Research Article

Journal of Clinical & Experimental Immunology

Immunological Exploration of Primary Metabolite Extracted From Aqueous Stem Extract of Caralluma Fimbriata

Amit Gupta[1]* and Bharat Shinde[1,2]

[1]*Department of Immunology and virology, Vidya Pratishthan's School of Biotechnology (VSBT) Baramati, Maharashtra, India*

[2]*Principal, Arts, Science and Commerce College, Baramati, Maharashtra, India*

***Corresponding author**
Amit Gupta, Assistant Professor/Senior Scientist, Department of Immunology and virology, India; Tel: 08308881506; E-mail: amitvsbt@gmail.com; amitgupta@vsbt.res.in.

Submitted: 10 Sep 2016; **Accepted**: 22 Sep 2016; **Published**: 27 Sep 2016

Abstract

Objective: *The objective of our study is to determined its immunological property of primary (protein) metabolite extracted from aqueous stem extract of Caralluma fimbriata against specific protein antigens (lactoferrin and Ovalbumin, OVA) pertaining to determine B cell production in vitro by ELISA (enzyme linked immunosorbent assay) and also measured T cell production in vitro using immature bone marrow cells of mice exposed with Incomplete freunds adjuvant (IFA) and Concanavalin (Con) A.*

Methods: *For these studies, estimation of protein from stem extract of Caralluma fimbriata were determined and also determining the total cellular (protein) content in immature bone marrow cells along with variable concentration of Caralluma fimbriata in presence or absence of IFA. In addition, lactoferrin (100 µg/well) and weak antigen i.e. ovalbumin (OVA; 100 µg/well) pertaining to antibody (IgG) production were determined in vitro through Elisa and also measured its proliferative response using Con A (2.5 µg/ml).*

Results: *The results showed that aqueous stem extract of Caralluma fimbriata showed the presence of protein (0.698 mg/ml; 10 µl) content and also showed the enhancement in total cellular (protein) content in presence or absence of IFA which is determined through NanoDrop method. In addition, this aqueous stem extract showed enhancement in anti-lactoferrin and anti-OVA IgG titre in vitro at higher doses as compared to control but there is sudden decline in bone marrow cell proliferation containing Con A at higher doses.*

Conclusion: *Our data suggest that aqueous stem extract of Caralluma fimbriata may help to raise antibodies in vitro against lactoferrin and OVA but sudden decline in Con A proliferative response in bone marrow cells. In other words, aqueous stem extract containing primary metabolite of Caralluma fimbriata could be a potent immune enhancer of B cells in vitro but inhibitor of T cells at higher doses.*

Keywords: *Caralluma fimbriata*; Lactoferrin; Ovalbumin; Bone marrow cells; Elisa.

Introduction

The evaluation of all these medicinal plant products are totally based on phytochemical and immunopharmacological approaches which lead to drug discovery and they are commonly referred and known as screening of natural products [1,2]. Recently, researchers focused on medicinal plant research and showed its awareness all over the world because of its medicinal properties [3]. These medicinal plants have played a crucial role in maintaining human health for thousands of years and provide valuable components in the form of medicines, beverages, cosmetics and dyes [3,4]. Most of the medicinal plant products in the form of secondary metabolites (i.e. flavonoids, terpenoids, saponin etc.) showed anti-inflammatory, anti-microbial, immunosuppressive activity etc. against specific as well as non-specific antigen [5-8]. In addition, these medicinal plant products are routinely used as raw material for extraction of active ingredients that were used actually in the synthesis and manufacturing of drugs for various purposes. So, using these medicinal plant products can provide an alternative to develop newly synthesized antibiotics, immunosuppressive drugs etc. with fewer side effects [9,10]. In other words, we focused on various medicinal plants which represents rich source of novel drug in the form of immunosuppressive and anti-inflammatory drugs.

Caralluma fimbriata (edible succulent cactus), medicinal plant belongs to the family *Apocynaceae* and genus *Caralluma* which comprised about 200 genera and 2500 species [11,12]. In addition, it showed various medicinal properties for the treatment of various disorders (i.e. Rheumatism, diabetes, leprosy, antiseptics

and disinfectants). Number of evidences were reported related to *Caralluma* extract and traditionally used in India as famine food and showed beneficial effect related to decline in fat calorie intake, feelings of hunger etc [11-13]. The present investigation was undertaken to study various parameters for establishing immunopharmacological profile of aqueous stem extract of *Caralluma fimbriata.*

Materials and Methods

Plant materials

Fresh stem of *Caralluma fimbriata* were collected in the month of September 2016 from Vidya Pratishthan's and this medicinal plant was identified by Botanist, Dr. Bharat Shinde, Principal, Vidya Pratishthan's Arts, Science and Commerce College, Vidyanagari, Baramati.

Preparation of aqueous stem extract

Fresh stem samples of plant *Caralluma fimbriata* were collected, dried in shady area and then crushed in mortar and pestle using liquid nitrogen (hard maceration, -196 ºC) to prepare fine powder. The powder (5g) was macerated and dissolved in phosphate buffered saline (PBS, pH 7.2, 50 ml) and then filtered it. Thereafter, filtrate was collected for qualitative based phytochemical analysis and immunopharmacological assays [14].

Animals

Animals (Swiss mice, body weight, 20 g; n = 3) were collected and perform dissection in Preclinical research centre, Vidya Pratishthan. All these studies were conducted under ethical guidelines with registration number and approved by animal ethics committee.

The mice were humanely sacrificed by cervical dislocation and then Femora were removed and cleaned with 70% ethanol. Thereafter, excess muscle and fat tissues were removed carefully without harming bone's integrity. For these studies, bone marrow cell suspension was obtained from the femora and tibia by flushing out with PBS containing 10 % fetal bovine serum (FBS), using a 26 gauge needle. All these cells were collected in 50 ml tube and then filter it. Finally, count the number of cells using hemocytometer.

Estimation of protein content

Fresh stem powder (2g) were taken in conical flask and add extraction buffer (i.e. 20 mM Tris HCl) dissolved in PBS. Incubate stem powder along with extraction buffer for 5 minutes at room temperature and then centrifuged (6000 rpm; 10 minutes at 4°C). Supernatant was collected after centrifugation and then add similar volume of ice cold acetone. Incubate the solution for 10-15 minutes at room temperature and then centrifuged [15]. Collect the pellet and washed with ice cold acetone to remove the pigments including lipids as well. Finally, protein concentration of stem powder (*Caralluma fimbriata*) was determined by using Nano drop method.

In addition, fresh bone marrow cells were cultured in 24 well plate for 48 h incubation along with variable doses of aqueous stem extract of *Caralluma fimbriata* (0.312 - 5 mg) in presence or absence of incomplete Freunds adjuvant (IFA). After incubation, collect all the cells including adhered ones in eppendorf tube. Centrifuge the samples at 15000 rpm and then collect the supernatant in order to determine the total cellular or protein content using NanoDrop 1000 A280 module. In NanoDrop, Beer-Lambert equation (A = E * b * c) is applied and used for all protein calculations to correlate absorbance with concentration.

Proliferation assay

In an effort to evaluate the effect of aqueous stem extract of *Caralluma fimbriata* on immature bone marrow cells of Swiss mice (using Concanavalin; Con A, 2.5 µg/ml) and determined its proliferation assay using MTT. For these studies, bone marrow cells (100 µl; 105 cells/well) were taken in 96 well tissue culture plate and then treated with variable concentration of aqueous stem extract (0.625 - 5 mg) of *Caralluma fimbriata*. Incubate the samples for 48 hrs at 37°C, 5% carbon dioxide incubator. After incubation, supernatant (100 µl) was discarded and then add fresh PBS containing fetal bovine serum (FBS, 10 %). Add MTT solution (5 mg/ml; 10 µl) and then incubate the plate for another 4 hrs at carbon dioxide incubator. Afterwards, formazan crystals will appear and settled at the bottom and dissolved in dimethyl sulphoxide (DMSO) solution after centrifuging and discarding the supernatant. The optical density (OD) was measured at 570 nm [16,17].

ELISA assay

Indirect Elisa was performed using standard lactoferrin capsule (1:1000 dilution) and OVA (100 µg/well, 0.2 ml) as coating antigen. Variable concentration of aqueous stem extract of Caralluma fimbriata were added and determined its anti-lactoferrin and anti-OVA titre. Anti-lactoferrin and anti-OVA serum were used as standard for the estimation of IgG antibody titre. Horse anti-serum used as secondary antibody and absorbance in the form of optical density measured at 450 nm [18,19].

Results

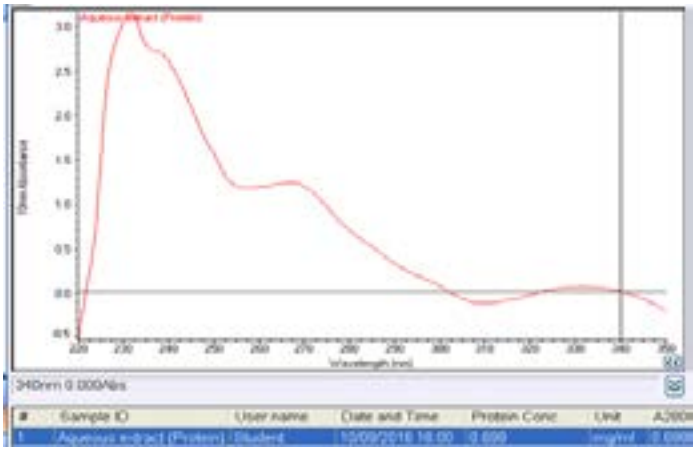
Estimation of primary (protein) and secondary metabolites

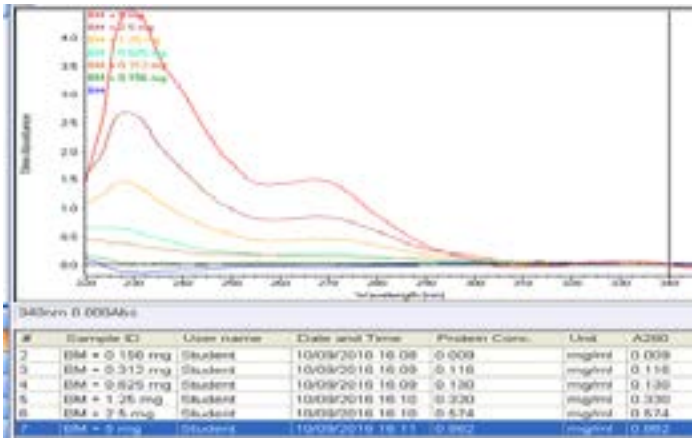
The results showed that aqueous stem extract showed the presence of protein content (10 µl, 0.698 mg/ml) which is determined through NanoDrop as shown in figure 1. In addition, total cellular (protein) content were also measured in bone marrow cells along with variable concentration of Caralluma fimbriata in presence or absence of IFA as shown in figure 1. The results showed that there is dose dependent enhancement in total cellular content as compared to control. In contrast, phytochemical analysis of Caralluma fimbriata revealed the presence of flavonoids, alkaloids and glycosides qualitatively.

Proliferation assay

The effect of aqueous stem extract of Caralluma fimbriata on Con A stimulated proliferative response in immature bone marrow cells of Swiss mice as shown in figure 2. At higher doses, there is markedly decline in Con A proliferation but there is enhancement in Con A proliferation at lower doses as compared to control.

Overall, the data indicates that Caralluma fimbriata inhibits T cell proliferation at higher doses but it also showed stimulatory effect at lower doses as well.





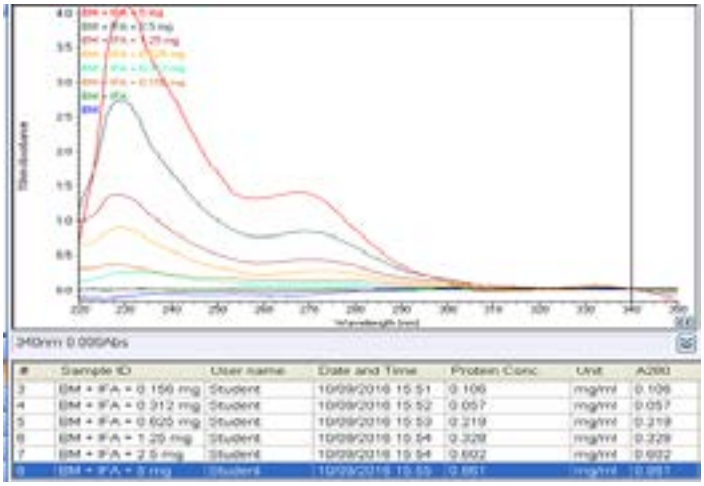


Figure 1: Caralluma fimbriata. **A)** Estimation of protein content from aqueous stem extract. **B & C)** represents total cellular (protein) content in bone marrow cells treating with aqueous stem extract in presence or absence of IFA. Protein concentration is expressed in mg/ml determined through NanoDrop method.

ELISA

Indirect Elisa assay was performed using lactoferrin and OVA as coating antigen as shown in figure 3. The results showed that aqueous stem extract of Caralluma fimbriata raised antibody

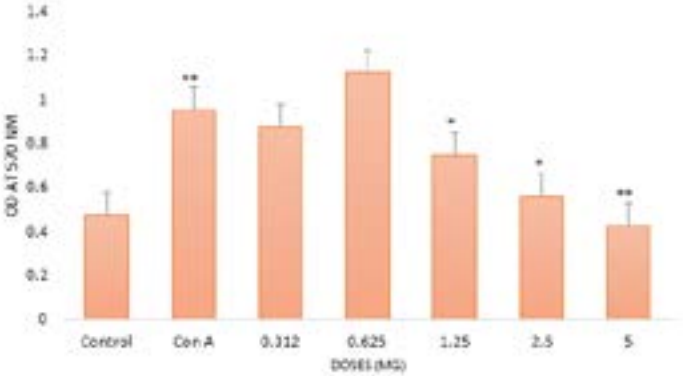


Figure 2: Effect of variable doses of Caralluma fimbriata on bone marrow cell proliferation assay using Con **A.** bone marrow cells (100 µl; 105 cells/well) were treated with variable concentration of aqueous stem extract (0.625 – 5 mg) along with Con A (as already described in materials and methods section). Values are expressed as Mean ± S.E. The difference between the control, standard and treated samples of flavonoids is determined by one way ANOVA test (Boniferroni multiple comparison test). *P<0.05; **P < 0.01 and ***P < 0.001.

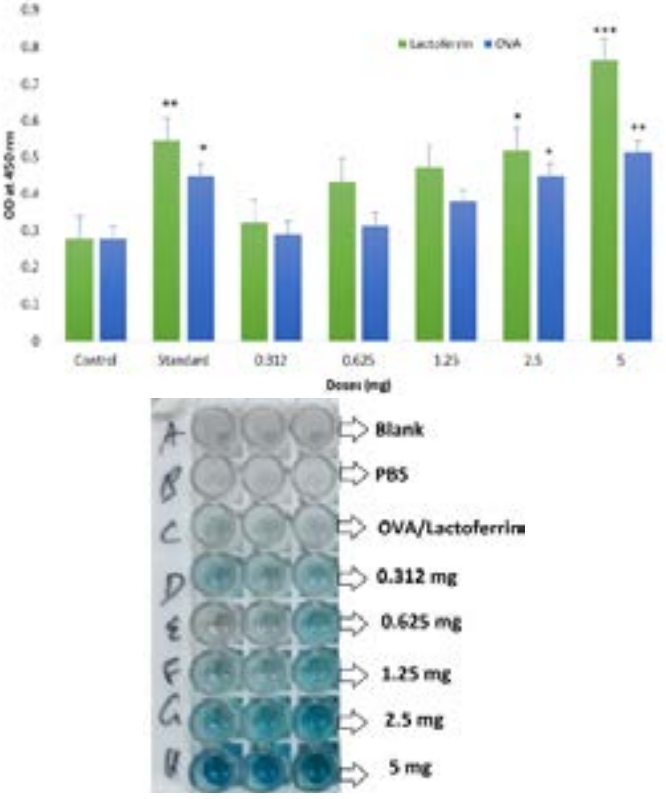


Figure 3: ELISA assay. Indirect Elisa was performed using standard lactoferrin and OVA (100 µg/well) as coating antigen. Aqueous extract of Caralluma fimbriata, anti-lactoferrin and anti-OVA serum from different companies were used for the estimation of anti-lactoferrin and anti-OVA antibody titre. Horse anti-serum used as secondary antibody and optical density measured at 450 nm. The difference between the control and standard is determined by one way ANOVA test. *P<0.05; **P < 0.01 and ***P < 0.001.

production at higher doses as compared to control. In other words, Caralluma fimbriata could be a potent enhancer of B cells against these specific protein antigens.

Discussion

As per the literature, more than 2500 medicinal plants of therapeutic value were mentioned as well as reported in the Ayurvedic and Unani systems of medicine. Out of these, most of them are considered under the category of immunosuppressive compounds. In the present study, we evaluated the effect of aqueous stem extract of Caralluma fimbriata against specific as well as non-specific antigens pertaining to determined B and T cell population.

For the last ten years, researchers reported number of immunosuppressive compounds which is generally involved in inhibiting T cell proliferation. The only difference in all these immunosuppressive compounds extracted naturally or synthetically but differ in their mechanism of action e.g. cyclosporine and betamethasone inhibit T cell proliferation [20].

As per our results indicated that the aqueous stem extract of Caralluma fimbriata not only inhibited T cell population in immature bone marrow cells containing Con A and IFA but there is enhancement in antibody titre at higher doses in vitro (against lactoferrin and OVA) as compared to control. From this data, it indicates that the presence of active immunosuppressive compounds in the aqueous stem extract of Caralluma fimbriata. In an effort to search for new immunosuppressants in the form of primary (protein) metabolites extracted from aqueous stem extract of Caralluma fimbriata. This study reported the effect of aqueous stem extract on T cell immune responses in mouse bone marrow cells against IFA and Con A. The results claimed that at higher doses of Caralluma fimbriata, there is dose dependent decrease in Con A proliferation. From this data, it indicates that Caralluma fimbriata suppress T cell proliferation.

In addition, there is significant enhancement in total cellular (protein) concentration at dose 0.625 mg of Caralluma fimbriata after treating with mouse bone marrow cells in presence of IFA and also showed enhancement in Con A proliferation at lower doses. From this data it may conclude that at lower doses it may showed immunostimulatory activity as well but at higher doses, there is dose dependent reduction in Con A proliferation. Suppressive effect of proliferation at higher doses in the aqueous stem extract of Caralluma fimbriata treated group indicates that the extract suppressed T cell immunity in a specific manner. These results suggest that Caralluma fimbriata may suppress cellular immune response and this study will have a way for future investigators to elucidate a suitable component for suppression the immune system to make an individual recovered from infectious diseases.

Conclusion

Caralluma fimbriata suppressed T cell responses (using Con A, T cell mitogen) at higher doses which is reported in immature bone marrow cells of Swiss mice. Further immunopharmacological studies were conducted in order to understand the mechanism of immunosuppression. In other words, Caralluma fimbriata should be effectively and applied used in different immunopathological conditions as a better anti-inflammatory and immunosuppressive agent.

Authors' Contributions

Dr Gupta designed the study, wrote the protocol and interpreted the data. Dr Shinde identified this medicinal plant. Both the authors read and approved the final manuscript.

Acknowledgements

We would also like to thank our organization (Vidya Pratishthan's School of Biotechnology) for conducting these studies.

References

1. Agarwal SS, Singh VK (1999) Immunomodulators: A review of studies on Indian Medicinal Plants and Synthetic peptides. Proc Indian Natl Sci Acad. 65: 179-204.
2. Gupta A, Khamkar PR, Chaphalkar SR (2014) Applications and uses of active ingredients from medicinal plants. Indian Journal of novel drug delivery 6: 106-111.
3. Thatte UM, Dahanukar SA (1988) Comparative study of immunomodulating activity of Indian medicinal plants, lithium carbonate and glucan. Methods Find Exp Clin Pharmacol 10: 639-644.
4. Agarwal SS, Singh VK (1999) Immunomodulators: A review of studies on Indian Medicinal Plants and Synthetic peptides. Proc Indian Natl Sci Acad 65: 179-204.
5. Gupta A, Chaphalkar SR (2016) Anti-inflammatory and immunosuppressive activities of flavonoids from medicinal plants. Journal of Herb Med Pharmacology 5: 121 -124.
6. Gupta A, Chaphalkar SR (2015) Potential immunosuppressive and anti-inflammatory activity of aqueous extract of Mangifera indica. Advanced herbal medicine 1: 47-54.
7. Gupta A, Chaphalkar SR (2016) Anti-inflammatory and anti-microbial activities of aqueous leaves extract of Butea frondosa. Journal of Herb Med Pharmacology 5: 07-11.
8. Gupta A, Chaphalkar SR (2015) Immunosuppressive activity of flavonoids isolated from Terminalia arjuna, Prosopis spicigera and Mimusops elengi. International Journal of Research in Pharmacy and Science 5: 14-17.
9. Jones FA (1996) Herbs--useful plants. Their role in history and today. Eur J Gastroenterol Hepatol 8: 1227-1231.
10. Gupta A, Khajuria A, Singh J, Bedi KL, Satti NK, et al. (2006) Immunomodulatory activity of biopolymeric fraction RLJ-NE-205 from Picrorhiza kurroa. Int Immunopharmacol 6: 1543-1549.
11. Kuriyan R, Raj T, Srinivas SK, Vaz M, Rajendran R, et al. (2007) Effect of Caralluma fimbriata extract on appetite, food intake and anthropometry in adult Indian men and women. Appetite 48: 338-344.
12. Sudhakara G, Mallaiah P, Sreenivasulu N, Sasi Bhusana Rao B, Rajendran R, et al. (2014) Beneficial effects of hydro-alcoholic extract of Caralluma fimbriata against high-fat diet-induced insulin resistance and oxidative stress in Wistar male rats. J Physiol Biochem 70: 311-320.
13. Dutt HC, Singh S, Avula B, Khan IA, Bedi YS (2012)

Pharmacological review of Caralluma R.Br. with special reference to appetite suppression and anti-obesity. J Med Food 15: 108-119.

14. Shaikh AC, Gupta A, Chaphalkar SR (2016) Quantitative phytochemical credential of Nakshtra plants and distribution analysis of aldehydes pertaining to LC-MS. International Journal of Chemistry and Pharmaceutical Sciences 4: 267-274.
15. Gupta A, Shah AP, Chaphalkar SR (2016) Extraction of proteases from medicinal plants and their potential as anti-viral targets. Journal of Biotechnology and Biomaterials 6: 1000228.
16. Gupta A, Chaphalkar SR (2016) Cytotoxic and antiviral activity of Acacia catechu on human peripheral blood mononuclear cells. Indonesian Journal of Pharmacy 27: 111-116.
17. Gupta A, Chaphalkar SR (2016) Evaluation of the proliferative activity of aqueous extracts from three medicinal plants in murine spleen cells. European Journal of Biological research 6: 170-175.
18. Gupta A, Chaphalkar SR (2015) Immunoadjuvant potential of Azadirachta indica against rabies, hepatitis and DPT vaccine antigen. International Journal of Medical and pharmaceutical sciences 5: 1-5.
19. Gupta A, Potdar J, Chaphalkar SR (2016) Screening of medicinal plants through ELISA against Pacillomyces liacinus and Trichoderma viride. World Journal of Pharmacy and Pharmaceutical sciences (2278–4357) 5: 829-837.
20. Morioka N, Sze LL, Mortan DL, Irie RF (1993) A protein fraction from aged garlic extracts enhances cytotoxicity and proliferation of human lymphocytes mediated by interleukin-2 and concanavalin A. Cancer Immunol Immunother 37: 316-322.