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# Effect of MLS® Laser Therapy for the treatment of experimentally induced acute tendinopathy in sheep – a preliminary study.

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## ABSTRACT

Tendon injuries are common in human athletes and sport horses. Unfortunately, traditional treatments are limited in their ability to completely heal injured tendons. Recent advances in low-level laser therapy (LLLT) have shown promising results. This study evaluated the effect of Multiwave Locked System (MLS®) laser therapy in collagenase-induced tendon lesions in sheep. Six animals were randomly assigned to two groups, with group 1 receiving ten MLS® Laser Therapy treatments at 5 J/cm<sup>2</sup> on the left hind limb and group 2 receiving the same number of treatment at 2.5 J/cm<sup>2</sup> on left hind limb. The right hind limb was considered a control for both groups. Clinical follow-up, ultrasonography and histological examinations were performed on the injured tendons.

Clinical and histological evaluations demonstrated that using a therapeutic dose less than 5 J/cm<sup>2</sup> resulted in an anti-inflammatory effect. Moreover, the histological examinations showed a statistically significant reduction in cell number in both treated groups and a significant decrease in vascularization in the treated tendons in group 2. MLS® Laser Therapy appears to be an effective tool to improve collagen fiber organization in the deep digital flexor tendon.

## INTRODUCTION

Overuse tendinitis and other tendon injuries are common among athletes [1,2] and represent a frequent cause of lameness in sport horses [3,4]. In the human medical field, Low Level Laser Therapy (LLLT) has been used to treat acute and chronic musculoskeletal

pain and foster wound healing [5]. However, few studies have evaluated its effectiveness in treating patients with acute tendonitis and other tendinopathies [6].

As demonstrated in the literature, LLLT acts on two phases of the healing process [7]. First, it reduces PGE2 concentrations and inhibits cyclo-oxygenase [8,9,10,11]. Secondly, it modulates fibroblast metabolism and collagen deposition due to its anti-inflammatory effect. Histological changes observed in tendons receiving LLLT include increased collagen production [12], improved collagen bundle organization [13,14] and an increased number of small blood vessels [5,15].

Animal models are commonly utilized in tendon disorder research [16] and the collagenase-induced tendinitis model has been used to study acute inflammatory responses [8]. This model has been used in rats, sheep and horses, and mimics a traumatic tendon injury [17,18]. The sheep is recognised as a model for human and equine orthopaedic injuries, including tendinopathy, due to the similar connective tissue structure of the flexor tendons in these species [19,20,21]. Numerous authors have described the positive effects of LLLT in experimental trials in rats [9,16,22,23], mice [13,24] and rabbits [12]. However, to our knowledge, there are no studies investigating the effect of LLLT on experimentally induced tendinitis in sheep. This preliminary study was designed to investigate the effect of MLS® (Multiwave Locked System) laser therapy on an experimental model of collagenase-induced tendinitis in sheep in order to evaluate a specific treatment for human and animal athletes.

## MATERIALS AND METHODS

This study was approved by the University Ethics Committee for Animal Experimentation (CEASA) and by the Italian Ministry of Health on 17 May 2010 (DM no. 97/2010-B). Six healthy adult female Bergamasca sheep weighing 50-60 Kg were included in the study. Prior to enrollment,

clinical and ultrasound examinations were performed to confirm tendon integrity.

A defect was produced in the deep digital flexor tendons (DDFT) of both hind limbs by collagenase injection as previously described [21,25]. Intravenous administration of 10 µg/kg of medetomidine (Sedator®, Ati srl Ozzano dell'Emilia, Italy), and 2 mg/kg of propofol (Rapinovet® Intervet Italia, Peschiera Borromeo, Italy) were used to anaesthetize the animals. After aseptic disinfection and placement in lateral recumbency, 500 IU of sterilized bacterial collagenase type 1A (C-9891; Sigma, Milan, Italy) in 0.13 ml of saline solution was injected bilaterally (left and right hind limbs) into the DDFT under ultrasound guidance. A 23-gauge needle was used to perform the injection. The needle was introduced 15 cm distal to the calcaneal bone and was inserted into the full thickness of the DDFT using a lateral approach with the hock joint flexed at 90°. A suture was applied close to the injection site to mark the precise location for treatment and tendon harvesting. Antibiotic therapy using amoxicillin-clavulanic acid (Synulox® Pfizer Italia, Rome, Italy) at a dose of 12.5 mg/kg SC was started and continued for 5 consecutive days. Buprenorphine (Temgesic® RB Pharmaceuticals, Slough, UK) at a dose of 0.01 mg/kg IM BID for 5 days was used to provide analgesia.

Seven days after collagenase injection, the 6 sheep were divided into two groups (group 1 and 2) and treated using MLS® Laser Therapy. The MLS® Laser Therapy was performed using an Mphi veterinary laser device (ASA, Arcugnano-VI, Italy), equipped with combined, synchronized and overlapping continuous and pulsed emissions from a single handpiece. Continuous emissions or continuous interrupted emissions were produced by an InGa(Al)As diode laser with the following parameters: wavelength of 808 nm, peak power of 1000 mW for continuous wave, mean power of 500 mW for continuous interrupted wave, spot area of 3.14 cm<sup>2</sup>, spot diameter of 2 cm. Pulsed emissions were produced by an InGaAs/

GaAs diode with the following parameters: wavelength of 905 nm, peak power of 25 W, mean power of 54 mW at 1500 Hz, pulsed wave, spot area of 3.14 cm<sup>2</sup>, spot diameter of 2 cm. Following the protocol of Bjordal and Lopes-Martins (2013), the applications were performed daily for 5 days followed by 2 days of no treatment and then daily for 5 additional days [26]. All treatments were conducted by the same individual. Scan modality was based on the size and shape of the treatment area (Fig. 1). The equipment was calibrated before the start of every session using the Powermeter Ophir Nova II Display S/N 573995. In group 1, the left hind DDFT received MLS® laser treatment at a dose of 5 J/cm<sup>2</sup>. In group 2, the left hind DDFT received MLS® laser treatment at a dose of 2.5 J/cm<sup>2</sup>. The right hind DDFT was considered an internal control (without treatment) for both groups.



Fig. 1 MLS® Laser Therapy

Sheep in both groups were monitored daily by evaluating the circumference, swelling and heat of the limb at the point of injury. Pain on limb palpation and degree of lameness were also assessed using a previously developed scoring system ranging from a grade of 0 to 4 [27]. Tendon thickness and echogenicity of the wound area were evaluated ultrasonographically [17,28]. Ultrasound examinations, using a GE Medical System LOGIQ P5 machine and linear 6-10 MHz probe, were performed 7, 21 and 37 days after lesion creation.

At day 37 after tendon lesion creation (30

days after the first laser treatment), the animals were sedated and anesthetized as previously described. The animals were subsequently euthanized using an intravenous injection into the auricular vein of 10 ml of a combination of drugs approved for euthanasia (Tanax®, Intervet, Milan, Italy). After euthanasia, the tendons were surgically removed from the calcaneus to the end of the metatarsal region and the DDFT of both hind limbs harvested for histological analysis. Tendons were removed 5 cm proximally to 5 cm distally of the lesion previously marked by a cutaneous surgical stitch. Harvested DDFTs were cut into 1 cm pieces and the proximal–distal orientations were marked. Tissue samples for histology were fixed in 4% paraformaldehyde (PFA) and embedded in paraffin. Sections were cut into 5 µm slices, mounted on microscope slides and stained using Harris hematoxylin and eosin (HE). Sections were analyzed for cell density, vascularization and tissue organization using specific markers to evaluate fibroblast and tissue/matrix organisation characteristics. A quantitative analysis was performed to compare differences in cell number between groups. Differences in vascularization were also evaluate by looking at the ratios of blood vessel areas. Three segments were processed from each tendon, with 5 slides taken from each segment and three microscopic fields examined per slide, resulting in a total of 540 fields evaluated.

Analyses were performed using STATISTICA 9 (StaSoft) software, and data were assessed for normality using a Shapiro–Wilk test. Differences among the experimental groups within each sampling were evaluated using a Kruskal–Wallis Test. In all analyses, a  $p < 0.05$  value was considered significant.

## RESULTS

After the collagenase 1A injection, an inflammatory reaction with a mild localized thickening of the DDFT was detected in all subjects. Lameness, ranging from grade 3 to 4, and pain (detected by palpation) remained evident for the first 3-5 days. A localized

increase in temperature around the point of injury was detected manually for the first 3 days. From day 7 of the MLS® Laser Therapy, an inflammatory reaction was observed in the treated limbs of group 1, with about 1 centimeter increase in wound circumference and an increase in the temperature of the metatarsus (Fig.2); whereas no worsening of lameness or pain was observed. This inflammatory response was not observed in the treated limbs of group 2.



Fig. 2 Inflammatory reaction observed in the treated limbs of group 1 during MLS® Laser Therapy in left limb: A after collagenase induction, B 7 days after MLS® Laser Therapy

A progressive reduction in limb circumference was observed by the end of treatment for both groups. Limb circumference returned to a value close to the starting size only in group 2. In addition, local temperature, lameness and pain decreased in all subjects. The treated left DDFT showed a more rapid reduction in local inflammation compared to the right DDFT in all sheep. Ultrasound examinations detected the presence of a lesion in the DDFT 7 days after lesion creation and during the entire follow-up period. Better collagen-fiber alignment and more uniform filling of the lesions were observed in the treated limbs compared to the control limbs. During the follow-up period, group 1's treated limbs showed a marked thickening of the DDFT compared to the control limbs. Histological analysis revealed that there was disorganization of the extracellular matrix, increased vascularization and increased cell density in the DDFTs of the control limbs. In contrast, the sections

obtained from tendons treated with MLS® Laser Therapy showed a more uniform and organized extracellular matrix, a lower number of cells and a better realignment of collagen fibers. The quantitative analysis revealed a significant decrease in fibroblasts in the treated legs compared to the control legs in group 1 (5 J/cm<sup>2</sup>). However, the ratio of the vessel areas did not differ between the control and treated tendons. In group 2 (2.5 J/cm<sup>2</sup>), the treated legs also had a decrease in fibroblast number compared to the control legs. A significant decrease in vascularity was observed in the treated tendons compared to the control tendons.

## DISCUSSION

This is the first experimental study to evaluate the effect of LLLT on the tendon healing process in a sheep model. We evaluated the effects of two different doses of MLS® Laser Therapy in the acute phase of induced tendon lesions in order to determine a suitable therapeutic range for physiotherapy in human and veterinary medicine. The latest reviews on the effectiveness of LLLT in human medicine<sup>5,6</sup>, highlighted the need to identify a specific treatment protocol for tendinopathy. Several authors [13,14,15,16,23,29,30,31] have reported the efficacy of LLLT in increasing the calcaneal tendon's mechanical properties as well as increasing the alignment of collagen fibers and angiogenesis, with doses between 3 and 5 J/cm<sup>2</sup>. In the present animal model study, we initially decided to use 5 J/cm<sup>2</sup>, which is the average value reported in the literature for treatment of acute tendinitis in human medicine. A 50% reduction in radiant fluence was elected for the second group (2.5 J/cm<sup>2</sup>) because the clinical symptoms and ultrasonographical data demonstrated an increase of inflammatory response in group 1 during and after MLS® treatment. The tendon circumference in the first treatment group did not return to normal after a month of follow-up. In contrast, in the second group, the tendons' external morphology returned to the physiological state by the end of the trial. In particular, the clinical manifestations of two

sheep worsened slightly in the initial treatment phase, with an increase in circumference at the injection site, a local rise in temperature and a slow remission of symptoms. The aggravation of the inflammatory condition, observed during the applications of MLS® Laser Therapy in group 1, appears to indicate that a dose of 5 J/cm<sup>2</sup> was excessive for treatment of acute tendinitis with this type of laser emission. Instead, the dose of 2.5 J/cm<sup>2</sup> (group 2) appears to be suitable to produce an anti-inflammatory and biostimulating effect on tendon healing. Results from histological examinations indicated that both treatments induced a statistically significant decrease in cell number, although the values only returned to normal in the second group. Moreover, the MLS® dose of 2.5 J/cm<sup>2</sup> (group 2) caused a significant decrease in blood vessel area and better improvement of collagen fiber organization in deep digital flexor tendon compared to group 1 and the control group.

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## AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist

# Application of MLS® therapy on a disc herniation with radiculopathy: a case study.

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## ABSTRACT

This paper presents the case of a 17 year old female who suffered from severe lumbalgia and radiculopathy into the left leg. The purpose of this clinical case was to determine the effects of NIR laser therapy on pain associated with disc herniation. The source used was a Multiwave Locked System (MLS®) MLS® Laser. The instrument consisted of two assembled laser diodes with synchronized emissions at 808 and 905 nm, respectively. The technique utilized to apply the laser radiation was the global approach using both the ultrahead and handpiece to deliver the treatment. Laser application was initially performed three times per week for the first course of treatment and decreased in frequency for the remainder of care according to the patient's reduction in subjective findings. The patient's progress was measured by the Visual Analogue Scale (VAS). A percentage improvement scale was also utilized to determine the percent improvement in pain and range of motion. The results showed steady improvement of pain relief during the seven month course of treatment. At nine months the pain was not present. The

results indicate that MLS® laser therapy has beneficial effects on pain associated with disc herniation.

## INTRODUCTION

Low back pain can occur from many sources. Differentiating lumbalgia due to disc herniation is best done with radiological imaging, specifically magnetic resonance imaging (MRI) [1]. The associated pain can manifest in the area of injury or become radiating in nature, traveling down the pathway of the affected nerve roots [2, 3]. Orthopedic testing including Valsalva's, straight leg raiser (SLR), range of motion (ROM) and other tests can help with a positive differential diagnosis of pain associated with disc herniation [4].

The purpose of the investigation was to determine if laser therapy administered by a dual wavelength NIR laser (M6-MLS® laser) could be an effective treatment on a 17 year old female patient who suffered from severe lumbalgia and radiculopathy associated with a L4-L5 disc herniation.

## MATERIALS AND METHODS

A 17 year old female patient presented to Orland Park Laser Therapy Center with a

chief complaint of severe lumbalgia with associated radiculopathy into the left buttock, left posterior thigh and lower leg to the ankle.

Pertinent history of the case included a high speed rear end collision one year prior in which the patient was a passenger in the rear seat of the vehicle. There was no pain in the lower back post motor vehicle accident.

## Subjective findings included

Sharp shooting pain L4-L5 bilateral with radiculopathy into the left buttock, left posterior thigh and lower leg. The pain was 6 months in duration from the first appointment and was getting progressively worse. The pain was rated at a 10+ on the VAS scale. The pain was constant and resulted in marked impairment. The pain affected sleep, all daily activities and the ability to sit for more than three minutes without severe pain. The patient missed fifty percent of school days two months prior to seeking treatment.

## Objective findings included

Severe pain in lower lumbar area and down left leg on flexion, extension, lateral bending and right rotation. Decrease in all ranges of motion of lumbar area due to pain and muscle spasm. Positive orthopedic tests included Adam's, Kemp's, Valsalva's, Soto hall and Braggard's. A decrease in L4-L5 dermatomal sensation was noted with pinwheel testing. Weakness was noted on left leg extension and flexion during muscle testing. Surface electromyography (SEMG) testing revealed severe bilateral paraspinal muscle spasm at the L1 thru S1 level.

## Radiology findings

MRI without contrast revealed L4-L5 disc herniation in the left paracentral region affecting multiple nerve roots. A central disc herniation was noted at the L5-S1 level with no nerve root involvement. Initial diagnosis was lumbar disc herniation

Number of treatments	Dates	Hz	Time	Intensity	VAS	Improvement Scale	Comments of Improvement
1-12	Dec 5 <sup>th</sup> -30 <sup>th</sup>	2000 2000	10:00 4:00	100% 100%	Started= 10 Finished= 5	Finished 50%	After 4 Treatments 30% (sleeping better, less pain, moving around better)
13-24	Jan 2 <sup>nd</sup> -Feb 8 <sup>th</sup>	2000 2000	12:00 4:00	100% 100%	Started= 5 Finished= 3	Finished 70%	Continued to notice decrease in pain except for sitting in class at school. Started exercises in this phase
25-36	Feb 11 <sup>th</sup> -Mar 29 <sup>th</sup>	CW 2000 2000	12:00 2:00 4:00	100% 100% 100%	Started= 3 Flared= 5 Finished= 2	Finished 80%	Had a flare up back to 5 pain (due to overuse). Increased technique to CW for 5 treatments. Went back to 2000 Hz, pain level again decreased and improved. Added motion with hand piece to low back. Sitting long periods much easier, not missing school.
37-48	Apr 8 <sup>th</sup> -June 10 <sup>th</sup> *Suspended treatment for one week to see subjective results*	900 2000 2000	12:00 4:00 2:00	50% 100% 100%	Started= 4 Finished= 2	Finished 90%	Added dynamic with TP to left lower Lumbar in pain areas 30 seconds each 4 spots doing flexion. NO PAIN 80% of days. Went to prom and danced, went on roller coasters at six flags without our consent but was PAIN FREE.
49-54	June 17 <sup>th</sup> -July 29 <sup>th</sup>	900 2000 2000	12:00 4:00 2:00	50% 100% 100%	Started= 2 Current= 1	Current 95%	No pain issues, mild cramping noted with overuse. Helped brother move, which included, carrying boxes and bending over with little to no pain. Increased exercising to 1 hour + per day with no issues.

Table 1: Treatment Schedule  
 Legend: "VAS" = Visual Analogue Scale  
 "TP" = Trigger Point  
 "HZ" = Hertz  
 "CW" = Continuous Mode Operation

with associated lumbar segmental dysfunction, lumbalgia and radiculopathy. Prior treatment included local injections of Tosdal, Tylenol, prescription anti-inflammatories and prescription pain medications with no pain relief. Physical therapy utilizing low back exercises and treadmill therapy intensified the pain. Discussions with Orthopedic surgeon and pain specialists included steroid injections, Celebrex and surgery. This is when the patient decided to seek care at Orland

Park Laser Therapy Clinic. The initial treatment plan consisted of laser therapy utilizing a dual wavelength NIR laser ( M6 MLS® laser, ASA srl, Arcugnano, Vicenza, Italy). The treatment was initially applied 3 times per week for 4 weeks. Application of the treatment was done using the global approach, which consisted of the following parameters and mode: 2000Hz at 100% intensity for 10 minutes, using the ultrahead directly over the L2 thru S1 lumbar spine and paraspinal area. The handpiece was used over the L4-L5 area at 4 points, 1 minute each, with 2000Hz and 100% intensity. Following the initial 4 weeks of treatment with the M6-MLS laser low back stability exercises were added to begin an exercise

rehabilitation program. Flexion-distraction technique utilizing the Cox flexion-distraction method was also added to treatment to aid in the resorbtion of disc material and increase the pliability of the lower back soft tissue [5].

Table 1 shows the continuation of care over a 7 month period utilizing the MLS® laser. Note that after the 24th treatment motion was added to the treatment utilizing the handpiece to trace the sciatic nerve in the left leg.

**RESULTS**

The patient went from a pain level of 10 on the VAS scale on initial evaluation to a pain level of 1 over the 7 month period of treatment. ROM study revealed

normal ranges of motion post treatment with no pain associated on any ranges. All orthopedic, dermatomal and muscle tests, including SEMG testing, were normal on patient's final evaluation.

A 90 day follow-up revealed no pain in the lower back or left leg unless sitting in class for longer than 2 hours without getting up to stretch.

### DISCUSSION

Severe lumbalgia with radiculopathy caused by disc herniation is usually progressive in nature and can lead to many debilitating limitations including the inability to exercise which can accelerate further degenerative changes leading to increased pain, weight gain and other problems [6].

In this case study, the patient had total remission of pain in the lumbar and left leg areas. Initially the only treatment administered to the patient was M6-MLS® laser which showed a 50% decrease in pain over 4 weeks. At this point exercise was added which allowed the patient to lose 50 lbs. over the course of her treatment possibly contributing to her final results. Other possible contributing factors include Cox flexion-distraction technique and rehabilitative exercises.

### CONCLUSION

In this case study, the M6-MLS® laser proved to be effective in decreasing the amount of pain associated with nerve root irritation caused by disc herniation. In addition to the analgesic effect, MLS® therapy may also have long term effects on this condition related to its ability to increase microcirculation and lymphatic drainage causing a decrease in chronic inflammation [7] and edema [8] associated with chronic symptoms of prior injuries. Furthermore, effects on cell energy metabolism leading to increased ATP production [9,10] and efficient ATP utilization [11], may aid in the body's natural ability to heal. Another advantage

of the M6-MLS® laser is no side effects or heat generated during treatment, making it a more pleasant experience for the patient therefore increasing patient compliance.

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# Effect of high power dual wavelength NIR laser emission in a rat model of compressive pain.

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## ABSTRACT

Chronic pain condition, such as neuropathic pain, is one of the most important health problems worldwide and, due to its uncertain etiology and poor response to therapy, it represents an important challenge for medicine. Although, nowadays, there are many drugs for the treatment of chronic pain, their use is limited by the frequent side effects and, in some cases, ineffectiveness. Thus, the search for new therapeutic strategies which minimize this important problem is actually growing.

Since many years, laser therapy has been used as a physical therapy for pain relief and has become increasingly popular because it is non-invasive and no side effects have been reported after treatment. However, its true effectiveness is still controversial because of the counteracting results reported in literature.

In the present study we investigated the effectiveness of a high power, dual wavelength NIR laser source in producing fast analgesic effect in a rat model of neuropathic pain, induced through loose ligation of the

sciatic nerve. Twelve animals were included in the study and two treatment protocols were tested, one performed daily, the other every 48 hours, with a total of 5 applications. Both protocols used were able to statistically increase the pain threshold of the ipsilateral paw (the one with loose ligation of the sciatic nerve), starting 5 min after their first applications. The anti-hyperalgesic effect of laser treatment terminated 60 min after application. It started again, showing the same anti-hypersensitivity profile, during the subsequent applications.

The treatment protocol tested in this in vivo study in animal model might be applied to give the patient immediate pain relief and combined with treatments aimed to reduce inflammation, thus leading to analgesic effects that lasts over time.

## INTRODUCTION

Pain is a serious health and social problem worldwide. Statistics is alarming and in many countries the difficulty of the patient to access to care is added. Chronic pain causes personal afflictions, social costs

and, if not treated, leads to depression, reduced performance and inability to work. Depending on its origin, chronic pain can be classified as inflammatory or neuropathic. Neuropathic pain can be initiated or caused by a primary lesion or dysfunction in the central nervous system (CNS) or the peripheral nervous system (PNS). Millions of people worldwide are suffering from this chronic condition that, as mentioned above, can compromise their engaging in daily activities [1,2].

The etiology of neuropathic pain is very heterogeneous and the underlying pathophysiology is very complex, so it is a very difficult condition to treat [2,4]. Often, the available drugs have limited therapeutic potential in the management of the chronic pain and can induce risks for the patients due to side effects.

Because universally effective therapy for neuropathic pain does not exist and the interest for studies aimed at exploring new therapeutical strategies is growing, neuropathic pain research has been explored with different animal models where intentional damage is inflicted to the sciatic nerve, branches of spinal nerves or in the spinal cord [3,5]. Although these models are not completely representative of the neuropathic pain condition in humans, the development of such experimental models is essential, not only for the detection of new analgesic drugs or therapy, but also for a better understanding of pain syndromes that are difficult to manage clinically [6]. Chronic constriction injury (CCI) of the sciatic nerve with loose ligatures is the most widely used model of neuropathic pain [7,8]. The model simulates the clinical condition of chronic nerve compression in nerve entrapment neuropathy [3].

Conservative treatments, which consist in modifying the pain-precipitating activity, biomechanical correction with physiotherapy, the use of antidepressants, analgesics and/or steroids, are common forms of therapy in the case of neuropathic pain [9,10]. However, treatments with drugs

have undesirable side effects. Therefore, physical therapies which minimize the risk for side effects have been advancing [11]. Among the resources available for treating neuropathic pain within the field of physical therapies, laser therapy has become increasingly popular, due to a large body of evidence that supports its anti-inflammatory [12,15], analgesic [11, 16, 17, repairing and restoring effects [18,21]. Clinical studies on the effects of laser therapy on injured nerves have revealed an increase in nerve function and improved capacity for myelin production [22]. Laser therapy has also been shown to be effective in promoting axonal growth in injured nerves in animal models [23,26]. However, there is controversy about the effectiveness of laser therapy for producing analgesia in cases of neuropathic pain, which may be related, in most cases, to a lack of reproducibility and the doubtful quality of certain studies.

Although many studies using laser therapy reported improvements in symptoms and pain relief, the mechanisms that underlie the analgesic effect are still unclear.

In the case of neuropathic pain, the analgesic effects of laser therapy may be due to the local release of neurotransmitters such as serotonin [27], increased mitochondrial ATP production [28], increased release of endorphins [29], or anti-inflammatory effects [30].

The present study was aimed to evaluate the effectiveness of a high power, dual wavelength NIR laser source in inducing fast analgesic effect in a model of neuropathic pain induced through loose ligation of the sciatic nerve.

## MATERIALS AND METHODS

### Animals

Male Sprague-Dawley rats (Harlan, Varese, Italy), weighing 200–250g at the beginning of the experimental procedure, were used for all the experiments. Animals were housed in the Centro Stabulazione Animali da Laboratorio, University of Florence and used no earlier than 1 week after their

arrival. Four rats were housed per cage (size 26×41cm); animals were fed with standard laboratory diet and tap water ad libitum, and kept at 23±1 °C with a 12-hour light/dark cycle, light at 7am. All animal manipulations were carried out according to the European Community guidelines for animal care (DL 116/92, application of the European Communities Council Directive of 24 November 1986; 86/609/EEC). The ethical policy of the University of Florence complies with the Guide for the Care and Use of Laboratory Animals of the US National Institutes of Health (National Institutes of Health Publication No. 85-23, revised 1996; University of Florence assurance number: A5278-01). Formal approval to conduct the described experiments was obtained from the Animal Subjects Review Board of the University of Florence. All efforts were made to minimize animal suffering and to reduce the number of animals used

### Induction of peripheral mononeuropathy by CCI

Neuropathy was induced according to the procedure described in [31]. Briefly, rats were anaesthetized with 2% isoflurane. Under aseptic conditions, the right (ipsilateral) common sciatic nerve was exposed at the level of the middle thigh by blunt dissection. Proximal to the trifurcation, the nerve was carefully freed from the surrounding connective tissue, and 4 chromic catgut ligatures (4-0, Ethicon, Norderstedt, Germany) were tied loosely around the nerve with about 1-mm spacing between ligatures. After hemostasis was confirmed, the incision was closed in layers. The animals were allowed to recover from surgery and then housed one per cage with free access to water and standard laboratory chow. Another group of rats were subjected to sham surgery in which the sciatic nerve was only exposed but not ligated.

### Laser treatments

Treatments have been performed with a Multiwave Locked System laser (MLS laser,

ASA Srl, Vicenza, Italy). It is a commercially available laser source built in compliance with EC/EU rules, which received FDA clearance and is widely used in clinics. MLS laser is a class IV, NIR laser with two synchronized sources (laser diodes). The two modules have different wavelengths, peak power and emission mode. The first one is a pulsed laser diode, emitting at 905 nm, with 25 W peak optical power; each pulse is composed of a pulse train (100 ns single pulse width, 90kHz maximum frequency). The frequency of the pulse trains may be varied in the range 1-2000 Hz, thus varying the average power delivered to the tissue. The second laser diode (808 nm) may operate in continuous (power 1 W ) or frequenced (repetition rate 1-2000 Hz) mode, 500 mW mean optical power output, duty ratio 50% independently of the repetition rate. The two laser beams work simultaneously, synchronously and the propagation axes are coincident.

14 days after the sciatic nerve ligation, animals were randomly distributed into three groups containing 4 animals each:

- group 1 (control), animals not exposed to laser treatment;
- group 2 (laser treated group I), animals exposed to laser radiation. The treatment was performed daily, every 24 h, for 5 consecutive days and it consisted in the irradiation of two points, one located in the region where the ligation was performed and the other at level of the paw joint. Each point was treated for 23s with the following laser parameters: 10 Hz, 25% intensity, 1 J/cm<sup>2</sup>, 3.171 J/tot.
- group 3 (laser treated group II), the treatment was very similar to that administered to the group I, but the animals of the group II were treated every 48 h. Also in this case the treatment was repeated 5 times.

Paw pressure test was performed before laser treatment and 5 min, 30 min, 1 h, 3 h from treatment, after. All the treatments have been performed by a single experienced operator.

All control animals were submitted to the same procedures and handling used for laser-exposed animals.

### Paw pressure test

The nociceptive threshold in the rat was determined with an analgesimeter (Ugo Basile, Varese, Italy), according to the method described by [32]. Briefly, a constantly increasing pressure was applied to a small area of the dorsal surface of the hind paw using a blunt conical mechanical probe. Mechanical pressure was increased until vocalization or a withdrawal reflex occurred while rats were lightly restrained. Vocalization or withdrawal reflex thresholds were expressed in grams. Rats scoring below 40 g or over 75 g during the test before starting the experiment (25%) were rejected. An arbitrary cut-off value of 100 g was adopted. The data were collected by an observer who was blinded to the protocol.

### Statistical analysis

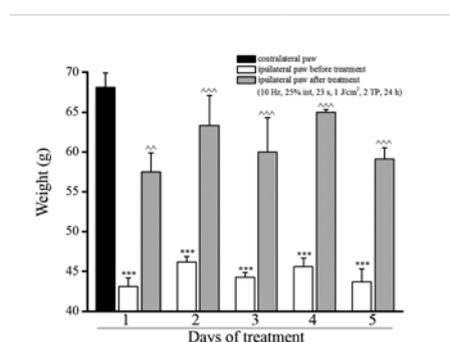
Behavioural measurements were performed on 5 rats for each treatment carried out in 2 different experimental sets. Measurements were taken in duplicate at least 1 min apart, the responses of both left and right paws were measured. For behavioural experiments One-Way analysis of variance (ANOVA) followed by Fisher's protected least significant difference procedure were used.

Data were analyzed using the "Origin 9.0" software (OriginLab, Northampton, MA, USA). Differences were considered significant at a  $P < 0.05$ .

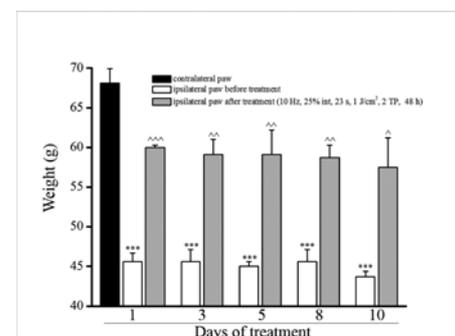
## RESULTS

CCI induces a mononeuropathy characterized by hyperalgesia that appears about 3 days after nerve injury and reaches a plateau from 7 up to 30 days. Laser treatment started 14 days after surgery and, in the first protocol adopted, its application was performed once a day for 5 consecutive days. The nociceptive threshold was measured by Paw Pressure test before and after (5 min,

30 min, 1 h, 3 h) laser application. In Figure 1, the effect of laser treatment 30 min after its application was shown. On day 1, before laser treatment, rats underwent CCI showed a nociceptive threshold significantly reduced, tolerating  $43.1 \pm 1.1$  g on the ipsilateral paw in comparison to the contralateral (nonoperated) paw ( $68.0 \pm 1.8$  g). Laser treatment significantly reduced hypersensitivity starting 5 min after application (data not shown) reaching a peak at 30 min (Figure 1; day 1,  $57.5 \pm 2.4$  g). The effect terminated at 60 min. The anti-hyperalgesic effect was no longer recorded after 24 h (day 2, ipsilateral paw before treatment) but a new administration was able to induce an anti-hypersensitive effect comparable to that obtained the previous day. Similar effects were observed also on days 3, 4 and 5 of laser treatment (Fig. 1). The nociceptive threshold of the contralateral paw did not change during the entire duration of the experiment (not shown).



**Figure 1.** Pain: noxious stimulus, Paw pressure test. Repeated applications of laser (10 Hz, 23s, 25% int, 1 J/cm<sup>2</sup>, 3.171 Jtot, 2 treated points, 24 h) inhibit pain behaviours induced by Chronic Constriction Injury (CCI) in the rat. Peripheral mononeuropathy was induced by CCI of the right sciatic nerve (ipsilateral). Laser treatment was performed daily for 5 consecutive times starting 14 days after surgery. Behavioural measurements were conducted before and after (5 min, 30 min, 1 h and 3 h) laser treatment. The values of the ipsilateral paw before and 30 min after laser application were shown. Each value represents the mean  $\pm$  sem of 5 rats per group. \*\*\* $P < 0.001$  vs the contralateral paw; ^^ $P < 0.01$  and ^^ $P < 0.001$  vs the ipsilateral paw before treatments.



**Figure 2.** Pain: noxious stimulus, Paw pressure test. Repeated applications of laser (10 Hz, 23s, 25% int, 1 J/cm<sup>2</sup>, 3.171 Jtot, 2 treated points, 48 h) inhibit pain behaviours induced by Chronic Constriction Injury (CCI) in the rat. Peripheral mononeuropathy was induced by CCI of the right sciatic nerve (ipsilateral). Laser treatment was performed every 48h for 5 times starting 14 days after surgery. Behavioural measurements were conducted before and after (5 min, 30 min, 1h and 3h) laser treatment. The values of the ipsilateral paw before and 30 min after laser application were shown. Each value represents the mean  $\pm$  sem of 5 rats per group. \*\*\* $P < 0.001$  vs the contralateral paw; ^ $P < 0.05$ , ^^ $P < 0.01$  and ^^ $P < 0.001$  vs the ipsilateral paw before treatments.

In Figure 2 is depicted the effect of laser treatments performed every 48 h. The paw hypersensitivity of CCI animals (ipsilateral,  $45.6 \pm 1.1$  g vs contralateral,  $68.1 \pm 1.8$  g), recorded on day 1 before laser treatment, was significantly increased 30 min after laser application, reaching a value of  $60.0 \pm 0.3$  g in the rat Paw pressure test (Figure 2). The effect onset was 5 min after application and vanished at 60 min (data not shown). Forty-eight h after, the CCI-dependent pain threshold alteration ( $45.6 \pm 1.5$  g) was newly improved by the 2nd laser application ( $59.1 \pm 1.9$  g, day 3) for 30 min. The efficacy of laser treatments was verified on days 5, 8 and 10.

## DISCUSSION

The present data show the anti-hypersensitivity effect of two different laser treatments in a rat model of peripheral neuropathy induced by sciatic nerve ligation (CCI). CCI induces a damage characterized by pain sensation correlated with evident tissue alterations [33,34]. Fourteen days after the unilateral loose ligation of the sciatic nerve, which

is one of the most frequently used model for the study of neuropathic pain and its treatments [35,36], rats showed a high degree of hyperalgesia against mechanical stimulus (Paw Pressure test). Laser treatments were performed daily (first protocol adopted) and every 48 hours (second protocol adopted) with a total of 5 applications for treatments. Both protocols used were able to statistically increase the pain threshold of the ipsilateral paw starting 5 min after their first applications. The anti-hyperalgesic effect of laser treatments terminated 60 min after application and started again, showing the same anti-hypersensitivity profile, during the subsequent applications. Accordingly, a recent work evaluated the effect of 660 and 980 nm low level laser therapy on neuropathic pain relief in the same model of CCI in the rat. Laser application (energy dose 4J/cm<sup>2</sup>), started the first day after surgery and continued for 2 weeks, increased significantly thermal and mechanical threshold. In particular, the laser radiation with 660 nm wavelength had better therapeutic effects with respect to the laser radiation with 980 nm wavelength [37]. However, the efficacy of 980 nm laser therapy was also demonstrated in CCI-induced neuropathy by Jameie et al.[38]. Moreover, these authors highlighted a synergistic effect between the simultaneous use of laser and CoQ10 on pain relief [38]. The reduction of oxidative damages has been postulated as one of the main mechanisms following the exposure to laser therapy, which induces an increase in SOD activity, thus leading to decrease tissue damages and promote the healing process [39,41]. The efficacy of 660 nm GaAlAs laser at energy dose of 9 J/cm<sup>2</sup> in significantly reducing CCI-allodynia was demonstrated by Hsieh et al. [42], who found decreased levels of HIF-1 $\alpha$  and IL-1 $\beta$  in treated animals and hypothesized an anti-inflammatory effect of the therapy. It is noteworthy

that previous research demonstrated the ability of the laser source used in the present study to increase the expression of NLRP10 [43], a protein which inhibits the inflammasoma decreasing the IL-1 $\beta$  production. The effectiveness of laser therapy in improving neuropathic pain symptoms was further demonstrated by [44], applying a low power laser with 830 nm laser and energy density 4J/cm<sup>2</sup> to an experimental model of sciatica in rats. Clinical studies on carpal tunnel syndrome also showed significant improvement in pain and nerve conduction in patients undergoing low-level laser therapy over the carpal tunnel area [45,46].

### CONCLUSION

The results of this study demonstrate that the application of a high power, dual wavelength (808 nm + 905 nm), NIR laser in a model of neuropathic pain induced a statistically significant increase in the pain threshold. The anti-hyperalgesic effect of laser treatment occurred immediately after treatment and terminated 60 min after application. Subsequent applications were able to reproduce the effect, showing the same anti-hypersensitivity profile. The effectiveness of red-NIR low power laser radiation in reducing neuropathic pain was demonstrated by several studies. It is noteworthy that in the present study, where a high power, dual wavelength NIR laser has been used, the pain relief has been obtained with very short exposure time (23 sec) and lower energy density (1J/cm<sup>2</sup>) in comparison with that applied in other studies, ranging from 3 to 9 J/cm<sup>2</sup>. The treatment protocol tested in this study might be applied to give the patient an immediate pain relief and might be combined with treatments aimed at reducing the inflammatory component to have an analgesic effect that lasts over time.

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# Effects of high-intensity laser on gonarthrosis.

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## ABSTRACT

Osteoarthritis of the knee joint, which is also called gonarthrosis, can be classified as primary and secondary. The primary role in primary gonarthrosis is played by heredity, systemic factors, local mechanic problems and chronic overloading; in secondary gonarthrosis, the primary role is played by post-traumatic and post-inflammatory conditions, and, more rarely, conditions following septic arthritis.

The aim of this study was to verify the effectiveness of high intensity laser therapy (HILT) administered by a high-power Nd:YAG laser (HIRO 3.0), in alleviating pain and increasing the range of motion in the affected joint of patients suffering from arthrosis of the knee joint of higher grades. The study comprised 50 patients with diagnosed grade II to III arthrosis. WOMAC questionnaire was applied to evaluate the effect of laser therapy.

## INTRODUCTION

### Clinical picture of gonarthrosis

A typical symptom of gonarthrosis is pain upon exertion in the affected location. At first it occurs after a major exertion, or as start-up pain at the beginning of the movement. Later there is resting pain which interferes with sleep. Short periods of morning stiffness develop into functional deterioration; the extent of movements in the joint gradually decreases. Objective findings (through palpation,

auscultation) are crepitations and tenderness to palpation. Typical deformities develop, i.e. varosity or valgosity, which are accompanied by varying degrees of flexion contractures in severe cases. The system of ligaments in the location of deformity becomes relaxed. The range of motion is limited to a various degree, later it may lead even to semiankylosis, or even to ankylosis, almost always in a functionally unfavorable position [1].

The bone under the cartilage responds to load by rebuilding its structure (i.e. sclerotization), which leads to the formation of growths (osteophytes). It is a compensatory mechanism, which ultimately limits the mobility of the joint.

Typical characteristics of osteoarthritis are limited function of certain joints and frequent asymmetry of the process. In the course of the disease, deterioration often comes up in the form of attacks, sometimes without apparent cause, which are subsequently followed by spontaneous remissions.

### Pain causing mechanisms in osteoarthritis

According with the heterogeneity and the different developmental stages of osteoarthritis in patients, the mechanism of pain development is not always the same. The following conditions are among the causes of pain in osteoarthritis: synovitis, tension of the joint capsule, periosteal elevations,

pain in ligament insertions (enthesopathy) and tendinopathy, muscle hypertonus, increased intraarticular pressure and bone microfractures. According to Pavelka [2]: "The primary location of structural changes in osteoarthritis is hyaline cartilage in the joint, however, the cartilage is aneural and avascular, so it cannot be the primary source of painful stimuli. Pain is caused by secondary changes in other articular tissues – synovial membrane, periosteum, ligament insertions, muscle insertions and the subchondral bone. A relatively frequent source of pain in osteoarthritis is synovitis. Inflammation has often clinical manifestations, which can be used to verify its presence. The joint is usually swollen and results tender to palpation, sometimes even warm. Swelling of the joint may be due both to effusion and thickening of the synovial membrane. If inflammation is present, pain sometimes tends to be present at rest and the quality of the symptom is similar to pain in inflammatory rheumatic diseases. The presence of inflammation can be documented by the presence of exudation. However, cell content in exudation is usually lower than 2000 cell/μl, because the intensity of inflammation is much lower than, e.g., in rheumatoid arthritis. Histological analysis shows that inflammation is present in the synovial membrane, but it is often limited to specific regions, its intensity is lower than in rheumatoid arthritis (RA) and thickening of the synovial membrane is not as extensive as in primarily inflammatory rheumatic diseases. A significant analgesic effect of orally administered NSAIDs or intra-articular glucocorticoids can be also explained by the presence of inflammation.

Bone microfractures in subchondral bone may represent another source of pain. Experimental studies showed changes in the quality of subchondral bone in osteoarthritis and led to several studies which applied drugs showing activity in bone (e.g. risedronate, sodium ranelate) in the effort to achieve both symptomatic and structural effects in osteoarthritis. The results of these

studies have not been positive so far, but some of them have not been finished yet." Another source of pain, occurring particularly during the night in hip and knee, might be increased intraosseous pressure, which apparently occurs as a result of venous obstruction. Pain can also derive from the joint capsule, due to increased intra-articular pressure caused by intra-articular expansion, if there is an effusion, or synovial hypertrophy, or mechanical problems caused, e.g., by the instability of the joint, which can lead to direct stimulation of mechanoreceptors in the joint capsule, or furthermore, focal ischemia in the joint capsule. However, pain in joints also may be caused by intraarticular periosteum, which may be mechanically irritated by osteophytes. Apart from intra-articular structures, pain may also originate in extraarticular (e.g. periarticular) structures. Such conditions include bursitis, tendonitis, enthesopathy or enthesitis and stretched ligaments. An important source of pain in osteoarthritis may be the muscles, their weakness or dysbalance." [2].

Last but not least, pain is influenced by psychological and social factors.

## MATERIALS AND METHODS

### General therapeutic scheme

The source used was a high power Nd:YAG laser (Hiro 3.0, ASA srl, Arcugnano, Vicenza, Italy). The treatment was divided in three phases:

**First phase** – manual scanning at higher speed (10 cm/1 s). It was divided into three subphases and characterized by increasing intensity and reduced frequency of the beam.

**Second phase** – treatment of trigger points. The handpiece was placed statically and directly on the trigger point for a maximum period of 7 seconds.

**Third phase** - slow scanning, instrument parameters remain the same as in the first phase.

Beyond the treatment on the diseased joint, this protocol also includes the treatment of

First Phase	Second Phase	Third Phase
Quick scanning	Treatment of trigger points	Slow scanning

Tab I: General Scheme

Parameters of treatment			
Phase	Intensity (mJ/cm <sup>2</sup> )	Frequency (Hz)	Scanning speed
First	360 mJ/cm <sup>2</sup>	10Hz	Higher
Second	810 mJ/cm <sup>2</sup>	25Hz	Static treatment
Third	1780 mJ/cm <sup>2</sup>	30Hz	Slow

Tab II: General parameters of treatment

the surrounding muscles, to address any contractures or secondary problems arising as a consequence of the joint disease. During the treatment, the muscle groups receive about 60% of the total energy supplied, while about 40% of it reaches the diseased area and the region where pain irradiates.

### Treatment protocol

Although in the available literature there is no standardized procedure for the application of laser therapy in gonarthrosis, protocols similar to the one reported above have proved to be effective for degenerative chondropathies and gonarthrosis [3,4]. During the first and third phases, the tissues adjacent to the joints and associated muscles were treated. In the second phase, the trigger points were treated. The last step was the treatment of the knee joint by slow scanning in 6 optical windows, which was performed using a DJD head:

- Anteromedial and lateral window – the patient flexing the knee joint up to 90°
- Posteromedial and lateral window – the patient was lying on his stomach, with his knee extended
- Medial and lateral edge of patella – the patient's knee was extended

In each optical window, 500 J were supplied, for a total of 3000 J considering all the treated windows. This treatment phase was performed in contact scanning mode, using an handpiece (mod. DJD - Degenerative Joint Disease, ASA srl, Arcugnano, Vicenza, Italy) specifically designed to minimize the energy loss due to reflection and convey the emission (spot Ø 5 mm) into the intra-articular spaces.

The patients received a series of 10 applications every other day.

### Statistical analysis – comparison of WOMAC index before and after the treatment

The effectiveness of the therapy was assessed by WOMAC questionnaire.

Statistical comparison of the answers of a group of respondents before and after the therapy was performed using the paired t-test. It is testing the null hypothesis, which is the agreement of the averages before and after the therapy. An alternative hypothesis is that there are statistically significant differences between the averages, which is assessed at the significance level of 5%. The t-test was computed by Microsoft Excel and the resulting p-value was compared to the significance level of  $\alpha = 0.05$  (i.e. 5%). If  $p > \alpha$ , the null hypothesis remains valid and no significant differences are proved. If  $p < \alpha$ , then the null hypothesis is rejected in favor of the alternative hypothesis, i.e. that there are statistically significant differences between the average answers of the respondents before and after the therapy, which is proved on the significance level of 5%.

### RESULTS

#### Comparison of WOMAC index before and after the treatment

The full comparison of all individual components of the WOMAC questionnaire, in particular the indexes WOMAC-A, WOMAC-B and WOMAC-C and the summary WOMAC indexes, before and after laser therapy, is presented in Table III.

The averages reported in Table III are arithmetic averages of the respondents' answers to each question or component of the WOMAC questionnaire.

The column "p-value" shows the results of the t-test. It can be observed that in all cases the resulting value  $p < \alpha$ . This means that all individual components of the WOMAC questionnaire, including the partial indexes WOMAC-A, WOMAC-B, WOMAC-C, and even the summary WOMAC index, showed statistically significant differences between

Component of WOMAC questionnaire	Average before the therapy	Average after the therapy	p-value	Relative decrease (before/after)	Absolute difference (before/after)
1	2.24	1.72	0.000	77%	0.52
2	3.02	2.22	0.000	74%	0.80
3	3.48	2.38	0.000	68%	1.10
4	2.02	1.72	0.000	85%	0.30
5	2.34	1.94	0.000	83%	0.40
6	3.78	2.68	0.000	71%	1.10
7	2.88	2.28	0.000	79%	0.60
8	3.30	2.44	0.000	74%	0.86
9	3.38	2.50	0.000	74%	0.88
10	2.82	2.12	0.000	75%	0.70
11	2.22	1.74	0.000	78%	0.48
12	3.12	2.40	0.000	77%	0.72
13	2.08	1.76	0.000	85%	0.32
14	2.60	2.06	0.000	79%	0.54
15	2.56	2.04	0.000	80%	0.52
16	2.50	1.96	0.000	78%	0.54
17	2.68	2.00	0.000	75%	0.68
18	2.34	1.86	0.000	79%	0.48
19	1.64	1.42	0.002	87%	0.22
20	3.10	2.22	0.000	72%	0.88
21	1.72	1.38	0.000	80%	0.34
22	2.10	1.62	0.000	77%	0.48
23	3.46	2.48	0.000	72%	0.98
24	2.56	1.94	0.000	76%	0.62
WOMAC-A	13.10	9.98	0.000	76%	3.12
WOMAC-B	6.66	4.96	0.000	74%	1.70
WOMAC-C	44.18	33.94	0.000	77%	10.24
WOMAC	63.94	48.88	0.000	76%	15.06

Table III: Comparison of average values before and after the high-intensity laser therapy

the average answers of patients before and after the therapy on the significance level of 5% (and even 1%).

In summary: using the t-test it was proved that with a probability of 99%, the high-intensity laser therapy significantly reduced pain, stiffness and problems with normal daily activities.

The column "relative decrease" shows the proportion of average answers before and after treatment expressed as percentages.

E.g. in question No. 6, this proportion is 71%, which means that after treatment the respondents rated the feeling of joint stiffness after awakening in the morning at 71% of the initial stiffness prior to therapy, that is an improvement of 29%.

The column "absolute difference" is computed as the average difference between the values before and after the therapy. In question No. 6 mentioned above, there was an improvement by 1.1 points.

Graph1: WOMAC - average responses

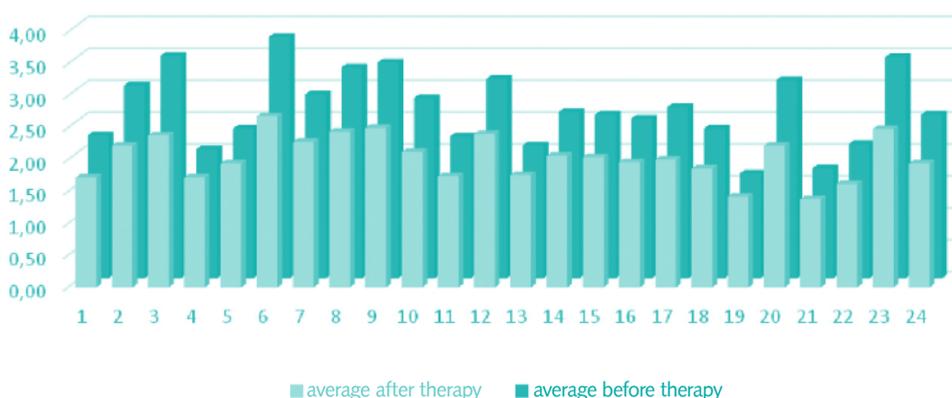


Fig.1 Results of WOMAC questionnaire

The best results (the greatest difference when comparing the state before and after the therapy) were found in questions 3 (pain at night in bed), 6 (joint stiffness after awakening in the morning) and 23 (performing heavy housework). Nevertheless, the alleviation of pain and joint stiffness is noticeable also in all other components of the WOMAC questionnaire, which is very clear in Fig 1.

### Conclusion from the statistical analysis of data

The assessment of each of the individual components of the WOMAC questionnaire, i.e. in partial indexes WOMAC-A, WOMAC-B, WOMAC-C, and even the summary WOMAC index, showed statistically significant differences between the average answers of patients with gonarthrosis before and after the therapy with a high-intensity NIR Nd:YAG laser on the significance level of 5% (and even 1%). After the HILT, there was a statistically significant decrease in the WOMAC, WOMAC-A, WOMAC-B, WOMAC-C indexes of the respective components.

Using the t-test it was proved that, with a probability of 99%, HILT significantly reduced pain, stiffness and problems with normal daily activities.

The summary WOMAC index on average decreased by 24%.

### DISCUSSION

The main objective of this work was to evaluate the effectiveness of HILT in gonarthrosis.

Etiology of gonarthrosis is a result of a complex interaction of interrelated biological, mechanical and biochemical factors. Effects of these factors result in impaired integrity and gradual loss of the cartilage. The final effect is joint destruction [5].

Osteoarthrosis of the knee joint (gonarthrosis) significantly reduces the quality of life. A typical clinical picture of gonarthrosis includes pain upon exertion, localized at the site of pathology, later also pain at rest and at night.

The patients included in the present study were selected based on pre-diagnosed grade II - III gonarthrosis. In order to assess the effectiveness of therapy, WOMAC questionnaire was chosen, which contains components dealing with pain, stiffness, and finally with problems in activities of daily living.

The study resulted in the following findings:

- An analgesic effect of a high-intensity laser was observed after the first application.

- Pain at rest and at night alleviated soon.
- None of the patients noticed any side effect of the HILT.

The aim of the present study was to test three hypotheses:

Hypothesis 1: Application of HILT has a positive effect on the mobility of the knee joint affected with arthrosis.

In the component related to stiffness after awakening in the morning, the resulting proportion is 71%. It means that, after the therapy, the respondents

assessed the feeling of joint stiffness after awakening in the morning at 71% of the stiffness before therapy, with an improvement of 29%. In this component, the improvement was 1.1 points.

Hypothesis 2: Application of HILT alleviates pain caused by gonarthrosis.

Alleviation of pain and reduction of joint stiffness can be observed in all components of the WOMAC questionnaire.

Hypothesis 3: The effect of the therapy has a positive influence on the quality of life and functional self-sufficiency.

The assessment of each of the individual components of the WOMAC questionnaire, i.e. partial indexes WOMAC-A, WOMAC-B, WOMAC-C, and even the summary WOMAC index, showed statistically significant differences between the average answers of patients before and after the therapy, with significant improvements in the quality of life

Therefore, according to the results obtained, the three hypotheses are verified.

The effectiveness of HILT in the treatment of musculoskeletal disorders was confirmed by various studies [6,13]. Buda et al. [14] investigating the effects of HILT in the treatment of patellar tendinopathy in sportsmen, demonstrated that patients who were treated with HILT showed accelerated recovery.

The findings here presented are in agreement with those obtained by other authors. Valent [4] demonstrated improvements in the function of the knee

joint in 74% of the patients and 66% of the patients reported alleviation of pain. Viliani [3] found that HILT was effective in treating pain and functional disorders related to gonarthrosis. A remarkable statistically significant improvement was found after the treatment and no side effects were observed.

The advantages of laser therapy are simple application, relatively low operating costs and easy device operation.

The results of this study demonstrate the positive effect of HILT on gonarthrosis and confirm hypotheses and objectives of this research.

## CONCLUSIONS

The data obtained in this research clearly state a positive effect of HILT on the symptoms of osteoarthritis. The conclusion of this research is that a therapy using this method could contribute to an improvement in availability and extend the healthcare options in this area

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## APPENDIX

### Likert scaling

Likert scaling is a method used to determine the level of agreement or disagreement with a statement with which the respondents are confronted in a survey. Likert scale, which was created in 1932, represents one of the most reliable techniques in the measurement of opinions. As this is a one-dimensional method, the essence of the problem investigated should be limited to one issue. In order to make it possible to identify the respondent on the scale, it is necessary not only to polarize the scale in an appropriate manner, but, of course, also to formulate the confronting statements in an appropriate, not misleading manner.

### WOMAC algofunctional index

One of the most frequently used algofunctional indexes for gonarthrosis is the WOMAC index (Western Ontario and MacMaster Universities Osteoarthritis Index). The WOMAC index consists of three parts [15]:

WOMAC-A: 5 questions concerning various types of pain

WOMAC-B: 2 questions concerning the stiffness of knee joints

WOMAC-C: 17 questions concerning the activities of daily living

The WOMAC questionnaire evaluates pain, stiffness, functional disorders and summary index of algofunctional disability. Quantitative assessment of answers is divided into categorical, which is determined using a Likert scale, and continuous, which is determined using an analogue scale. WOMAC is the most frequently used tool for the assessment of algofunctional disability in gonarthrosis in Czech clinical studies [15].

# Guide for Authors

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## PREPARATION OF MANUSCRIPTS

Manuscripts must be written in clear, concise, grammatical English. Authors unfamiliar with English usage are encouraged to seek the help of English-speaking persons in preparing their manuscripts. Manuscripts should be double-spaced.

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The title page (page 1) should include:

- A concise and informative title (capital bold font; not exceeding 120 characters)
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- The name of the corresponding author, with complete address, e-mail address, telephone and fax numbers

## ABSTRACT

Each paper must be preceded by an abstract (page 2) that summarizes in no more than 250 words a brief introduction, the aim of the study, materials and methods; main results and conclusions. It shouldn't contain any reference.

## KEYWORDS

After the abstract, in the same page, a list of 4-6 keywords should be supplied for indexing purposes.

## INTRODUCTION

The introduction should describe the state of the art, give a short review of pertinent literature, state the purpose of the investigation. It should be as concise as possible, without subheadings.

## MATERIALS AND METHODS

The “materials and methods” section should follow the introduction and should provide enough information to enable the experiments to be reproduced.

Patients (clinical studies): typology of patients (age, sex,...), criteria for enrolment in the study, etc.

Experimental model: cellular, animal, etc.

Instruments: laboratory instruments used for the research.

Methodology: protocols and evaluation mode.

"In the case that laser sources are considered, authors are requested to specify all the necessary technical data pertinent to the experiment(s): laser type and wavelength, emission mode (continuous, pulsed), laser power (peak and average power in case of pulsed emission), laser beam dimensions, beam intensity (Watt/cm<sup>2</sup> spot area), total energy dose on the irradiated area in a single treatment (J/cm<sup>2</sup>), duty cycle. In case of laser treatment of cultured cell models, as well as in vivo and ex vivo treatments, authors are requested to specify the dimensions of the treated region, treatment duration and timing modalities (e.g. one session, multiple sessions)."

Data analysis: data-analysis method, statistical analysis.

## RESULTS

This section should describe the outcome of the study without any comment. Data should be presented as concisely and clear as possible.

## DISCUSSION

The discussion should be an interpretation of the results and their significance, also with reference to works by other authors. The relevance of the results in the research and clinical applications should be explained.

## CONCLUSIONS

They should be concise and effective, with reference to possible involvements in the future.

## ACKNOWLEDGEMENTS

Concise acknowledgements may be addressed to persons, public and private organizations, companies.

## REFERENCES

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1. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. *Nature*, 2003, 423: 337-342.

Reference to a book:

2. Michaeli W. Extrusion Dies. Hanser Publishers, Munich, Vienna, New York, 1984.

Reference to a chapter in an edited book:

3. Gmünder FK, Cogoli A. Effect of space flight on lymphocyte function and immunity. In: Fregly MJ, Blatteis CM, eds. *Handbook of Physiology*. Oxford:University Press, 1996, vol. 2, pp 799-813.

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