Objective: to evaluate the effects of low-intensity laser AsGa (904 nm) and microcurrent in the healing process of cutaneous wounds in rats. Method: forty-eight female Wistar rats were submitted to surgery to make a cutaneous wound on the back with a metallic punch of 2.13 cm² of area. They were divided into 4 groups: Laser Group (treatment with AsGa low intensity laser from 904 nm to 4 J/cm²), Micro Group (treatment with microcurrent at 80 µA for 12 minutes), Laser + Micro Group (treatment with the same parameters of the Laser and Micro groups), and Control Group (without treatment), and received daily treatment for 21 consecutive days. Results: Laser Group animals presented better wound healing (0.0056 cm²), followed by Laser + Micro (0.0084 cm²), Micro (0.0109 cm²) and Control (0.0197 cm²) groups. Conclusion: laser treatment was more efficient in the healing of cutaneous wounds in rats. Descritores: Lasers; Wound Healing; Electric Stimulation, Transcutaneous.

RESUMO
Objetivo: avaliar os efeitos do laser de baixa intensidade AsGa (904 nm) e da microcorrente no processo de cicatrização de feridas cutâneas em ratos. Método: quarenta e oito ratos Wistar, fêmeas, foram submetidos à cirurgia para confecção de ferida cutânea no dorso com um punho metálico de 2,13 cm² de área. Logo após, foram divididos em quatro grupos: Grupo Laser (tratamento com laser de baixa intensidade AsGa de 904 nm a 4 J/cm²), Grupo Micro (tratamento com microcorrente a 80 µA por 12 minutos), Grupo Laser+Micro (tratamento com os mesmos parâmetros dos grupos Laser e Micro) e Grupo Controle (sem tratamento), e receberam tratamento diário por 21 dias consecutivos. Resultados: os animais do Grupo Laser apresentaram melhor cicatrização da ferida (0,0056 cm²), seguidos pelos grupos Laser+Micro (0,0084 cm²), Micro (0,0109 cm²) e Controle (0,0197 cm²). Conclusão: o tratamento com laser foi mais eficiente na cicatrização de feridas cutâneas em ratos. Descritores: Laser; Cicatrização; Estimulação Elétrica Transcutânea.

ABSTRACT
Objective: to evaluate the effects of low-intensity laser AsGa (904 nm) and microcurrent in the healing process of cutaneous wounds in rats. Method: forty-eight female Wistar rats were submitted to surgery to make a cutaneous wound on the back with a metallic punch of 2.13 cm² of area. They were divided into 4 groups: Laser Group (treatment with AsGa low intensity laser from 904 nm to 4 J/cm²), Micro Group (treatment with microcurrent at 80 µA for 12 minutes), Laser + Micro Group (treatment with the same parameters of the Laser and Micro groups), and Control Group (without treatment), and received daily treatment for 21 consecutive days. Results: Laser Group animals presented better wound healing (0.0056 cm²), followed by Laser + Micro (0.0084 cm²), Micro (0.0109 cm²) and Control (0.0197 cm²) groups. Conclusion: laser treatment was more efficient in the healing of cutaneous wounds in rats. Descritores: Lasers; Wound Healing; Electric Stimulation, Transcutaneous.
INTRODUCTION

The professional who provides assistance to wounded patients performs an extremely important activity, exercises a holistic approach to the patient, follows the evolution of the lesion, guides and dresses the wound, with a technical domain.1 2 The treatment of wounds is dynamic and depends on the each moment of the evolution of the healing phases. There are numerous options for coverages and adjuvant treatments available in the market, such as coverage with new technologies, herbal treatment, adjuvant treatment through a hyperbaric chamber, laser, ultrasound and fadioic current, among others.3

The financial resources of the patient or health unit, the need for continued use of the dressing, including home care, and the evaluation of benefits and costs are some of the aspects to be considered when choosing the type of treatment and coverage, which should be appropriate to the nature, location and size of the lesion. Although there is a wide variety of adjuvant treatment and coverage, only one type of coverage does not meet the requirements for all types of skin wounds, and adjuvant treatment is often required.4 7

Studies on the use of low-power laser therapy have opened a new frontier for the clinical treatment of many disorders, which began to be used in the process of regeneration and functional recovery of peripheral nerve lesions in the 1970s. There were several reports and disagreements about the results obtained.5 7

The microcurrent is a non-invasive therapy modality that uses low amperage current of the order of microamperes (μA), with alternating positive and negative polarity every three seconds. Its therapeutic effects are related to increased cellular metabolism, stimulation of tissue repair and regeneration, normalization of local pH, and increased synthesis of proteins (collagen and elastin), promoting the revitalization and rejuvenation of the skin.8 The effects of stimulation by microcurrent also include increased O2 uptake, increased amino acid transport, and increased membrane transport.7 8 Microcurrent accelerates the production of adenosine triphosphate (ATP) by up to 500%, which is responsible for protein synthesis and tissue regeneration due to its participation in all the energetic processes of the cell.7 8

Microcurrent electrical nerve stimulation (MENS) is indicated for wound healing, postoperative, skin rejuvenation, tissue flaccidity, burn recovery, cutaneous innovation (inducing increase in the number of fibroblasts and alignment of collagen fibers, potentiating the lymphatic circulation, reducing edema), treatment of stretch marks and elimination of cellular metabolic.9

The application of this technique can be performed either manually or automatically. In manual application, the professional slowly moves two previously wetted styli electrodes. It is best suited for people who have more time and who need special attention, for example, people in the stress phase. The automatic application consists of the placement of fixed electrodes at predetermined points of the body surface, with consequent choice of a program more suitable for the case to be treated. Because it was a faster therapeutic treatment, the automatic mode was chosen for the accomplishment of this study.

OBJECTIVE

- To evaluate the effects of low-intensity AsGa laser of 904 nm and of the microcurrent treatment in the healing process of cutaneous wounds in rats.

METHOD

This is a quantitative, longitudinal, prospective, descriptive, analytical, experimental, interventional and controlled study carried out at the José Manoel Lopes Experimental Surgery Center, the University of Vale do Sapucaí (UNIVAS), Pouso Alegre, MG. The study was approved by the Ethical Commission on Animal Use (CEUA) of the University of Vale do Sapucaí (Protocol Number 208/14).

There were 48 Wistar rats participating (Rattus norvegicus Albinus, Rodentia Mammalia), female, aged 2 to 3 months, eutrophic, weighing between 200 and 300 grams and without malformation, from the vivarium and Experimental Surgery Center José Manoel Lopes of UNIVAS. The study had the criterion of not including male rats or weighing less than 200 grams and greater than 300 grams.

The rats were divided into four groups of 12 animals each: Laser Group (low-intensity laser therapy with 904 nm AsGa laser), Micro Group (electrotherapy with 80 μA microcurrent), Laser + Micro Group (low-intensity laser therapy associated with electrotherapy with microcurrent) and Control Group (carbopol gel).

Before the experiment, the animals were housed for 10 days in polypropylene cages,
containing one animal per cage, with light and dark cycle of 12 hours in a room with exhaustion control, with a standard feed of the vivarium and water ad libitum. During the experiment, the animals remained under these same conditions.

For the surgical procedure of wound production, the animals received general anesthesia with intramuscular injection ketamine hydrochloride (85 mg/kg body weight) associated with xylazine hydrochloride (2.0 mg/kg body weight).

After the anesthesia, the epilation was done by digital traction of an area of 4 x 6 cm with its center in the dorsal median line initiated in the inferior margin of the scapula. Then, epileptic area antisepsis was performed with 2% chlorhexidine gluconate solution. The area of skin excision for wound production in the transverse plane was demarcated, with its center in the dorsal midline following caudally, starting at 1.0 cm from the lower margin of the scapula, by a surgical excision of the skin in shape circular of 2 cm² of area, with the aid of a punch of 2.13 cm² of area. Skin existence was then performed to the muscular fascia, with the number 15 scalpel. After surgery, the animals received 0.1 mg for each gram of paracetamol weight (200 mg/ml) orally. For postoperative analgesia, this drug was diluted in 250 mL of water in the dose for 24 hours and administered to the animals next to ad libitum hydration. Treatment was then initiated in each study group according to the proposed objective.

The treatment was applied daily, always at the same time, for 21 days in a row. The wounds were treated as described below:

Before the treatment, the wound was sanitized with 20 mL of 0.9% saline heated at 36.5°C by syringe with a continuous stream directly onto the surface and dried with a sterile gauze folded into four parts.

Laser therapy of the lesions was performed with a low-intensity laser of gallium arsenide (AsGa) of 904 nm (red), with a power of 30 mW, at a dose of 4 J/cm². The laser was applied in a punctual way, at 4 points on the margin of the wound previously marked, distant from each other by 1 cm. Each demarcated point received a Laser application.

The device used for microcurrent electrotherapy was Neurodyn Multicorrentes (IBRAMED, São Paulo, SP, Brazil). Its application was performed daily for 21 days, always at the same time, in automatic application mode, with the intensity of 80 μA, frequency of 60 Hz, for 12 minutes. For the application, two silicone electrodes were used for individual use, cut in the size of 300x150 mm, which was coupled to the caudal and cranial margin of the wound. Carbopol gel was used as the transmission medium for the electric current.

After treatment of each group, the wound was covered with a 100% carbopol gel layer to 100%; the adhesive film was used on the gel (Opsite Flexifix, Smith & Nephew Trade in Medical Products, Diadema, SP, Brazil), extended in the region where the trichotomy was performed.

The 4x6 cm epilated area received a new trichotomy every 5 days, with the exception of a 0.5 cm band around the wound.

Data were collected by planimetry of the wound area. The first evaluation occurred shortly after the surgical procedure on day 0 and the remainder on days 3, 6, 9, 12, 15, 18 and 21. The measurement of the wound area was measured by a perimeter decal, two sterile polypropylene films placed so as to overlap the wound. By a fine-tipped brush and indelible ink, the contour of the wound was made, following its margins. Soon after, the film that came in contact with the wound was discarded and the other was scanned and used to measure the area through the AutoCAD program.

On day 21, after resection of the skin for histological examination, the animals were sacrificed by injection of 10% potassium chloride into the mediastinum. The bodies of the rats were discarded in an infectious trash bag, sent to the infectious garbage shelter and stored in a refrigerator until collection.

The data were recorded in SPSS software for Windows, version 15.0, which compared the evolution of the wound areas in the seven moments, among all the groups, studied. The Kruskal-Wallis test was used to compare the four groups. To compare two or three groups, the Mann-Whitney test was used to evaluate the percentage of healing of each wound, the percentage Delta test (△%), and the Chi-Square calculation was applied to conduct an adjustment quality test to show whether there is, in fact, a statistically significant difference between the percentages of wound areas and whether the sample is suitable for the results obtained. For all analyses, the significance level of P <0.05 was considered.

RESULTS

By the evaluation of the planimetry, it was observed that, of all the groups, the animals submitted to the laser therapy treatment...
presented the greatest regression of the wounds.

When laser microcurrent treatments were associated, it was possible to observe that this one obtained an inferior result to the laser and superior to the microcurrent.

For all four treatments, a value of $p<0.05$ was obtained, which leads to rejection of the null hypothesis that the percentages do not present statistical difference.

From the Chi-square test of adjustment adequacy, it was concluded that the percentages are in fact different for each step of the treatments, according to Table 1.

Comparing the area of the wounds between the four groups, they did not present significant differences when to the size of the wound made. Regarding the wounds regression, the groups showed statistically significant differences between the means of the wound sites from day 15 of observation, with the laser treatment presenting better results, observed through the lower mean values of the areas at different moments, and following this tendency until the last day of observations (Table 2).

### Table 1. Mean, median, standard deviation and percentage delta of the wound areas for the different groups at different times. Pouso Alegre (MG), 2014, Brazil.

<table>
<thead>
<tr>
<th>Days</th>
<th>Micro Group (n=12)</th>
<th>Laser Group (n=12)</th>
<th>Micro+Laser Group (n=12)</th>
<th>Control Group (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (cm$^2$)</td>
<td>Median (cm$^2$)</td>
<td>SD (cm$^2$)</td>
<td>SD (cm$^2$)</td>
</tr>
<tr>
<td>3</td>
<td>2.044</td>
<td>2.009</td>
<td>0.345</td>
<td>3.057</td>
</tr>
<tr>
<td>6</td>
<td>1.653</td>
<td>1.618</td>
<td>0.217</td>
<td>1.36</td>
</tr>
<tr>
<td>9</td>
<td>0.892</td>
<td>0.848</td>
<td>0.379</td>
<td>0.619</td>
</tr>
<tr>
<td>12</td>
<td>0.413</td>
<td>0.319</td>
<td>0.238</td>
<td>0.157</td>
</tr>
<tr>
<td>15</td>
<td>0.203</td>
<td>0.159</td>
<td>0.144</td>
<td>0.076</td>
</tr>
<tr>
<td>18</td>
<td>0.094</td>
<td>0.093</td>
<td>0.034</td>
<td>0.078</td>
</tr>
<tr>
<td>21</td>
<td>0.01</td>
<td>0.009</td>
<td>0.003</td>
<td>0.019</td>
</tr>
<tr>
<td>$\chi^2$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$p$-value</td>
<td>&lt; 0.0001*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$\chi^2 = $ Chi-square test. * $p < 0.05$. SD = Standard Deviation; $\Delta% =$ Percentage delta.
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Table 2. Mean and Δ% Delta wound areas for different groups at different times. Pouso Alegre (MG), 2014, Brazil.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Micro (n=12)</th>
<th>Laser (n=12)</th>
<th>Laser+Micro (n=12)</th>
<th>Control (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days</td>
<td>Mean (cm²)</td>
<td>Δ%</td>
<td>Mean (cm²)</td>
<td>Δ%</td>
</tr>
<tr>
<td>3</td>
<td>2.044</td>
<td>20.232</td>
<td>2.165</td>
<td>2.040</td>
</tr>
<tr>
<td>6</td>
<td>1.653</td>
<td>35.399</td>
<td>1.552</td>
<td>43.217</td>
</tr>
<tr>
<td>9</td>
<td>0.892</td>
<td>64.407</td>
<td>0.867</td>
<td>66.602</td>
</tr>
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<td>0.413</td>
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<tr>
<td>18</td>
<td>0.094</td>
<td>96.159</td>
<td>0.040</td>
<td>98.360</td>
</tr>
<tr>
<td>21</td>
<td>0.010</td>
<td>99.568</td>
<td>0.005</td>
<td>99.781</td>
</tr>
</tbody>
</table>

|                      | Mean (cm²)   | Δ%           | Mean (cm²)         | Δ%            |
| 3                    | 36.383       | 2.036        | 34.844             | 0.877         |
| 6                    | 46.570       | 1.390        | 55.287             | 0.254         |
| 9                    | 77.803       | 0.573        | 81.389             | 0.030*        |
| 12                   | 90.939       | 0.302        | 90.116             | 0.080         |
| 15                   | 96.158       | 0.157        | 94.791             | 0.021*        |
| 18                   | 98.242       | 0.076        | 97.467             | 0.0001*       |
| 21                   | 99.720       | 0.019        | 99.357             | 0.0001*       |

*Kruskal-Wallis Test. *P < 0.05. Δ% = Percentage delta.

DISCUSSION

The use of low-power laser therapy in wound treatment as a therapeutic technology has shown significant growth. The curative properties of laser radiation together with the safety of treatment, seem to be the main factors responsible for this growth, justifying the interest of biomedical researchers to investigate the mechanisms of action and the therapeutic effects of low power laser.\textsuperscript{10,11}

Laser therapy has an effect on the process of epithelialization of the lesion, by reducing the healing time. This response enables the individual to return to his or her routine activities faster. However, some mechanisms involved in this response remain obscure, especially regarding the effects of laser on the mitochondrial respiratory chain and oxidative stress biomarkers.\textsuperscript{1,8} Several studies have shown that the use of low-power laser in the treatment of wound has an anti-inflammatory effect, promoting the reduction of pain and acceleration of cell proliferation and healing process, depending on the wavelength, dose, and local condition. The low-power laser has the ability to penetrate the tissues, acting on the synthesis of collagen, stimulating the local circulation, influencing the proliferation of fibroblasts, osteoblasts, and epithelial cells, and increasing the mitotic activity of epithelial cells.\textsuperscript{10,15}

When comparing the effects of low-intensity laser and microcurrent, this study showed efficacy in the treatment of cutaneous wounds in rats. However, there was a difference between the groups, and the group that received laser therapy presented better results, followed by the laser therapy group associated with the microcurrent and the group treated by microcurrent. All study groups obtained better results than the Control Group.

Authors investigated the effects of different types of low-intensity laser therapy on cutaneous flaps of rats, noting that the application of the low-intensity laser presented a significant result in the healing of these wounds.\textsuperscript{16-17}

A study in which the authors investigated the healing of epithelial tissue in rats treated with low-intensity laser therapy for 21 days, with evaluation at treatment days 7, 14 and 21, showed that after 7 days of treatment, there was little difference compared to the control group.\textsuperscript{18} On evaluation after 14 and 21 days of treatment, the difference was significant, evidencing the efficacy of the group treated with low intensity laser.\textsuperscript{19}

In this study, the low-intensity laser of 904 nm was used at a dose of 4 J/cm², and significant results were identified when compared to the other groups. There was a significant difference from day 9 to day 21 of treatment.

A study was conducted to evaluate the effect of the 904 nm low-intensity laser on surgical wound healing in rats and concluded that the treatment reduced the intensity of the inflammatory reaction and influenced the dynamics of the immune response, inducing a change in the leukocyte infiltration pattern.\textsuperscript{20}

In another study, the authors compared the use of microcurrent in isolated and associated with Jatropha curcas L. in rats and observed, through histological analysis, that the groups treated with microcurrent (10 μA for 2 minutes) and pinion oil obtained better results than the control group and the group that received only Jatropha oil.\textsuperscript{21}

The microcurrent was used in another study in an isolated and combined form with Aloe Vera gel in wounds produced in rats and the groups treated with microcurrent, isolated or associated with Aloe Vera, showed early onset of the proliferative phase.\textsuperscript{22}

Studies using microcurrent in the tissue healing of third-degree burns in rats showed faster, more orderly healing and a better inflammatory response compared to the control group.\textsuperscript{23}

A study using laser and microcurrent in the healing of thermal wounds in rats, isolated and associated, showed positive and similar
results in the therapies when used alone, but did not obtain the same results when associated.²⁴

The several studies suggest that although the laser and the microcurrent separately are beneficial for tissue healing, combining these treatments with other agents seems to decrease the therapeutic action of the same. The results recommend attention during wound treatment and indicate that the therapeutic use of these modalities independently may be the best course of action.

The mechanisms of cellular and biophysical action associated with the combined use of therapeutic resources deserve further investigation to obtain a complete understanding of the phenomena analyzed.

CONCLUSION

The low-intensity AsGa laser treatment at 904 nm was more efficient in the healing of cutaneous wounds in rats compared to the treatment with microcurrent at 80 μA, either alone or in combination with laser therapy. All study groups presented better results than the Control Group.

REFERENCES


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