academicJournals

Vol. 8(20), pp. 727-730, 25 May, 2014
DOI: 10.5897/JMPR2014.5359
Article Number: 145202F44838
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plant Research

Short Communication

Gastroprotective and immunoadjuvant activities of butanolic extract of *Calliandra haematocephala*

Antony de Paula Barbosa

Instituto de Pesquisas de Produtos Naturais, Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro, CEP 21941-971 Rio de Janeiro, Brazil.

Received 12 January, 2014; Accepted 16 May, 2014

Calliandra haematocephala **(Leguminosae) is a native species found in tropical America. This plant is widespread and cultivated with ornamental purposes in gardens and parks. Previous studies with other species of this genus confirmed some pharmacological properties, such as antiinflammatory, anticonvulsant, immunomodulatory, and mainly antiulcerogenic activity. Phytochemical investigations have been carried out on the constituents of this genus and demonstrated the presence of tannins, flavonoids and saponins. In order to confirm the ethnopharmacological use of this species, a phytochemical screening was performed with a butanolic extract and its gastroprotective and immunoadjuvant properties were evaluated. The gastroprotective effects were analyzed by measuring acute gastric lesions induced by acidified ethanol, using cimetidine as reference compound. The immunoadjuvant activity was evaluated against ovalbumin antigen, since the delayed type hypersensitivity reaction was measured as an *in vivo* assay of cellular immune response. In both experiments, the results of the biological properties were corroborated, which justifies the use of this plant in traditional medicine as stomach protector and immunomodulatory.**

Key words: *Calliandra haematocephala*, gastroprotective effects, immunoadjuvant activity.

INTRODUCTION

The genus *Calliandra* (Fabaceae) contains 132 species. Most of them are native of America, but few are of Asia and Africa (Tani et al., 1998). *Calliandra haematocephala* (Leguminosae) is native from tropical Americans, usually cultivated in gardens for ornamental purposes. Previous studies with other species of this genus confirmed some pharmacological properties, such as antiinflammatory, anticonvulsant, immunomodulatory, and mainly anti-ulcerogenic activity. Aqueous extracts of the branches of *Calliandra anomala* are used as an antimalarial and antifebrile agent on Mexico (Zeid et al., 2006). *Calliandra pulcherrima*, *Calliandra brevifolia* and *C. haematocephala* are related native species found in Tropical America. These evergreen plants are non invasive, but widespread ornamental species cultivated in gardens and parks. In Brazil, the aqueous extract of the aerial parts of *C. pulcherrima* is used as a remedy for malaria and leishmaniasis (Da Silva et al., 2005). Phytochemical investigations have been carried out on the constituents of this genus and demonstrated the presence of tannins,

*Corresponding author. E-mail: antonybarbosa@hotmail.com.

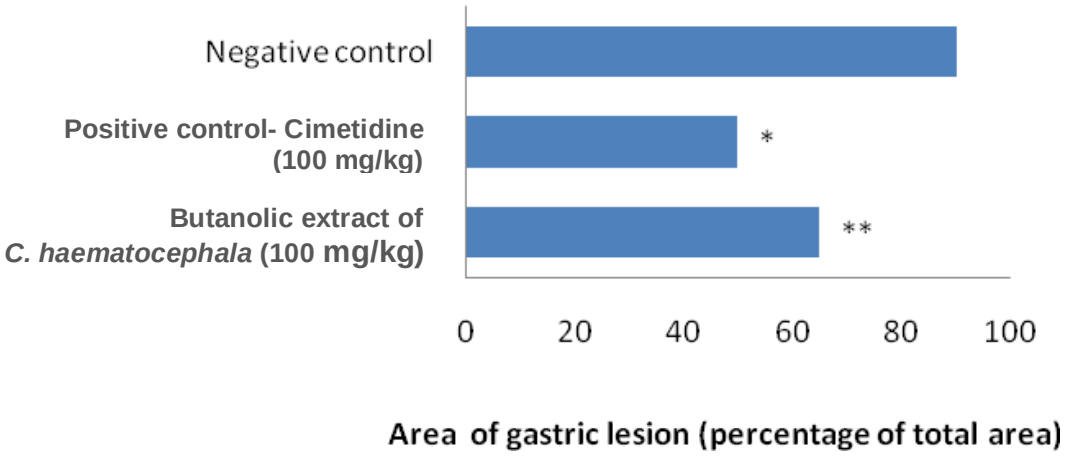

Figure 1. Area of gastric lesion (percentage of total area) in Swiss mice using cimetidine as positive control in comparison with the butanolic extract of *C. haematocephala*. Results are mean ± standard error of mean (SEM) (n=5); *p<0.05, **p<0.01 significantly different to the saline control. Student's t-test.

flavonoids and saponins (Zeid et al., 2007). In this work, the gastroprotective effects and the immunoadjuvant activity of the butanolic extract from the aerial parts of *C. haematocephala* were reported.

MATERIALS AND METHODS

Extraction

The aerial parts of the plant (200 g) were extracted with MeOH (1 L) for 72 h. The extract was concentrated under reduced pressure, and the resulting aqueous phase was shaked with *n*-BuOH (water/*n*-BuOH (1:1) v/v). The resulting organic phase was evaporated in vacuum to give a crude material (15 mg), which was used in both experimental models as the following.

Gastroprotective effects

Antiulcerogenic activity was evaluated by measuring acute gastric lesions induced by acidified ethanol. Male Swiss mice (three months old, 25 to 35 g) in groups of five were fasted for 24 h before the experiment and administered orally with 1 ml of pure water as the negative control, or butanolic extract (100 mg/kg), or the reference compound cimetidine (100 mg/kg) dissolved in vehicle as positive control. One hour after the treatments, all animals received orally 200 µl of acidified ethanol solution (0.3 M HCl/EtOH) to induce gastric lesions. The animals were killed after 1 h treatment with the ulcerogenic agent and the stomachs removed, opened along the greater curvature and rinsed with physiological saline to determine the lesion damage. The degree of gastric mucosal damage was evaluated from digital pictures using a computerized image analysis system. The percentage of the total lesion area (hemorrhagic lesions) to the total surface area of the stomach was defined as the ulcer index (Hamauzu et al., 2008).

Immunoadjuvant activity

Male Swiss mice (three months old) were subcutaneously immunized twice at weekly intervals with 100 µl saline (SAL) as the control group or 100 µg ovalbumin (OVA) mixed with 100 µg of each adjuvant dissolved in 100 µl of saline as vehicle. Delayed type hypersensitivity (DTH) responses were assessed by measuring the increment in the right footpad; thicknesses were after subcutaneous challenge with 100 µg OVA in 100 µl saline a week after the second immunization. The footpad thickness was measured with a spring-loaded dial gauge before and 24, 48 and 72 h after injection. Injecting each animal with 100 µl saline in the left hind footpad served as controls. The ovalbumin specific responses were obtained by subtracting the response to OVA challenge in unimmunized control mice (Mowat et al., 1991).

RESULTS AND DISCUSSION

Gastroprotective effects

The butanolic extract exhibited a moderate control of gastric lesions (35% of inhibition at 100 mg/kg), provoking an inhibition of the development of the hemorrhage and necrotic aspects of tissue injury; how-ever, showing lesser activity than the reference compound at the same dosage (50% of inhibition at 100 mg/kg) (Figure 1). The intensity of gastric ulcers was quantified by the percentage of the injury area in relation to the control group. The results obtained confirm the gastroprotective activity of the butanolic extract of *C. haematocephala*, which probably interfere with the ulcerogenic mechanism, through the synthesis or degradation reduction of prostaglandins responsible, along with other factors, for the gastric cytoprotection.

Immunoadjuvant activity

Several important biological properties have been attributed to saponins. Since the original observation that certain saponins cause substantial enhancement of immune responses when given together with an antigen in a vaccine, their use as adjuvants received special attention.

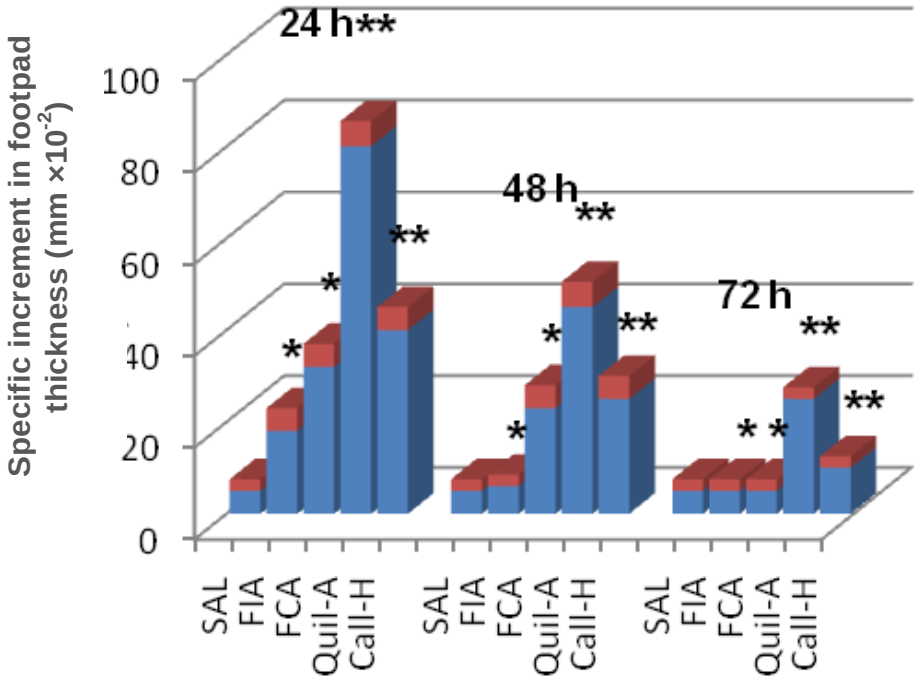

Figure 2. Delayed type hypersensitivity responses after two subcutaneous immnunizations with 100 µg of ovalbumin and 100 µg of each adjuvant. Results are mean ± standard error of mean (SEM) (n=5); *p<0.05, **p<0.01 significantly different to the saline control. Student's t-test.
Abbreviations: SAL, saline solution; FCA, Freund's complete adjuvant; FIA, Freund's incomplete adjuvant; Quill-A, commercial extract of *Quillaja saponaria*; Call-H, butanolic extract of *C. haematocephala.*

The immunoadjuvant action of saponins can be observed through the cellular responses that promote the fixation of complement factors and the release of certain cytokines such as interleukin (IL)-2 and interferon (IFN), and also the humoral response, resulting in increased circulation and secretion of antibodies and cytokines such as IL-4, IL-5, IL-6 and IL-10. In order to investigate the biological properties of the butanolic extract from the aerial parts of *C. haematocephala*, it was evaluated for immunoadjuvant activity and compared with adjuvants commonly used in experimental models. The immunodadjuvant property was evaluated against ovalbumin antigen, since the delayed type hypersensitivity reaction was measured as an *in vivo* assay of cellular immune response. Mice immunized with ovalbumin conjugated with extracts showed remarkable responses greater than those when the antigen was combined with commercial adjuvants. This response developed rapidly after immunization and persisted at lower levels for at least three days. The results obtained suggest the relevant adjuvant potential of the butanolic extract from *C. haematocephala* in comparison with the commercial extract of *Quillaja saponaria*, a commonly used adjuvant for experimental vaccine formulations (Figure 2) (Marciani et al., 2000).

Conflict of Interest

Authors declare no conflict of interest.

REFERENCES

Da Silva BP, Soares JBRC, De Souza EP, Palatnik M, De Sousa CBP, Parente JP (2005). Pulcherrimasaponin, from the leaves of *Calliandra pulcherrima*, as adjuvant for immunization in the murine model of visceral leishmaniasis. Vaccine 23:1061-1071.
Hamauzu Y, Irie M, Kondo M, Fujita T (2008). Antiulcerative properties of crude polyphenols and juice of apple, and *Chinese quince* extracts. Food Chem. 108:488-495.
Marciani DJ, Press JB, Reynolds RC, Pathak AK, Pathak V, Gundy LE, Farmer JT, Koratich MS, May RD (2000). Development of semisynthetic triterpenoid saponin derivatives with immune stimulating activity. Vaccine 18:3141-3151.
Mowat AM, Donachie G, Reid, Jarrett O (1991). Immune-stimulating complexes containing Quil A and protein antigen prime class I MHC-restricted T lymphocytes in vivo and are immunogenic by the oral route. Immunology 72:317-322.
Tani C, Ogihara Y, Takeda T (1998). Studies on the constituents of *Calliandra anomala* (Kunth) Macbr. IV. Structure analysis by HPLC retention time and FAB-MS spectrum. Chem. Pharm. Bull. 46:723-

725.
Zeid AA, Hifnawy M, Saleh M, Sleem A, Mohamed R (2006). Flavonoids, volatiles and biological activities of the aerial parts of *Calliandra haematocephala* Hassk. Planta Med. 72(11): 1080-1080.
Zeid AHS, Hifnawy MS, Sleem AA, Mohamed RS (2007). Chemical constituents and anti-inflammatory activity of the aerial parts of *Calliandra haematocephala* Hassk. Planta Med. 73(9): P_009.