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# Buriti oil associated with de use of therapeutic ultrasound on the regenerative process of peripheral nerve injury in rats

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## Abstract

Peripheral nerve lesions are a common cause of disability and may result in loss of motor and / or sensory functions, representing a significant clinical problem with unsatisfactory treatment options. A widely studied alternative in the context of tissue regeneration are the essential oils. The objective of this study was to evaluate the efficacy of Buriti oil associated with therapeutic ultrasound in the functional recovery of rats. There were 18 male Wistar rats (250 and 300g), divided into three groups: control, submitted to ultrasound and another subjected to ultrasound associated with Buriti oil. Subsequently, the right sciatic nerve of rats suffered a controlled injury by strangling a portable device with a dead weight of 5,000g / 10 minutes. Samples were collected before treatment (control) and 24 h, 7, 14 and 21 days after sciatic nerve compression. These tests consisted of filming the gait of each animal on the bottom of the glass walkway and analyzed the IMAGE-J® program. Measurements were the lengths of the legs (left back), and the distance between the toes. It has been shown that the combination of Buriti oil with ultrasound was more effective in neuromotor regeneration of rats after axonomy compared to single ultrasound therapy.

**Keywords:** Therapeutic ultrasound. Buriti Oil. Peripheral nerve injury.

# Óleo de buriti associado ao uso de ultrassom terapêutico em processo regenerativo de injúria em nervo periférico de ratos

## Resumo

As lesões nervosas periféricas são uma causa comum de incapacidade, podendo resultar em perda de funções motoras e/ou sensoriais, representando um problema clínico significativo com opções de tratamento insatisfatórias. Uma alternativa amplamente estudada no contexto da regeneração de tecidos são os óleos essenciais. O objetivo deste estudo foi avaliar a eficácia do óleo de Buriti associado ao ultra-som terapêutico na recuperação funcional de ratos. Foram 18

ratos Wistar machos (250 e 300g), divididos em três grupos: controle, submetidos a ultra-som e outro submetido a ultra-som associado ao óleo de Buriti. Posteriormente, o nervo ciático direito de ratos sofreu uma lesão controlada estrangulando um dispositivo portátil com um peso morto de 5.000g/10 minutos. As amostras foram coletadas antes do tratamento (controle) e 24 h, 7, 14 e 21 dias após a compressão do nervo ciático. Estes testes consistiram em filmar a marcha de cada animal no fundo da passarela de vidro e analisaram o programa IMAGE-J®. As medidas foram os comprimentos das pernas (costas esquerda), e a distância entre os dedos. Demonstrou-se que a combinação de óleo de Buriti com ultra-som foi mais eficaz na regeneração neuromotora de ratos após axonomose em comparação com a terapia de ultra-som sozinha.

**Palavras-chave:** Ultrassom. Óleo de Buriti. Lesão de nervo periférico.

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## INTRODUCTION

The peripheral nerve lesions are a major health problem, originated from several causes, such as accidents caused by motor vehicles, penetrating trauma by firearm or bladed weapon and stretch or crush after falling. Approximately 1% to 2% of patients treated at first aid centers have this type of injury which is one of the most significant forms of morbidity and disability currently[1].

After the peripheral nerve lesion, starts a series of degenerative events, with the fragmentation of the axon and the myelin sheath. Hours following, starts the regeneration process[2].

The recovery of peripheral nerves, even after applying reconstruction techniques, it is usually not complete, depending on factors such as the nature of the injury and the level of injury, denervation time, type and diameter of the affected fibers, the age of the affected individual and other individual variables[3].

Most knowledge about regeneration of peripheral nerves and functional recovery was generated through experimental studies, in which the variables are controlled to ensure the reliability of the results, so as to analyze the efficacy of new therapeutic approaches. Under experimental conditions, the recovery of peripheral nerve lesions is mainly studied by electrophysiological techniques, histology and morphometry. Although electrophysiological

and morphological parameters are useful, it is important to know the degree of functional recovery they imply[4].

Among the therapeutic approaches analyzed can highlight the use of ultrasound, phototherapy, treadmill exercise, electrical stimulation of low frequency, among others[1].

A little approach used for functional recovery of peripheral nerve injuries is the use of oils, specifically in the present work, the Buriti oil associated with phonophoresis, which, through its healing properties and stimulating tissue repair, we intend to demonstrate their efficiency optimization of injury recovery in peripheral nerves[5]. To this end, this research has the general objective to glimpse the positive effects of Buriti oil use associated with therapeutic ultrasound in the process of functional recovery of rats gait after peripheral nerve injury to the sciatic nerve. For which use are the specific objectives: define the peripheral nerve injury, characterize the Sciatic Functional Index (SFI) and analyze the gait of rat, define the mechanism of therapeutic ultrasound and phonophoresis and show the characteristics and properties of Buriti oil.

## MATERIALS AND METHODS

### Ethical aspects and animals

The research project was approved by the Ethics Committee on Animal Use (CEUA / FACID) number 080-14, based on Resolution of Law No. 11,798 of October 2008. The experiment was conducted with 18 adult animals *Rattus norvegicus* species *Wistar* variety, weighing between 250 and 300 grams, clinically healthy, obtained in the vivarium of the Faculty Integral Differential – FACID/DeVry. During the experimental period, the animals were housed in collective cages with six animals each, with free access to food and water, being kept for 48 hours in the new environment for acclimatization before the surgical procedure. Specimens were randomly distributed in three experimental groups of six per group, each group corresponding to a different evaluation of treatment, as follows:

- Control group (CG): containing six rats with injury resulting from the sciatic nerve crush without the use of any therapeutic resource.
- Ultrasound group (UG): formed by six rats with lesions of the sciatic nerve crush and treated with therapeutic ultrasound gel and water based.

- Buriti oil and therapeutic ultrasound group (BUG): formed by six rats with lesions of the sciatic nerve crush and treated with therapeutic ultrasound gel and burity oil base.

**Obtaining the vegetable material and extraction of Buriti oil**

The fruits of Buriti (*Mauritia flexuosa L.*), used for oil extraction, were collected after natural fall in the municipality of Santa Filomena, Piaui, being washed in running water, depulped manually and baked at a temperature of approximately 60°C to 80°C. After this process the supernatant was collected and baked again for complete dehydration. When cold, the oil obtained was packed to an amber container with a lid and labeled.

**Surgical Procedure**

The animals were anesthetized with a mixture of 10% Ketamine (0.1 ml / 100 g body weight) and 2% Xylazine (0.07 ml / 100 g body weight) given intraperitoneally. The lateral side of the thigh was routinely prepared For operation (tricotomy and antisepsis with 20% alcohol-iodine solution). The right sciatic nerve was approached through a straight cutaneous cutaneous incision on the side of the thigh and exposed to its total length, from the emergency under the maximal gluteus muscle to its trifurcation at the knee level. For crushing the sciatic nerve, you are using a portable device with a deadweight load of 5 kg. This device is characterized by being a faster, easier and more reliable milling process than the load used. The apparatus consists of an animal support platform with a main body receiving the animal support bracket, a nerve support base, a pressure application shaft, a lever for driving the weight and positioning the nerve at the base of And a spring to keep the Lever in balance. The load was applied for 10 minutes. After the lesion was produced, the animal was removed from the crushing device, the nerve placed in its anatomic bed and the surgical wound was sutured.

**Data Collection**

Soon after the production of nerve injury, the animals of the three groups were subjected to test for the evaluation of motor function. This constituted to examine the opening of the toes of the hind legs during walking by a glass walkway, which was filmed using a digital camera for later analysis of the footprints by the SFI, using the software Image J® to quantify the SFI parameters in five stages: preoperative period, 1 day postoperative (PO) without having been done any treatment in groups, 7th postoperative day after being subjected to five treatment

sessions, on the 14th postoperative day, after being subjected to ten sessions treatment and on the 21st postoperative day, after being subjected to 15 treatment sessions. The SFI was calculated by measuring the length parameter of footprint (PL, print length), full opening of the fingers (TS, toe spread, transverse distance between the 1st and 5th fingers) and opening of the intermediate fingers (IT, intermediate toes) transverse distance between the 2nd and 4th fingers. The reference points of each parameter were marked with the mouse, one parameter at a time, and the SFI was automatically calculated by Exel program. The data obtained by recording the footprints were placed in the following equation:

EPL = footprint length of the experimental paw;

NPL = length of the normal paw footprint;

ETS = distance between the first and the fifth toe of the experimental paw;

NTS = distance between first and fifth toe of the normal paw;

EIT = distance between the second and the fourth toe of the experimental paw;

NIT = distance between the second and the fourth toe of the normal paw.

For therapy, the UG animals were subjected to sessions with UST associated with water-based gel. Ultrasound used was a Sonopulse of Special Ibramed® regulated in pulsed mode with frequency of 1MHz, intensity 0.5W/cm, transmission frequency of 100 Hz pulses, with free cycle of 50% and ERA 1cm$^2$ by direct coupling method, with circulatory movement, for two minutes, once a day, and five days a week. The BUG animals were submitted to ultrasound sessions with the same mark and the same parameters UG applied with gel Buriti oil base.

**Statistical Analysis**

The SFI data were subjected to statistical tests by analysis of variance (ANOVA), considering significant results with P less than 0.05. The results were expressed by mean and standard deviations between the control group, treated with ultrasound and treated with ultrasound and Buriti oil in the preoperative period, immediate postoperative and 7, 14 and 21 days postoperatively.

**RESULTS AND DISCUSSION**

In order to analyze the Buriti oil action in recovery to neural function of the peripheral nerve by traumatic injury, we evaluated the role of gait after injury of the sciatic nerve crush in laboratory animals, it is important to better understand the pathophysiology of this condition.

The parameters of the SFI gait function are important as they check the effectiveness of Buriti in peripheral neural recovery because their values reflect well the level of functionality and nerve integrity.

The data obtained through the gait analysis were statistically analyzed by arithmetic mean and standard deviation. Evaluation of SFI showed loss of motor function close to -100 in the three groups after completion of the injury, which made that there were major changes in the rats gait pattern. The animals are not supported in the operated leg, with the foot drop and complete adduction of the fingers as a result of severe crush injury. The record of footprints was performed in different periods, preoperative, 7, 14 and 21 days postoperatively. The SFI mean values and standard deviations of the groups are described in Table 1.

**Table 01:** Mean values of the Sciatic Functional Index (SFI) of the evaluated groups.

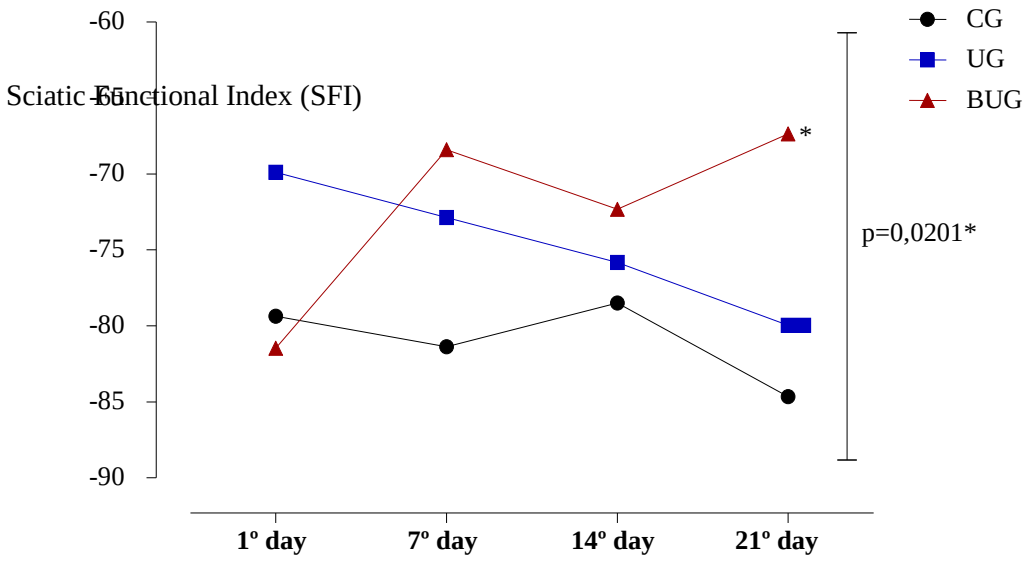
| VARIABLE | CG | UG | BUG |
|---|---|---|---|
| 1st day | - 79,36±3,66 | - 66,56±4,02 | - 81,47±2,02 |
| 7th day | - 81,37±3,81 | - 72,87±3,79 | - 68,40±5,34 |
| 14th day | - 78,49±4,92 | - 75,82±2,43 | - 72,32±6,32 |
| 21th day | - 84,66±3,99 | - 79,95±4,35 | - 64,02±5,49 |

CG: control group, UG: ultrasound group, BUG: ultrasound group with Buriti oil
Source: Research data.

The results obtained with the SFI of the three groups at 7, 14 and 21 postoperative days were respectively: control group -81.37, -78.49, -84.66; ultrasound group -72.87, -75.82, -79.95 and the Buriti oil group -68.40, -72.32, -64.02.

The comparative statistical analysis (ANOVA) of the functional index comparing the three groups through the SFI of the 95% and $p<0.05$ was shown that after 21 days there was statistically significant differences in functional improvement of gait, not being found such a result in the other periods of the experiment, as shown in graph 1, suggesting the effectiveness of Buriti oil association with therapeutic ultrasound regarding the use of ultrasound gel water-based.

Graph **1** Effect of the Buriti oil (*Mauritia flexuosa*) and/with association with ultrasound, on the effect of the sciatic functional index.




Crushing injuries are relatively frequent occurrence, from a clinical point of view, therefore we know the injury in more detail than those currently available in the medical literature[8]. Thus, they are virtually ideal model for experimental use, particularly in research on the use of therapeutic modalities of lesions of the peripheral nerves. Specialized literature is relatively abundant in publications focusing on aspects of the regeneration of peripheral nerves, using models of crushing injury. However there is no a pattern of crushing injury, as each author uses a different technique or equipment, so that it is highly questionable the reproducibility of each method[9].

In the recovery of tissue injury, the inflammatory process is needed for initiating the regeneration process, for promoting the exudation of leukocytes, which destroy devitalized tissue, tissue debris and necrotic tissue. However, intense inflammatory reaction can damage the healing process by promoting edema, excessive amount of exudate[5]. Furthermore, there is the production of free radicals also damage the reparative process to cause damage to cellular components such as proteins and DNA itself[10].

Despite the wide divergence of parameters and no elucidation of the mechanism of action, there seems to be consensus that ultrasound can exert pro-inflammatory action[11]. Thus, the ultrasonic wave acts as a potentiator of the inflammatory response, promoting the release of histamine, macrophages and monocytes, accelerating the normal cellular phase of inflammation.

The increase occurred due to the smooth movement of interstitial fluid that can increase the phagocytosis rate, the movement of particles and cells by increasing the release of chemical

mediators in the inflammatory process such as histamine and serotonin, showing no anti-inflammatory characteristics as usually think[12].

Pereira et al., in an experiment using therapeutic ultrasound did not show a significant improvement in function of the gait rats after 21 days of treatment. This finding was similar to the present study, which was found a significantly relevant difference between the CG and UG as functional recovery gait of animals. These changes corroborate the idea that the lack of significance between the CG and UG may result from this pro-inflammatory mechanism of ultrasound[6].

However, other studies using similar methodology to arrive discordant findings, in which ultrasound was effective in functional recovery of rats gait. Jatte et al. studied the effects of ultrasonic radiation of low intensity applied over the spinal cord in the regeneration of the sciatic nerve of rats for controlled crush injury, concluded that the therapeutic ultrasound of low intensity stimulating nerve regeneration, with significance during the 3rd week of treatment. The result, according to the authors, finds support in the fact that it is in these places that are the cell bodies of motor and sensory neurons[13].

Sganzella affirms in studies that low frequency ultrasound triggers a strong activity of Schwann cells, showing the promotion of peripheral nerve regeneration, obtaining favorable results for the lesion and the application of ultrasound in nerve regeneration and that the application of ultrasound therapeutic for nerve regeneration region concluded that the possible non-thermal effects seen by dilation of blood vessels, new vessel formation, improves local tissue nutrition, stimulating nerve sprouting, increase of the density of nerve fibers, which led to restoration of the tissue neural tube[14].

Monte-Raso et al. affirms that ultrasound accelerates the regeneration of nerve speed after a circumscribed lesion (moderate compression), an effect related to the intensity and the ultrasound application time, and was observed that high intensity, if applied for a prolonged period may have adverse effects[7].

However, this study did not include these results, suggesting that the ultrasound therapy isolated did not promote a significant recovery of the sciatic nerve over the 21 days after injury. Although treatment with ultrasound has not accelerated the repair process of the nerve fiber, you cannot dismiss the possibility that ultrasound has effects after traumatic injuries.

The Buriti oil therapy associated with therapeutic ultrasound showed statistical significance in the functional recovery of gait of rats, as evidenced, however, only on the 21st day of treatment. Already the lack of significance found between CG and BUG until the 14th

day after surgery can be credited to the change process undergone by the axon in the first hours after the injury.

According to Pereira et al., work comparing the use of ultrasound with the laser in the regeneration of the sciatic nerve in rats, found significant improvement in animal gait function from the 14th day of treatment with laser use, without improves equally observed with the use of therapeutic ultrasound at the same time. Say the authors, the cell body at that time starts to present a series of changes called for chromatolysis, characterized histologically by engorgement of cell degeneration of Nissl substance (rough endoplasmic reticulum of the neuron) and migration of the center nucleus to the periphery. These changes are aimed at the production of proteins (actin and tubulin) related to the regeneration of the cytoskeleton of the axon, to the detriment of the neurotransmitter production, being related to intracellular transport and handling of the growth cone at the expense of regenerative process. This result was similar to that found in this study, in which the functional improvement of gait with use of therapeutic ultrasound with Buriti oil is made from the 21st day, it can be inferred that the same reasons[6].

There is strong evidence in the literature showing the anti-inflammatory action and Buriti oil antioxidant characteristics that exert a protective effect on the recovery of motor function and nerve conduction. In studies with the purpose Burity oil (*Mauritia flexuosa L.*) in the healing cutaneous lesions in mice, it evidenced the histological cut of a smaller number of injuries polymorphonuclear cells with decreased inflammatory activity at the site of injury[15].

Studies about the treatment of nerve injuries caused by crushing of the sciatic nerve in rats with the use of other vegetable oils have also been effective in the recovery of motor nerve function and thus animal gait. Jiang et al. observed, after the use of frankincense extract, an extracted gum genre trees Boswellia (family *Burseraceae*) in post-crushing injury to the sciatic nerve in rats, showed a significant improvement in SFI values after seventh day of treatment compared to the control group. In another study, it was observed the effects of Epimedium extract in functional recovery of rats gait, was found significant recovery from gait function in animals treated with the herbal extract[16,17].

The oil extracted from the fruit pulp *M. flexuosa* presents significant amounts of carotenoids, vitamin E (a-tocopherol), unsaturated fatty acids such as oleic and linoleic acid[5]. Bataglion et al in a study of the analysis of Burity oil properties revealed a great antioxidant capacity, indicating that the oil comprises a rich source of compounds with potential for various applications. Such properties indicate their ability to protection and regeneration nervous[10].

The thermal and mechanical effects of therapeutic ultrasound can lead to physical and chemical changes of biological tissues, favoring the penetration of active principles present in the topical use substances. The heating of the area to be treated may increase the absorption of the drug in the tissues because there is an increase in blood flow, dilated hair follicles, reduced skin resistance and increases the kinetic energy of the drug, thus facilitating absorption. The ultrasound, for these effects has the ability to increase the penetration through the skin on average 4 to 5 cm deep[18].

Muniz et al. reported factors inherent to the drug and the mode of application that can influence the results of the research such as biological factors related to the skin, epidermal thickness, weight, age, blood flow, metabolic conditions of the body, hydration and skin application area. Corroborating the above mentioned factors, these have all seen not to influence the results of the research[19].

In a study comparing the use of ultrasound and the use of ultrasound associated with calendula gel in the treatment of experimental muscle injury in rats, it was found that the group that was associated ultrasound to calendula had a significantly lower lesion area, due to their larger amount of fibroblasts, neovascularization and myoblasts in the control group and the group that only used ultrasound[20].

Maia Filho et al. studied action of aloe gel combining the therapeutic ultrasound in action in the acute inflammation process in the paw of rats, found superior action in reducing the inflammatory edema with this therapy than using only ultrasound therapeutic[11].

Thus, it is possible to verify, through studies that ultrasound promoted a potentiation of Buriti oil effects on sciatic nerve injury by increasing its concentration at the site of injury.

However, it is not known exactly which oil component is responsible for optimizing the nerve recovery. It can be inferred that it is a joint action of fatty acids, carotenoids, vitamins present in Buriti oil.

The research goals were achieved, for according to the results demonstrated that the oil Buriti associated with therapeutic ultrasound showed efficacy in functional improvement of gait of the animals, which reflects the degree of sciatic nerve regeneration of rats, seen through the SFI values, which were higher in the group submitted to ultrasound with buriti oil, compared to the control group and the group using ultrasound.

These results indicate the new research with the aim of find new options for the treatment of peripheral nerve injuries.

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