

Immunopharmacological Activity of *Zingiber officinale* on Human Peripheral Blood Mononuclear Cells

Amit Gupta^{1*} and Sushama R Chaphalkar²

¹Department of Immunology, Vidya Pratishthan's School of Biotechnology, Baramati, District Pune, Maharashtra India

²Director, Vidya Pratishthan's School of Biotechnology, Baramati, District Pune, Maharashtra India

*Corresponding author's Email: amitvsbt@gmail.com; amitgupta@vsbt.res.in

ABSTRACT: There are number of medicinal plant products which have been traditionally used in India including various other subcontinents for various kinds of ailments or diseases. In spite of these treatments, there is an urgent need for those substances which showed anti-inflammatory as well as anti-viral activity. To achieve this objective, our group focused on the rhizome portion of *Zingiber officinale* was screened for anti-inflammatory activity against specific antigen (hepatitis B vaccine, HBsAg) and also observed its antiviral activity against new castle disease virus (NDV) in human peripheral blood mononuclear cells (PBMC). The results showed that the aqueous rhizome extract of *Zingiber officinale* showed remarkably decline in the number of CD14 monocyte count with exposure of HBsAg and NDV as compared to control group. In addition, this extract also showed drastically reduced in the level of antigen specific HBsAg and NDV proliferation at higher doses as compared to control. NDV and HBsAg used as standard for these studies and the results showed that there is drastically increased in CD14 monocytes count and proliferation assay as compared to control. Overall, the results showed that the aqueous rhizome extract of *Zingiber officinale* showed anti-inflammatory as well as anti-viral activity.

Keywords: *Zingiber officinale*, anti-inflammatory, anti-viral, NDV

ORIGINAL ARTICLE
PII: S2322-47891500003-5
Received 16 Jun. 2015
Accepted 25 Jun. 2015

INTRODUCTION

There are number of medicinal plant products i.e. leaves, root, stem etc. which have potential to showed number of immunopharmacological activities such as anti-inflammatory (Gupta et al., 2014 a,b,c; Chen et al., 1999), anti-viral (Gupta et al., 2014d) etc. These medicinal plants serve as rich sources of chemical constituents which can be used for a variety of prophylactic and therapeutic purposes (Yeap et al., 2011; Gupta et al., 2014e,f). These activities could be due to the presence of active phytochemicals i.e. alkaloids, flavonoids, terpenoids, glycosides, saponin etc present in the medicinal plants (Gupta et al., 2014d). However, most of the medicinal plant products with antiviral activity is still not known and these plants are capable of acting therapeutically in various infections (viral, bacterial, parasitic etc.) has raised optimism about the future of phyto-antiviral agents. Most of the anti-viral products are of synthetic based origin or analogs of natural products possess chemical as well as therapeutic applications (Gupta et al., 2014d). In the present study, our group focused on medicinal plants research techniques for anti-inflammatory as well as antiviral drug discovery and development.

One of the natural medicinal plant i.e. *Zingiber officinale* (family, Zingiberaceae) commonly known as ginger which is commonly used as well as effective against cough, cold and congestion (Kikuzaki et al., 1991; Sharma

and Gupta, 1998; Bone et al., 1990). During literature survey of *Zingiber officinale*, this natural medicinal plant showed various immunopharmacological activities such as anti-emetic (Bone et al., 1990) and chemo-protective effects (Katiyar et al., 1996). In addition, natural medicinal plant, ginger showed number of medicinal uses i.e. effective in lowering or reducing the cholesterol level in case of person with heart problems; daily intake of ginger reduced the level of migraine problem (Katiyar et al., 1996) etc. In the present study, our group focused on natural medicinal plants i.e. *Zingiber officinale* showed anti-inflammatory as well as anti-viral activity in human peripheral blood mononuclear cells (PBMC) using specific antigen HBsAg and NDV.

MATERIAL AND METHODS

Assemblage and preparation of aqueous extract

Zingiber officinale rhizome was purchased from the local market at Baramati, District Pune, Maharashtra, India and used for various immunopharmacological studies. One kilogram fresh *Zingiber officinale* rhizome was cleaned and washed under running tap water, cut into small pieces and macerated with liquid nitrogen to prepare fine *Zinger officinale* powder. Fifty gram of *Zingiber officinale* powder was dissolved in 50 ml phosphate buffered saline and centrifuged at 8000 rpm for 10 minutes. The clear

supernatant was separated and referred as *Zingiber officinale* rhizome aqueous extract.

Qualitative analysis of aqueous extract

Different qualitative tests were performed in order to determine the presence of secondary metabolites present in the rhizome aqueous extract of *Zingiber officinale*. The results clearly indicate that the rhizome aqueous extract showed the presence of alkaloids (Acetic acid and Ethanolic extraction); terpenoids (Absolute Alcohol extraction test); flavonoids (Methanolic extraction test), saponin (Diethyl ether and n-Butanolic extraction test) and glycosides (glacial acetic acid and ferric chloride test).

Segregation and proliferation of NDV in embryonated chicken eggs

Specific pathogen free chicken eggs were purchased from Venkys India Ltd, Pune (Maharashtra, India). The allantoic cavity route of embryonated chicken specific pathogen free eggs (9-11 day old) was used for segregation and proliferation of NDV from field samples (Gupta et al., 2014c). These pathogen free eggs were observed through candling at different time intervals. During observation of these eggs, bigger sized embryos (area without blood vessels; 3 - 4 mm below the air cell) selected for inoculation. After disinfection of egg shell with spirit, 200 μ l of supernatant was inoculated at 45^o angle into embryonated chicken eggs. The motility of embryo was observed every 4 hours by candling. After the death of embryos, amnio-allantoic fluid was harvested and checked for presence of virus (determination through haemagglutination test).

Determination of anti-inflammatory and antiviral activity in human PBMC using CD14 monocyte surface marker and lymphocyte proliferation assay using HBsAg and NDV

Informed consent letter was collected from the healthy volunteers prior to blood collection only if the participants are healthy and does not show any signs or symptoms of asthma exacerbation or respiratory infection or any other illness.

To evaluate the effect of rhizome aqueous extract of *Zingiber officinale* on the proliferation of PBMC, cell suspension (5×10^6 cells/ml, 100 μ l) was pipetted into 96 well plates in the presence of rhizome aqueous extract (1.25 – 10 mg, 50 μ l) of *Zingiber officinale* including with or without exposure of HBsAg (2 μ g/ml, 50 μ l) or NDV (1:100 dilution, 50 μ l) cultured at 37 °C for 48 h, the plates were centrifuged at 1400 x g, 5 min and the supernatant was discarded and add fresh fresh complete media. Incubate 96 well plates for 24 h and then suddenly add 10 μ l of MTT solution (2.5 mg/ml) were added to each well and incubated for 4 h. The plates were centrifuged at 5000 rpm for 5 minutes and then the supernatant was discarded. Adding DMSO solution to the formazon crystals and the absorbance was evaluated in an ELISA reader at 570 nm

(Bernas T and Dobrucki, 2002; Gupta et al., 2015a; Gupta et al., 2014g). HBsAg used as standard for anti-inflammatory studies whereas NDV for anti-viral studies.

In another set of experiment, PBMC were again co-cultured for 48 h with serial dilutions of rhizome aqueous extract of *Zingiber officinale* along with HBsAg or NDV for flow cytometric analysis. 100 μ l of PBMC cell suspension (5×10^6 cells/ml) was taken in each tube. Afterwards, add FITC labeled CD14 monoclonal antibody (3 μ l) was added directly to the cells. Incubated all the PBMC samples with HBsAg or NDV in dark for 30 minutes at carbon dioxide incubator. Subsequently, add red cell lysis solution was added and then incubated for 15 minutes at room temperature. All the samples were centrifuged and the supernatant was aspirated and sample was given three washings of PBS (pH 7.4). The resulting stained cell pellet was resuspended in 2000 μ l of PBS and run on a FACS Calibur, flow cytometer (Davey and Kell, 1996; Gupta et al., 2015b; Legendre et al., 2001). The gating (forward and side scatter) applied for data acquisition and analyzed through flow cytometer using cell quest software.

Statistical analysis

Values are expressed as Mean \pm S.E. The difference between the control and treated groups of *Zingiber officinale* is determined through One way Anova test i.e. Boniferroni multiple comparison test.

RESULTS

Effect of rhizome aqueous extract of *Zingiber officinale* on lymphocyte proliferation assay in human PBMC using HBsAg and NDV

The effect of variable doses of rhizome aqueous extract of *Zingiber officinale* on human PBMC proliferation assay using HBsAg (2 μ g/ml, 50 μ l) and NDV (1:100 dilution, 50 μ l) as shown in Figure 1. The results showed that the rhizome aqueous extract significantly reduced the PBMC proliferation assay at higher doses (10 mg) as compared to the HBsAg and NDV control group. HBsAg and NDV used as standard for these studies and the results indicated that there is significant increase in PBMC proliferation assay as compared to control. Overall, rhizome aqueous extract of *Zingiber officinale* at higher doses showed anti-inflammatory as well as anti-viral activity as compared with control group.

Effect of rhizome aqueous extract of *Zingiber officinale* on CD14 monocyte surface marker in human PBMC using HBsAg and NDV

The effect of variable doses of rhizome aqueous extract of *Zingiber officinale* on CD14 monocyte surface marker in human PBMC using HBsAg and NDV as shown in Figure 2. HBsAg and NDV used as standard for these immunopharmacological (anti-inflammatory and anti-viral) studies. The results showed that the rhizome aqueous

extract significantly inhibited the CD14 monocyte surface marker in human PBMC at higher doses (10 mg) as compared to the HBsAg and NDV control group. In

comparison with HBsAg and NDV, rhizome aqueous extract of *Zingiber officinale* showed anti-inflammatory as well as anti-viral activity as compared with control group.

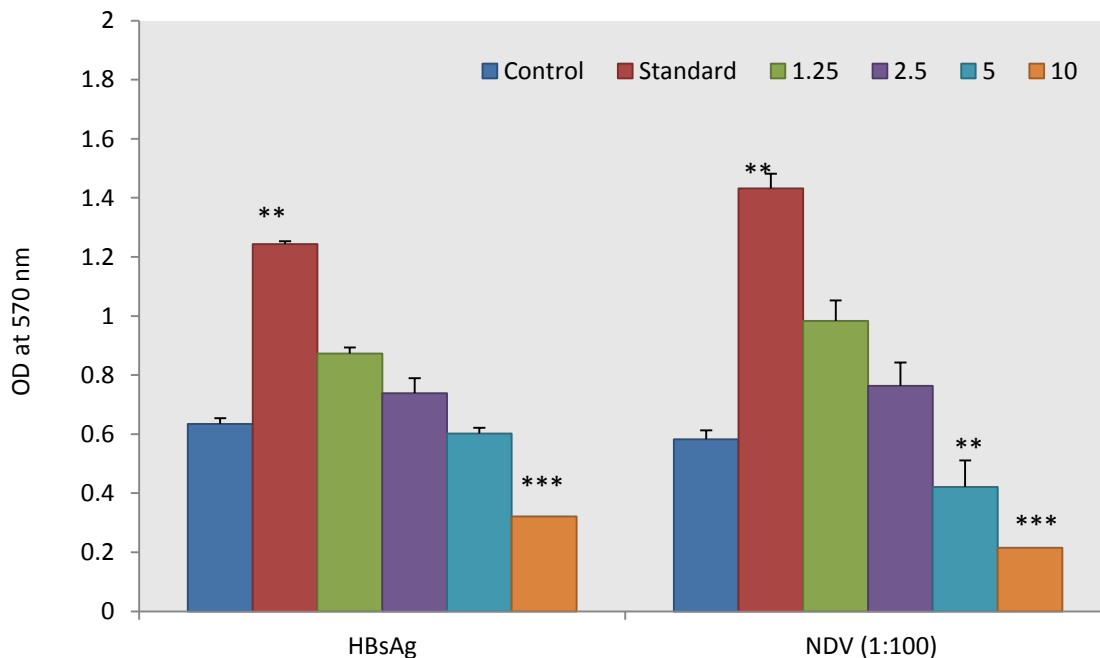


Figure 1. Effect of variable doses of rhizome aqueous extract of *Zingiber officinale* on human PBMC proliferation. PBMC (10^5 cells/ml) were cultured with variable concentration of rhizome aqueous extract (1.25 – 10 mg) along with HBsAg and NDV. Cells were incubated for 48 h and proliferation was measured by MTT assay. Values are expressed as Mean \pm S.E. The difference between the control and treated groups is determined by One way ANOVA test (Bonferroni multiple comparison test). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

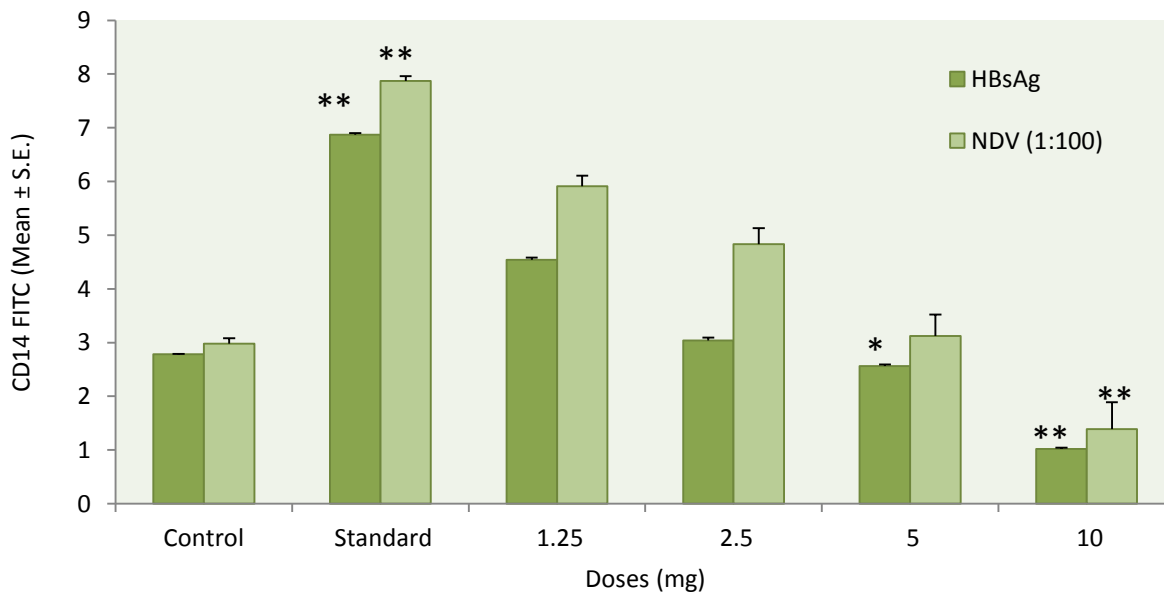


Figure 2. Effect of variable doses of rhizome aqueous extract of *Zingiber officinale* on CD14 monocyte surface marker in human PBMC proliferation. Values are expressed as Means \pm S.E. The difference between the control and treated groups is determined by One way ANOVA test (Bonferroni multiple comparison test). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

DISCUSSION

Medicinal plants have always caught the attention of immunopharmacologists in order to develop the anti-inflammatory as well as antiviral drugs against number of diseases (Gupta et al., 2014 a,b). These medicinal plants are a rich source of primary (protein) as well as secondary metabolites such as alkaloids, flavonoids, tannins and terpenoids that are present and showed number of antimicrobial activities (Gupta et al., 2014d). To further understand the mechanism of secondary metabolites present in the rhizome aqueous extract of *Zingiber officinale* and tested against specific antigen i.e. HBsAg for anti-inflammatory and NDV for anti-viral studies. Hopefully, in the future these medicinal plants especially *Zingiber officinale* can serve as an important source for developing new anti-inflammatory as well as anti-viral drugs.

Zingiber officinale showed an enormous number of immunopharmacological activities i.e. neuroprotective activity and colon cancer have facilitated the extent of further research related to this natural medicinal plant for finding out less toxic and more potent drugs for the better treatment of those diseases (Ghosh, 2011). On the basis of these activities, our objective of our study is to estimate the anti-inflammatory and anti-viral activity of rhizome aqueous extract of *Zingiber officinale* on human PBMC against HBsAg and NDV using proliferation assay by MTT and also determined the CD14 monocyte surface marker which is determined through flow cytometry. The use of flow cytometry for the determination of CD14 surface marker is very useful as well as reproducible for determining the immune status of healthy human of both sexes and different age groups. Moreover, flow cytometry is important as it can provide standard profiles of immune cell elements for males and females. Thus, it provides essential data for evaluating the strength, direction and result of the immune response to antigens, as well as for therapy in specific cases and prevention in cases of exposure to pathogens (Gupta et al., 2015c,d,e).

This study focused on the influence of rhizome aqueous extract of *Zingiber officinale* that have shown anti-inflammatory as well as anti-viral activity on HBsAg and NDV induced PBMC proliferation. The results obtained from this study indicated that rhizome aqueous extract of *Zingiber officinale* exerted an anti-inflammatory as well as anti-viral activity on HBsAg and NDV-stimulated proliferation of human PBMCs with a dosage-dependent relationship. It may be suggested that the PBMC (separated by means of gradient centrifugation) provides relatively accurate as well as reliable information regarding anti-inflammatory and anti-viral activity of treated as well as control samples in human immune system (Gupta et al., 2015e). In this study, the results showed that the rhizome aqueous extract of *Zingiber officinale* significantly inhibited the population of HBsAg and NDV population at higher doses as compared to HBsAg and NDV control

group. HBsAg and NDV used as standard for these anti-inflammatory and anti-viral studies. The results showed that HBsAg showed significantly enhancement of PBMC proliferation as compared to control group. Overall, the data suggests that rhizome aqueous extract of *Zingiber officinale* showed anti-inflammatory as well as anti-viral activity.

To further determined these immunopharmacological activities, correlation exists between PBMCs in treated rhizome aqueous extract of *Zingiber officinale* and standard HBsAg and NDV. In spite of the fact, CD14 (55 kDa glycoprotein with multiple leucine-rich repeats) is widely used as a useful marker molecule for monocytes and macrophages which is determined through flow cytometry (Davey and Kell, 1996). However, the data demonstrate a significant decrease in CD14 monocyte surface marker in PBMC after incubation with variable doses of rhizome aqueous extract of *Zingiber officinale*. HBsAg and NDV used as standard for these studies and the results showed that there is enhancement of CD14 monocyte surface marker as compared to control group. The results showed that *Zingiber officinale* at higher doses showed anti-inflammatory as well as anti-viral activity as compared to control group.

Further investigation should be considered in the effect of rhizome aqueous extract of *Zingiber officinale* on other immune parameters such as macrophage activity, NK cell activity including cell signaling and cytokine production.

CONCLUSION

In the present study, we found that the rhizome aqueous extract of *Zingiber officinale* significantly inhibited the production of CD14 monocyte surface marker and PBMC proliferation assay using HBsAg and NDV. To further continue these studies, our group now will focus on the *in vivo* assessment of the biological activity of these rhizome aqueous extracts and on the chemical identification of the major active components responsible for the anti-inflammatory and anti-viral activity in the efficacious extracts.

Acknowledgement

Authors are thankful to Dr Gandhi and Mr. Shekhar, *Mangal Pathology Laboratory*, Baramati region, District Pune, Maharashtra, India for providing EDTA human blood samples for research work and also thankful to Dr H Pawar (Veterinary Doctor) for collection of NDV samples under the project of Biovillage- Sustainable development through Biotechnology.

REFERENCES

- 1) Gupta A, Khamkar PR and Chaphalkar SR (2014a). Anti-inflammatory activity of aqueous extract of

- Mimusops elengi* on human whole blood and peripheral blood mononuclear cells. *Int. J. Current Trends Pharmac. Res.*, 2(5): 494 – 501.
- 2) Chen YJ, Dai YS and Chen BF, et al (1999). The effect of tetrandrine and extracts of *Centella asiatica* on acute radiation dermatitis in rats. *Biol. Pharm. Bull.*, 22: 703-706.
 - 3) Gupta A, Khamkar PR and Chaphalkar SR (2014b). Immunomodulatory and anti-inflammatory activity of aqueous extract of leaf, stem and root of *Ficus religiosa* on human whole blood and peripheral blood mononuclear cells. *Int. J. Med. Pharmac. Res.*, 2(4): 732 - 739.
 - 4) Gupta A, Khamkar PR and Chaphalkar SR (2014c). *In vitro* anti-inflammatory activities of root aqueous extract of *Mesua ferrea* in human whole blood and peripheral blood mononuclear cells using flow cytometry. *Int. J. Pharm. Life Sci.*, 5(10): 3903 – 3909.
 - 5) Gupta A, Jagtap RB and Chaphalkar SR (2014d). Anti-viral activity of *Azadirachta indica* leaves against Newcastle disease virus: A study by *in vitro* and *in vivo* immunological approach. *Int. J. Current trends Pharm. Res.*, 2(6): 494 - 501.
 - 6) Yeap SK, Alitheen M, Noorjahan B, Ho W, Abdul Rahman O, Abdul Manaf A, et al (2011). Immunomodulatory role of *Rhaphidophora korthalsii* methanol extract on human peripheral blood mononuclear cell proliferation, cytokine secretion and cytolytic activity. *J. Med. Plants Res.*, 5(6): 958- 965.
 - 7) Gupta A, Khamkar PR and Chaphalkar SR (2014e). Applications and uses of active ingredients from medicinal plants. *Ind. J. Novel Drug Del.*, 6(2): 106 – 111.
 - 8) Gupta A, Khamkar PR and Chaphalkar SR (2014f). Review on medicinal plants to target and inhibit the epidermal growth factor receptor signaling in cancer and tissue repair therapy. *Int. J. Adv. Pharm. Biol. Chem.*, 3(1): 210- 213.
 - 9) Kikuzaki H, Kobayashi M and Nakatani N (1991). Constituents of Zingiberaceae, Diarylheptanoids from Rhizomes of *Zingiber officinale*. *Phytochem.*, 30: 3947-3952.
 - 10) Sharma SS and Gupta YK (1998). Reversal of cisplatin-induced delay in gastric emptying in rats by ginger (*Zingiber officinale*). *J. Ethnopharm.*, 62 (1): 49-55.
 - 11) Bone ME, Wilkinson DJ, Young JR, McNeil J and Charlton S (1990). The effect of ginger root on postoperative nausea and vomiting after major gynecological surgery. *Anaesth.*, 45 (8): 669 – 671.
 - 12) Katiyar SK, Agarwal R and Mukhtar H (1996). Inhibition of tumor promotion in SENCAR mouse skin by ethanol extract of *Zingiber officinale* rhizome. *Cancer Res.*, 56 (5): 1023 – 1030.
 - 13) Bernas T and Dobrucki J (2002). Mitochondrial and nonmitochondrial reduction of MTT: interaction of MTT with TMRE, JC-1, and NAO mitochondrial fluorescent probes. *Cytomet.*, 47 (4): 236 – 242.
 - 14) Gupta A and Sushama R Chaphalkar (2015a). Anti-inflammatory activity of aqueous extract of leaves of *Prosopis spicigera*. *Int. J. Res. Pharm. Life Sci.*, 3(1): 829 – 834.
 - 15) Gupta A and Sushama R Chaphalkar (2014g). Immunorestorative and anti-inflammatory activity of leaf aqueous extract of *Calotropis gigantean* using flow cytometry. *Int. J. Drug Disc. Herbal Res.*, 4(4): 761 – 765.
 - 16) Davey HM and Kell DB (1996). Flow cytometry and cell sorting of heterogeneous microbial populations: the importance of single-cell analyses. *Microbiol. Rev.*, 60: 641 -696.
 - 17) Gupta A and Sushama R Chaphalkar (2015b). Flow cytometric evaluation of anti-proliferative and anti-inflammatory activity of plant viruses: TMV tobacco plant. *J. Int. Res. Med. Pharmaceu. Sci.*, 5(4): 169 - 175.
 - 18) Legendre L, Courties C and Troussellier M (2001). Flow cytometry in oceanography 1989--1999: environmental challenges and research trends. *Cytomet.*, 44:164-72.
 - 19) Ghosh AK (2011). ZINGIBER OFFICINALE: A natural gold. *Int. J. Pharma Bio. Sci.*, 2(1): 283 – 294.
 - 20) Gupta A and Sushama R Chaphalkar (2015c). Exploring the immunogenicity potential of plant viruses used as vaccine antigen: Tobacco plant. *J. of Int. Res. Med. Pharmaceu. Sci.*, 5(4): 118 -127.
 - 21) Gupta A and Sushama R Chaphalkar (2015d). Rapid detection of immunosuppressive activity of aqueous extract of *Calamus rotang* using flow cytometry. *J. Med. Chem. Drug Disc.*, 2: 01- 08.
 - 22) Gupta A and Sushama R Chaphalkar (2014e). Inhibitory potential of aqueous extract of *Ficus bengalensis* on human peripheral blood mononuclear cells. *J. Pharma Res.*, 3(11): 250 – 253.