Proliferative effect of aqueous extract of *Hyptis fructicosa* on liver regeneration after partial hepatectomy in rats[1]

Efeito proliferativo do extrato aquoso da *Hyptis fructicosa* na regeneração hepática após hepatectomia parcial em ratos

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ABSTRACT

PURPOSE: To evaluate the effect of aqueous extract of *Hyptis fructicosa* on hepatic regeneration after partial hepatectomy in rats.

METHODS: Sixteen rats were divided in two groups: C (Control Group) and HF (Whose rats received aqueous extract of *Hyptis fructicosa* during 4 days using the dose of 100 mg/kg/day). On the consecutive day of this treatment, the animals of both groups underwent hepatectomy of about 67% of liver. Twenty four hours later, they were sacrificed, and the remaining mass of liver was removed and prepared to be studied through the PCNA immunohistochemical technique.

RESULTS: The liver regeneration index of HF group was 53.56 ± 18.91%, while in C group was 21.12 ± 8.29% (p=0.0003).

CONCLUSION: These results show that the administration of aqueous extract of *Hyptis fructicosa* using the dose of 100mg/kg/day increased the hepatocyte proliferation in the group HF.

Key words: Plants, Medicinal. Hepatectomy. Proliferating Cell Nuclear Antigen. Liver Regeneration. Hyptis. Rats.

RESUMO

OBJETIVO: Avaliar o efeito do extrato aquoso da Hyptis fructicosa sobre a regeneração hepática após hepatectomia parcial em ratos.

MÉTODOS: Dezesseis ratos foram divididos em dois grupos: C (grupo controle) e HF (ratos que receberam o extrato aquoso da Hyptis fructicosa durante quatro dias na dose de 100mg/kg/dia). No dia consecutivo deste tratamento, os animais de ambos os grupos foram submetidos a hepatectomia de aproximadamente 67% do fígado. Vinte e quatro horas depois, eles foram sacrificados, e que a massa restante do fígado foi retirado e preparado para ser estudado através da técnica de imuno-histoquímica PCNA**.**

RESULTADOS: O índice de regeneração hepática do grupo HF foi 53,56 ± 18,91%, enquanto no grupo C foi de 21,12 ± 8,29% (p=0,0003).

CONCLUSÃO: Estes resultados mostram que a administração do extrato aquoso da Hyptis fructicosa na dose de 100mg/kg/dia aumentou a proliferação de hepatócitos no grupo HF.

Descritores: Plantas Medicinais. Hepatectomia. Antígeno Nuclear de Célula em Proliferação. Regeneração Hepática. Hyptis. Ratos.

Introduction

Hyptis fructicosa Salzm ex Benth is a plant from the Lamiaceaea family[1] and is popularly known in Brazil as "Alecrim do Campo" or "Alecrim de Tabuleiro". This specie is about 0.5-1.5 m high and its leaves are aromatic, rhomboid and simple, and its flowers are clustered into axillary inflorescences, hermaphrodite and pentamer. It is popularly used to treat pain. Besides, it has been shown antinociceptive effect in mice[2]. Two quinoids were isolated, from this plant, with antimicrobial and antineoplastic effect against Gram-positive microorganisms and mouse Erlich carcinoma studies, respectively[3].

Studies using different species from the same gender were performed. It has been shown that the *Hyptis suaveolens* has positive effect on healing wounds[4]. The *Hyptis ovalifolia* has presented antimicrobial effect[5]. Also, the *Hyptis pectinata* has enhanced liver regeneration[6-8]. However no article about the effect of *Hyptis fructicosa* on liver regeneration was found.

Considering that there are no studies defining the effects of this plant on liver, this research aimed at assessing the effects of *Hyptis fructicosa* leaves aqueous extract on liver regeneration after 67% partial hepatectomy in rats.

Methods

Hyptis fruticosa (Salzm. ex Benth.) Lamiaceae aerial parts were collected in September 2010, at "Feijão village" (10º56´S, 37º05´W), state of Sergipe, Brazil. The species was identified and authenticated by Dr. Adauto Souza Ribeiro. A voucher specimen of the plant (number ASE 01137) was deposited in the Herbarium of the Universidade Federal de Sergipe (UFS), Brazil. Its leaves were dried in an oven with air renewal and circulation (model MA-037) at 37°C until complete dehydration. Dried *Hyptis fructicosa* leaves were triturated in a blender until a finely granulated powder was obtained. The extract was obtained from this powder (100g) by adding distilled water (3:10, w/v) under constant shaking for 4h at 35°C, followed by filtration (pH 6.0). The filtrate was lyophilized (aqueous extract) and stored at 5°C. At the time of use, the extract was resuspended in distilled water at the desired concentrations.

The study was carried out on 16 male Wistar albino rats (300 to 450g). They were fed with a standard pellet diet and water *ad libitum* and kept at biotherium under natural light-dark cycle and environment temperature and humidity. All rats received humane care and the study protocol complied with the institution guidelines. All animals were randomly assigned to two groups, which consisted of eight rats each: CG, Control Group (n=8), whose rats received water daily for 4 days and HF, whose rats received aqueous extract of *Hyptis fructicosa* (HF group) during 4 days using the dose of 100 mg/kg/day. This dose showed no acute toxicity[2]. On the consecutive day of this treatment, the animals of both groups underwent hepatectomy of about 67% of liver.

Surgical procedure

The operations were always performed at the same time in order to avoid the influence of the changes in circadian cycle. All operations were performed under ether anesthesia. In order to improve anesthesia performance and prevent problems such as air pollution, unstable anesthesia level and anesthetic wastefulness, an ether vapourizator for little rodents was used[9,10]. The median and left lateral lobes of the liver (corresponding to 67% of the organ) were excised[11]. After surgery, the rats were on a normal diet for the whole experimental period. Twenty four hours later, they underwent a new operation to remove the remaining liver. Then they were sacrificed.

Liver regeneration analysis

Liver regeneration was evaluated by immunohistochemical staining for proliferating cell nuclear antigen (PCNA) using monoclonal primary anti-PCNA antibody (PC-10; DAKO A/S, Glostrup, Denmark) on formalin-fixed and paraffin-embedded liver tissues. Sections were cut at 4 μm, mounted on poly- L-lysine-coated glass slides, deparaffinized, rehydrated in an increasing alcohol series, placed in phosphate-buffered saline (PBS), and treated with 2% hydrogen peroxide in methanol for 15 min to block endogenous peroxidase activity. This was followed by incubation with PC-10 monoclonal primary antibody, diluted 1:40 in PBS for 120 min at 25°C. The sections were then incubated for 35 min with a biotinylated horse anti-mouse immunoglobulin. The reaction product was detected with an avidin–biotin–peroxidase complex and diaminobenzidine was used as a chromogen substrate. Positive and negative controls were used to assess and control the staining procedure. Sections were examined blindly at high power (400×), and 20 fields were chosen at random. Nuclear labeling indexes for PCNA (positive nuclei/total number of counted nuclei) were determined by evaluation of at least 1000 hepatocyte nuclei, as previously described[12,13]. The specimens were processed by a pathologist who did not know which group he belonged to the animal.

Statistical analysis of data

All data are expressed as mean ± standard deviation. Statistical comparisons among the groups were performed by

Mann-Whitney test. Probability values lower than 0.05 were considered to be statistically significant.

Ethic

This research was approved by the Ethic Committee in Researches using Animals from Universidade Federal de Sergipe – UFS, with the protocol number 36/2005.

Results

All data presented below were assessed 24h after 67% partial hepatectomy. The plant extract had been administered daily during 4 days, using the dose of 100 mg/kg of *Hyptis fructicosa* leaves aqueous extract, as previously explained.

Table 1 shows the individual results of liver regeneration index by each rat of both groups which was assessed by the proliferating cell nuclear antigen (PCNA) using monoclonal primary anti-PCNA antibody. Figure 1, shows the liver regeneration index in group HF was 53.56% ± 18.91, significantly increased compared to control group that presented index of 21.12 % ± 8.29 (p= 0.0003).

TABLE 1 - Data presented as means and standard deviation (SD). Results of individual liver regeneration index. Liver regeneration was assessed by proliferating cell nuclear antigen (PCNA) 24 hr after partial hepatectomy.

Control Group		HF Group	
Rat 1	11.72	Rat 1	46.69
Rat 2	18.51	Rat 2	41.50
Rat 3	20.41	Rat 3	35.31
Rat 4	23.39	Rat 4	43.01
Rat 5	25.37	Rat 5	83.05
Rat 6	16.15	Rat 6	54.90
Rat 7	14.96	Rat 7	41.39
Rat 8	38.45	Rat 8	82.68
Mean	21.12 ± 8.29	Mean	53.56 ± 18.91

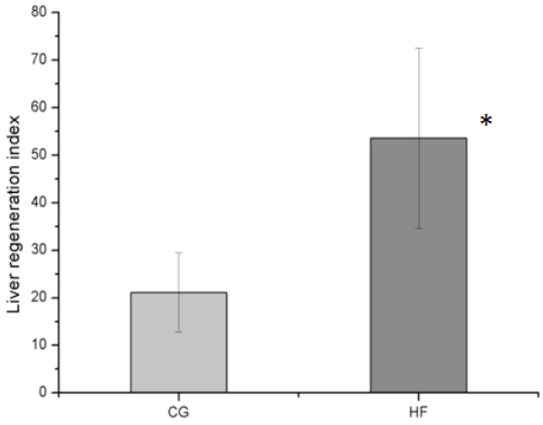

FIGURE 1 - Data presented as means and standard deviation of mean of liver regeneration index. The aqueous extract of *Hyptis fructicosa* (oral administration during 4 days before surgery) caused a significant increase at this parameter. Liver regeneration was assessed by proliferating cell nuclear antigen (PCNA) 24h after partial hepatectomy. Statistical comparisons among the groups were performed by Mann-Whitney test *(p=0.0003).

Discussion

There are several studies intended to comprehend the regeneration mechanism. It is known that some substances affect this process of regeneration. One study showed that the immunosuppressant tacrolimus has stimulatory effect on the process of liver regeneration after partial hepatectomy in rats[14]. An important number of growing factors can stimulate hepatoyte DNA synthesis, on primary culture, as HGF (*hepatocyte growth factor*), TGF-α (*transforming growth factor-α*) and EGF (*epidermal growth factor*). Some hormones participate on the liver regeneration process. The Norepinefrin acts on adrenegic α-1 receptors and amplifies the mitogenic signals of EGF and HGF[15]. The estradiol hexahydrobenzoate also promotes liver regeneration in rats submitted to a 70% hepatectomy[16]. The insulin together with glucagon stimulates the hepatocyte regeneration either *in vitro*[17] or *in vivo*[18,19]. The α2b interferon inhibits the hepatocyte proliferation when administered after 2 or 12h of the hepatectomy[20].

Also, anatomical factors can interfere. For instance, the hepatic branch of left vagus is important for the maintenance of the regenerative ability of liver, and the hepatic vagotomy promotes a delay in DNA synthesis[21,22]. Partial portal vein arterialization (PPVA) is a method of iatrogenic promotion of liver regeneration in the setting of acute liver failure treatment[23]. The partial occlusion of hepatic venous drainage in rats subjected to partial hepatectomy prolongs the proliferation of liver cells when compared to animals with veins of normal caliber[24]. These data shows that liver regeneration is a complex phenomenon affected

by different factors.

In our study the aqueous extract of Hyptis fructicosa augmented the liver regeneration in about 2.5 times compared with the control group. Former studies presented similar results using the extract of another plant, the *Hyptis pectinata*[6-8,25]. As these plants belong to the same gender, it is possible that both plants share factors that can stimulate the liver regeneration, although the mechanism of this effect is yet unknown. As the *Hyptis fucticosa* is a phytotherapic, it may contain different substances which perhaps produce different effects. For instance, studies show that it has either antimicrobial effect against Gram-positive microorganisms and antineoplastic against mouse Erlich carcinoma[26]. Another study showed that *Hyptis fucticosa* has anti-inflammatory and antioxidant[27].

A study presented an antinociceptive effect of this plant[2]. This effect is similar to the effect presented by the *Hyptis pectinata* in others studies[28]. As the antinociceptive effect of *Hytpis fructicosa* was reversed by naloxone, it also has been suggested that this may involve the activation of opiod receptors[2]. Another study shows that the liver of rats with cholestasis express the preproenkephalin mRNA, which codes for the endogenous opioid peptide Met-enkephalin. Furthermore, Met-enkephalin immunoreactivity is detected in hepatocytes and in proliferating bile ductules in the cholestatic rat liver. This suggests that the opiods may have some effect on cholestatic liver and participate on liver regeneration[29]. Thus, it is plausible that the *Hyptis fructicosa* effects on liver may be related with the activation of opioid receptors.

In contrast, the diterpenoid Horminone, a substance found in *Hyptis fructicosa* plants[26], was suggested to be toxic to the liver in rats[30]. Other substances found in this plant are the taxodione and hyptol[26], however no publication about the effects of these substances on liver cells was found.

As there are few publications about the *Hyptis fructicosa* and the mechanism of liver regeneration may be influenced by different factors, new studies are necessary in order to isolate the substances presents and elucidate the mechanism which this plant enhance the liver regeneration.

Conclusion

The present study shows the aqueous extract of *Hyptis fructicosa* leaves at dose of 100mg/kg/day can stimulate the hepatic regeneration in rats.

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