ISSN: 2319-6505

Available Online at http://journalijcar.org

International Journal of Current Advanced Research
Vol 4, Issue 5, pp 87-91, May 2015

International Journal of Current Advanced Research

ISSN: 2319 - 6475

RESEARCH ARTICLE

USE OF FLOW CYTOMETRY TO MEASURE THE IMMUNOSTIMULATORY ACTIVITY OF AQUEOUS EXTRACT OF *JASMINUM AURICULATUM*

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A R T I C L E I N F O

Article History:

Received 13th, April, 2015
Received in revised form 16th, April, 2015
Accepted 20th, May, 2015
Published online 28th, May, 2015

Key words:

Jasminum auriculatum; aqueous extract; immunostimulatory

A B S T R A C T

The objective of our study is to examine the immunostimulatory activity of leaves aqueous extract of *Jasminum auriculatum* against specific hepatitis B vaccine antigen on human whole blood using flow cytometer. Human whole blood were treated with variable doses of aqueous extract (0.5 – 30 mg/ml) of *Jasminum auriculatum* and evaluated the lymphocytes, monocytes and granulocytes count and observed the forward (shape and size) and side scatter (granularity of the cell) which is evaluated through flow cytometer and also determined its hemolytic activity. The results showed that the leaves aqueous extract of *Jasminum auriculatum* showed increased in the number of monocytes and granulocytes count which is evidenced through the retain and decline in the level of forward and side scatter on human whole blood. At high doses of aqueous extract i.e. 30 mg/ml showed hemolytic activity as compared to control. The results showed that the aqueous extract (leaves) of *Jasminum auriculatum* showed immunostimulatory activity against specific vaccine antigen.

INTRODUCTION

The term medicinal plants includes various types of plants used in herbalism and played an essential as well as important role in the development of human culture e.g. religions (Hindu, Datura, worship of shiva; Indian hindu god). Most of the natural as well as synthetic based drugs are directly or indirectly derived from medicinal plants [1, 2, 3]. Recently, the demand or requirment of medicinal plants is enormously increasing in both developing and developed countries [1, 2]. As per the World Health Organization (WHO), it is estimated that more than 80% of the population of developing countries is totally rely on traditional medicines derived from plants for their primary health care needs and most of these species of medicinal plants are under threat to become extinct. Recently, number of primary and secondary metabolites [1] extracted from medicinal plants and provides an important source for the discovery of novel pharmacologically active compounds against number of intracellular as well as extracellular infections or diseases [1, 2]. The medicinal plant product i.e. leaves, roots and stem including flowers has its own medicinal importance or its use which is scientifically approved and some of which needs to be proved. The exact criteria or reason or requirement for selecting these medicinal plants showed wide acceptance of herbal products or medicines are being cheaper, lesser side effects and being natural in origin [3].

Out of these medicinal plants, *Jasminum auriculatum* (also called as needle flower jasmine, English; Juhi, Hindi; Yoothika, Sanskrit and Jai, Marathi) is small, evergreen [4, 5, 6], climbing shrub which belongs to the class Magnoliopsida-Dicotyledons; order- *Scrophulariales* and family Oleaceae (Olive family). *Jasminum auriculatum* is widely distributed in India, Nepal and Sri Lanka [4] and showed number of medicinal uses of plant e.g. roots are useful in skin diseases i.e. ring worm [5, 6]; flowers are generally used to make medicine and used as flavoring agent in foods e.g. frozen dairy desserts; beverages; gelatins; puddings etc and also provides beneficial effects such as aphrodisiac [5]; anti-septic [6]; aromatherapy [7] etc. Jasmine oil can also be extracted or obtained from flowers and is generally used in perfumery and its leaves are used for the treatment of mouth ulcers [7, 8]. In contrast, *Jasminum auriculatum* also showed number of immune pharmacological activities such as wound healing activity [7]; diuretic activity [8]; antilithiatic activity [9] etc. In recent years, further studies have been carried out to explore this medicinal plant for its potential in various immune pharmacological activities. In this study, our group explores the immune stimulatory effect of leaves aqueous extract of *Jasminum auriculatum* using specific hepatitis vaccine antigen.

MATERIALS AND METHODS

Collection of Plant Material

The fresh leaves of *Jasminum auriculatum* were collected

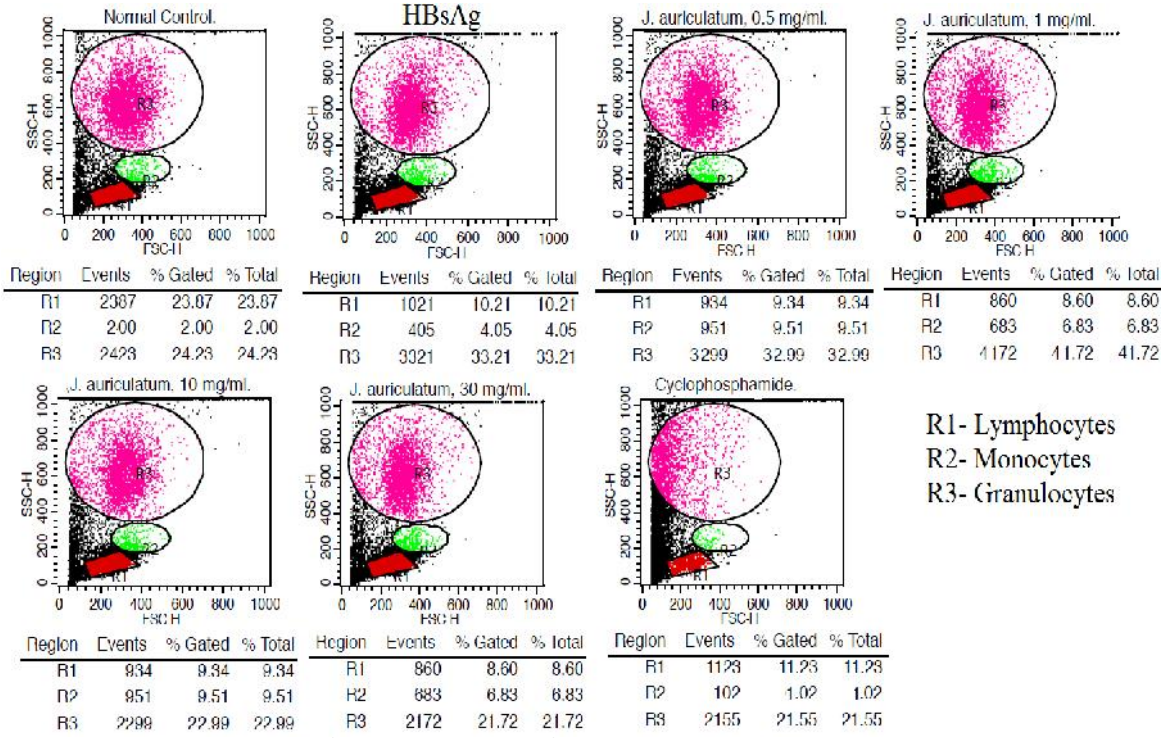

Figure 1 Flow cytometric analysis of leaves aqueous extract of *Jasminum auriculatum* on lymphocytes, monocytes and granulocytes count. Data acquisition of 10000 events and fraction or separation of cell populations representing different phenotypes analyzed using cell quest software

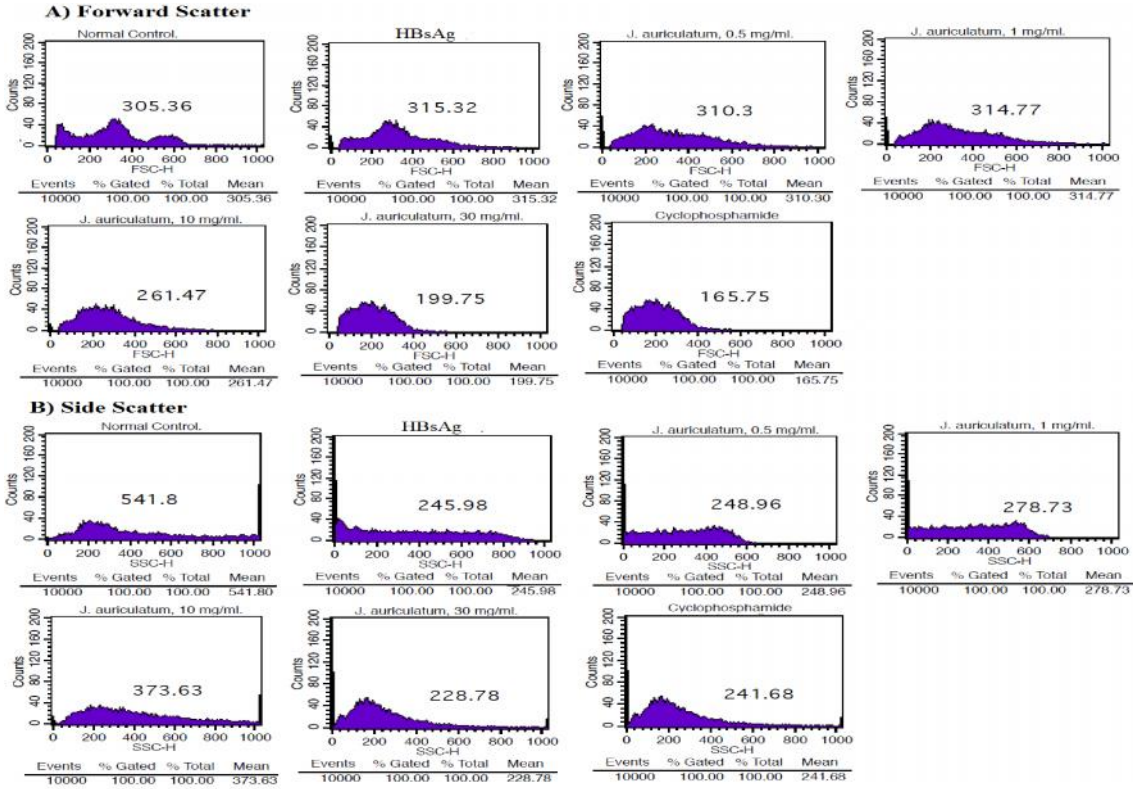

Figure 2 Flow cytometric analysis of leaves aqueous extract of *Jasminum auriculatum* using forward and side scatter. Data acquisition of 10000 events and fraction or separation of cell populations representing forward and side scatter using cell quest software.

from the garden of Vidya Pratishthan's in the morning, between January and February 2015 in Baramati region, District Pune, Maharashtra, India.

Preparation of aqueous extract

The plant leaves of *Jasminum auriculatum* were gathered and washed it with distilled water to remove the debris/dust from the leaves and cut into small pieces and then dried into a shady area. Afterwards, the plant leaves were weighed and macerated with liquid nitrogen to prepare the fine powder and then used for aqueous extract preparation for immunological studies.

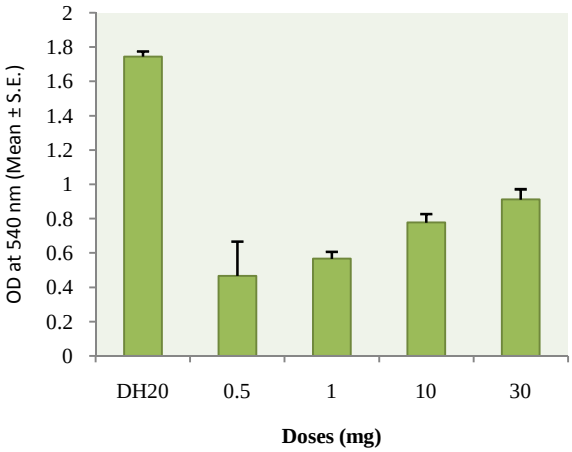

Figure 3 Hemolytic activity of leaves aqueous extract of *Jasminum auriculatum* on human erythrocytes. Data are represented as Mean ± S.E. of human whole blood samples. Distilled water and phosphate buffered saline used as positive and negative control

Phytochemical Screening and extraction

The test procedures were carried out to measured or detect the secondary metabolites using qualitative and quantitative based assay. During qualitative based assay, the aqueous extract of *Jasminum auriculatum* showed the presence of terpenoids (using acetic anhydride test); flavonoids (alkaline reagent test) and phenolics ($FeCl_3$ test). On the other hand, high performance thin layer chromatography (HPTLC) is used for the detection of quantity of secondary metabolites present in the aqueous extract using mobile phase ethyl acetate: n Butanol at the ratio (6:4). The resolution was obtained at 220 nm and the peaks at retardation factor (Rf) value is 0.97 (terpenoids), 0.8 (flavonoids) and phenolics (0.51 – 0.89, 3.10 – 5.10 µg).

Estimation of blood counts and analyzed the forward (FSC) and side scatter (SC)

The human anti-coagulant EDTA blood samples (control) included in this study were collected from *Mangal Pathology Laboratory*, Maharashtra, India and analyzed at the VSBT, Baramati, Maharashtra, India, between January to February 2015.

For the estimation of variable doses of aqueous extract (0.5 – 30 mg/ml, 100 µl) of *Jasminum auriculatum* along with hepatitis (20 µg/ml, 10 µl) vaccine antigen in human whole blood (100 µl). Cyclophosphamide (5 mg/ml, 100 µl) used as standard for these studies. Incubate the samples for 2 h at 37 °C in carbon dioxide incubator. After incubation, add ACK lysing solution/red cell lysis buffer/FACS lysing solution is added and incubated the sample for 10 minutes. Afterwards, centrifuge the sample at 1800 rpm for 10 min at 4 °C and the supernatant was discarded and washed two times with phosphate buffered saline (PBS) and then analyzed through flow cytometer (FACS Calibur) for the estimation of lymphocytes, monocytes and granulocytes count and also measured the forward (shape and size) and side scatter (granularity of the cell) gating applied for data acquisition of 10000 events of cell populations representing different phenotypes analyzed using cell quest software [10, 11].

Preparation of erythrocytes suspension and determined its hemolytic activity

EDTA human whole blood samples were collected from pathology laboratory. Add 2-3 ml PBS is added into the EDTA human blood and then centrifuged at 2500 rpm for five minutes at 4°C in a refrigerated centrifuge. After centrifugation, the supernatant (i.e. plasma) was discarded and the red blood cell pellets were washed continuously two to three times with phosphate buffer saline solution by centrifugation at 2200 rpm for 7 minutes at 4°C.

For this experiment, 1% washed human red-blood cell suspension (10^6 cells/ml, 100 µl) dissolved in phosphate buffer saline (PBS; pH 7.2) was used throughout the experiment. Aqueous extracts of leaves of *Jasminum auriculatum* (0.5 – 30 mg/ml, 100 µl) containing different concentrations along with a fixed volume of washed 1% human red-blood cell suspension. Distilled water (DH_20) used as positive control containing only human whole blood. The result for each aqueous extract concentration (0.5 – 30 mg/ml) of leaves of *Jasminum auriculatum* was interpreted qualitatively *in vitro* hemolytic action either being present or absent [12]. The optical density was measured at 540 nm.

Statistical analysis

Data are reported as means ± standard error (S.E).

RESULTS

Effect of aqueous extract on blood counts

The effect of the aqueous extract of leaves of *Jasminum auriculatum* on lymphocytes, monocytes and granulocytes count using flow cytometry as shown in Figure 1. The results showed that the aqueous extract at a dose range of 0.5 and 1 mg/ml showed drastically increased in the number of monocytes and granulocytes count as compared to control. Cyclophosphamide used as standard for these studies and the results showed that there is enormous loss of monocytes and granulocytes count as compared to control.

Effect of aqueous extract on forward and side scatter using flow cytometry

These studies suggest that the aqueous extract of leaves of *Jasminum auriculatum* showed inhibitory action in forward (shape and size) and side (granularity of the cell) scatter at higher doses i.e. 30 mg/ml. At a dose range of 0.5 and 1 mg/ml, the aqueous extract will retain the level or number or mean of forward scatter as compared to control but there is drastically reduction in side scatter. To clarify these studies, live cells has higher forward scatter and lower side scatter where as dead cells has higher side scatter and lower forward scatter. It means our aqueous extract at a dose range of 0.5 and 1 mg/ml showed immunostimulatory activity as compared to control and standard. Cyclophosphamide used as standard for these studies and there is drastically reduction in forward and side scatter as compared to control.

Effect of aqueous extract on hemolytic activity

The hemolytic activity of aqueous extract of leaves of *Jasminum auriculatum* as shown in Figure 3**.** In *Jasminum*

auriculatum, the results showed that the hemolytic activity was observed at higher doses (30 mg/ml) as compared to control. In this study, we used distilled water and phosphate buffered as positive and negative control.

DISCUSSION

In the last thirty years, number of primary as well secondary metabolites isolated from medicinal plants which is already reported [1,2] and showed immune stimulatory potential against number of specific as well as non-specific antigen. The term immune stimulation comprises a group of prophylactic (to prevent) as well as therapeutic (healing of disease) concept which aims at the stimulation of our specific as well as nonspecific immune system [13]. In the present study, we examined the immune stimulatory effect of leaves aqueous extract of *Jasminum auriculatum* against specific i.e. hepatitis B vaccine antigen using flow cytometry. In the present study, our group focused on the complexity of immune pharmacological activities present in traditional medicinal plant i.e. *Jasminum auriculatum* used in India and help to further our understanding of mechanisms for action and why most of the medicinal plants are used to treat individual diseases [2].

To achieve this objective, we evaluated the immune stimulatory activity of leaves aqueous extract of *Jasminum auriculatum* against specific i.e. hepatitis B vaccine antigen using human whole blood containing lymphocytes, monocytes and granulocytes count, forward and side scatter and also determined its hemolytic activity. In human whole blood, the increased in the number of monocytes and granulocytes count in human whole blood treated with variable doses of aqueous extract which is confirmed through flow cytometric analysis and the results showed that the leaves aqueous extract of *Jasminum auriculatum* showed immune stimulatory activity as compared to control and standard.

Flow cytometry is generally used for measuring the properties of the cell suspended in a stream of fluid as they pass through one, two or three lasers. This instrument is generally used for measuring the immune phenotyping i.e. detection of cell surface molecules e.g. cluster of differentiation (CD), cell cycle analysis, cell viability, total protein, enzyme activity, gene expression etc [14, 15]. The identification of cells present in a fluid is measured through light scatter properties as it has been shown that there is a direct relationship between forward scattered light and cell volume and this has become common practice in flow cytometry. The scattering of light i.e. coherent light source (488 nm, blue) using forward scatter (small angle scattering between 0.5 – 5° C and measured its shape and size of the cell) and side scatter (large angle scattering between 15 – 150° C dark field and measured its complexity and granularity of the cell). The main advantage of this instrument is to measure the live cells and dead cells in the form of forward and side scatter. For flow cytometric analysis, dead cells have higher side scatter and lower forward scatter where as live cells have higher forward scatter and lower side scatter [16, 17]. In this study, the results showed that the leaves aqueous extract of *Jasminum auriculatum* showed slightly reduction in the level of side scatter and retained the number of forward scatter as compared to control where as cyclo phosphamide showed inhibitory effect on forward (shape and size) and side scatter (granularity) as compared to control. In addition, hemolytic activity is done at higher doses. Overall the results showed immune stimulatory activity.

CONCLUSION

The result obtained from the experiment it is concluded that the leaves aqueous extract of *Jasminum auriculatum* having good immune stimulatory activities. The results of leaves aqueous extract support the traditional use of this plant in immune stimulatory conditions and suggest the presence of biologically active components which may be worth further investigation and elucidation.

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