left	Mean	3.4000	2.4000	2.2000	2.8000	1.4000	1.4000	1.8000
	Std. Deviation	.54772	.54772	.44721	.44721	.54772	.54772	.44721
Total	Mean	3.6000	2.7000	2.3000	2.6000	1.6000	1.5000	1.5000
	Std. Deviation	.51640	.48305	.48305	.51640	.51640	.52705	.52705

The results of Friedman's test showed that the trend of left and right score change between the right knee and the left knee is significant. The right knee VAS parameter between before and after the fifth month (p value 0.002, and 0.035) was decreasing significant. The right knee VAS parameter between before and after the sixth month (p value 0.000 and 0.004) was decreasing significant. Therefore, the first significant decreasing effect on the right knee VAS parameter appears after the fifth month.

The left knee VAS parameter between before and after the fifth month (p value 0.001, and 0.027) was decreasing significant. The left knee VAS parameter between before and after the sixth month (p value 0.001 and 0.021) was decreasing significant. Therefore, the first significant decreasing effect on the left knee VAS parameter appears after the fifth month.

DISCUSSION

Knee osteoarthritis (OA) is a common cause of knee pain in the elderly. These patients may experience knee swelling, pain and weakness, cartilage degeneration, bone spur formation and knee bursitis as common causes. Treatment for knee osteoarthritis can be divided into surgical and non-surgical options (Mubark, 2021, 2022).

A surgical procedure such as total knee replacement may not be possible for some patients and they can receive injections of autologous platelet-rich plasma (PRP), exosome, stem cells from various sources, exosomes. Researchers are continually looking for non-surgical, disease-modifying options to treat knee osteoarthritis (Mubark, 2022).

Because of the long-term side effects of nonsteroidal anti-inflammatory drugs (NSAIDs), it is increasingly recommended to avoid injections of these drugs in the treatment of osteoarthritis (Ding, 2002; Wieland *et al*., 2005; Zhang *et al.*, 2019).

Low-intensity radiation at specific wavelengths (low-level laser therapy (LLLT)) is known to cause cell proliferation and differentiation. The energy density required for LLLT is very low, typically at the level of 2-4J/cm2, and the transmitted power is less than 0.5 Watt. Laser light is absorbed by chromophores and photoreceptors identified in the mitochondrial respiratory chain, causing mitochondrial stimulation. Mitochondrial photoreceptor molecules absorb laser light, triggering a series of photochemical reactions that induce changes in cellular metabolism, including protein signaling. Through a series of stimuli in the mitochondrial respiratory chain, adenosine triphosphate (ATP) is formed and LLLT stimulates tissue metabolism and modulates inflammatory pathways (Andrade *et al*., 2014; Brosseau *et al*., 2000; Chen *et al.*, 2022; Weber, Mehran, *et al.*, 2023).

Many studies suggest that introducing laser fibers from the skin surface into the knee joint can provide better treatment effects for the surrounding cartilage and tissue (Ailioaie et al., 2014; Chen *et al*., 2022; Muschter & Whitfield, 1999; Michael Weber; Weber, 2011; Weber, Mehran, *et al*., 2023). Many studies show that LLLT has been shown to stimulate tissue metabolism and regulate inflammatory pathways when combined with PRP (Ghidini *et al*., 2021; Gonçalves *et al.*, 2021; Ozaki *et al*., 2016; Prodromos *et al*., 2018). The amount of transforming growth factor beta (TGF-β) can be increased by LLLT irradiation. TGF-β is important for cartilage integrity and can act as a powerful tool to prevent or repair cartilage damage. Exosomes contain many growth factors and anti-inflammatory factors. Exosomes are small vesicles with a diameter of 50 to 150 nm that contain specific proteins, lipids and nucleic acids such as DNA, mRNA, miRNA and other small non-coding fragments. In recent years, evidence has demonstrated the basic functions and mechanisms of exosomes derived from various cells and extracellular fluids, such as stem cells, immune cells, progenitor cells, and stem cells bone marrow (BMSC), etc (Chen *et al*., 2019; Dompe *et al*., 2020; Eroglu *et al*., 2021; Pinto *et al*., 2021).

On the other hand, some studies have reported success with laser treatment while others have found it ineffective. Ineffective treatment may be due to the laser traveling a short distance through soft tissue. We can solve this problem not by increasing the output power but by using single-use optical fibers to transmit photons to the target area (Chen *et al*., 2022). This study aimed to find out the effectiveness of the combination of exosome injection and intra-articular (IA) laser compared with exosome injection in the treatment of knee osteoarthritis (OA). The WOMAC and VAS osteoarthritis index can be used to evaluate the functional status of knee osteoarthritis. The results of this study showed that after at least 6 months in both knees, functional status improved, and inflammation was significantly reduced. The first improvements occurred after the fourth month and in all volunteers, we saw a significant improvement in function and the patient's rate of improvement was significant. By studying the VAS parameters between the left knee and right knee, we can expect faster results by combining extracorporeal and intra-articular laser therapy. This is a preliminary study for further investigation.

CONCLUSION

Low-level laser therapy (LLLT) using a 20-minute infrared (808nm) intra-articular laser applied to the knee joint has been shown to be beneficial in the treatment of knee osteoarthritis (grade 3) in elderly volunteers. year old. The treatment effect can last at least 4 months. Therefore, intra-articular LLLT may be a viable option in the treatment of elderly patients with moderate knee osteoarthritis when these patients are averse to surgery and intra-articular exosome injection, as previous studies show similar effects in combination with PRP (Chen *et al*., 2022; Prodromos *et al*., 2018, 2019; Tan *et al.*, 2021). Beneficial effects of PBM have been shown in various physiological and pathological processes (Chen *et al*., 2019). In fact, the laser produces a photobiomodulatory effect that can accelerate wound healing because it improves cell viability through mitochondria stimulation of exosomes and cell membrane photoreceptors. as well as ATP production (Chen *et al*., 2019; Ghidini *et al*., 2021; Gonçalves *et al*., 2021). It may also play an important role in promoting cell proliferation, differentiation and migration. and restore cell metabolism (Yoo, 2020). Different mechanisms of action of PBM therapy in irradiated tissues have been identified, including regulation of histone acetylation and NFκB expression, some signaling activation such as ERK, SIRT1, PI3K/AKT and NF-kB, which releases growth factors, stimulates extracellular matrix production, reduces inflammation, promotes platelet activation and aggregation, accelerates cell proliferation and migration, improves cell activity and survival, improves Antioxidant function, preserve the mitochondrial homeostasis of exosomes, promote the biogenesis and mitochondrial transfer of exosomes (Pinto *et al*., 2021; Yoo, 2020). In general, PBM, due to its effects, can improve the adaptive capacity of cells to cope with cell damage during disease development (Asgari *et al*., 2024; Pinto *et al*., 2021). Therefore, this type of therapy may allow the development of new clinical approaches and combinations to achieve better results in treatment procedures. We can summarize the effect of PBM on the mitochondria of exosomes. PBM can affect the mitochondria of exosomes through four main pathways: 1: increase mitochondrial transfer, 2: enhance mitochondrial membrane potential, 3: regulate oxidative stress and 4: increase ATP synthesis. Pathways 3 and 4 can induce several signals that lead to inhibition of apoptosis and cell survival. We hope that this study will open an effective non-surgical treatment for osteoarthritis using a combination of intra-articular laser and exosome or stemcell therapy.

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References

- Ailioaie, L. M., Litscher, G., Weber, M., Ailioaie, C., Litscher, D., & Chiran, D. A. (2014). Innovations and challenges by applying sublingual laser blood irradiation in juvenile idiopathic arthritis. *International Journal of Photoenergy*, *2014*.
- Andrade, F. d. S. d. S. D., Clark, R. M. d. O., & Ferreira, M. L. (2014). Effects of low-level laser therapy on wound healing. *Revista do Colégio Brasileiro de Cirurgiões*, *41*, 129-133.
- Asgari, R., Mehran, Y. Z., micheal Weber, H., Weber, M., Golestanha, S. A., Kazerouni, M. H., Panahi, F., Mohammadi, P., & Mansouri, K. (2024). Management of oxidative stress for cell therapy through combinational approaches of stem cells, antioxidants, and photobiomodulation. *European Journal of Pharmaceutical Sciences*, 106715.
- Brosseau, L., Welch, V., Wells, G., Tugwell, P., De Bie, R., Gam, A., Harman, K., Shea, B., & Morin, M. (2000). Low level laser therapy for osteoarthritis and rheumatoid arthritis: a metaanalysis. *The Journal of rheumatology*, *27*(8), 1961-1969.
- Chen, H., Wu, H., Yin, H., Wang, J., Dong, H., Chen, Q., & Li, Y. (2019). Effect of photobiomodulation on neural differentiation of human umbilical cord mesenchymal stem cells. *Lasers in medical science*, *34*, 667-675.
- Chen, J.-L., Hsu, C.-C., Chen, W. C., Peng, Y.-N., Chen, C. P., & Suputtitada, A. (2022). Intra-articular laser therapy may be a feasible option in treating knee osteoarthritis in elderly patients. *BioMed Research International*, *2022*.
- Ding, C. (2002). Do NSAIDs affect the progression of osteoarthritis? *Inflammation*, *26*, 139-142.
- Dompe, C., Moncrieff, L., Matys, J., Grzech-Leśniak, K., Kocherova, I., Bryja, A., Bruska, M., Dominiak, M., Mozdziak, P., & Skiba, T. H. I. (2020). Photobiomodulation—underlying mechanism and clinical applications. *Journal of clinical medicine*, *9*(6), 1724.
- Eroglu, B., Genova, E., Zhang, Q., Su, Y., Shi, X., Isales, C., & Eroglu, A. (2021). Photobiomodulation has rejuvenating effects on aged bone marrow mesenchymal stem cells. *Scientific reports*, *11*(1), 13067.
- Ghidini, G., Mori, D., Pulcini, S., Vescovi, P., & Sala, R. (2021). Photobiomodulation with a 645 nm diode laser of Saos-2 cells and platelet-rich plasma: The potential for a new mechanism of action. *Photobiomodulation, Photomedicine, and Laser Surgery*, *39*(2), 86-93.
- Gonçalves, A. B., Bovo, J. L., Gomes, B. S., Pigoso, A. A., Felonato, M., Esquisatto, M. A. M., de Jesus Lopes Filho, G., & do Bomfim, F. R. C. (2021). Photobiomodulation (λ= 808nm) and platelet-rich plasma (PRP) for the treatment of acute rheumatoid arthritis in wistar rats. *Journal of Lasers in Medical Sciences*, *12*.
- Iyer, S. R., Scheiber, A. L., Yarowsky, P., Henn III, R. F., Otsuru, S., & Lovering, R. M. (2020). Exosomes isolated from platelet-rich plasma and mesenchymal stem cells promote recovery of function after muscle injury. *The American Journal of Sports Medicine*, *48*(9), 2277-2286.
- Liu, X., Wang, L., Ma, C., Wang, G., Zhang, Y., & Sun, S. (2019). Exosomes derived from platelet-rich plasma present a novel potential in alleviating knee osteoarthritis by promoting proliferation and inhibiting apoptosis of chondrocyte via Wnt/β-catenin signaling pathway. *Journal of orthopaedic surgery and research*, *14*(1), 1-16.
- Mubark, H. (2021). Autologous Expanded Mesenchymal Stem Cell Implantation for Orthopedics Conditions as a Non-Surgical Approach. *Biomedical Journal of Scientific & Technical Research*, *39*(2), 31175-31177.
- Mubark, H. (2022). Autologous Expanded Mesenchymal Stem Cell Implantation for

Orthopedic Conditions as a Non-Surgical Approach: A Recent Study. *Issues and Developments in Medicine and Medical Research Vol. 3*, 62-69.

- Muschter, R., & Whitfield, H. (1999). Interstitial laser therapy of benign prostatic hyperplasia. *European urology*, *35*(2), 147-154.
- Ozaki, G. A. T., Camargo, R. C. T., Koike, T. E., Garcia, T. A., Castoldi, R. C., Pereira, J. D. A. d. S., Constantino, C. J. L., & Camargo Filho, J. C. S. (2016). Analysis of photobiomodulation associated or not with platelet-rich plasma on repair of muscle tissue by Raman spectroscopy. *Lasers in medical science*, *31*, 1891-1898.
- Pinto, H., Goñi Oliver, P., & Sánchez-Vizcaíno Mengual, E. (2021). The effect of photobiomodulation on human mesenchymal cells: a literature review. *Aesthetic Plastic Surgery*, *45*, 1826-1842.
- Prodromos, C. C., Finkle, S., Dawes, A., & Dizon, A. (2018). Intra-articular laser treatment plus Platelet Rich Plasma (PRP) significantly reduces pain in many patients who had failed prior PRP treatment. Mechanisms of Photobiomodulation Therapy XIII,
- Prodromos, C. C., Finkle, S., Dawes, A., & Dizon, A. (2019). Intra-articular laser treatment plus platelet rich plasma (PRP) significantly reduces pain in many patients who had failed prior PRP treatment. *Medicines*, *6*(3), 75.
- Tan, S. H. S., Kwan, Y. T., Neo, W. J., Chong, J. Y., Kuek, T. Y. J., See, J. Z. F., Wong, K. L., Toh, W. S., & Hui, J. H. P. (2021). Intra-articular injections of mesenchymal stem cells without adjuvant therapies for knee osteoarthritis: a systematic review and meta-analysis. *The American Journal of Sports Medicine*, *49*(11), 3113-3124.
- Weber, M. Interstitial and intraarticular laser therapy-attractive new therapeutic option for the treatment of spinal diseases and advanced joint osteoarthritis.
- WEBER, M. Intravenous and interstitial photodynamic laser therapy: New options in oncology.
- Weber, M. (2011). Intravenöse und interstitielle Lasertherapie: Eine neue Option in der Onkologie. *Akupunkt. Aurikulomed*, *37*, 32-34.
- Weber, M., Ghanbarzadeh, A., Mehran, Y. Z., Weber, H. M., Kazerouni, S. H., Mehran, F. Z., Hosseini, S., Kazerouni, A. A., Golestanha, S. A., & Rahbar, N. (2023). Combination of Photodynamic Therapy and Multi-Wavelengths Photobiomodulation in Chronic Wound Healing Disorders: Case series.
- Weber, M., Mehran, Y. Z., Kazerouni, S. M. H., Aghaiee, S., Weber, H. M., Ghanbarzadeh, A., Mehran, F. Z., & Parnian, S. (2023). Innovative Laser Therapy in Chronic Wound Healing Disorders; Multi Wavelengths Transdermal and Interstitial Laser Therapy. *therapy*, *19*(25), 26.
- Wieland, H. A., Michaelis, M., Kirschbaum, B. J., & Rudolphi, K. A. (2005). Osteoarthritis—an untreatable disease? *Nature reviews Drug discovery*, *4*(4), 331-344.
- Wong, K. L., Zhang, S., Wang, M., Ren, X., Afizah, H., Lai, R. C., Lim, S. K., Lee, E. H., Hui, J. H. P., & Toh, W. S. (2020). Intra-articular injections of mesenchymal stem cell exosomes and hyaluronic acid improve structural and mechanical properties of repaired cartilage in a rabbit model. *Arthroscopy: The Journal of Arthroscopic & Related Surgery*, *36*(8), 2215-2228. e2212.
- Yoo, S. H. (2020). Effect of photobiomodulation on the mesenchymal stem cells. *Medical Lasers; Engineering, Basic Research, and Clinical Application*, *9*(2), 119-125.
- Zhang, R., Ma, J., Han, J., Zhang, W., & Ma, J. (2019). Mesenchymal stem cell related therapies for cartilage lesions and osteoarthritis. *American journal of translational research*, *11*(10), 6275.
- Zhang, Y., Wang, X., Chen, J., Qian, D., Gao, P., Qin, T., Jiang, T., Yi, J., Xu, T., & Huang, Y. (2022). Exosomes derived from platelet-rich plasma administration in site mediate cartilage protection in subtalar osteoarthritis. *Journal of Nanobiotechnology*, *20*(1), 1-22.