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Revisión | Review

Larrea divaricata Cav.: Scientific evidence showing its beneficial effects and its wide potential application.

[*Larrea divaricata* Cav.: evidencia específica mostrando sus efectos beneficiosos y su amplia aplicación potencial]

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Abstract
Larrea divaricata Cav. (jarilla) (Zygophyllaceae) is used in Argentinean folk medicine. It contains nordihydroguaiaretic acid (NDGA), a lignan with nefrotoxic and hepatotoxic effects. The presence of NDGA converts controversial the use of *L. divaricata*. The amount of NDGA is higher in alcoholic extracts than in aqueous extracts (AE). The last 20 years had a great advance on the use of AE to treat different conditions in a safe manner. In this review, we present the scientific results tending to confirm the potential beneficial effects of the AE on human health. The anti-proliferative effects of AE of *L. divaricata* have been assayed on a tumor lymphoid line (BW 5147) and the pathways involved in such effects were described. The anti-microbial activity was determined by tests for bacteria and fungus. The anti-inflammatory activity was assayed by using carrageen and TPA induced-inflammation tests. The Immunomodulatory effects were investigated "in vivo" and "in vitro" on mice. Sub-fractions of aqueous extracts were obtained and analyzed. The immunogenicity of proteins from crude AE was characterized and antioxidant and nutritional activity were studied. The effect of an AE on hair loss was assayed. In summary, AE from *L. divaricata* has pharmacological activities including anti-microbial, anti-inflammatory, anti-cancer effects.

Keywords: *Larrea divaricata* Cav., jarilla, potential mechanism, aqueous extract, anti-tumor effect.

Resumen
Larrea divaricata Cav. (Jarilla) (Zygophyllaceae) es utilizada en medicina popular Argentina. Contiene ácido nordihidroguaiarético (NDGA), un lignano con efectos nefrotóxicos y hepatotóxicos. La presencia de NDGA hace controvertido el uso de *L. divaricata*. La cantidad de NDGA es mayor en extractos alcohólicos que en extractos acuosos (EA). Los últimos 20 años han tenido un gran avance en el uso de EA para el tratamiento de diferentes condiciones en forma segura. En esta revisión se presentan resultados científicos que confirman los efectos potencialmente beneficiosos de los EA sobre la salud humana. Los efectos anti-proliferativos se han ensayado en una línea de tumor linfoide, así como los mecanismos involucrados. La actividad anti-microbiana se determinó usando pruebas para bacterias y hongos. La actividad anti-inflamatoria fue evaluada mediante el uso de inflamación inducida por carragenina y TPA. Los efectos inmunomoduladores fueron investigados "in vivo" e "in vitro" en ratones. Sub-fracciones de los EA fueron obtenidos y analizados. La inmunogenicidad de las proteínas del EA crudo se caracterizaron y se estudiaron las actividades antioxidantes nutricionales. Además, se ensayó el efecto del EA en la caída del cabello. En resumen, los EA de *L. divaricata* presentan actividades farmacológicas como por ejemplo, anti-microbiana, anti-inflamatoria y efectos anti-cancerígenos.

Palabras claves: *Larrea divaricata* Cav., jarilla, mecanismo potencial; extracto acuoso; efecto antitumoral.

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INTRODUCTION

Plant extracts have been used in folk medicine since remote times until now. Already 2000 years B.C, the Sumerian civilization used poppy and other 700 medicinal preparations (Cambría, 1993). Hypocrates, 500 years b.C mentioned 300-400 medicinal plants. In the first century a.C, Discorides organized "The Materia Medica", a catalog of medicinal plants. Even in the Bible there is a description of about 30 medicinal plants (Kanba *et al*., 1998; Lewis, 2001). In this sence; pharmaceuticals have been with us less than 150 years. If our ancestors left Africa via the Holy Land 2000 years ago (for faith-based literalists), maybe a million years ago (for the less literal), then our genes, tracing back to our African/Holy Land ancestors, have had at least ten times more temporal experience with biblical herbs (e.g., cinnamon, coriander, cumin, dill, garlic, grape, mint, milk thistle, myrrh, olive, onion, pomegranate, saffron, turmeric, and the like). Besides, our bodies may even require many of them. Pharmaceuticals and synthetic food additives are relatively new to our genes. (Duke *et al*., 2008)

On the other hand, around 80% of the world´s population, mainly in non-developed countries, has in natural products as the first option for medication in Primary Health Care (Núñez-Sellés *et al*., 2007).

Larrea divaricata Cav. (Zygophyllaceae) is widely distributed in north-west, center, and south-east of Argentine (Discole *et al*., 1940). It is an evergreen shrub of 3 to 6 feet (0.9 to 1.8 m) tall. The small, dark green, resinous leaves are borne mostly at the tips of twigs and the rest of the branches are bare. Small yellow flowers appear throughout the year following rains. The fruit is a small woolly ball (Fig. 1).

In folk medicine, *L. divaricata* have the following claims and uses: healing sores and wounds, rheumatism, inflammation of respiratory and intestinal tract, gastric disturbance, venereal diseases, tonic, corrective, antiseptic, stimulating expectorant, emetic, (Waller and Gisvold, 1945), arthritis, cancer (Lambert *et al*., 2002), tuberculosis (Copp and Pearce, 2007; Rodriguez-Fragoso et al., 2008; Tyler and Foster, 1999) common cold (Tyler and Foster, 1999) and rubefascient (Del Vitto *et al*., 1997). *Larrea divaricata* contains NDGA (Mabry *et al*., 1977), a lignan with reported nefrotoxic and hepatotoxic effects (Arteaga *et al*., 2005). Therefore, the presence of NDGA makes controversial the use of *L. divaricata* to treat diseases by which their uses and applications were limited.

Figure 1. *Larrea divaricata* Cav. is an evergreen shrub of 0.9 to 1.8 m tall. The small, dark green, resinous leaves are borne mostly at the tips of twigs and the rest of the branches are bare. The flowers are yellow and the fruit is a small ball.

NDGA has an inhibitory effect on inflammatory cytokines during the acute phase and can act as a scavenger of free radicals generated during inflammatory processes (Schreck *et al*., 1992). NDGA is soluble in ethanol, slightly soluble in hot water, and insoluble in cold water (Coy and Gisvold, 1944**).** These properties reduce significantly the amount of NDGA in aqueous extracts (AE) compared with alcoholic extracts (Obermeyer *et al*., 1995; Davicino *et al*., 2006; Davicino *et al*., unpublished results).

The chemical composition of *Larrea divaricata* has been studied previously. It was found to contain principally phenolic compounds such as NDGA**,** essential oils (limonene, camphene, borneol and alcanfor) (Mabry *et al*., 1977), dihydroguaiaretic acid, norisoguaiacin, 3′-demethoxyisoguaiacin and 20 flavonoids (Mabry *et al*., 1977). The presence of saponins and leucoantocianidines was also reported (Bandoni *et al*., 1971**).** Among the flavonoids, O-glycosides of quercitin, myricetin and a C-glycoside of apigenin were found (Timmerman *et al*., 1979). Davicino et al. (unpublished results) showed the presence of a polar derivate from NDGA in an AE obtained from this plant.

During long time *Larrea divaricata* Cav. was confused with *Larrea tridentata*. Initially they were considered to be sub species but now they are separated into two different species. In fact, they have

different active compounds (Horm and Gisvold, 1945; Sakakibara *et al*., 1976) and distinct genetic composition (Palacio and Hunziker, 1972; Yang, 1970). Because of this, the majority of studies about toxic effect of *Larrea spp.* are referred to *L. tridentata.* The toxicity of *L. tridentata* was observed after the consumption of leaf preparation (Katz and Saibil, 1990; Gordon *et al*., 1995; Batchelor *et al*., 1995). In these preparations 5-10 % of NDGA was found; as NDGA has been considered to be principally nephrotoxic and hepatotoxic, the toxicity of the plant was attributed mainly to this compound (Stickel *et al*., 2000). The toxicity of *L. divaricata* is discussed below, in relation to its NDGA content.

The question then is: it is possible to use the AE from *L. divaricata* to treat the diseases related with it use in folk medicine?

In this review we present scientific reports tending to confirm the potential benefit of the AE obtained from *L. divaricata,* on human health and also we propose a novel mechanism by which AE could be exert its anti-tumor effect.

PHARMACOLOGY

Anti-tumor effects

Chemoprevention by medicinal plants has received growing attention in recent years as a promising approach in controlling the incidence of cancer (Mettlin, 1997). It has been demonstrated that an AE of *L. divaricata* has a dual effect upon the tumor lymphoid cells BW 5147: an anti-proliferative activity at high concentrations and a pro-proliferative activity at low concentration. This cellular line corresponds to a murine immature T-lymphoma with H-2k haplotype and αβ T cell receptor (αβTCR). These cells express CD3, CD4, CD28 and β-adrenoreceptors (Criado and Rojo, 2000; Gorelik *et al*., 2002; Wegener *et al*., 1992; Clavreul *et al*., 2000).

In previous studies, it is demonstrated that the anti-proliferative action of the AE of *L. divaricata* on BW5147 cells is related to an increase in cAMP levels, this effect appeared not to be mediated by the activation of receptors coupled to cAMP (beta adrenergic receptors and histaminergic receptors) (Anesini *el al*., 1996). It is important to note that these cells show a poor expression of β-adrenoreceptors (Cremaschi *et al*., 1991), moreover these receptors are uncoupled to the adenylate cyclase system. This last is related to the phosphorylation performed by protein kinase C (PKC) (Cremaschi *et al*., 2000). The increase in cAMP exerted by *L. divaricata* extract could be related to the inactivation of the PKC previously observed. The BW 5147 cells expressed mainly PKCζ , this is a calcium independent isoform (Gorelik *et al*., 2002; Hirai and Chida, 2003; Moscat and Diaz-Meco, 2005) meanwhile, these cells have barely detectable levels of the β isoform from PKC, with similar levels of α and δ isoforms, and no detectable levels of the γ and θ isoenzymes. Generally, cellular proliferation is mainly calcium independent so, PKC activity involves calcium-independent isoforms. Based on these facts, it is possible that the increase in PKC β isoform observed during mitogenic stimulation of T cells would allow an efficient but limited activation, leading to differentiation to immune effectors cells, while low levels of this isoenzyme, along with high levels of the ζ isoform, would be responsible for the hyperproliferative pattern of BW5147 cell line (Gorelik *et al*., 2002). *L. divaricata* extract could inhibit directly the ζ isoform of PKC. By the other hand, eicosanoids such as leukotrienes and prostaglandins have been related to the modulation of cell proliferation through the action exert not only on cAMP level, but also on PKC activation (Nishizuka, 1988; Parker *et al*., 1989). For example, leukotriene B4 (LTB4) exerts a pro-proliferative effect on BW5147 cell line, by deceasing cAMP levels and by the induction of PCK. In a recent publication, Davicino *et al*. (2011) were able to show that the AE has a dual effect on cell proliferation: first increases cAMP levels and inhibits PKC, producing a depleting effect on proliferation, but on the other hand induces LTB4 production, which antagonizes the anti-proliferative effects. However, the end result is a clear anti-proliferative effect. They conclude that the effect of the extract on leukotrienes decreases the anti-proliferative action, and the extract exerts an anti-proliferative effect due to cAMP production and PKC inhibition.

It is known that the treatment of cells with H_2O_2 may causes activation of NF-κB, phenomenon related to apoptosis and to a decrease in cell proliferation by the induction of NO level. In lymphocytes, H_2O_2 is produced from mitochondria during respiration and can diffuse to cytosol (Rezaei *et al*., 2008). In the first step, superoxide free radical is simultaneously reduced and oxidized (dismutated) to form hydrogen peroxide and oxygen. This is accomplished by superoxide dismutase (SOD). In the second step, hydrogen peroxide is converted into water and oxygen. There is increasing evidence to suggest that H_2O_2 generated at low levels during normal cell

signaling may act as a second messenger (Lee and Esselman, 2001). Recently, Davicino *et al.* (2009a) advanced on the studies and analyzed the participation of H_2O_2 in the anti-proliferative effect of *L. divaricata* on BW 5147 cells. Different assays were performed showing that the inhibition of cell proliferation was related to activation of ERK1/2 pathway and the subsequent translocation of NF-κB to the nucleus leading to the induction of iNOS (inducible nitric oxide sintase) and the consequent increase of NO level, which in turn activates P-38 pathway leading to apoptosis. Moreover, it was observed that BW5147 lymphocytes need to have low levels of H_2O_2 to allow the proliferation, this requirement can be accomplished by maintaining a low Mn^{2+}SOD and high peroxidase (Px) activities. *L. divaricata* increased the activity of SOD which leads to increase H_2O_2 (Davicino *et al*., 2010).

On the other hand, it is known that H_2O_2 is capable of affecting the function of proteins by oxidizing thiol groups in Cys amino acid residues to sulfenic acid (Lee and Esselman, 2001). In Jurkat T lymphocytes, H_2O_2 enhanced the phosphorylation level of ERK1/2 caused by T cell receptor (TCR) stimulation. Sub-millimolar concentrations of H_2O_2 induced phosphorylation of ERK1/2 and MEK1/2 without antigenic stimulation, leading to cell proliferation. Interestingly, when intracellular cAMP levels were increased, it inhibits upstream signaling pathways activated by H_2O_2, resulting in decrease of H_2O_2-induced MEK1/2 phosphorylation. H_2O_2 also inhibits ERK-dephosphorylating phosphatases (PTPs) that may be dissociated from ERK1/2 by PKA phosphorylation, leading to overall enhancement of ERK1/2 phosphorylation by cAMP (Lee and Esselman, 2001). Besides, CD28 co-stimulation produces H_2O_2 by 5-lipoxygenase (5-Lox), resulting in IL-2 gene expression through NF-kB activation. Treatment of cells with low concentrations of H_2O_2 was previously shown to enhance IL-2 gene expression after TCR activation, as well as mitogenically induced T cell proliferation (Hehner *et al*., 2000; Los *et al*., 1995). When BW5147 cells are treated with high concentrations of AE of *L. divaricata* (100-1000 μg/ml) probably it produces high amounts of mitochondrial H_2O_2 by Mn^{2+}SOD stimulation (Davicino *et al*., 2010). The H_2O_2 produced may act on the PTPs that inhibit ERK1/2 and thus lead to production of NO that finally triggers apoptosis. The extract also increases the intracellular cAMP which could act promoting the anti-proliferative action of H_2O_2. *L. divaricata* could directly activate adenylate cyclase to produce more amount of cAMP. In accord to these findings, we propose in Figure 2 an overall review of the potential mechanism by which AE obtained from *L. divaricata* could exert the anti-proliferative effects on BW5147 cells.

Although it has been demonstrated the anti-proliferative activity of *L. divaricata*, Anesini *et al.*, (1999), by using inhibitors, demonstrated that at low concentration (0.5 μg/ml) showed pro-proliferative effect, which was due to the activation of lipoxygenase metabolism. They also reported the effects of an AE from *L. divaricata* in comparison with the effect of NDGA on proliferation of BW5147 cells (Anesini *et al*., 2001). Considering the amount of NDGA detected in the extract, the anti-proliferative activity of *L. divaricata* should not be attributed to NDGA. According to the preceding paragraph it can be suggested that the increase in BW 5147 cells proliferation exerted by low concentration of *L. divaricata* extract (0.5 μg/ml) could be related to a compound that stimulates the production of H_2O_2 which in turn induces proliferation through the pathway of arachidonic acid. This observation is supported on previously results, that shows that low concentrations of H_2O_2 (10^{-8} - 10^{-13} M) leads to BW5147 cells to proliferate and high concentrations of H_2O_2 (10^{-3} - 10^{-5} M) induces cells to apoptosis (Davicino *et al*., 2009a). The authors also showed that BW5147 cells do not display SOD activity and possessed elevated levels of superoxide (Davicino *et al*., 2009a). This observation suggests that these cells are less sensitive to superoxide than to H_2O_2.

The anti-tumor activity "in vivo" of an AE of *L. divaricata* was studied on spontaneous and chemically induced carcinomas. The extract exerted anti-tumor action at 25 mg/kg when was administered to a pregnant rat with a spontaneous mammary carcinoma and also the anti-tumor activity was shown when mammary carcinoma was induced with N-nitroso-N-methylurea (NMU) in female rats (Anesini *et al*., 1997). In this study from a total of 75 tumors, a regression of 10 tumors (13%), a stabilization of 60 tumors (80%) and an increment of 5 tumors (6%) were observed. None of the 80 control tumors decreased (0%) and only 12 remained static (15%). In this case the extract increases the survival time of the animals in comparison with control ones (Anesini *et al*., 1997).

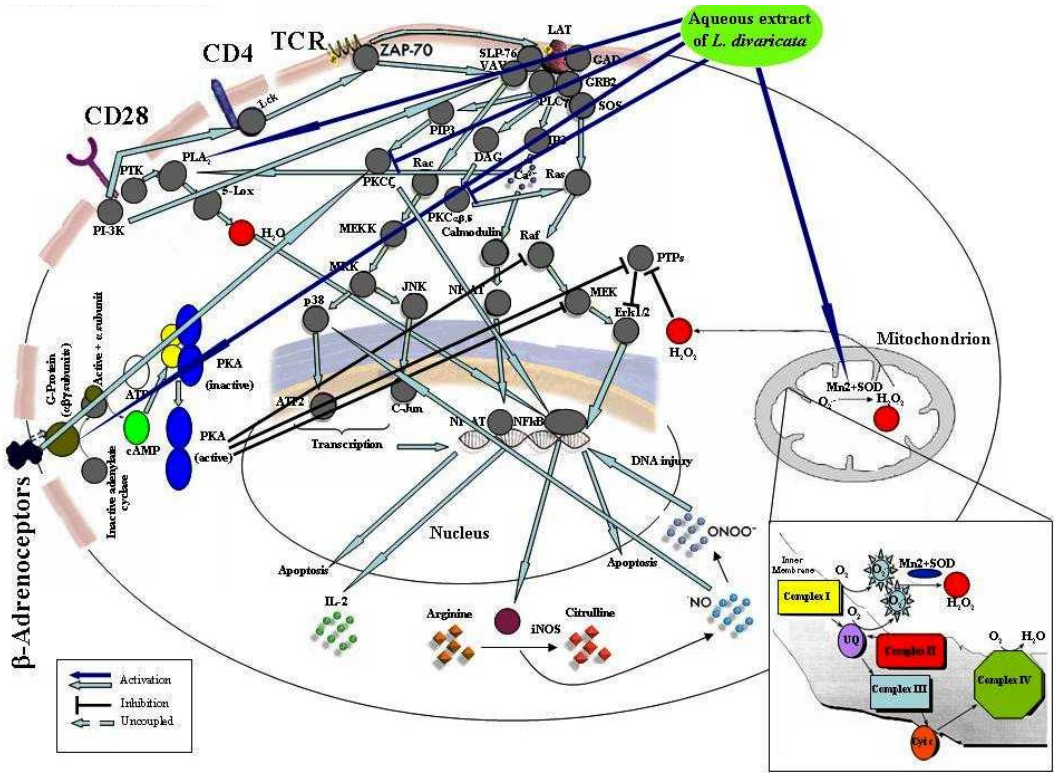

Figure 2. Proposed mechanism for pharmacological action of aqueous extract obtained from *Larrea divaricata* Cav. on BW5147 tumor cells.

Finally, Bongiovanni *et al*. (2008) demonstrated that methanolic and methane dichloride extracts of aerial parts of *Larrea divaricata* exhibited a pronounced cytotoxic effect by arresting cell viability at a level of 35% against the MCF-7 cell line (a human breast adenocarcinoma), while only a weak cytotoxic effect was observed for the aqueous extract. Apoptosis measured by flow cytometry revealed that methanolic and methane dichloride extracts resulted in a rapid cell plasma membrane perturbation and triggered cellular death.

Anti-microbial effects.

The studies performed with plant extracts in the field of infections reveled that plants are potential sources of anti-microbial agents. It was shown that, an AE obtained from *L. divaricata* displays remarkable activity against *Staphylococcus aureus* obtaining an equivalence of 15.2 ± 1.0 µg/ml with Cephazolin used as reference antibiotic (Anesini & Perez, 1993). Stege *et al*. (2006) showed that cold extract, infusion and decoction of *L. divaricata* has inhibitory activity at 40–100 µg/ml against clarithromycin and metronidazole susceptible and resistant *Helicobacter pylori* strains. Besides, these results support the popular use of *L. divaricata* in gastric disturbances such as gastric ulcers and gastric cancer associated with *H. pylori*. On the other hand, it has been demonstrated the antifungal activity of an AE of *L. divaricata* at 100 mg/ml against *Saccharomyces cereviceae* (Davicino *et al*., 2007a) which is a common colonizer of mucous membranes causing both superficial and invasive deep infections (Aucott *et al*., 1990; Jones *et al*., 2002).

The ethanolic extract also inhibits the *in vitro* growth of the following fungi and yeasts: *Lenzites elegans*, *Schizophyllum commune*, *Pycnoporus sanguineus*, *Ganoderma applanatum*, *Fusarium oxysporum*, *Penicillium notatum*, *Aspergillus niger*, and *Trichorerma* spp. A fraction obtained from this ethanolic extract (Fr. B) has several phenolic compounds that are responsible of the cytotoxicity observed on *P. sanguineus*, *L. elegans* and *G. applanatum* (Quiroga *et al*., 2004). Although ethanolic extracts have better fungicidal effects than aqueous extracts, little is known about the potential toxic effects on humans, due to its higher content of NDGA.

Anti-inflammatory activity

Although in folk medicine, *L. divaricata* is widely used as anti-inflammatory (Del Vitto *et al*., 1997), there are few works documenting this activity. Pedernera *et al*. (2006) determined the anti-inflammatory effect of *L. divaricata* but using only a methanol extract. Others authors observed that *L. divaricata* at 33.4 mg/kg decreases carrageen and TPA induced-inflammation in mice. In these animals a decrease of TNF-alpha, IL-6 and eosinophil and an increase of IL-10 were observed (Davicino *et al*., unpublished results). These results support that the anti-inflammatory effect exerts by *L. divaricata* could be due to a decrease of pro-inflammatory cytokines and an increase of anti-inflammatory cytokines.

Immunomodulatory effects

The impact of the immune system in human ailments is enormous. In this sense, autoimmune diseases, type I diabetes mellitus, systemic lupus erythematosus, multiple sclerosis, solid tumors, hematologic malignancies, infectious diseases, asthma and various allergic conditions are mediated by immune system. Immunological diseases are growing at epidemic proportions that require aggressive and innovative approaches to develop new treatments. Drugs commonly use in immune therapy can modulate the immune response in three ways: immunosuppression, tolerance, and immunostimulation. The essence of immunomodulation is that a pharmacological agent acting under various dose and time regimens displays an immunomodulating effect (Flemming, 1985; Kowalozyk-Bronisz and Paegelow, 1986). The immunomodulating action is reversible and requires maintaining the dose of a preparation. Natural adjuvant, synthetic agents, antibody reagents are used as immunosuppressive and immunostimulating agents. There are major limitation to the general use of these agents such as increased risk of infection and generalized effect throughout the immune system (Diasio and LoBuglio, 1996). The search for new bioactive molecules in plants is of great interest considering the diversity of chemical entities produced by them (Gottlieb *et al*., 1996) and the need of new medicines for more effective treatment of pathologies with lower toxic effects (Oliveira Costa et al., 2008). Some studies related to immunomodulatory activity were done with an AE of *L. divaricata.* Aqueous extracts, decoction and infusion from *L. divaricata* were investigated for immunomodulating activity on mice peritoneal macrophages (MPhi). The results showed that *L. divaricata* stimulates MPhi activation at 0.2 mg/ml whereas it shows a clear pro-apoptotic activity at higher concentrations (1 and 4 mg/ml). The dual effects found are relevant, considering the folk use of this plant to activate the immune system (Davicino *et al*., 2006). Moreover, the author shows that the apoptosis triggered by *L. divaricata* is a consequence of cell activation and that the effects are independent of NDGA. They concluded that this "activation and death" could be the mechanism of *L. divaricata* to exert the anti-tuberculosis effect known in folk medicine (Davicino *et al*., 2007b). With the aim to identify the active compounds of the extract, the same authors made a fractionation of it. Sub-fractions without and with low concentrations of NDGA were obtained and analyzed on mouse macrophages. They observed that, a free NDGA sub-fraction (F1) at 100 μg/ml is capable to activate macrophages and decreases NO level, all together suggested an anti-inflammatory action. These results indicate that NDGA was not the compound responsible of the immunomodulatory action exerts by the AE of *L. divaricata* (Martino *et al*., 2010). On the other hand, these authors studied in healthy mice the immunomodulatory potential of AE of *L. divaricata* "in vivo". They found that the decoction and infusion are able to prime Mphi "in vivo" and to induce full activation "in vitro" at 0.5 mg/kg. These finding contributed to characterize the biological activity of *L. divaricata* and to understand the ability of its extracts to enhance immune responses (Davicino *et al*., 2007c).

Fractions obtained from *L. divaricata* were assessed "in vivo" as well (Martino *et al*., 2011). The authors showed that F1 induces a state of pre-activation on MPhi "in vivo", which is enhanced by the presence of *Candida albicans*. They postulate that F1 could be a good candidate to treat disseminated candidiasis by enhancing the innate immune system. Besides, Davicino *et al*. (2008) studied the mechanisms by which an AE of *L. divaricata* releases oxygen reactive species "in vivo". They showed that the extract at 0.5 mg/kg increases the expression of dectin-1 leading to increased superoxide production.

On the other hand, Mattar *et al*. (2009) characterized the immunogenicity of proteins from a partially purified crude aqueous extract (JPCE) of *L. divaricata* and evaluated the cross reaction between JPCE and bacterial proteins of *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, and *Klebsiella pneumoniae* using a mouse anti-JPCE serum. Bacterial proteins showed a strong reaction with the anti-JPCE serum, suggesting by this way that,

proteins from *L. divaricata* could be used as immune stimulants.

Antioxidant effects.

Taking into account that free radicals are involved in several diseases, including cancer, central nervous system alterations and inflammatory pathologies, the antioxidant activity of an AE of *L. divaricata* was studied.

Anesini *et al*. (2004) demonstrated that an AE of *L. divaricata* induces peroxidase secretion by β_1 adrenoreceptors stimulation in submandibulary glands of female rats in a dose-dependent manner, reaching a maximum activity at 300 μg/ml. This effect was not related to NDGA as it decreases the peroxidase secretion. As well as, both the AE and NDGA increases total peroxidase activity, the maximum effect of the AE is 6.4 times higher than NDGA. Taking together these results, NDGA could be one of the active compounds related to the increase of total peroxidase activity, but other compounds in the AE could be enhanced by NDGA. On the other hand, Turner *et al*. (2011) determined and compared the antioxidant activity of AE of *L. divaricata* and NDGA at different concentrations (0.01-10000 μg/ml). The extract presents peroxidase (Px), catalase (CAT) and superoxide dismutase (SOD) like activities. Meanwhile, CAT activity is related to the presence of NDGA, SOD activity is not. Also, the extract presents inhibitory action of lipid peroxidation as well as free radical scavenger effect. Both activities are not related to the presence of NDGA. The fact that the extract presented stimulatory effect on peroxidase secretion as well as peroxidase like activity is very important as peroxidase is an oral enzyme implicated in the defense of oral cavity, moreover protects against reactive oxygen species (ROS). ROS in oral cavity are related to the inflammatory events as tissue destruction during periodontitis. Also, ROS in the inhaled cigarette smoke have been suggested to induce dysplasic lesions of the mucosa which then are transformed into "in situ" carcinoma lesions eventually resulting in full-blown infiltrating and metastasizing cancer. In accord to said above Turner *et al*. (2008) postulated the use of AE of *L. divaricata* as extract or as pharmaceutical preparations (mouth rinse, dental cream, gels and powders) as oral antioxidant, as well as a agent destined to prevent illnesses, among others, cancer and peritonitis in human.

Other studies

The effect of an AE of *L. divaricata* was assayed on hair loss. Hair loss is a distressing condition for an increasing number of men and women and it is of great importance; therefore, to develop new therapies for the treatment of this diseases (Rho *et al*., 2005). Davicino *et al*. (2009b) assayed the effects of *L. divaricata* on hair growth and proposed the topical use of association between decaffeinated coffee and *L. divaricata* AE in at least 1000 mg/ml as a possible therapeutic agent destined to induce hair growth in human and in animals.

Toxicity studies

About toxicity studies performed with the AE of *L. divaricata* there are only two evidences. In one study NDGA, isolated from *L. divaricata* resin, as well as the resin were administered to mice, suggesting that NDGA was the main constituent related to the toxic effects observed with the resin (Ríos *et al*., 2008). In another study, Davicino et al. (unpublished results) fed mice with a dose of 33.4 mg/kg of an AE obtained from *L. divaricata* and demonstrated that mice increased weight, consumed more food and preferred food with extract. The extract did not modify the weight and histology of several organs and did not present stimulant or depressant activity. Besides, the extract did not present hepatotoxicity and nephrotoxicity and did not modify the levels of cholesterol and triglycerides, in fact no toxicity signals were observed. In this study also, the acute toxicity of NDGA and the AE were examined. The authors determined that the lethal dose 50 (LD_{50}) of NDGA was 60 mg/kg meanwhile, the AE exerted a value of 40.000 mg/kg (Davicino et al., unpublished results). By other way, the NDGA amount determined by HPLC in the AE was 0.3- 0.7 w/w. In 33.4 mg/kg of extract the animals are ingesting between 0.1-0.2 mg/kg of NDGA. So, the low toxicity observed could be related to the fact that, the dose administered was very low in comparison with $LD_{50.}$

CONCLUSIONS

Numerous natural products from traditional medicinal plants have been introduced in the development of new drugs. The objective of this review has been to show the advances in the exploration of aqueous extracts of *L. divaricata* as potential therapeutic agent. The emphasis is put in the fact that the extract does not possess pharmacologically active amounts of NDGA. In Table 1 are summarized the pharmacological actions of the different extracts of *Larrea divaricata*.

With the current information, it is evident that the AE from *L. divaricata* has pharmacological activities including anti-microbial, anti-inflammatory, anti-cancer effects. The broad applications of AE of this plant make it a potential medicine for the treatment of several diseases. However, more studies are needed to determine the human safety and efficacy of the extract to use it in medicine. For these reasons, extensive pharmacological studies, referring to pharmacodynamic and pharmacokinetic should be performed in humans. Also, a phytochemical study will be a focus for future studies.

Table 1. Pharmacological effects of aqueous extract obtained from *Larrea divaricata* Cav.

Aqueous extract of *L. divaricata*
1. Anti-tumor effects: -***In vitro*** - BW5147 cells -***In vivo*** -Spontaneous mammary carcinoma in rats. -Mammary carcinoma induced with NMU in rats.
2. Anti-microbial effects: -*Staphylococcus aureus* -*Helicobacter pylori* -*Saccharomyces cereviceae*
3. Anti-inflammatory activity: -Decreases inflammation in mice: -Decreases levels of TNF-α. -Decreases levels of IL-6. -Decreases eosinophil. -Increases IL-10.
4. Immunomodulatory effects: -Stimulates macrophages activation *in vitro* in mice. -Shows pro-apoptotic activity at higher concentrations. -A sub-fraction from aqueous extract without NDGA activates mice macrophages and decreases NO level. -Primes macrophages *in vivo* in mice. -Increases the expression of dectin-1 and increases superoxide production. -Proteins from aqueous extract presents cross reaction with bacterial proteins.
5. Antioxidant effects: -Induces peroxidase secretion by β_1 adrenoreceptors stimulation in submandibulary glands in rats. -Presents peroxidase, catalase and superoxide dismutase like activities. -It could be used as oral antioxidant as well as agent destined to prevent cancer and peritonitis in human.
6. Toxic effect: -No toxicity signals were observed in mice.
7. Effect on hair growth: -Induces hair growth in mice.

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