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Antioxidant properties of *Baccharis trimera* in the neutrophils of Fisher rats

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ABSTRACT

Ethnopharmacological relevance: *Baccharis trimera* (Less.) (Asteraceae) is a native plant of Brazil. Also known as "carqueja", it has been popularly used to treat liver diseases, diabetes, as well as digestive disorders. Other studies have described the hepatoprotective, antioxidant and anti-inflammatory activities of the species.
Aim of the study: The aim of the present study was to investigate the antioxidant properties of *Baccharis trimera* in the neutrophils of Fisher rats in both *in vitro* and *in vivo* experimental models.
Material and methods: In the *in vitro* assay, the neutrophils of male rats were isolated and incubated with *Baccharis trimera* extract at concentrations of 0.5, 5.0 and 50.0 μg/mL. In the *in vivo* assay, male rats were first treated with crude extract 600 mg/kg body weight of *Baccharis trimera* or with 50 mg/kg body weight of quercetin (reference substance) and then treated with 835 mg/kg of acetaminophen (APAP) after 24 h.
Results: The hydroethanolic extract of *Baccharis trimera* reduced the release of reactive oxygen species in the neutrophils in both the *in vitro* and *in vivo* experimental models. Therefore confirming its antioxidant effect.
Conclusion: The results of this study confirm the antioxidant effect of Baccharis trimera.

1. Introduction

The genus *Baccharis* (Asteraceae), which probably originated in South America, consists of approximately 500 species (Carneiro and Fernandes, 1996). Several *Baccharis* species are commercially used in folk medicine as antiseptics and anti-inflammatory agents, and to treat both gastric ulcers and skin sores (Verdi et al., 2005).

Baccharis trimera (Less.) DC is popularly known as 'carqueja' in Brazil. Infusions, decoctions, and tinctures of its aerial parts are used in Brazilian popular medicine (Grance et al., 2008) for the treatment of liver and gastrointestinal tract illnesses (Soicke and Leng-Peschlow, 1987; Gamberini et al., 1991), inflammatory processes (Gené et al., 1996), and diabetes (Oliveira et al., 2005). Because of these biological effects, research on the chemical composition of *Baccharis trimera* was conducted and demonstrated that this plant has many bioactive compounds, such as flavonoids, diterpenes and triterpenes (Verdi et al., 2005). Triterpenes, along with a saponin described by Gené et al. (1996), have been reported to be primarily responsible for the anti-inflammatory activity (Dela-Loggia et al., 1994; Akihisa et al., 1996), while the flavonoids, due to their antioxidant activity, have been linked to protecting the body against reactive oxygen species (ROS) (Rodrigues et al., 2009).

It is well-known that radicals of oxygen or reactive oxygen species (ROS) can be generated in the organism during metabolic processes due to ambient stimulations or during the "burst" of phagocytic cells in an infection by the action of NADPH oxidase (Emerit and Chaudiere, 1989; Babior, 2000). ROS are able to oxidize various biomolecules including DNA, proteins, carbohydrates, fatty acids and many other cellular structures (Bagchi and Puri, 1998). There is evidence that ROS may be involved in more than 100 diseases and nosologic events, such as cancer, arteriosclerosis, rheumatoid arthritis and post-ischaemic organ injury (Halliwell, 1994). In many of these events, the origin of ROS seems to be directly related to the activation of neutrophils (Weiss, 1989).

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Neutrophils are polymorphonuclear leukocytes that play a crucial role in defending the body against bacteria, fungi and protozoa. They are the principal effector cells of innate immunity. The role of neutrophils (PMNs) in ROS production in the physiopathology of various inflammatory illnesses has drawn interest with the discovery of new compounds to modulate this process (Middleton Jr. et al., 2000).

Based on the widespread use of carqueja in folk medicine and the neutrophils' (PMNs) role in the progression of various diseases, we now report the results obtained for the *in vitro* and *in vivo* evaluation of the possible antioxidant effects of the hydroethanolic extract from *Baccharis trimera* on neutrophil reactive species generation, which was triggered by opsonized zymosan and assessed by luminol-enhanced chemiluminescence.

2. Material and methods

2.1. Chemicals

Acetaminophen (APAP) (200 mg/mL) was obtained from Janssen-Cilag Pharmaceuticals. Trypan blue, quercetin, luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) and zymosan were purchased from Sigma–Aldrich, St. Louis, MO. The Leukopaque and Monopaque gradients were from Bion LTDA, Brazil. The kit for measuring serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was from Diagnostic Labtest, Brazil.

2.2. Animals

The Laboratory of Experimental Nutrition from the Federal University of Ouro Preto (UFOP) provided the male albino Fisher rats used in the experiment; the animal were approximately 12 weeks old and weighed about 180 g. All animals were kept in individual cages placed in an environment with controlled temperature, light and humidity, and received both commercial rat chow and water *ad libitum*. This work was carried out in accordance with the international standards of animal protection and with the ethical principles of the Brazilian College of Animal Experimentation, and was approved by the Ethics Committee on Animal Use (CEUA) of UFOP (OF 01/2009 and OF 011/2009).

2.3. Collection of plant material

The aerial parts of *Baccharis trimera* were collected during April 2008 in the city of Ouro Preto, Minas Gerais, Brazil. The specimen, voucher number OUPR 22.127, was identified by professor Viviane R. Scanlon and deposited in the Herbarium José Badini – UFOP.

2.4. Preparation of extract

After identification, the aerial parts of the plant were dried in a ventilated oven, sprayed in a mechanical mill and stored in plastic bottles. To obtain the hydroethanolic extract, approximately 100 g of the plant were extracted with distilled water and 70% alcohol at a ratio of 1:1 for 24 h. Vacuum filtration and evaporation of the solvent in a rotavap was then performed. The crude extract formed was then diluted with phosphate buffered saline (PBS, pH 7.4) to concentrations of 50.0, 5.0 and 0.5 μg/mL that were used *in vitro* and the concentration of crude extract 600 mg/kg body weight that was used *in vivo*. The methodology for the extract preparation was based on the work of Grance et al. (2008) with some modifications. The quercetin, used as positive control, was resuspended in distilled water and given to the animals, through gavage, at a concentration of 50 mg/kg body weight, according to Miltersteiner et al. (2003).

2.5. Isolation of polymorphonuclear leukocytes

Blood was obtained by bleeding of the brachial plexus and was collected in heparinized tubes. The neutrophils were then isolated using two gradients of different densities, Monopaque ($d = 1.08$) and Leukopaque ($d = 1.12$), according to the procedure of Bicalho et al. (1981) with minor modifications. The cell viability of each sample was always greater than 95% as determined by the exclusion test with trypan blue.

2.6. Opsonization of zymosan particles

Zymosan was opsonized by adding 900 mL of PBS (pH 7.4) to 100 mL of zymosan (13 mg/mL) as done in the study by Nogueira-Machado et al. (2003). The solution was then centrifuged for 2 min at 200 × *g*. The supernatant was discarded and the pellet was resuspended in 200 μL of fresh autologous serum and 800 mL of PBS (pH 7.4). This solution was incubated for 30 min in a 37 °C water bath and shaken at 10 min intervals. Subsequently, the solution was centrifuged again for 2 min at 200 × *g*, the supernatant discarded and the pellet resuspended in 500 μL of PBS. The volume of opsonized zymosan with serum (ZC3b) used for the experiments was 50 μL.

2.7. In vitro test

A chemiluminescence assay for the luminol amplified by activation of phagocytic cells was carried out to assess the generation of ROS as described by Chaves et al. (2000). To perform the experimental procedure, 1×10^6 neutrophils/1 mL of PBS were incubated with 500 μL of luminol (10^{-4} M) for 10 min in siliconized glass tubes. A luminol [Sigma Co.] stock solution was made by dissolving 1.77 mg of luminol in 1.0 mL of dimethyl sulfoxide (DMSO) to give a final concentration of 10^{-2} M. Before use this solution was diluted further to 10^{-4} M in PBS (pH 7.4). The photons emitted in this range were recorded each minute by the internal printer of the luminometer. After running the reaction for the initial 10 min, PBS (basal) or 50 μL of opsonized zymosan particles (13 mg/mL) or *Baccharis trimera* (Bt) extract was added. For all tests, the final volume was adjusted to 700 μL with PBS (pH 7.4). The chemiluminescence measurements were performed in a luminometer (Lumat, LB 9507, Berthold, Germany). The chemiluminescence was recorded for 30 min, which was enough time to observe the peak. The results were expressed in relative light units/min (RLU/min).

2.8. ROS generation analysis – in vivo test

2.8.1. Assessment of liver injury induced by APAP

To evaluate the liver injury induced by APAP, 10 rats were divided into two groups of five animals according to the treatment received. The control group (C) received 1.0 mL of PBS by gavage and the APAP group received a single dose of 835 mg/kg of APAP (200 mg/mL), also by gavage. The dose of APAP was used as a reference in the work of Yen et al. (2008). At different time intervals samples of blood were collected via the ocular plexus to quantify the activity of ALT and AST in response to the high dose of APAP.

2.8.2. Treatment of rats with Baccharis trimera extract and quercetin

Thirty rats were divided into six groups of five animals according to the treatment received. The control group (C) received 1.0 mL of PBS, group *Baccharis trimera* (Bt) received 600 mg/kg of the *Baccharis trimera* extract, group quercetin (Que) received 50 mg/kg of quercetin, the acetaminophen (APAP) group received a single dose of 835 mg/kg of acetaminophen, group *Baccharis trimera* + acetaminophen (Bt + APAP) first received 600 mg/kg of the *Baccharis trimera* extract and an hour later, a single dose of

835 mg/kg of acetaminophen and group quercetin + acetaminofen (Que + APAP) first received 50 mg/kg of the quercetin and an hour later a single dose of 835 mg/kg of acetaminophen. All treatments were administered by gavage. Twenty-four hours after the dose of APAP, the animals were anesthetized and blood was collected by bleeding from the brachial plexus into heparinized tubes for the isolation of neutrophils. Chemiluminescence assays were performed with neutrophils from each experimental group and were recorded for 30 min at 1-min intervals. At the end of the tests, we obtained curves of 30 min, and the values were expressed in relative light units/min (RLU/min).

2.9. Statistical analysis

The data were expressed as mean ± standard deviation (SD). All data were subjected to a normality test. After determining that they were following a normal distribution, we chose to use Student's *t* test. *p* value <0.05 was considered significant. Tests were performed with GraphPad Prism version 4.00 for Windows (San Diego, CA, USA).

3. Results

3.1. In vitro assays: the effect of hydroethanolic Baccharis trimera on the modulation of reactive oxygen species in rat neutrophils stimulated with zymosan

To evaluate the effect of the *Baccharis trimera* extract on the modulation of reactive oxygen species in neutrophils, we tested three different concentrations of the extract. Our results showed that extracts of *Baccharis trimera* at concentrations of 50.0, 5.0 and 0.5 μg/mL were able to significantly inhibit the release of ROS as measured by the chemiluminescence assay. This inhibition is dose-dependent, since the extract concentration of 50.0 μg/mL displayed a high percentage of inhibition (96.8%) accompanied by low viability (48.0%), while the extract concentration of 0.5 μg/mL displayed a low percentage of inhibition (51.0%) accompanied by high viability (95.0%). However, the concentration of 5.0 μg/mL inhibited 85.0% of ROS release while maintaining cell viability above 93.0% (Fig. 1).

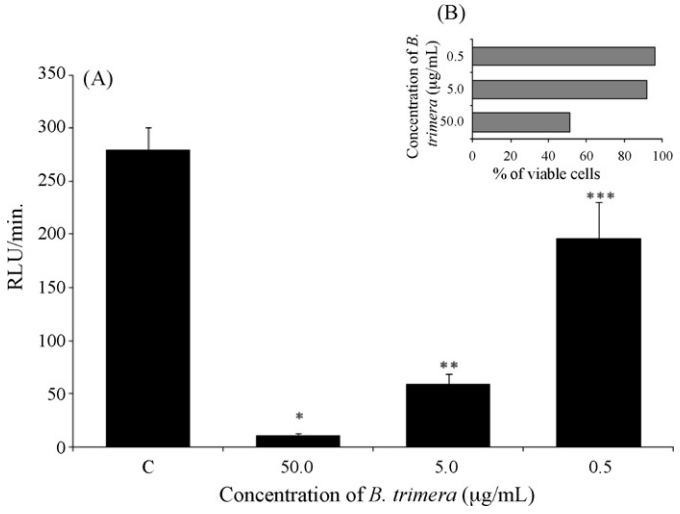

Fig. 1. Graph A represents the effect of the hydroethanolic extract of *Baccharis trimera* on ROS production by rat neutrophils. The data are expressed as mean ± standard deviation. $^{*}p = 0.00005$ in relation to the control, $^{**}p = 0.00003$ in relation to the control, $^{***}p = 0.05$ in relation to the control. Graph B represents the percentage of living cells after the experiments had been carried out.

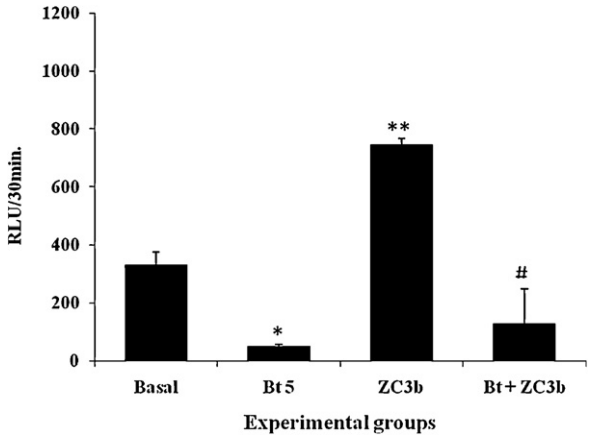

Fig. 2. Effect of *Baccharis trimera* extract on ROS modulation of the neutrophils *in vitro*. Values are expressed as the mean and the vertical bars indicate the standard deviation. $^{*}p = 0.00003$ in relation to the control, $^{**}p = 0.003$ in relation to the control, $^{\#}p = 0.001$ in relation to ZC3b.

The results in Fig. 2 show that the concentration of 5.0 μg/mL of extract was able to significantly inhibit the release of ROS as measured by the chemiluminescence assay when compared to basal neutrophil metabolism. Neutrophils incubated with ZC3b particles were able to significantly increase the production of ROS. However, when neutrophils were pre-incubated with *Baccharis trimera* extract and then incubated with ZC3b particles, there was a significant reduction in ROS release when compared to cells incubated with only ZC3b particles.

3.2. Activity of alanine aminotransferase (ALT)

As seen in Fig. 3, the control group (C) that received PBS displayed low ALT activity (9.59 ± 1.86 IU). In contrast, animals that received an oral treatment of 835 mg/kg of APAP demonstrated higher activity of this enzyme, although this increase was only significant after the first 6 h, and reached its peak 24 h (142.21 ± 5.67 IU) after the administration of APAP. Forty-eight hours after APAP dose of ALT, the activity began to decline, approaching 72 h (26.55 ± 6.15 IU), with values significantly lower than those found in the first 24 h, indicating that the endogenous mechanisms of repair may have been activated.

As seen in Fig. 4, the control group (C) that received PBS displayed low AST activity (81.84 ± 10.50 IU). As was seen with the ALT activity, AST activity also increased after the administration of APAP with a significant increase after the first 6 h, and reaching its peak 24 h (241.03 ± 42.20 IU) and 48 h (247.16 ± 20.50 IU) after the administration of APAP.

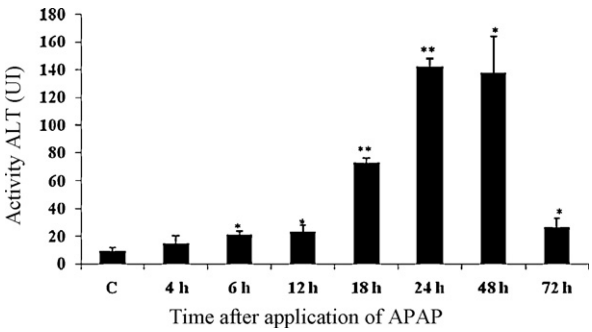

Fig. 3. Activity of serum alanine aminotransferase (ALT) measured at the indicated times after a single dose of APAP (835 mg/kg, po). Data are represented as the mean ± standard deviation. $^{*}p < 0.05$ compared to the control, $^{**}p < 0.001$ compared to the control.

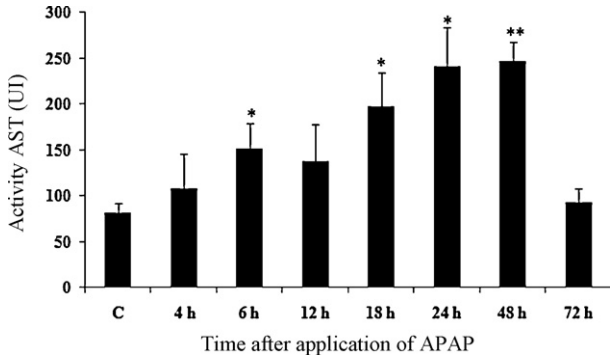

Fig. 4. Activity of serum aspartate aminotransferase (AST) measured at the indicated times after a single dose of APAP (835 mg/kg, po). Data are represented as the mean ± standard deviation. $^{*}p < 0.05$ compared to the control, $^{**}p < 0.001$ compared to the control.

3.3. *In vivo test: the effect of the hydroethanolic extract of Baccharis trimera on the modulation of reactive oxygen species in the neutrophils of rats treated with APAP*

The results in Fig. 5 show that the oral administration of APAP induced a significant increase in ROS production (1768 ± 447 RLU) when compared to the control (329 ± 283 RLU). In animals pretreated with the *Baccharis trimera* extract or quercetin and later with APAP, we also observed a significant reduction in ROS production (917 ± 344 or 52 ± 33 RLU, respectively) when compared with the neutrophils of rats receiving APAP only. Only the animals treated with *Baccharis trimera* extract alone showed no significant reduction in the production of ROS. However, animals treated with quercetin alone showed significant reduction in the production of ROS.

4. Discussion

Studies with *Baccharis trimera* report that this species has important antioxidant and anti-inflammatory properties. In this context, to examine the role of *Baccharis trimera* on the ROS modulation in neutrophils, we tested three different concentrations of the extract in order to achieve greater inhibition and cell viability. After the tests, we found that different concentrations of *Baccharis trimera* extract were able to reduce the release of ROS in neutrophils. This reduction was dose-dependent, since an extract concentration of 50.0 μg/mL showed the highest inhibition of ROS compared to an extract concentration of only 0.5 μg/mL. However, despite the higher potential for inhibition, the extract concentration of 50.0 μg/mL caused a greater mortality of the cells, corroborating the fact that some chemicals present in the plant are actually toxic when in excess. Given this result, we used the intermediate concentration of 5.0 μg/mL *in vitro*, which was able to inhibit approximately 85% of ROS production while maintaining cell viability above 93%.

The incubation of neutrophils with opsonized particles of zymosan induced a significant increase in the production of ROS. The ZC3b particles trigger both FcR and CR receptors. This activation leads to the generation of various ROS by these cells. The initial ROS produced by activated neutrophils is the superoxide anion ($O_2^{\bullet -}$), which is generated by the NADPH oxidase complex (Dávalos et al., 2009). *Baccharis trimera* extract at a concentration of 5.0 μg/mL was able to reduce ROS release, even if administered after stimulation with zymosan**.** In stimulated neutrophils, the inhibition of chemiluminescence may be mediated by three main mechanisms: cell death, the scavenging of ROS or the inhibition of enzymes involved in the signal transduction pathways of ROS generation in these cells (Van Dyke and Castranova, 1987). The first possibility was initially discarded because the cell viability of each sample was always greater than 93% as determined by the exclusion test with trypan blue. However, the results do not distinguish whether the antioxidant properties found in this study are due to scavenging of ROS through interactions between the antioxidants present in the extract and the oxidant species released, or to the inhibition of enzymes involved in the signal transduction pathways of ROS generation.

The antioxidant properties found in this study are in agreement with the literature data, which show a similar effect on other parameters for different extracts from *Baccharis trimera* and other species of this genus, such as *Baccharis illinita*, *Baccharis platypoda* (Brighente et al., 2007), *Baccharis articulata* (Oliveira et al., 2003), and *Baccharis grisebachii* (Tapia et al., 2004). In face of this effect, research on the chemical composition of *Baccharis trimera* showed that the ethanol extract of the plant is a mixture of five flavonoids: quercetin, luteolin, nepetin, apigenin and hispidulin (Soicke and Leng-Peschlow, 1987), which are known for their antioxidant properties, such as donating hydrogen atoms to the radicals (Jovanovic et al., 1998; Merken and Beecher, 2000). These compounds can also modulate the activity of enzymes present in mammals, such as cytochrome P450 and antioxidant enzymes (Ferguson, 2001). Therefore, it is likely that the antioxidant activity produced by the

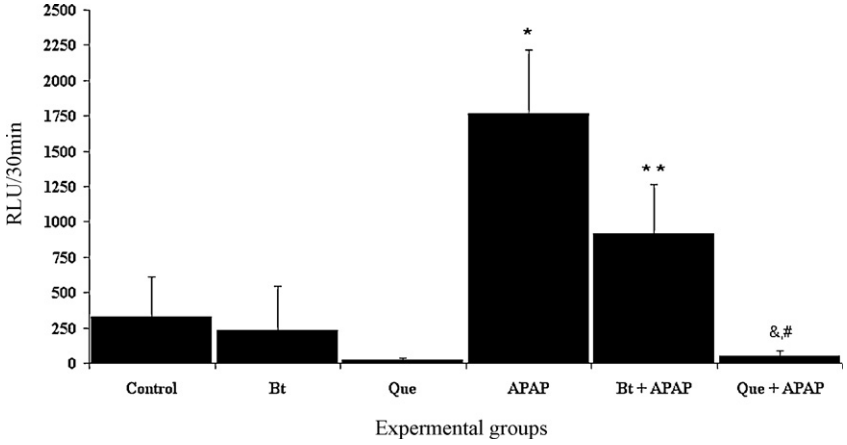

Fig. 5. Effect of the hydroethanolic extract of *Baccharis trimera* and quercetin on the production of ROS in the neutrophils of rats 24 h after treatment with APAP. The rats were treated with a 600 mg/kg dose of *Baccharis trimera* or 50 mg/kg dose of quercetin 1 h before administration of APAP. $^{*}p < 0.0001$ compared to the control, $^{**}p < 0.01$ compared to APAP, $^{\&}p < 0.001$ compared to APAP, $^{\#}p < 0.01$ compared to Bt + APAP.

Baccharis trimera hydroethanolic extract is partly due to the action of flavonoids.

The results from the first *in vitro* studies were used to guide *in vivo* experiments designed to test the inflammation induced by APAP. To achieve this goal, the first step was to determine at what time after the APAP dose we would find more liver damage and, consequently, increased recruitment of neutrophils. Studies using APAP as a model of inflammation, such as those by Liu et al. (2006), Lawson et al. (2000) and Smith et al. (1998), demonstrate that the administration of a toxic dose of APAP leads to neutrophil recruitment and a consequent increase in ROS generation. In this context, this study has demonstrated through the activity of ALT and AST that the administration of a sublethal dose of APAP led to significant liver injury after a period of 24 h. These observations corroborate other studies that found similar effects with high doses of APAP (Smith et al., 1998; Yen et al., 2008).

APAP is known as an analgesic and antipyretic drug that is safe in therapeutic doses. However, in high amounts it can damage the liver and kidneys and even cause death in humans and laboratory animals (Jollow et al., 1974). Therapeutic doses of APAP are rapidly metabolized in the liver, mainly through glucuronidation and sulfation. A small portion is oxidized by cytochrome P450 2E1 to generate a highly reactive and cytotoxic intermediate, N-acetyl-p-benzoquinone imine (NAPQI) (Vermeulen et al., 1992; Lee et al., 1996), which is rapidly conjugated by liver glutathione (GSH) to generate a non-reactive water-soluble product, mercapturic acid.

Although the role of neutrophils in the pathophysiology of APAP-induced hepatotoxicity is still controversial (Jaeschke, 2005), studies show that a high dose of this drug is able to induce neutrophil recruitment, culminating in the increased production of ROS (Bautista et al., 1994; Zhang et al., 1994).

It is possible to examine the close relationship between the free radicals produced by neutrophils and the hepatotoxicity induced by APAP by using this model to elucidate the *in vivo* antioxidant effects attributed to *Baccharis trimera*.

With regard to this assay, we observed that the *Baccharis trimera* extract had a tendency to reduce basal ROS production in neutrophils. On the other hand, the *Baccharis trimera* extract significantly reduced ROS production in the neutrophils of animals pretreated with the extract and then with APAP. *In vivo* experimental results suggest that the *Baccharis trimera* extract can act systemically. In this case, a possible mechanism for oxidant species modulation is the inhibition of the enzymes responsible for neutrophil ROS production. Since the analyses were performed 24 h after treatment with the extract, it was sufficient time to induce changes in the expression pattern of enzymes such as NADPH oxidase. However, other studies are needed to confirm this hypothesis and to clarify the exact point of action of these antioxidant compounds. The antioxidant properties of *Baccharis trimera* were also confirmed by further analysis of the thiobarbituric acid reactive substance (TBAR) levels, which indicated that animals treated with *Baccharis trimera* extract and then with APAP (2.87 ± 1.04) showed lower TBAR levels than animals that received APAP alone (5.01 ± 0.93) (results not shown). As discussed above, this effect can be attributed to the flavonoids, as noted for quercetin in this study and by others who observed the pronounced antioxidant activity and a great ability to modulate enzymes, NADPH oxidase (Dávalos et al., 2009).

In this context, by reducing the production of ROS in neutrophils, *Baccharis trimera* extract seems to be acting in order to minimize any further damage to the liver. This immunomodulatory role demonstrated by the treatment with *Baccharis trimera* extracts was also demonstrated by Paul et al. (2009), who found that carrageenan induced a significant reduction in the number of neutrophils in rat pleurisy.

The data obtained in this study showed significant *in vitro* and *in vivo* antioxidant activity of the *Baccharis trimera* hydroethanolic extract, which is capable of reducing the release and/or production of ROS in neutrophils. In this context, the natural compounds present in *Baccharis trimera* can protect the body from free radicals and slow the progress of many chronic diseases. Although we have not analyzed the effect of other cell types and cytokines involved in the inflammatory process on the ability of *Baccharis trimera* extract to modulate the release and/or production of free radicals in neutrophils, the main cells recruited during inflammation, this plan ensures that, at least in part, there is also an anti-inflammatory function. To substantiate this idea, further studies are needed in order to understand the role of *Baccharis trimera* extract in the inflammatory process.

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References

Akihisa, T., Yasukawa, K., Uma, H., Kasahara, T., Yamanouchi, S., Takido, M., Kumaki, K., Tamura, T., 1996. Triterpene alcohols from the flowers of Compositae and their anti-inflammatory effects. Phytochemistry 43, 1255–1260.

Babior, B.M., 2000. Phagocytes and oxidative stress. American Journal of Medicine 109, 33–44.

Bagchi, K., Puri, S., 1998. Free radicals and antioxidants in health and disease. Eastern Mediterranean Health Journal 4, 350–360.

Bautista, A.P., Spolarics, Z., Jaeschke, H., Smith, C.W., Spitzer, J.J., 1994. Antineutrophil monoclonal antibody (1F12) alters superoxide anion release by neutrophils and Kupffer cells. Journal of Leukocyte Biology 55, 328–335.

Bicalho, H.M.S., Gontijo, M.C., Nogueira-Machado, J.A., 1981. A simple technique for simultaneous human leuckocytes separation. Journal of Immunology 40, 115–116.

Brighente, I.M.C., Dias, M., Verdi, L.G., Pizzolatti, M.G., 2007. Antioxidant activity and total phenolic content of some Brazilian species. Pharmaceutical Biology 45, 156–161.

Carneiro, M.A.A., Fernandes, G.W., 1996. Herbivoria. Ciência Hoje 20, 35–39.

Chaves, M.M., Rocha-Vieira, E., Lima e Silva, R., Nogueira-Machado, J.A., 2000. Increase of reactive oxygen (ROS) and nitrogen (RNS) species generated by phagocyting granulocytes related to age. Mechanisms of Ageing and Development 119, 1–8.

Dávalos, A., De La Peña, G., Sánchez-Martín, C.C., Guerra, M.T., Bartolomé, B., Lasunción, M.A., 2009. Effects of red grape juice polyphenols in NADPH oxidase subunit expression in human neutrophils and mononuclear blood cells. British Journal of Nutrition 102, 1125–1135.

Dela-Loggia, R., Tubaro, A., Sosa, S., Becker, H., Saar, S.T., Isaac, O., 1994. The role of triterpenoides in the anti-inflammatory activity of *Calendula officinalis* flowers. Planta Medica 60, 516–520.

Emerit, J., Chaudiere, J., 1989. In: Miguel, J., Quintanilha, A.T., Weber, H. (Eds.), Handbook of Free Radicals and Antioxidants in Biomedicine, vol. 1. CRC Press, pp. 162–192.

Ferguson, L.R., 2001. Role of plant polyphenols in genomic stability. Mutation Research 475, 89–111.

Gamberini, M.T., Skorupa, L.A., Souccar, C., Lapa, A.J., 1991. Inhibition of gastric secretion by a water extract from *Baccharis triptera*, Mart. Memórias do Instituto Oswaldo Cruz 86, 137–139.

Gené, R.M., Cartana, C., Rarella, T., Cañigueral, S., 1996. Anti-inflammatory and analgesic of *Baccharis trimera*: identification of its active constituents. Planta Medica 62, 232–235.

Grance, S.R.M., Teixeira, M.A., Leite, R.S., Guimarães, E.B., Siqueira, J.M., Filiu, W.F.O., Vasconselos, S.B.S., Vieira, M.C., 2008. *Baccharis trimera*: effect on hematological and biochemical parameters and hepatorenal evaluation in pregnant rats. Journal of Ethnopharmacology 117, 28–33.

Jaeschke, H., 2005. Role of inflammation in the mechanism of acetaminophen-induced hepatotoxicity. Expert Opinion on Drug Metabolism & Toxicology 1, 389–397.

Jollow, D.J., Thorgeirsson, S.S., Potter, W.Z., Hashimoto, M., Mitchell, J.R., 1974. Acetaminophen-induced hepatic necrosis. Pharmacology 12, 251–271.

Jovanovic, S.V., Steenken, S., Simic, M.G., Hara, Y., 1998. Antioxidant properties of flavonoids: reduction potentials and electron transfer reaction of flavonoid radicals. In: Rice Evans, C., Parcker, L. (Eds.), Flavonoid in Health and Disease. Marcel Dekker, New York, pp. 137–161.

Halliwell, B., 1994. Free radicals, antioxidants and human disease: curiosity, cause or consequence? Lancet 344, 721–724.

Lawson, J.A., Farhood, A., Hopper, R.D., Bajt, M.L., Jaeschke, H., 2000. The hepatic inflammatory response after acetamoniphen overdose: role of neutrophils. Toxicological Sciences 54, 509–516.

Lee, S.S.T., Buter, J.T.M., Pineau, T., Fernandez-Salguero, P., Gonzalez, F.J., 1996. Role of CYP2E1 in the hepatotoxicity of acetaminophen. The Journal of Biological Chemistry 271, 12063–12067.

Liu, Z.X., Han, D., Gunawan, B., Kaplowitz, N., 2006. Neutrophil depletion protects against murine acetaminophen hepatotoxicity. Hepatology 43, 1220–1230.

Merken, H.M., Beecher, G.R., 2000. Measurement of food flavonoids by high performance liquid chromatography. Journal of Agricultural and Food Chemistry 48, 577–599.

Middleton Jr., E., Kandaswami, C., Theoharides, T.C., 2000. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacological Reviews 52, 673–751.

Miltersteiner, A., Miltersteiner, D., Filho, N.P., Frota, A.R., Ely, P.B., Zettler, C.G., Marroni, C.A., Marroni, N.P., 2003. Uso de quercetina a longo prazo em ratos cirróticos. Acta Cirúrgica Brasileira 18, 232–237.

Nogueira-Machado, J.A., Lima e Silva, F.C., Mares-Guia, M.L., Costa, D.C., Chaves, M.M., 2003. Discrimination between granulocytes from type I and type II diabetes patients by calorimetry. Thermochimica Acta 395, 115–120.

Oliveira, A.C.P., Endringer, D.C., Amorim, L.A.S., Brandão, M.G.L., Coelho, M.M., 2005. Effect of the extracts and fractions of *Baccharis trimera* and *Syzygium cumini* on glycaemia of diabetic and non-diabetic mice. Journal of Ethnopharmacology 102, 465–469.

Oliveira, S.Q., Dal-Pizzol, F., Gosmann, G., Guillaume, D., Moreira, J.C.F., Schenkel, E.P., 2003. Antioxidant activity of *Baccharis articulata* extracts: isolation of a new compound with antioxidant activity. Free Radical Research 37, 555–559.

Paul, E.L., Lunardelli, A., Caberlon, E., Oliveira, C.B., Santos, R.C.V., Biolchi, V., Bastos, C.M.A., Moreira, K.B., Nunes, F.B., Grace, G., de Oliveira, J.R., 2009. Anti-inflammatory and immunomodulatory effects of *Baccharis trimera* aqueous extract on induced pleurisy in rats and lymphoproliferation *in vitro*. Inflammation 32, 419–425.

Rodrigues, C.R.F., Dias, J.H., Mello, R.N., Richter, M.F., Picada, J.N., Ferraz, A.B.F., 2009. Genotoxic and antigenotoxic properties of *Baccharis trimera* in mice. Journal of Ethnopharmacology 125, 97–101.

Smith, G.S., Nadig, D.E., Kokoska, E.R., Solomon, H., Tiniakos, D.G., Miller, T.A., 1998. Role of neutrophils in hepatotoxicity induced by oral acetaminophen administration in rats. Journal of Surgical Research 80, 252–258.

Soicke, H., Leng-Peschlow, E., 1987. Characterization of flavonoids from *Baccharis trimera* and their antihepatotoxic properties. Planta Medica 53, 37–39.

Tapia, A., Rodriguez, J., Theoduloz, C., Lopez, S., Feresin, G.E., Schmeda-Hirschmann, G., 2004. Free radical scavengers and antioxidants from *Baccharis grisebachii*. Journal of Ethnopharmacology 95, 155–161.

Van Dyke, K., Castranova, V., 1987. Cellular Chemiluminescence, vol. 1. CRC Press, Boca Raton, pp. 162–192.

Verdi, L.G., Brighent, I.M.C., Pizzolatti, M.G., 2005. Gênero *Baccharis (Asteraceae)*: aspectos químicos, econômicos e biológicos. Química Nova 28, 85–94.

Vermeulen, N.P.E., Bessems, J.G.M., Vandestraat, R., 1992. Molecular aspects of paracetamol-induced hepatotoxicity and its mechanism-based prevention. Drug Metabolism Reviews 24, 367–407.

Weiss, S., 1989. Tissue destruction by neutrophils. New England Journal of Medicine 320, 365–379.

Yen, F.L., Wua, T.H., Lin, L.T., Cham, T.M., Lin, C.C., 2008. Nanoparticles formulation of *Cuscuta chinensis* prevents acetaminophen-induced hepatotoxicity in rats. Food and Chemical Toxicology 46, 1771–1777.

Zhang, J.X., Jones, D.V., Clemens, M.G., 1994. Effect of activation on neutrophil-induced hepatic microvascular injury in isolated rat liver. Shock 4, 273–278.