WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 4, Issue 2, 1063-1071. Research Article ISSN 2277– 7105

FLOW CYTOMETRIC ANALYSIS OF IMMUNOADJUVANT ACTIVITY OF *EMBLICA OFFICINALIS* ON HUMAN WHOLE BLOOD

Amit Gupta*[1] and Sushama R Chaphalkar[1, 2*]

[1]Department of Immunology, Vidya Pratishthan's School of Biotechnology (VSBT) Vidyanagari, Baramati, District Pune, Maharashtra, India.

[2]Director, Vidya Pratishthan's School of Biotechnology (VSBT) Vidyanagari, Baramati, District Pune, Maharashtra India..

Article Received on
26 Nov 2014,

Revised on 21 Dec 2014,
Accepted on 15 Jan 2015

***Correspondence for Author**
Dr. Amit Gupta
Department of Immunology, VSBT, Baramati, District Pune, Maharashtra, India.

Dr. Sushama R Chaphalkar
Director, VSBT, Baramati, District Pune, Maharashtra, India.

ABSTRACT

In this study, alkaloid fraction from *Emblica officinalis* was evaluated for its potential ability as an adjuvant effect on the immune responses to hepatitis and diphtheria-pertussis-tetanus (DPT) antigen on human whole blood using flow cytometry. Cells were treated with variable doses of alkaloid fraction (0.625 – 2.5 mg) in presence or absence of hepatitis and DPT vaccine antigen. Hepatitis and DTP vaccine containing alum used as standard for these studies. The results showed that the DPT mediated blood counts were significantly enhanced by alkaloid fraction at lower doses (0.625 mg) compared with DPT control group. Moreover, alkaloid containing hepatitis antigen showed no adjuvant effect on human whole blood.

KEYWORDS: *Emblica officinalis*, hepatitis, DPT, alum.

INTRODUCTION

A body of evidence showed that numerous bioactive molecules isolated as well as purified from various medicinal plants have shown immunomodulation and anti-cancer effects. Previous studies have shown that bioactive molecules evoke stronger immune responses (antibody titre and cytokines) than alum or others.[1, 2, 3, 4, 5] Thus, the bioactive molecules from various medicinal herbs are becoming an attractive material as pharmaceutical products and may provide an opportunity to develop a new adjuvant for vaccine antigen. Indeed, a wide range of bioactive molecules have been isolated from various medicinal plants and these molecules have been shown to possess immunomodulatory

activity through their ability to modulate macrophage function.[3, 4] Appropriate enhancement of innate immune functions by bioactive compounds can then augment host defense responsiveness. Thus, because of their low toxicity and high potency of these molecules, plant bioactive molecules represent one of the ideal or best candidates for therapeutics with immunomodulatory action.

Vaccine adjuvant research totally belongs to immunopharmacological field that is advancing very fast or rapidly, reflecting at a very high rate in which new or proposed adjuvant candidates are being discovered. The requirement as well as challenge for safe and effective adjuvant for current vaccine antigen cannot be overstated. Newly developed or proposed vaccines are usually based on target antigen, they are usually weak immunogenic, costly and induce poor immunological responses. However, adjuvants can override such immunological inadequancy and help in mounting protective immune responses.[1, 2, 6, 7] There is a significant interest in developing as well as synthesizing the new vaccine adjuvant that combine the safety advantages of subunit or recombinant protein based vaccines with enhanced efficacy. However, many of them are not highly immunogenic whether administered parenterally or mucosally and approved adjuvants are ineffective in triggering the innate as well as adaptive immunity, thus the identification of plant based adjuvant candidates capable of facilitating antigen delivery to immune responsive cells and functioning as adjuvants.[3, 4, 5]

Emblica officinalis (commonly known as Indian gooseberry or amla) belongs to the family Euphorbiaceae.[8] Various part of this medicinal plant are used in the treatment of number of diseases such as respiratory disorders, diabetes, heart and eye disorder, scurvy and ageing.[9, 10, 11] In addition, this medicinal plant also has anti-bacterial and astringent properties. The number of preclinical studies has shown that *Emblica officinalis* exhibits strong antioxidant activity;[8] immunomodulatory;[9] anti-inflammatory; antiulcer; hepatoprotective and anticancer actions.[10, 11] However, there is very little information about the clinical evidence to support the use of *Emblica officinalis* as an adjuvant for vaccine antigen.

To achieve the objective for adjuvant development against bacterial infections is normally described in three groups of vaccines. Firstly, it involves well established and commonly used vaccines such as BCG and DPT vaccines. Secondly, it includes newer vaccines or vaccines that are under development, for instance vaccines against number of pneumococcal diseases, *Haemophilus influenzae* and enteropathogenic *E. coli*. Thirdly, it covers vaccines whose realization at present appears to be difficult or hardly feasible, e.g. vaccines against

enterotoxins of enteropathogenic organisms; gonorrhea or organisms of hospital infections. In this short communication, our group focused on the alkaloid isolated from the *Emblica officinalis* on human whole blood was treated with first group of well established vaccines such as DPT (for eliminating bacterial infections) and also analyzed the effect on hepatitis vaccine (for eliminating viral infections) as well.

The extract powder obtained from the fruits of *Emblica officinalis* was subjected to qualitative and quantitative investigation of metabolites through HPTLC. The solvents and other purified reagents, HPTLC plates (10 x 10 cm) were purchased from Qualigens and Merck. The result is depicted in Fig. 1. The following HPTLC figure for the extract powder of *Emblica officinalis* sample whose 10 µl was injected shows six peaks at Rf values 0.04, 0.20, 0.56, 0.62, 0.65, 0.80. The maximum concentration is obtained at Rf value 0.20 indicating presence of phytoconstituents eluting out at 200 nm with the mobile phase ethyl acetate and n-butanol.

In order to find out the adjuvant activity of alkaloid fraction with hepatitis vaccine and DPT vaccine antigen (Serum Institute of India, India) containing alum. Human blood samples were collected from *Mangal Pathology laboratory*, Baramati, District Pune, Maharashtra, India at different time intervals for flow cytometric studies. Human whole blood was treated with 20 µg hepatitis and DPT formulated with one of the following delivery vehicles: Phosphate buffered saline and alkaloid fraction (0.625, 1.25 and 2.5 mg). Phosphate buffered saline-treated sample were included as control. Control adjuvant (standard) was alum (0.5 and 1.45 mg). In every experiment, one tube was kept as control, whilst another tube received standard vaccine for comparison of the study and authenticity of the experiment.

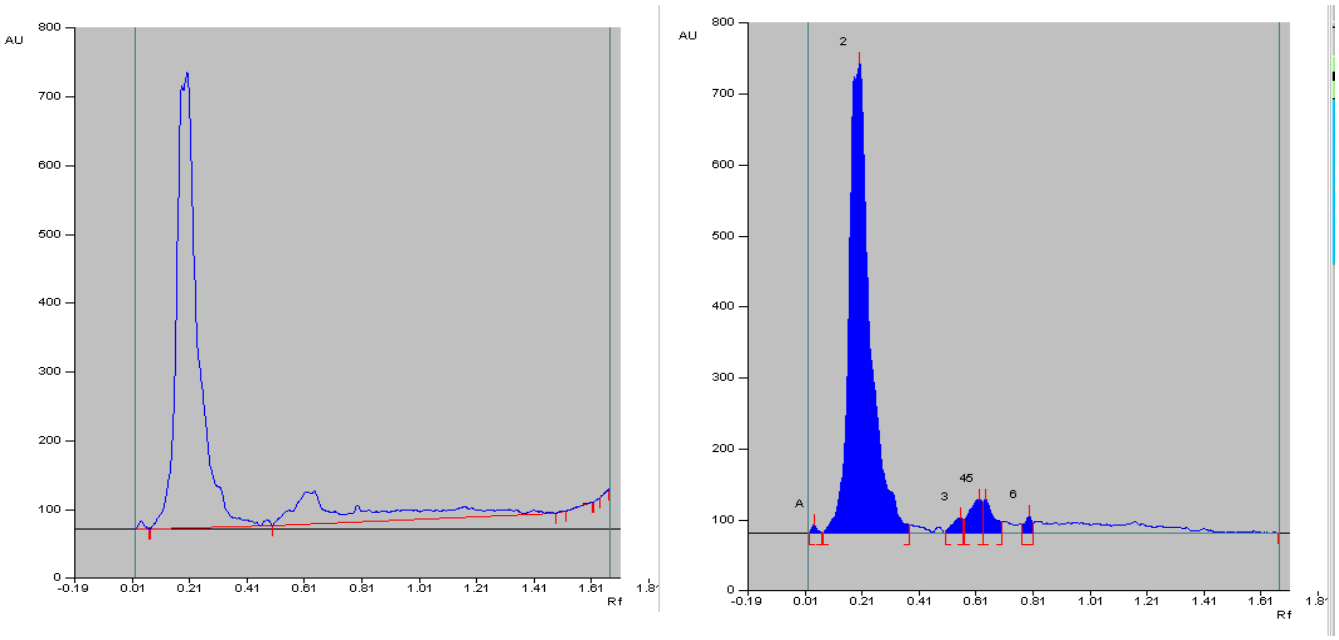
Flow cytometry analysis of human whole blood for examine and counting the number of cells i.e. lymphocytes, monocytes and granulocytes count which are suspended in a stream of fluid. To examine the forward and side scatter gating of human whole blood with variable doses of alkaloid ranging from 0.625 – 2.5 mg for data acquisition of 10000 events and fraction or separation of cell populations representing different phenotypes analyzed using cell quest software. Briefly, 50 µl of human whole blood was pipetted directly into a falcon tube containing phosphate buffered saline or with containing concentrations of alkaloid and then incubated at carbon dioxide incubator (37 °C, 5 % CO_2) for 2 h. After incubation, RBCs were lysed using 2 ml of red cell lysis buffer and incubated the sample for 30 minutes. After

centrifugation at 1800 rpm for 10 minutes, the supernatant was removed or aspirated and washed two- three times with phosphate buffered saline. After centrifugation, pellet dissolved in PBS and observed the cells through flow cytometer.[12, 13, 14]

Hepatitis and DPT vaccine is commonly used as a model for specific T and B cell mediated immune function. When this human whole blood were firstly exposed to hepatitis and DPT vaccine containing alum, it is possible that protein antigens such as hepatitis and DPT activate both B and T cells population, which results in the induction a highly efficient B cell differentiation pathway through specific structures (germinal centers, GCs) in which antigen-specific B cells proliferate and differentiate into antibody-secreting plasma cells or memory B cells.[7] The short life span of these plasma cells results in a small increased in the blood count, which eventually returns to baseline levels or control. In secondary immune responses, challenging exposure to proposed adjuvant candidate alkaloid from *Emblica officinalis* reactivates immune memory and results in a rapid increase of blood count especially lymphocytes, monocytes and granulocytes count. However, the addition of alum or alkaloid in human whole blood, the results showed that the alkaloid fraction in case of DPT vaccine showed remarkable increased in the number of blood counts as compared to alum containing DPT and control (Fig .2) where as in hepatitis vaccine, alkaloid showed negligible effect on human whole blood (Fig. 2). Furthermore, presence of alkaloid on human whole blood in presence or absence of DPT or hepatitis vaccine, it showed (Fig. 3) at higher doses showed rapidly decline in blood counts. In contrast, alum is one of the approved adjuvant for human use and it increased only humoral response and poorly elicited the cell mediated immunity. The main challenge for the science of adjuvants is how to selectively induce the appropriate type of immune response to protective antigens and to provide optimal effect against each type of infection.[3, 4]

In this study, DPT vaccine is the most effective method in the prevention and control of this disease i.e. Corneybacterium diphtheria, Bacillus pertussis and clostridium tetani and it has been used successfully for decades.[7] The primary vaccination of infants against these disease does not always afford long term protection suggest the need for a better immune strategy to maintain adequate levels of specific immunity to these diseases.[7] There are multiple antigens which is already present in the DPT vaccine encoded as combined vaccines. To increase the immunogenicity of DPT vaccine antigen, alum is used as an adjuvant for human use. The relatively poor efficacy of DPT vaccines in immune responses, especially in

animals has limited their practical use. However, we provide the strategy to increase the efficacy of these antigens or to improve the immune response induced or supplementing with the alkaloid isolated from *Emblica officinalis*. In this study, protein antigen i.e. DPT in adjuvant alum or alkaloid was used as a boosting agent and significantly enhanced immune response. Blood count especially lymphocytes, monocytes and granulocytes at lower concentration elicited in the group protein DPT containing alkaloid boosted showed significant increases than those in group protein boosted containing alum and control. This prime and boost strategy of enhancement of lymphocytes, granulocytes and monocytes count has proven to be very useful in improving the immunogenicity of DPT vaccines against number of diseases.



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.03	1.3	0.04	11.3	1.39	0.07	0.3	102.4	0.39
2	0.08	0.3	0.20	662.6	81.62	0.38	11.1	23601.5	89.88
3	0.50	0.8	0.56	20.6	2.53	0.57	19.3	359.9	1.37
4	0.57	18.7	0.62	46.4	5.72	0.64	43.2	1034.9	3.94
5	0.64	43.2	0.65	46.8	5.76	0.70	15.8	859.3	3.27
6	0.77	11.8	0.80	24.1	2.97	0.81	14.5	300.4	1.14

Fig 1. HPTLC of aqueous extract of *Emblica officinalis*.

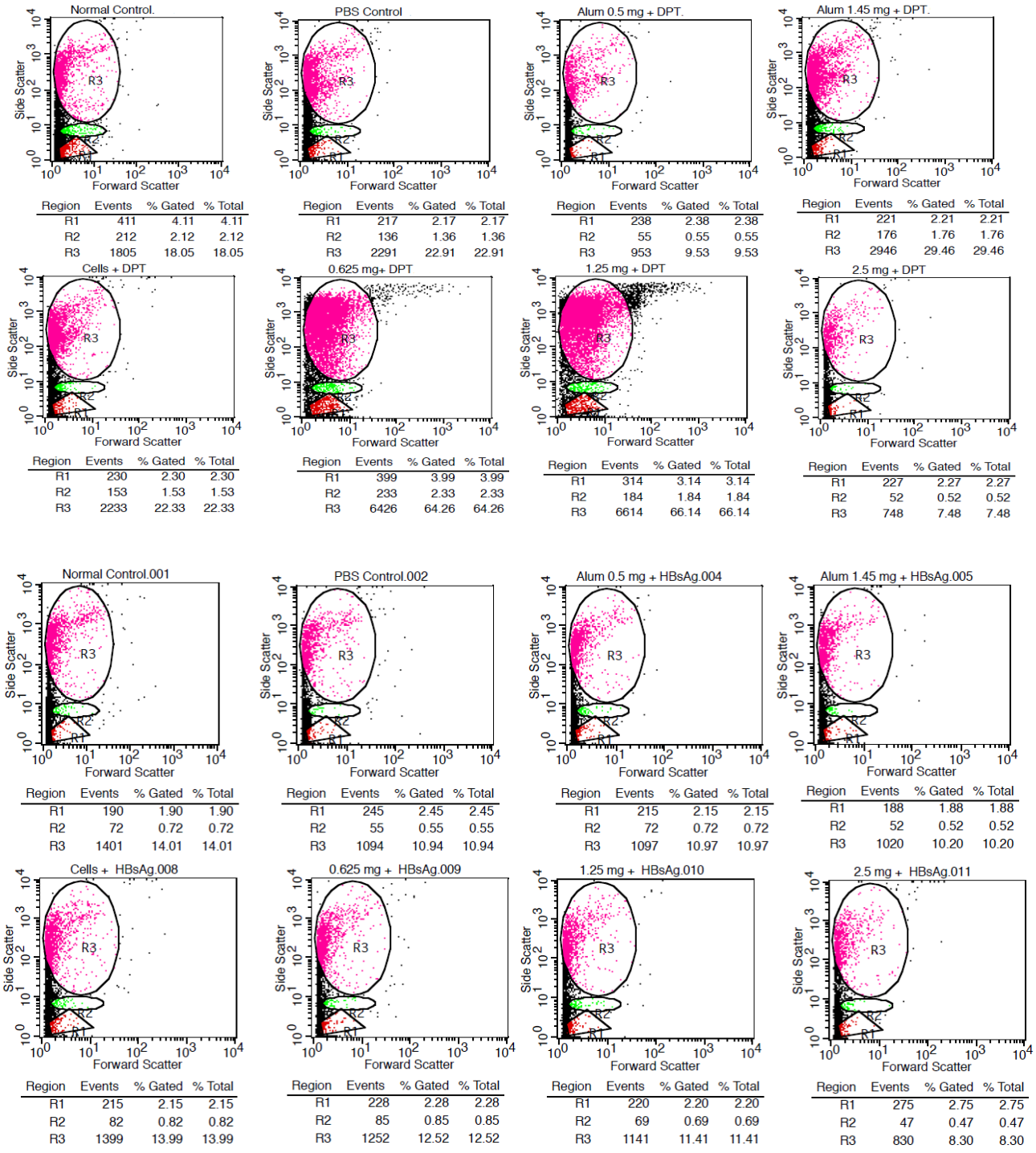


Fig. 2. Effect of proposed adjuvant candidate alkaloid isolated from *Emblica officinalis* on hepatitis and DPT vaccine.

Human whole blood was incubated with variable doses of alkaloid (0.625, 1.25 and 2.5 mg/ml) in presence or absence of alum. After incubation, cells were lysed using lysis buffer and washed with phosphate buffered saline. After centrifugation, pellet dissolved in PBS and observed the cells i.e. lymphocyte, monocyte and granulocyte count through flow cytometer

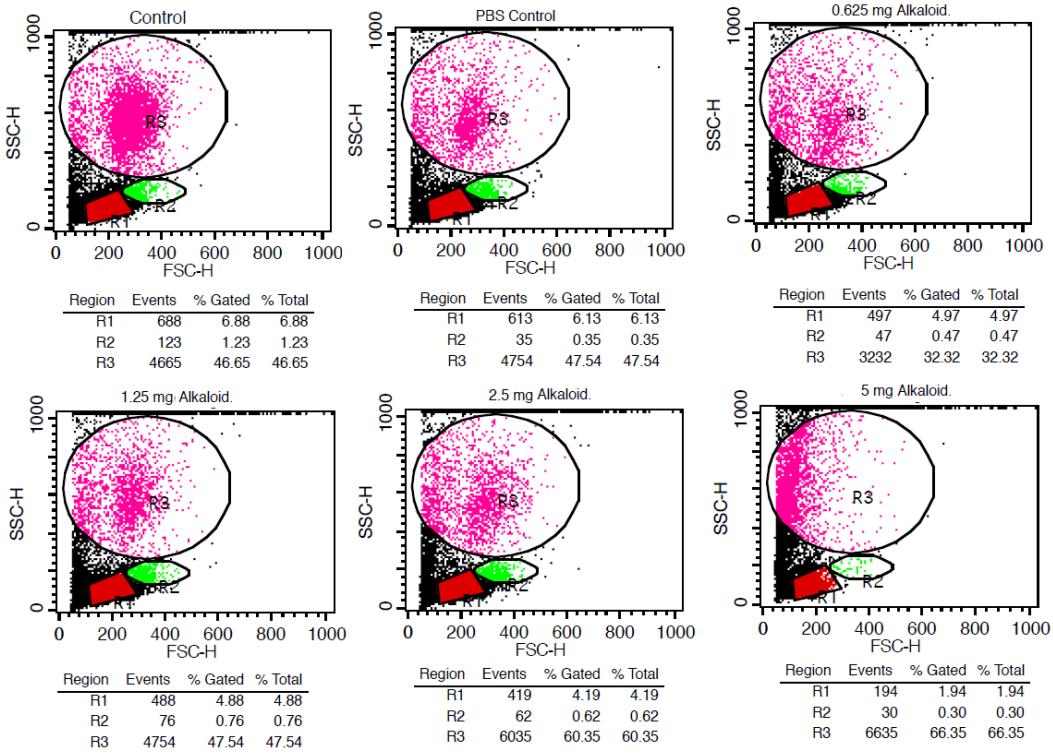


Fig.3. Effect of alklaoid on human whole blood.

Human whole blood was incubated with variable doses of alkaloid (0.625, 1.25 and 2.5 mg/ml). After incubation, cells were lysed using lysis buffer and washed with phosphate buffered saline. After centrifugation, pellet dissolved in PBS and observed the cells i.e. lymphocyte, monocyte and granulocyte count through flow cytometer.

CONCLUSION

Alkaloid fraction is a strong Th2 adjuvant for DPT vaccine antigen in human whole blood. Alkaloid-adjuvanted with DPT vaccine antigen is better than that of DPT adjuvanted with alum. The DPT-specific blood counts induced by alkaloid fraction associated with increased in the number of lymphocytes, monocytes as well as granulocytes count. Furthermore, alkaloid fraction at higher doses showed rapidly decline in the number of blood counts. Thus, alkaloid fraction is a potent enhancer of antigen-specific humoral immune responses, thus showing promise as immune adjuvant for vaccines against extracellular infectious agents such as bacteria, protozoa etc.

ACKNOWLEDGEMENT

Authors are thankful to Dr Gandhi and Mr. Shekhar, *Mangal Pathology Laboratory*, Baramati region, District Pune, Maharashtra, India and Devdan Chopade, research associate VSBT Baramati for giving and collecting human samples for research work.

REFERENCES

1. Liu FX, Sun S, Cui ZZ. Analysis of immunological enhancement of immunosuppressed chickens by Chinese herbal extracts. J Ethnopharmacol, 2010; 127: 251- 56.
2. Ragupathi G, Yeung KS, Leung PC, Lee M, Lau CB, Vickers A, Hood C, Deng G, Cheung NK, Cassileth B, Livingston P. Evaluation of widely consumed botanicals as immunological adjuvants. Vaccine, 2008; 26: 4860-65.
3. Yang L, Hu Y, Xue J, Wang F, Wang D, Kong X, Li P, Xu W. Compound Chinese herbal medicinal ingredients can enhance immune response and efficacy of RHD vaccine in rabbit. Vaccine, 2008; 26: 4451- 55.
4. Zhang X, Zhang X, Yang Q. Effect of compound mucosal immune adjuvant on mucosal and systemic immune responses in chicken orally vaccinated with attenuated Newcastle-disease vaccine. Vaccine, 2007; 25: 3254- 62.
5. Atal CK, Sharma ML, Kaul A and Khajuria A. Immunomodulating agents of plant origin: Part 1. Preliminary screening. J Ethnopharmacol, 1986; 18: 133 – 141.
6. Gupta A, Khajuria A, Singh J, Bedi KL, Satti NK, Prabhu Dutt, Suri KA, Suri OP and Qazi GN. Immunomodulatory activity of biopolymeric fraction RLJ-NE-205 from *Picrorhiza kurroa.* International Immunopharmacology, 2006; 6 (10): 1543- 49.
7. Abbas AK and Lichtman AH. Basic immunology: functions and disorders of the immune system (2nd ed.).WB Saunders Philadelphia, 2004.
8. Pardeshi S, Dhodapkar R, Kumar A. Molecularly imprinted microspheres and nanoparticles prepared using precipitation polymerisation method for selective extraction of gallic acid from *Emblica officinalis.* Food Chemistry, 2014; 146: 385 – 393.
9. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian medicinal plants. New Delhi, India: CSIR; 2002.
10. Dhar ML, Dhar MM, Dhawan BN, Mehrotra BN, Roy C. Screening of Indian plants for biological activity. Part I. Indian J of Exp Biol, 1968; 6: 232 – 247.
11. Tumer RA. Screening methods in pharmacology. New York, USA; Academic Press, 1944; 303.
12. Gupta A, Khamkar PR and Chaphalkar SR. Anti-inflammatory activity of aqueous extract of *Mimusops elengi* on human whole blood and peripheral blood mononuclear cells. International Journal of Current trends in Pharmaceutical research, 2014; 2(5): 494 – 501.
13. Gupta A, khajuria A, Singh J, Singh S, Suri KA and Qazi GN. Immunological adjuvant effect of *Boswellia serrata* (BOS 2000) on specific antibody and cellular response to ovalbumin in mice. International Immunopharmacology, 2011; 11(8): 968 – 975.

14. Gupta A, Jagtap RB and Chaphalkar SR. Anti-viral activity of *Azadirachta indica* leaves against Newcastle disease virus: A study by *in vitro* and *in vivo* immunological approach. International Journal of Current trends in Pharmaceutical research, 2014; 2(6): 494 - 501.