

Proliferative effect of medicinal plants and laser on liver regeneration. A considerable experimental model: from an experimental model to clinical applications¹

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INTRODUCTION

The use of medicinal plants to treat human diseases has been performed for millenniums. Nowadays, it is known that 80% of the world population have already taken medicinal plants and 30% were prescribed by physicians¹. In addition to this, it was estimated by the World Health Organization that 80% of the population of developing countries believe in traditional medicine (which consists of medicinal plants use in 85% of the cases) for primary health assistance². In China, particularly, 30 to 50% of all medicines used are originated from plants³.

In Brazil, there are around 55 thousand species of registered plants; 20% of them are supposed to have medicinal effects^{4,5}. Besides such diversity, there are only few studies describing the mechanisms correlated to their effect. Considering that the study of their effects may lead to the development of new and cheaper kinds of treatment for so many different diseases, we decided to assess the outcomes of the aqueous extract of 2 plants from northeastern Brazil on liver regeneration following 67% partial hepatectomy.

Sida cordifolia L. (Malvaceae) is popularly known as “malva-branca” (white mallow) or “malva-branca-sedosa” (silky white mallow) in Brazil. It grows as a bush of up to 2 m in height. The leaves are light green, cordiform, serrated and oval-elongated, and the pedunculated flowers are arranged in axillary or terminal racemes.

In traditional medicine, the plant is used for the treatment of stomatitis, blenorrhea, asthmatic bronchitis and nasal congestion⁶. In Brazil, it is also used due to its anti-inflammatory properties. Some studies have demonstrated the presence in the leaves of sympathomimetic amines, ephedrine and pseudoephedrine, vasocinone⁷ and

vasicine as major alkaloids⁸. Other studies also showed the presence of fatty oils, steroids, resin, resin acids, mucin and potassium nitrate in the plant⁹. The effectiveness of the extract of *Sida cordifolia* leaves as analgesic, anti-inflammatory (inhibition of prostaglandin synthesis) and hypoglycemic has been reported^{6,9}.

Hyptis pectinata (L.) Poit (Lamiaceae), popularly known in Brazil as “sambacaitá” or “canudinho”, is an herbaceous plant with opposing crossed, whole and aromatic leaves. Its flowers are small, clustered into axillary inflorescences, hermaphrodite, pentamer, strongly zygomorphous and bilabiate.

It is popularly used to treat rhinopharyngitis, nasal congestion, certain skin diseases, gastric disorders, fever and bacterial infections^{10,11}. In the state of Sergipe, it has been recommended for the treatment of inflammation, pain, cancer, bacterial infections and wound healing¹².

The essential oil of the plant contains 33 compounds. Monoterpenes are the most common (95,8%). The main constituents are *p*-cymene, thymol and β -terpinene. Together, they correspond to 68% of the total¹⁰. The presence of thymol is considered the main factor for the antiseptic property of this plant¹¹.

Besides the study of these 2 plants on liver regeneration, we decided to associate laser therapy with *Hyptis pectinata* leaves extract. Low intensity laser therapy has been widely used in all medical fields due to its therapeutic effects on reparative process. After penetration of the laser in the liver, stimulatory or inhibitory effects are common in biostimulation by light depending on the dose or wave length¹³⁻¹⁶. It seems to be more likely that laser target organelle is also the mitochondria^{15,17,18}. Biological responses of cells to laser radiation are caused by physical and chemical changes in photoacceptor molecules,

components of respiratory chains like cytochrome C oxidase and NADH-dehydrogenase¹⁹⁻²¹.

The study of these substances and laser on liver regeneration is extremely important due to the essential function of this organ. So, an accelerated liver regeneration process is required when this organ is submitted to partial hepatectomy in order to resect tumors; to improve its growth after transplantation from little donors; or to restore the hepatic mass following extensive injury. A rat model was chosen due to its similarity to the human body.

THE FACT AND NOT AN EPIPHENOMENON

Well, according to Stephenson²², all drugs exert a dose-dependent effect on their targets. However, this process depends on the amount of available receptors and the physical and chemical properties of the substances. This theory is applied to pharmacology. On the other hand, phytotherapy is not fully understood. It is possible that, at some concentrations, certain compounds of the plant extract could act in synergism and cause a significant effect. At lower or higher concentrations, these compounds could be antagonized by different ones.

Liver regeneration was stimulated by *Hyptis pectinata* at doses of 100 mg/kg. On the other hand, the enhancement effect was not observed by higher concentrations (200 and 400 mg/kg). This might be explained by hepatocellular membrane receptors saturation or a possible blockage of some stage of the metabolic reaction. It may lead to an uncoupled mitochondrial function which is able to cause relative decrease in liver regeneration and function, as verified in the copaiba oleoresin study¹⁴.

1. From several works developed in the Universidade Federal de Sergipe, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo (FMRP-USP) and Instituto de Física de São Carlos, Universidade de São Paulo (UFSC-USP)
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The association between *Hyptis pectinata* extract at 200 mg/kg and laser therapy brought about a significant increase in liver regeneration in comparison to each of them alone or their absence (control).

There are two main alternatives to be considered as a possible explanation for the observed effects. First, the used substance (*Hyptis pectinata*) may have a strong absorption of light. Therefore, it may be excited during irradiation, leading to the formation of more reactive radicals or molecules. These final products could be responsible for enhancing the metabolic activity and hence the final rate of cells regeneration. In this case, we can consider *Hyptis pectinata* as one of the target for laser effects, working as an added chromophors

The second possibility relies on the already observed effect of laser enhancement of hepatic regeneration^{15,23}. It exerts its effect on the natural existent chromophors of the hepatocytes by accelerating the metabolic level and resulting in a high mitotic rate. In this case, the presence of *Hyptis pectinata* may collaborate with the metabolic reaction sequence, arising an even higher level of reaction and, indirectly, causing

the observed enhanced effect. In this situation, the laser target is not the plant compounds but the already existent biomolecules which are potentialised by the association with extra molecules.

At last, *Sida cordifolia* leaves extract caused an important augmentation in liver regeneration at 100 and 200 mg/kg dose level.

In 1999, it was disclosed that the extract of *Sida cordifolia* leaves, which was proved to be anti-inflammatory, analgesic and hypoglycemic, augments insulin release after its administration⁹. The alcoholic extract of the plant, at 150, 300 and 600 mg/kg, was orally administered to Wistar rats. Then it was noticed a significant decrease in glucose levels. These findings suggest that *Sida cordifolia* stimulates insulin release by pancreatic β cells, acting like sulphonylureas⁹.

Knowing that insulin acts as an important co-mitogen^{24,25}, it can be thought that the augmented release of this hormone, stimulated by *Sida cordifolia*, can exert a positive role in the hepatocellular synthesis of DNA and, consequently, in the liver regenerating process at 100 and 200 mg/kg of the plant extract.

transport chain, on the import of substrates into the mitochondrial matrix and on the activities of ATP synthase and adenine nucleotide translocase³⁰. This reduction may be explained by the effect of the inhibitory compounds of the plant extract on the existent chromophors groups in the respiratory chain or on the activity of FoF1-ATPase, as happens with oligomycin. In our studies, the plant, in all concentrations used, caused no effect on mitochondrial oxygen consumption in state 4 respiration. State 4 represents the dissipation of the proton gradient and is in direct relationship with the coupling and phosphorylation process. And besides, it depends on the optimal activity and integrity of the respiratory chain (complexes I, III and IV) which extrudes protons from the mitochondrial matrix towards the intermembrane space. As a consequence, it generates the proton gradient. The plant extract caused a decrease in RCR by depressing state 3, while state 4 was unalterable. This means that the plant may not injure the mitochondrial inner membrane but reduces significantly the oxidative phosphorylation.

In conclusion, we found that *Sida cordifolia*, *Hyptis pectinata* and laser therapy in association cause an outstanding stimulation of liver regeneration. And besides, *Hyptis pectinata*, when studied *in vitro*, does not cause mitochondrial inner membrane injury.

We expect to have established a new perspective on the study of liver regeneration, medicinal plants and laser therapy. If this model could be put into practice in human beings, a new approach to treat liver diseases and to improve hepatic proliferation after injury would be developed. However, further studies are needed in order to determine the exact mechanisms related to our findings. Additionally, it is important to isolate the active compounds responsible for these results.

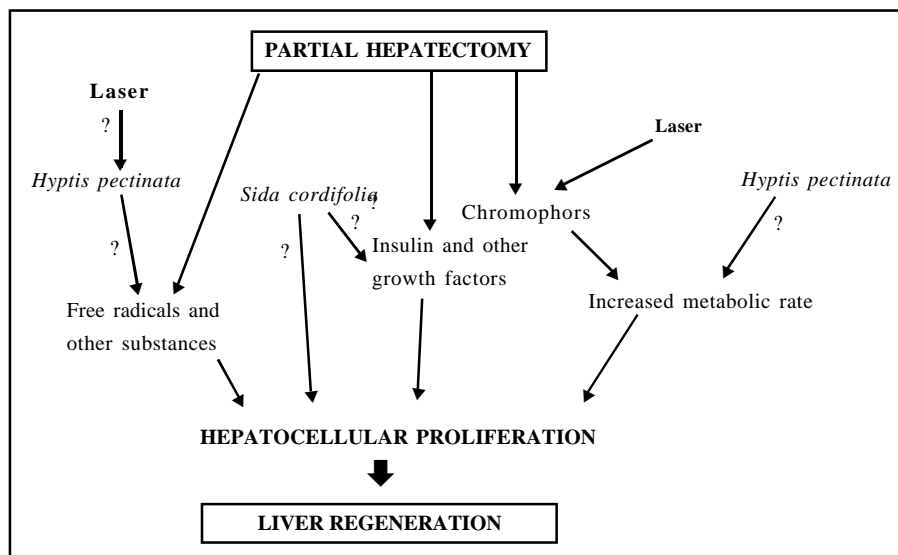


FIGURE 1: Flow chart disclosing possible and probable interactions of the substances involved in liver regeneration.

Concerning the study of *Hyptis pectinata* leaves aqueous extract on liver mitochondrial respiratory, it was found that it caused a statistically significant decrease in state 3 at 0.05, 0.1 and 0.2 mg/mg prot and RCR (respiratory control ratio) at 0.05, 0.1 and 0.2 mg/mg prot. Respiratory state 4 was not altered by the increasing concentrations.

Although the mechanism of biostimulation or inhibition remains to be elucidated, some recent studies indicate that mitochondria may be the target organelle of plants^{26,27}. The critical role played by mitochondria in the maintenance of cellular energy metabolism has long been recognized. The electron transport from the oxidation of NADH and reduced FADH₂ to O₂ is tightly coupled to the synthesis of ATP. This

transport occurs through protein-bound redox centers, from complex I (NADH-coenzyme Q reductase) or II (succinate-coenzyme Q reductase) to III (coenzyme cytochrome e reductase) and then to IV (cytochrome e oxidase). The free energy released by this transport is conserved by pumping out protons in order to create an electrochemical H gradient across the inner mitochondrial membrane. The electrochemical potential of this gradient is then harnessed in the synthesis of ATP by complex V (ATP synthase): this process is known as oxidative phosphorylation^{28,29}.

State 3 of mitochondrial respiration and RCR were reduced as the concentration of *Hyptis pectinata* in respiration media was increased. State 3 is specially dependent on the electron

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4—ARTIGO ORIGINAL

Is CO₂ gas insufflator necessary for laparoscopic training in animals?¹

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Tiraboshi RB, Domingos ALA, Dias-Neto JA, Paschoal RM, Travassos J, Martins ACP, Suaid HJ, Cologna AJ, Tucci Jr S. Is CO₂ gas insufflator necessary for laparoscopic training in animals? *Acta Cir Bras* [serial online] 2003 vol 18 suppl 5. Available in www.scielo.br/acb.

ABSTRACT – Objective – To verify the efficacy and safety of compressed air to produce pneumoperitoneum for laparoscopic surgery in pigs for a training program of residence. **Methods** - Daland pigs weighing 15-17kg underwent general anesthesia and mechanical ventilation. They were divided in 3 groups: A – (38) the pneumoperitoneum was established with an automatic CO₂ insufflator, B – (7) as in A except the CO₂ gas was changed by compressed air, and C – (11) abdomen insufflation was obtained with compressed air directly from hospital pipe network system. Intra-abdominal pressure in all groups was kept between 12 and 15 mmHg. The laparoscopic procedures performed were distributed proportionally among groups: 20 bilateral nephrectomy, 20 dismembered pyeloplasty and 16 partial nephrectomy. Arterial blood sampling for gasometry was obtained before and 2h after establishment of pneumoperitoneum in 5 pigs of group C. **Results** – The cost of 25 4,5kg CO₂ container used in group A was R\$ 3,150.00 (US\$ 1,050.00). The mean length time of surgeries in groups A, B and C were respectively: 181±30min, 196±39min e 210±47min (p>0.05). Respiratory alkalosis occurred in 3 out of 5 pigs of group C. No animal exhibited signs of gas embolism or died during surgery. **Conclusion** – The use of compressed air for laparoscopy in pigs was safe, reduced costs and did not require the use of an automatic gas insufflator.

KEY WORDS – Laparoscopy. Nephrectomy. Pyeloplasty. Partial nephrectomy. Pneumoperitoneum. CO₂ gas. Compressed air.

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