J HerbMed Pharmacol. 2016; 5(2): 85-88.

Journal of HerbMed Pharmacology

Journal homepage: http://www.herbmedpharmacol.com

Anti-inflammatory and anti-microbial activities of aqueous leaves extract of *Butea frondosa*

Amit Gupta*, Sushama R Chaphalkar

Vidya Pratishthan's School of Biotechnology (Research centre affiliated to Savitribai Phule Pune University), Baramati, District Pune, Maharashtra, India

ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 27 December 2015
Accepted: 23 February 2016

Keywords:
Butea frondosa
Anti-inflammatory
Anti-microbial
Hepatitis B vaccine

ABSTRACT

Introduction: *Butea frondosa* has been suggested to be very useful in treating inflammatory diseases but no scientific investigation has been done in such direction. In this study the anti-inflammatory and anti-microbial activities of leaves aqueous extract of *B. frondosa* were determined in infected and non-infected human whole blood against specific vaccine antigen, HBsAg.

Methods: In order to explore the anti-inflammatory and anti-microbial activities of *B. frondosa* (0.5–0 mg/mL; 50 µl), infected (virally) and non infected (control) human whole blood samples were stimulated with hepatitis B vaccine containing surface antigen (HBsAg, 20 µg/mL;10 µl) in order to determined its blood counts and proliferation assay.

Results: Aqueous leaves extract of *B. frondosa* (10 mg/mL; 50 µl) containing HBsAg inhibited the percentage count of monocytes as well as granulocytes population in both cases. In addition, this aqueous extract also reduced its proliferation rate at higher doses.

Conclusion: Aqueous leaves extract of *B. frondosa* possesses both anti-inflammatory and antimicrobial activities and might be used for these purposes.

Implication for health policy/practice/research/medical education:
Butea frondosa has anti-inflammatory and anti-microbial activities and might be used for these purposes.
Please cite this paper as: Gupta A, Chaphalkar SR. Anti-inflammatory and anti-microbial activities of aqueous leaves extract of Butea frondosa. J HerbMed Pharmacol. 2016;5(2):85-88.

Introduction

Medicinal plant products can be used as medicines by majority of cultures all around the world (e.g. Indian and Chinese medicines) and provide a source of new drugs to protect human beings against several diseases (1,2). These activities are due to the presence of various primary (carbohydrates, protein, lipids, etc) and secondary (flavonoids, glycosides, terpenoids, saponin, phenolics, etc) metabolites present in medicinal plants (3). According to the World Health Organization (WHO) more than 80% of the world's population still uses or relies on various traditional medicines from plant products against various infectious diseases, as one of their primary health care needs. Meanwhile, the majority of new drugs (70%) introduced in all over the world are derived from natural products, primarily from plants. Studying medicinal plants helps to understand their properties with reference to toxicity and protect humans and animals from natural poisons (1,4). There are approximately 5 lakh plant species occurring worldwide of which only 1% has phytochemically been explored with a great prospective for discovering novel bioactive compounds (1,2).

Butea frondosa (commonly known as palas; family *Fabaceae*), is a medicinal plant growing all over India with various medicinal purposes i.e. flowers (for fever, thirst and diarrhea); decoction of bark (for bleeding piles); paste seeds (for skin diseases, edema and eye diseases) (5,6). In contrast, palasonin (alkaloid) extracted from the seeds show anthelmintic effect especially in roundworm infestations (e.g. *Ascaris lumbricoides*). Recently, new alkaloids, namely butrin and isobutrin have been reported in flowers of *B. frondosa* (7,8). In view of this, a small amount of work has been done with respect to immunopharmacological activities. In this study, we focused on aqueous extracts of *B. frondosa* leaves for determining their anti-inflammatory and anti-microbial activities in infected and non-infected whole human blood against specific vaccine antigen (HBsAg).

***Corresponding author:** Dr. Amit Gupta, Assistant Professor, Department of Immunology and Virology, Vidya Pratishthan's School of Biotechnology, Baramati, Pin code 413133, District Pune, Maharashtra India.
Email: amitgupta@vsbt.res.in

Materials and methods

Collection of fresh leaves of *B. frondosa*

Fresh leaves of *B. frondosa* were collected from the udyan of Vidya Pratishthan's School of Biotechnology during the month of August 2015. They were then dried in a shady area and prepared in finely powdered form.

Phytochemical investigation

Five grams of above powder was macerated with mortar and pestle and then dissolved in phosphate buffered saline (PBS) (pH 7.2; 50 mL). The aqueous extracts were filtered and used to determine the presence of secondary metabolites. The phytochemical investigation of the aqueous extracts was carried out as per standard methods and revealed qualitatively the presence of phenolic compounds, flavonoids (by alkaline reagent test), saponin (by foam test), terpenoids (by acetic anhydride test) and glycosides. In addition, thin layer chromatography of the aqueous extract was performed using n butanol: acetic acid: water (5:3:2). The observed spots were then visualized using anisaldehyde-sulphuric acid reagent.

Human blood samples and estimation of lymphocytes, monocytes and granulocytes count using flow cytometer

Immunopharmacological studies were done using non-infected and infected EDTA anti-coagulant human blood samples (2-3 mL) collected from Mangal Pathology laboratory, Baramati.

To determine the effect of aqueous extracts of *B. frondosa* on whole human blood with various doses (0.5 to 30 mg/mL, 100 µL), pertaining to lymphocytes, monocytes and granulocytes count, were suspended in a stream of fluid using flow cytometry. In this study, EDTA infected and non-infected bloods (50 µL) were divided into two separate sets i.e. each exposed to various doses of aqueous extracts of *B. frondosa*. The samples were then incubated at 37°C for 2 hours. After centrifugation, the supernatant was separated out and washed with PBS. Finally, the pellets were dissolved in PBS and observed through flow cytometry. Data acquisition of 10 000 events representing different phenotypes was analyzed using CellQuest software (9,10).

Lymphocyte proliferation assay

Lymphocytes (10^6 cells/mL) of infected and non-infected samples of whole human blood were plated in 96-well flat = bottom plates (or tissue culture flasks) and incubated in the presence of hepatitis B vaccine (HBsAg, 20 µg/mL; 10 µL) along with serial dilutions of aqueous extracts of *B. frondosa* (0.5-10 mg/mL, 50 µL) at 37°C for 48 hours. After incubation, the 96-well flat-bottom plates were centrifugated (at 3000 rpm for 8 minutes). The supernatant (100 µL) was discarded and then added to an equal volume of fresh medium. The cells were then treated with 20 µL of MTT solution (5 mg/mL) and incubated for 4 hours. Again, the 96-well flat-bottom plates were spinned or centrifuged at 1500 rpm for 4 minutes with discarding the supernatant. 100 µL of DMSO solution was then added to the formazon crystals and the absorbance at 570 nm was measured using ELISA Reader (11,12).

Statistical analysis

One-way analysis of variance (ANOVA) tests (Bonferroni multiple comparison tests) were performed. All values were mentioned as mean ± standard error (SE).

Results

Effect of *B. frondosa* on human blood counts

The effect of aqueous extracts of *B. frondosa* on infected and non-infected whole human blood, pertaining to lymphocytes, monocytes and granulocytes count using HBsAg, was shown in Figure 1. As seen in both cases and at higher doses (10 mg/mL, 100 µL), there was an increase of the monocytes as well as granulocytes count as compared to the control, but at the same time an increase in the number of lymphocytes. From the data obtained, it appears that aqueous extracts of *B. frondosa* have anti-inflammatory as well as anti-microbial activities.

Effect of *B. frondosa* on lymphocyte proliferation assay

The effect of aqueous extracts of *B. frondosa* (0.5 to 10 mg/mL, 50 µL) on proliferation assay using human lysed infected and non-infected whole human blood along with HBsAg was shown in Figure 2. The results show that the aqueous extracts at higher doses (10 mg/mL, 50 µL) caused a decline in the proliferation rate as compared to the control. Overall the data obtained may confirm the inflammatory as well as anti-microbial activities of the aqueous extracts of *B. frondosa*.

Discussion

In recent times, focus on medicinal plant research (e.g. *B. frondosa*) has increased all over the world and a large body of evidence has collected to show immense potential of this medicinal plant used by various traditional systems. This study probably for the first time reveals the aqueous leaves extract of *B. frondosa* for its anti-inflammatory and antimicrobial activities. These activities were evaluated using whole human blood (infected and non-infected) and tested against hepatitis B vaccine containing surface antigen (HBsAg) pertaining to whole human blood counts (lymphocytes, monocytes and granulocytes count) and proliferation assay. The results showed that *B. frondosa* can decrease the count of monocytes as well as granulocytes including proliferation rate (at higher doses and in both cases i.e. infected and non-infected whole blood containing HBsAg). The significant anti-inflammatory and antimicrobial activities shown by the aqueous leaves extract of *B. frondosa* may be linked to flavonoids, terpenoids, glycosides and phenolics found qualitatively in the phytochemical screening. It is well known that flavonoids have inhibitory effects on enzymes and production of chemical mediators and phenolics and terpenoids can interfere with several components of the inflammatory cascade.

The findings of the current study were confirmed through

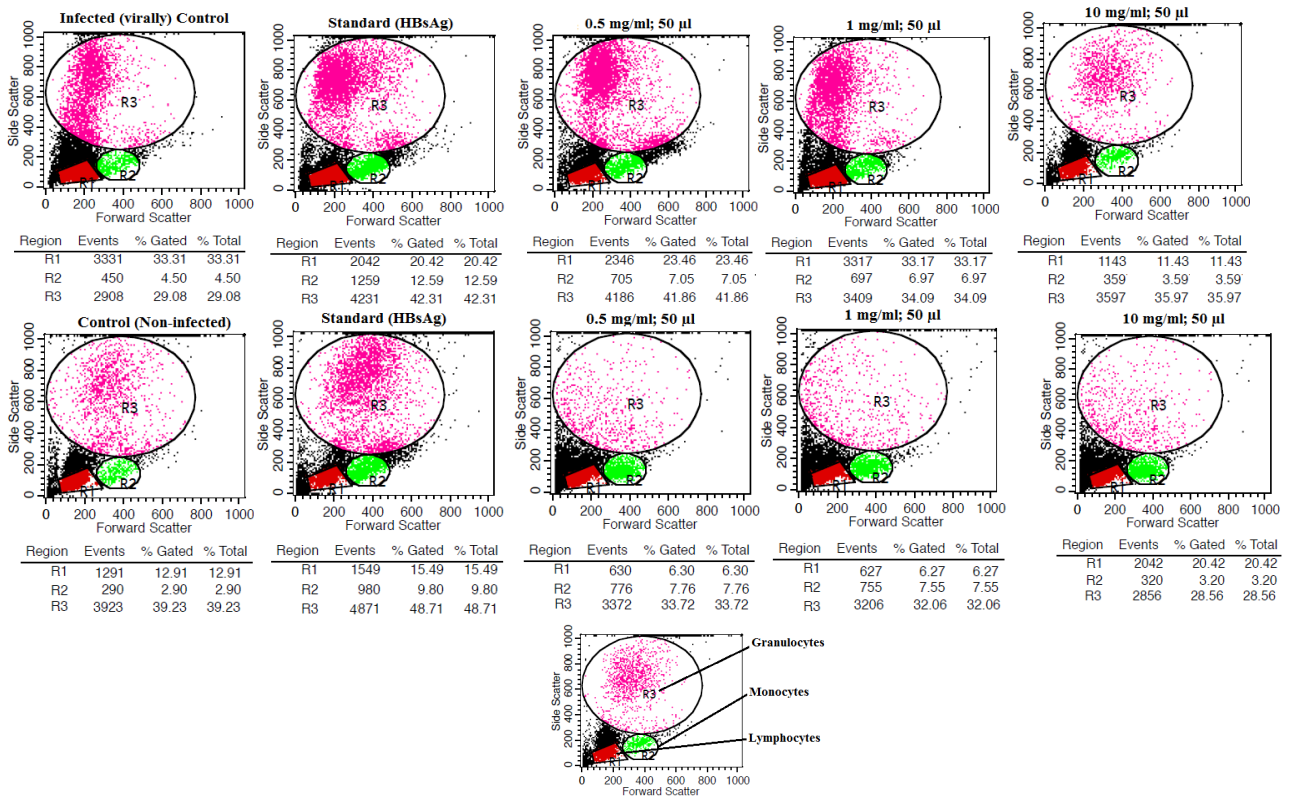

Figure 1. Effect of aqueous leaves extract of *Butea frondosa* on human whole blood (infected and non-infected) using flow cytometry i.e. forward scatter (FSC) and side scatter (SSC). Data acquisition of 10 000 events and fraction or separation of cell populations representing forward and side scatter using Cell quest software. R1, R2 and R3 denote lymphocytes, monocytes and granulocytes count.

flow cytometry and they provide some information related to the immune system. Nowadays the use of flow cytometry has grown qualitatively and quantitatively to determine the total cellular immune response against various antigens (13,14). The flow cytometric results obtained from the present study clearly indicate that aqueous leaves extract of *B. frondosa* have a declining effect in monocytes and granulocytes count (at higher doses and in both cases i.e. infected and non infected blood) stimulated with HBsAg, as compared to the control. The data represents that *B. frondosa* have anti-inflammatory as well as anti-microbial activities.

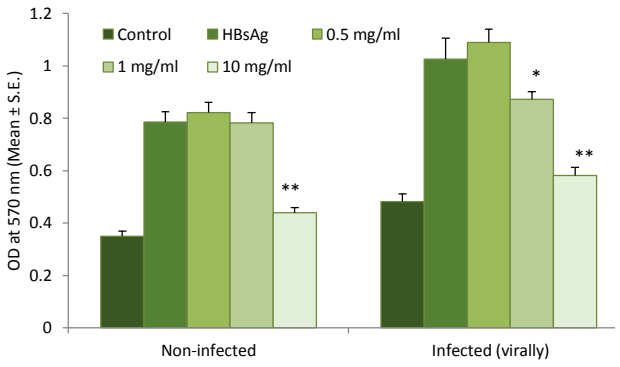

Figure 2. Effect of aqueous leaves extract of *Butea frondosa* on human whole blood using HBsAg. Lysed whole human blood (10^6 cells/mL, 100 µL) were cultured in triplicates in 96-well tissue culture plate along with various doses of aqueous leaves extract (0.5-10 mg/mL; 50 µL) in complete RPMI 1640 medium in presence of HBsAg (20 µg/mL; 10 µL). Proliferation was measured by MTT assay. Values are expressed as mean ± SE. The absorbance at 570 nm was measured using ELISA reader.

To estimate anti-proliferative activity of aqueous leaves extract of *B. frondosa* further immunopharmacological studies were done by lysing whole human blood (infected and non-infected) and lymphocyte proliferation assay using HBsAg. The capacity of aqueous leaves extract is to inhibit T cell immunity with respect to HBsAg (specific antigen). This can be shown by the reduction of lymphocyte proliferation response. The results indicate that the aqueous leaves extract of *B. frondosa* could significantly decrease the activation potential of T cells in lysed whole human blood. Overall, the data suggests that the aqueous leaves extract of *B. frondosa* show anti-inflammatory as well as anti-microbial activities.

Conclusion

This study suggests that the aqueous leaves extract of *B. frondosa* significantly inhibits the production of monocytes as well as granulocytes count including proliferation in both infected and non infected whole human blood. Further investigations of the aqueous leaves extract should be done through in vivo assessment for immunopharmacological studies in mice models with identification of the major active components responsible for anti-microbial and anti-inflammatory activities.

Acknowledgements

We would also like to thank Dr. Gandhi and Mr. Shekhar for providing infected (virally) and non-infected samples from Mangal Pathology laboratory, Baramati, Maharashtra, India.

Authors' contributions

Both the authors designed the study, wrote the protocol and interpreted the data. Both the authors read and approved the final manuscript.

Conflict of interests

Authors declare that they have no competing interests.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission and redundancy) were completely observed by authors.

Funding/Support

We are highly thankful to our organization i.e. Vidya Pratishthan's for conducting these immunological studies.

References

1. Maciel MA, Pinto AC, Veiga VF Jr, Grynberg NF, Echevarria A. Medicinal plants: the need for multidisciplinary scientific studies. Quim Nova. 2002;25(3):429-438.
2. Gupta A, Khamkar PR, Chaphalkar SR. Review on medicinal plants to target and inhibit the epidermal growth factor receptor signaling in cancer and tissue repair therapy. International Journal of Advances in Pharmacy, Biology and Chemistry. 2014;3(1):210-213.
3. Gupta A, Khamkar PR, Chaphalkar SR. Applications and uses of active ingredients from medicinal plants. Indian Journal of Novel Drug Delivery. 2014;6(2):106-111.
4. Scartezzini P, Speroni E. Review on some plants of Indian traditional medicine with antioxidant activity. J Ethnopharmacol. 2000;71(1-2):23-43.
5. Ramachandran S, Sridhar Y, Sam SK, et al. Aphrodisiac activity of Butea frondosa Koen. ex Roxb. extract in male rats. Phytomedicine. 2004;11(2-3):165-168.
6. Dixit P, Prakash T, Karki R, Kotresha D. Anti-obese activity of Butea monosperma (Lam) bark extract in experimentally induced obese rats. Indian J Exp Biol. 2012;50(7):476-483.
7. Sujith K, Ronald C, Darwin, Roosewelt C. Antidiabetic activity of methanolic extract of Butea frondosa leaves with its possible mechanism of action. Asian Journal of Pharmaceutical and Clinical Research. 2011;4(3):93-98.
8. Banji D, Banji O, Shanthmurthy M, Singh M. Antidiarrhoeal activity of the alcoholic extract of the leaves of *Butea frondosa* Koen. ex Rox. Indian J Pharm Sci. 2010;72(2):238-240.
9. Gupta A, Shah AP, Chaphalkar SR Immunopharmacological exploration of proteases from Catharanthus roseus on virally infected human whole blood. J Dis Glob Health. 2016;6(1):36-42.
10. Gupta A, Shah AP, Chaphalkar SR. Flow cytometric preliminary evaluation of immunosuppressive and cytotoxic effect of Catharanthus roseus on human whole blood. Journal of International research in Medical and Pharmaceutical Sciences. 2016;8(1):11-16
11. Gupta A, Chaphalkar SR. Immunopharmacological screening of aqueous root extract of Santalum album. J HerbMed Pharmacol. 2016;5(1):7-11.
12. Gupta A, Khajuria A, Singh J, Suri KA. Adjuvant effect of biopolymeric fraction from *Picrorhiza kurroa* to promote both Th1 and Th2 immune responses. Journal of Progressive Research in Biology. 2015;1(1):7-16.
13. Gupta A, Chaphalkar SR. Immunotoxicological studies of leaves aqueous extract of Tobacco plant (Nicotiana tabacum) using flow cytometer. Journal of International Research in Medical and Pharmaceutical Sciences. 2015;5(4):191-198.
14. Gupta A, Chaphalkar SR. Flow cytometric evaluation of anti-proliferative and anti-inflammatory activity of plant viruses: TMV tobacco plant**.** Journal of International Research in Medical and Pharmaceutical Sciences. 2015;5(4):169-175.