

Plant Science

SHORT COMMUNICATION

Anti-inflammatory properties and immunoadjuvant activity of *Samanea saman* extract

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, Brasil

Abstract

Samanea saman is a Leguminosae widely distributed in tropical regions, especially in Brazil. In folk medicine is used to treat skin infections, gastric inflammation and parasitic infestations, but there is no report in literature on its phytochemical. In order to confirm the utilization of this species in the traditional medicine, the anti-inflammatory activity of the chloroform extract and the immunoadjuvant activity of the butanolic extract were investigated. The chloroform extract exhibited a moderate control of the initial phase of inflammation, provoking an inhibition of edema formation similar to the reference compound dexamethasone. The butanolic extract showed a relevant adjuvant potential in comparison with the commercial extract of *Quillaja saponaria*, a commonly used adjuvant for experimental vaccine formulations.

Key words: Anti-inflammatory properties, immunoadjuvant activity, *Samanea saman*

Introduction

Samanea saman (Jacq.) Merr. is a tree, native of the New World, widely distributed in tropical regions, grown under shade and ornamental purposes. In folk medicine is called as Rain tree and is used to treat skin infections, gastric inflammation and parasitic infestations. There are several remedies prepared from various parts of this plant. The boiled bark is applied as a poultice to cure constipation. In the Philippines, a decoction of the inner bark and fresh leaves is used for diarrhea. In Venezuela, the roots are made into a hot bath for stomach cancer. In the West Indies, the seeds are chewed for sore throat (Staples and Elevitch, 2006). Preliminary qualitative phytochemical screening of *Samanea saman* were reported and showed the presence of tannins, flavonoids, steroids, saponins, cardiac glycosides and terpenoids (Prasad et al., 2008). In the Brazil studies on the constituents of *Samanea tubulosa*, an allopatric species from

Samanea saman, showed a significant amount of condensed tannin in its bark (Gonçalves and Lelis, 2001). Other substances with immunomodulating properties have been reported by isolation from plants of another genus, but from the same family. A complex triterpenoid saponin with adjuvant activity for immunization in the murine model of visceral leishmaniasis was isolated from *Calliandra pulcherrima* (Barbosa et al., 2008). Many researches on the constituents of the other species of this genus *Calliandra* have been conducted in an attempt to isolate substances with immunomodulatory properties. In this work, we will report the antiinflammatory properties of the chloroform extract of the leaves and the immunoadjuvant activity of the butanolic extract of the stem bark of this plant.

Materials and Methods

Antiinflammatory properties

Leaves of *Samanea saman* (100g) were extracted with chloroform for 72 hours. The extract was concentrated under reduced pressure, yielding a crude extract (50mg). The anti-inflammatory activity was determined by inhibition of carrageenan-induced mouse paw edema, using dexamethasone as reference compound. Fifteen male Swiss mice were divided into three groups. In the first group was administered 1mL of saline, which serves as negative control, in the second

Received 13 January 2014; Revised 12 June 2014; Accepted 13 June 2014; Published Online 01 July 2014

*Corresponding Author

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, Brasil

Email: antonybarbosa@hotmail.com

group was administered the butanolic extract (100mg/kg), and in the third group was administered dexamethasone (25mg/kg), the positive control. One hour after the oral administration an acute inflammation was produced by subplantar injection of 50mL of 1% freshly prepared colloidal suspension of carrageenan in physiological saline injected into subplantar region of the right hind paw of the mice. The footpad thickness were measured with a spring-loaded dial gauge before and 1 hour every during 5 hours after induction of inflammation (Wang et al., 2008).

Immuadjuvant activity

The stem bark of the plant (200g) was extracted with methanol (1L) for 72h. The extract was concentrated under reduced pressure, and the resulting aqueous phase was shaken with *n*-butanol [water/*n*-butanol (1:1) v/v]. The resulting organic phase was evaporated *in vacuo* to give a crude material (960mg), which was used in the immunization procedure. The presence of saponins in butanolic extract was confirmed by TLC employing assays using orcinol-H₂SO₄ as reagent cartridge. Male Swiss mice (three months old) were subcutaneously immunized twice at weekly intervals with 100μL saline (SAL) as the control group or 100μg ovalbumin (OVA) mixed with 100μg of each adjuvant dissolved in 100μL of saline as vehicle. Delayed type hypersensitivity (DTH) responses were assessed by measuring the increment in the right footpad thickness after subcutaneous challenge with 100μg OVA in 100μL saline a week after the second immunization. The footpad thickness was measured with a spring-

loaded dial gauge before and 24, 48 and 72h after injection. Injecting each animal with 100μL 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).

Statistical Analysis

The data obtained from the both test were expressed as mean \pm SEM and analyzed using one way ANOVA followed by Student's *t*-test. *P* values less than 0.05 and 0.01 ($P < 0.05$ and $P < 0.01$) were considered to be statistically significant.

Results and Discussion

Antiinflammatory properties

Crude extracts of *Samanea saman* showed anti-inflammatory activity confirmed in acute and chronic experimental models. Therefore, to confirm the use of this plant in traditional medicine for various inflammatory disorders, the anti-inflammatory potential of chloroform extract was available by inhibition of carrageenan-induced mouse paw edema using dexamethasone as reference compound. The increase in paw volume was observed in mice of the negative control group that received only the induction of inflammation by carrageenan. In the group that was administered the chloroform extract observed a significant decrease in the volume of paw edema of mice, thus suggesting an anti-inflammatory activity. However, that decreasing in paw volume of mice was not greater than the positive control dexamethasone (Figure 1).

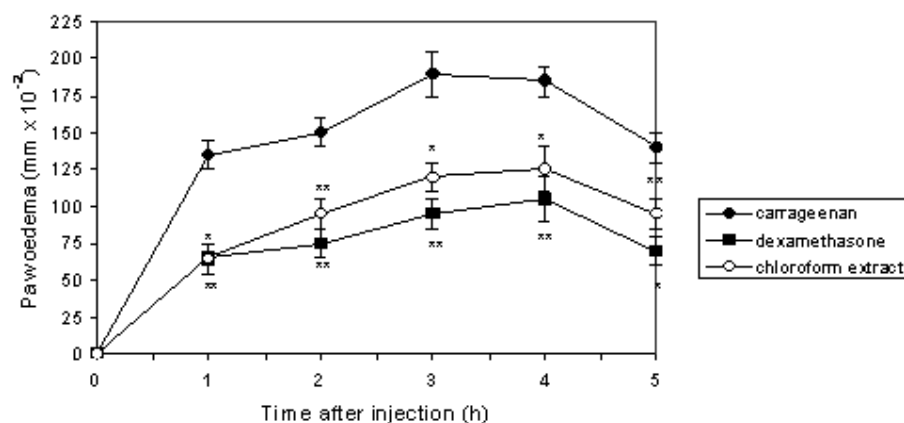


Figure1. Anti-inflammatory activity of the chloroform extract (100 mg/kg) and the reference compound dexamethasone (25 mg/kg) against mouse paw edema induced by carrageenan. Results are mean \pm S.E.M. (n = 5); * $p < 0.05$, ** $p < 0.01$, significantly different from the control group.

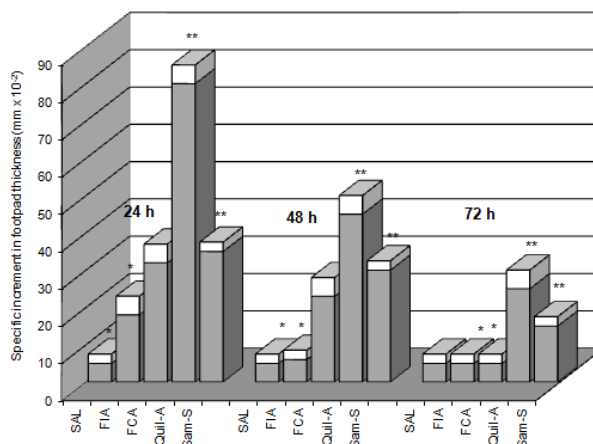


Figure 2. Delayed type hypersensitivity responses after two subcutaneous immunizations with 100µg of ovalbumin and 100µg of each adjuvant. Results are mean \pm S.E.M. (n=5); *p<0.05, **p<0.01 significantly different to the saline control. Student's t-test. Abbreviations: SAL, saline solution; FCA, Freund's complete adjuvant; FIA, Freund's incomplete adjuvant; Quil-A, commercial extract of *Quillaja saponaria*; Sam-S, butanolic extract of *Samanea saman*.

Immunoadjuvant activity

Several important biological activities have been attributed to the saponins, which are steroidal or triterpenoid molecules with one or more chains of sugars. Some saponins are known by the fact of presenting increased immune responses when administered together with antigens in vaccine models, featuring its adjuvant activity. Thus, in order to investigate the biological properties of the extract butanolic from the stem bark of *Samanea saman*, it was evaluated for immunoadjuvant activity and compared with adjuvant commonly used in experimental models. The immunoadjuvant 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 significant responses, however smaller than the commercial adjuvants from *Quillaja saponaria* and greater than other commercial adjuvants. This response developed rapidly after immunization and persisted at lower levels for at least three days (Figure 2) (Marciani et al., 2000).

Conclusion

The evaluation of the chloroform extract of *Samanea saman* shows an anti-inflammatory activity, however lower than the reference compound, dexamethasone. The results obtained suggest the relevant adjuvant potential of the butanolic extract from *Samanea saman* in comparison with the commercial extract of *Quillaja*

saponaria, a commonly used adjuvant for experimental vaccine formulations.

Acknowledgements

This work was financially supported by FUJB, CAPES, FINEP and CNPq.

References

- Barbosa, A. P., B. P. Da Silva and J. P. Parente. 2008. Brevifoliasaponin with Adjuvant Activity from *Calliandra brevifolia*. Z. Naturforsch. 63b:894–902.
- Gonçalves, C. A. and R. C. C. Lelis. 2001. Teores de tanino da casca e da madeira de cinco leguminosas arbóreas. Floresta e Ambiente, Seropédica 1:167-173.
- Marciani, D. J., J. B. Press, R. C. Reynolds, A. K. Pathak, V. Pathak, L. E. Gundy, J. T. Farmer, M. S. Koratich and R. D. May. 2000. Vaccine 18:3141-3151.
- Mowat, A. M., A. M. Donachie, G. Reid and O. Jarrett. 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(3):317-322.
- Staples, G. W. and C. R. Elevitch. 2006. *Samanea saman* (Rain tree). Species

- profiles for pacific island agroforest (www.traditionaltree.org), 1-15.
- Prasad R. N., S. Viswanathan, J. R. Devi, V. Nayak, V. C. Swetha, B. R. Archana, N. Parathasarathy and J. Rajkumar. 2008. Preliminary phytochemical screening and antimicrobial activity of *Samanea saman*. J. Med. Plants Res. 2:268-270.
- Wang, H., J. Gao, J. Kou, D. Zou and B. Yu. 2008. Anti-inflammatory activities of triterpenoid saponins from *Polygala japonica*. Phytomedicine 15:321-326.