

Original article

Immunomodulatory and antimicrobial activity of a polyherbal composition, Panchatulasi drops, derived from essential oils of five species of basil

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Abstract

Basil has been used for thousands of years in Ayurveda for its diverse healing properties. The juice of the leaves have strong expectorant properties, and is used in ailments of the respiratory system. This study investigated the immunomodulatory and antibacterial properties of Panchatulasi drops, a unique blend of essential oils from 5 different species of basil. The effect of the essential oils on proinflammatory mediators (IL-6 and TNF- α), and cell viability was determined in a dose dependent manner, using a rat splenocyte model. Immunomodulation via lipoxygenase inhibition was investigated, using lipoxygenase inhibitor screening kit. MIC against *Streptococcus pneumoniae* strains was determined by the broth dilution method with Cation adjusted Mueller-Hinton broth (CAMHB), and antibacterial efficacy was also tested using a neutropenic mouse model of lung infection against ATCC 700904 *Streptococcus pneumoniae* strain.

Panchatulasi drops demonstrated inhibition of IL-6 and lipoxygenase enzyme. It resulted in increased levels of TNF- α in a dose dependent manner in non-cytotoxic doses (up to 10 μ g/ml), although overall concentration decreased between 24 to 72 h. after LPS induction. The extracts exhibited good antimicrobial activity with an MIC of 32 μ g/ml and bacterial load reduction of 6.45 \pm 0.55 at 150 mg/kg bw, compared to 5.32 \pm 0.62 of amoxicillin (p<0.005). These studies provide significant evidence of immunomodulatory and antimicrobial properties of Panchatulasi drops. The formulation could, therefore, be developed as a potential therapy in inflammatory conditions such as respiratory infections and mucosal injury.

Key words: Immunomodulation, Ayurveda, essential oils, basil, Panchatulasi drops

1. Introduction

1.1 Immunomodulation in Ayurveda

The traditional Indian system of medicine, known as Ayurveda, has been used for generations in treatment of diseases associated with the immune system. Unlike the modern system of medicine, Ayurveda is more preventive in nature and is centered on maintaining a balance in the body. Medicinal plants prescribed by Ayurveda are known for enhancing the body's defense mechanism through their rejuvenating properties and immunomodulatory effect on the immune system (Mitra Mazumder *et al.*, 2012).

Immunomodulation is the regulation of host responses by stimulation or suppression according to different pathological or biological changes (Mukherjee *et al.*, 2014). The proper functioning of the immune system requires a balance between the two. This has been well established in Ayurveda using Rasayana therapy, one of

the eight branches of Ayurveda, which consists of rejuvenating plants or herbs with antimicrobial, anticancer, and immunomodulation effect.

One of these plants that has been used for the past several centuries as a complementary medicine to enhance immunity and metabolic functions is *Ocimum sanctum* Linn. (Basil). *O. sanctum* has been tested for anticancer activity (Seena *et al.*, 1993) and has also shown significant efficacy in treatment of patients with acute viral encephalitis, compared with steroids like dexamethasone (Das *et al.*, 1983). Other species found in India are *Ocimum gratissimum* Linn., *Ocimum basilicum* Linn., *Ocimum canum* Linn. and *Ocimum citriodorum* Linn. (hybrid of *O. basilicum* and *O. americanum* which is also found in India). The 5 species of *Ocimum* have been used in traditional practice to cure fever, fungal infections, respiratory infections, sunstroke and headache. They also have antipyretic, anti-inflammatory, antioxidant, stimulant, diuretic and demulcent properties (Bhasin, 2012; Tiwari *et al.*, 2016).

The formulation under investigation, Panchatulasi drops, is a liquid prepared with a unique blend of the 5 essential oils of basil. Constituents of basil, like eugenol, β -caryophyllene, carvacrol, linalool and limonene possess anti-inflammatory, virucidal, immunomodulatory, adaptogenic and mutagenic properties (Singh and Verma, 2010). This could, therefore, be a viable option for the

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treatment of immune related disorders in comparison with therapies which are prone to microbial resistance and are harmful to body. In the following study, the formulation Panchatulasi drops (Batch Number B2.3) was evaluated for its immunomodulatory activity on mouse splenocytes *via* its effect on cytokines (IL-6 and TNF- α) and lipoxygenase enzyme activity. The antimicrobial activity of the formulation was also investigated.

1.2 Cytokines in immune response

Cytokines are a large family of signaling proteins secreted by various cells of the immune system (lymphocytes, macrophages, monocytes), and are involved in regulation of immunity, inflammation and hematopoiesis. One of the mediators of immune response is IL-6, a pleiotropic cytokine involved in many diseases. It is increased in response to injury, inflammation, activation of cytotoxic T-cells, and differentiation of B-cells. Drugs that target IL-6 have been developed for various diseases including Castleman's disease, rheumatoid arthritis and other chronic inflammatory diseases (Tanaka and Kishimoto, 2012). Elevated levels of IL-6 have been detected in chronic lesions of patients with multiple sclerosis, and a preclinical study showed treatment with an anti-IL-6 receptor monoclonal antibody inhibited the development of experimental autoimmune encephalomyelitis (Serada *et al.*, 2008). TNF- α is another proinflammatory, pleiotropic cytokine that has broad immunomodulatory actions. It has been implicated in autoimmune inflammatory diseases, and anti-TNF- α treatments have shown therapeutic benefits in autoimmune arthritis, psoriatic arthritis, inflammatory bowel syndrome and other immune-mediated inflammatory diseases. Interestingly, however, TNF- α antagonism demonstrated 'deleterious' effects in multiple sclerosis. TNF- α inhibition stimulated the CNS immune response and increased disease activity (Lim and Constantinescu, 2010). The contrasting role of pleiotropic cytokines such as TNF- α suggests that the underlying mechanisms are complex and measuring the same under different conditions could elucidate some of these mechanisms.

1.3 Lipoxygenase and inflammation

Lipoxygenases (LOXs) are a family of iron-containing enzymes that catalyze the bioxygenation of polyunsaturated fatty acids to form hydroperoxides. They are the key enzyme involved in the synthesis of leukotrienes, implicated in many inflammatory and allergic diseases (Rackova *et al.*, 2007). A study showed that inhibition of 5-lipoxygenase (5-LOX), which is overexpressed in rheumatoid arthritis, significantly decreased the concentration of TNF-alpha-induced Interleukin-6 and chemo-attractant protein-1 in human synovial fibroblasts (Lin *et al.*, 2014). LOX inhibitors could, therefore, be potential therapeutic agents in inflammatory conditions.

1.4 Current anti-inflammatory therapeutics

Paclitaxel (Taxol) is an anticancer drug that functions by inhibiting mitosis at the G2/M phase. It has been used in chemotherapy for various types of cancer and solid tumors. Dexamethasone is a corticosteroid used in the management of inflammatory and autoimmune disorders including ocular, airways, skin and other central nervous system inflammation (Bucki *et al.*, 2010). Both drugs have been suggested as anti-inflammation agents, but are associated with adverse effects. Paclitaxel is associated with anaphylaxis, severe hypersensitivity reactions and resistance at

high doses (Peng *et al.*, 2014). Other side effects include hair loss, low blood count, nail disorders, diarrhea and peripheral neuropathy. Dexamethasone has metabolic effects that cause suppression of growth, immunity and the hypothalamic adrenal axis (Gligorov and Lotz, 2004; Deshmukh, 2007).

Considering the above, there is a need for further investigation of medicinal plants and evidence-based development of herbal drugs to treat inflammatory diseases and infections.

1.5 Study objective

Due to the side effects of standard anti-inflammatory medicines and ineffectiveness of many treatments for chronic autoimmune and respiratory tract infections (Doyle *et al.*, 1988), further research into basil essential oils could provide alternative safer treatments.

2. Materials and Methods

2.1 Chemicals

RPMI-1640, paclitaxel, dexamethasone, lipopolysaccharide from *E. coli* 0111:B4 and dimethyl sulfoxide were purchased from Sigma Life Sciences Ltd. Fetal Bovine Serum was purchased from GIBCO. AK lysis buffer and normal saline were of analytical grade.

2.2 Plant material

Leaves of the plant of *Ocimum* species, *viz.*, *O. sanctum*, *O. gratissimum*, *O. basilicum*, *O. citriodorum* and *O. canum* were obtained from the state of Uttarakhand, Haridwar and Chattisgarh, as per good agricultural and collection practices (GACP). They were in accordance with ethnobotanical assessment conducted by CSIR (Council of Scientific and Industrial Research). The formulation, Panchatulasi drops (Batch Number 2.3 also referred as test compound B2.3), produced using the essential oils derived from the plant leaves, was approved by the licensing authority of Uttarakhand under the Ministry of Health (Department of AYUSH).

2.3 Preparation of extracts of essential oil

For the preparation of the essential oil of species of *O. sanctum*, the fresh leaves (in lots of three kilos) were distilled with water from a large copper distilling apparatus fitted with a copper worm condenser. This was done till distillate changed from opalescent to perfectly clear. The distillate (5 liters) was shaken with petroleum ether in a large separator, and the upper layer was distilled after dehydration with anhydrous sodium sulphate until the petroleum ether no longer came over. The crude essential oil of the *O. sanctum* obtained is clear bright yellow with a characteristic odor of the plant, mixed with a strong note of cloves. Separation of phenolic and non-phenolic constituents of the essential oil was carried out using petroleum ether. The petroleum ether was removed from the crude distillate by heating on a water bath. Rectification of the phenolic constituent was done by fractional distillation under ordinary pressure. The same process was carried out for extraction of essential oils from *O. gratissimum*, *O. basilicum*, *O. canum* and *O. citriodorum*, respectively. All the essential oils obtained as well as the mixture were validated by GC-FID before testing for their activity.

2.4 Animals

Mice (BALB/c strain, male) having body weight 22-26 g were supplied by Harlan Laboratories, Indianapolis, USA. The animals were fed standard chow and *ad libitum*. The experimental procedures

were in accordance with the internationally accepted principles for laboratory animal use and care and; approved by Institutional Ethics Committee (IEC-2012) of Anthem Biosciences under the supervision of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Government of India.

2.5 Preparation of test drugs and reference compounds for *ex vivo* models

The initial stock solution for test compound B2.3 was prepared using 100% DMSO solution. The working stock solution was prepared by 1:1 dilution (50% DMSO concentration) with RPMI-1640 medium. The final stock solution (2 mg/ml) was prepared by 1:10 dilution with RPMI medium. 10 µl of above stocks was added into 180 µl of splenocyte cell suspension and LPS mix, in incubation wells to obtain 100, 10, 1, 0.1, 0.01, 0.001 and 0.001 µg/ml. The final DMSO concentration in plate was about 0.25%. The initial stock for reference compounds paclitaxel and dexamethasone was prepared using 100% DMSO. 10 µl of above stocks was added into 180 µl of cell suspension and LPS mix, in incubation wells to obtain 10, 1, 0.1, 0.01 and 0.001 µM. The final DMSO concentration in plate was 0.5%.

2.6 Splenocyte isolation and assay

The spleen was removed from male BALB/c mice aged 6-7 weeks (22-26 g) and placed into sterile culture dish containing plain RPMI-1640 medium. The splenocytes were extracted using cell strainer (70 µm) and centrifuged at 320 X g for 4 min. at 4°C. The pellet was re-suspended with 1 ml of RBC lysis buffer (ACK lysis), incubated at ambient temperature for 10 min. and washed twice with plain RPMI. Cells were counted in hemocytometer. Finally, pellets were resuspended in RPMI medium (with 2 µM glutamine, 10% heat-inactivated FBS and NaHCO₃). The counted cells were diluted with fresh RPMI medium to get 2×10⁶ cells/ml and 180 µl of cells suspension was added to the wells. Ten microliter of diluted LPS was added to 180 µl of cell suspension to test wells. The RPMI-1640 medium was added to control wells (No induction). Test compound (10 µl) from final stock solution (test compound B. No: 2.3) was added to 180 µl of cell suspension and LPS mix, incubation wells to obtain 100, 10, 1, 0.1, 0.01, 0.001 and 0.001 µg/ml. The final concentration DMSO was 0.25%. The same was done for reference compounds, and the final DMSO concentration in plate was about 0.5%. For control wells, 10 µl of RPMI medium containing 5% DMSO (No inhibition). Plates were then incubated in 5% CO₂ incubator for a period of 72 h. After 24, 48 and 72 h. of incubation, plates were centrifuged at 2000 RPM at 4°C for 10 min. The supernatant was removed (140 µl) without disturbing cell pellet and stored at (-80°C) for cytokine estimation. The cell pellet was taken for viability assay.

2.7 Cell viability assay and quantification of TNF-α and IL-6 in the supernatants

The cell viability of test compound was assessed by the mitochondrial dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) to purple formazan. The cell suspension test compound mixture, were incubated with MTT (5 mg/ml) for 2 h. at 37°C. The medium was removed by aspiration and formazan crystals were dissolved in DMSO. The extent of the reduction of MTT was quantitated by measurement of the absorbance at 570 nm. The percentage of cytotoxicity was calculated as:

Viability (%): (Test-Blank) / (Control-Blank) × 100

Quantification of TNF-α and IL-6 levels in supernatants

Since the level of TNF-α and IL-6 in the sample was high, the samples were diluted in such a way that they should come within the range of standard curve.

Cytokine detection

Enzyme Linked Immunosorbent Assay (ELISA) kit was used for quantitative determination of cytokines in supernatant of activated rat splenocytes. The appropriate capture antibody was obtained from R and D Systems, Inc USA. It was diluted in Phosphate Buffered Saline (PBS) and coated on a 96-well microtitre plate, 100 µl / well. After overnight incubation, each well was aspirated and washed thrice. The plates were blocked by 300 µl of reagent diluent and incubated at room temperature. The assay used a cytokine standard and biotinylated antibodies (streptavidin-HRP was added to wells) for capture and detection. The analysis was performed as per manufacturer's protocol. A standard curve was created by plotting the mean absorbance for each standard against the concentration and a best fit curve was drawn through the points. The reference standards of paclitaxel and dexamethasone were used in the study.

2.8 Lipoxygenase assay

Lipoxygenase inhibitor screening kit was used which was manufactured by Cayman Chemical (Cat No: 760700). Compound stock (10 mg/ml) solutions were prepared in 100% DMSO and 1:1 methanol water. Compound B2.3 was dissolved in 1:1 DMSO to get 50% DMSO final concentration. 100 µl of indomethacin and aspirin were used as controls. Blank, positive control, 100% initial activity and inhibitor wells were prepared in triplicates as per the protocol. The reaction was initiated by adding substrate (10 µl arachidonic acid) and the plate was incubated on a shaker for 5 min. After incubation, chromogen (100 µl) was added to each well to stop enzyme catalysis. The plate was covered and incubated for 5 min. on a shaker. After incubation, the absorbance was is read at 500 nm using a plate reader.

2.9 Method for MIC

The MIC against *Streptococcus pneumoniae* strains was determined by the Broth dilution technique with cation adjusted Mueller-Hinton broth (CAMHB) according to the technical procedures recommended by the Clinical Laboratory Standards Institute (CLSI). The test items (B No: 1.1, 1.2, 2.1, 2.2, 3.1 and 3.2, MC-OM/C11/011) were dissolved using 60% DMSO solution, and further dilutions were made with broth. The reference compounds ciprofloxacin, Vancomycin were dissolved in water as per the CLSI guidelines (Lorian Fifth Edition; CLSI Document M07-A9, 2012; CLSI Document M100-S17, 2007).

2.10 Antibacterial efficacy of test in neutropenic mouse model of lung infection against ATCC 700904 *Streptococcus pneumoniae* strain

ATCC 700904 *Streptococcus pneumoniae* was cultured in Sheep Blood agar and incubated at 5% CO₂ incubator for overnight at 35°C. The culture was diluted to provide a challenge inoculum, and volume of 20 µl was injected at time "0" through intranasal route. The challenge inoculum count was estimated prior to and after infection by modified Miles and Misra method. Mouse were sacrificed, lungs were isolated, tissue was processed and cultured for measuring bacterial load reduction. Amoxicillin was used as a standard control.

2.11 Statistical analysis

A simple statistical analysis was carried out to calculate the mean and the standard deviation. Standard deviations were calculated for the efficacy of the drug in five animal per group using excel spread sheet. Dunnett’s multiple comparison test (DMCT) was used to calculate the p value. The technique of one-way analysis of variance was also applied here in this present study.

3. Results

3.1 Cell viability by MTT assay

Cell viability increased at 48 h. and 72 h. for test compound B2.3 at a drug concentration of up to 10 µg/ml. At 100 µg/ml, the test compound showed a significant reduction in the concentration of the viable cells at 24, 48 and 72 h., while paclitaxel and dexamethasone did not show a significant reduction in the viable cell count compared to Batch No. 2.3 (Figure 1).

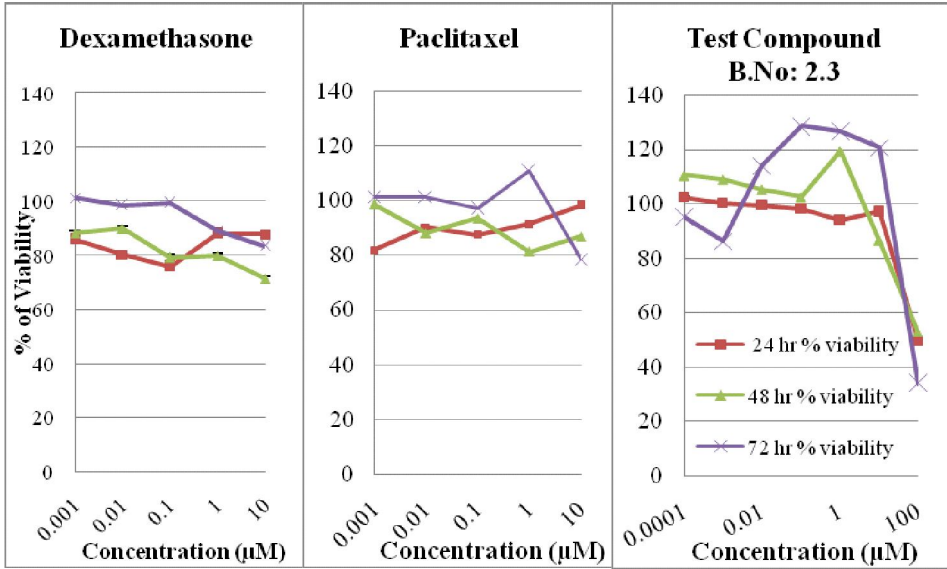


Figure 1: Cell viability comparison between 24, 48 and 74 h. for the dexamethasone, paclitaxel and the test compound.

3.2 TNF-α and IL-6 concentrations

The test compound Batch No. 2.3 at the dose of 10 µg/ml showed significant increase in the concentration of TNF-α at 24 h. The concentration of TNF-alpha was more at 48 h. and at 72 h. compared to cell control. A reduction in the concentration of TNF-α was obtained at 24, 48, 72 h. for 100 µg/ml. The reference compound paclitaxel, did not show significant inhibitory activity on TNF-α at 24 h. and showed an increase in TNF-α at 48 and 72 h. post

treatment. Dexamethasone on the other hand, showed a decrease in TNF-α concentration at all doses (Figure 2).

The test compound showed a reduction in concentration of IL-6 at 72 h. post treatment with doses of 0.1 µg/ml and higher. This effect was not as significant and dose dependent as dexamethasone, which showed substantial reduction in IL-6 concentrations at 24, 48 and 72 h. Paclitaxel did not show any effect on IL-6 at any of the time points measured (Figure 3).

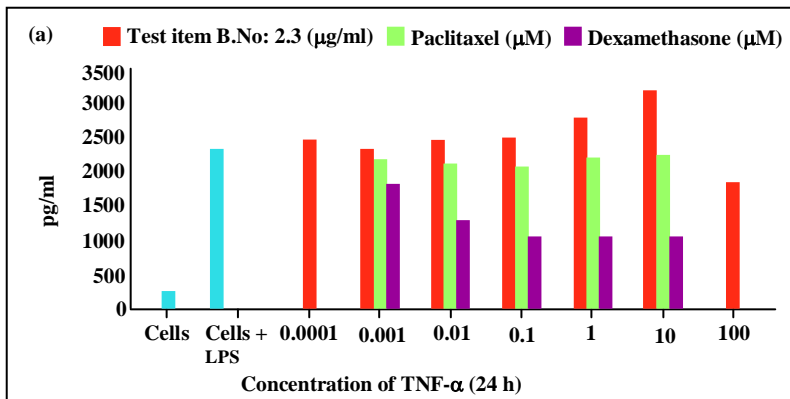


Figure 2(a): Bar chart showing concentration of test compound and controls, dexamethasone and paclitaxel, against the concentration of TNF-α at 24 h. after induction with LPS, determined using ELISA.

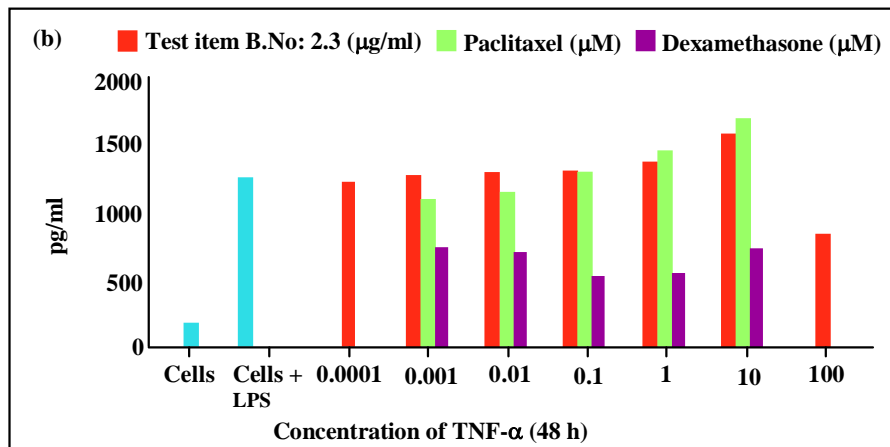


Figure 2(b): Bar chart showing concentration of test compound and controls, dexamethasone and paclitaxel, against the concentration of TNF-α at 48 h. after induction with LPS, determined using ELISA.

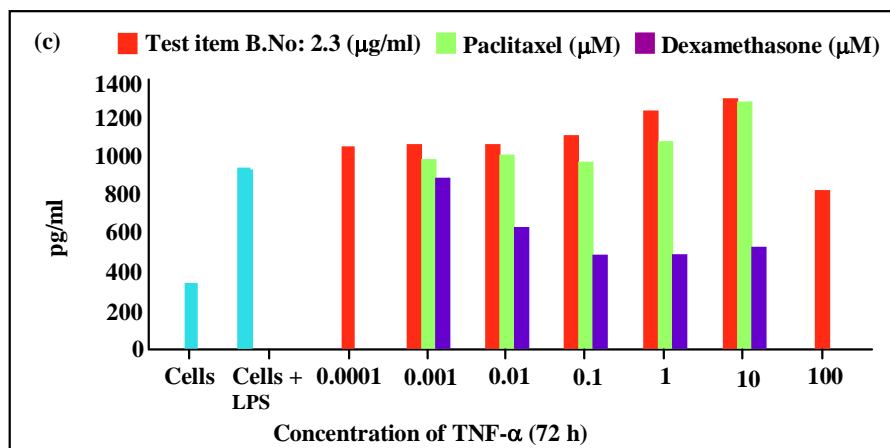


Figure 2(c): Bar chart showing concentration of test compound and controls, dexamethasone and paclitaxel, against the concentration of TNF-α at 72 h. after induction with LPS, determined using ELISA.

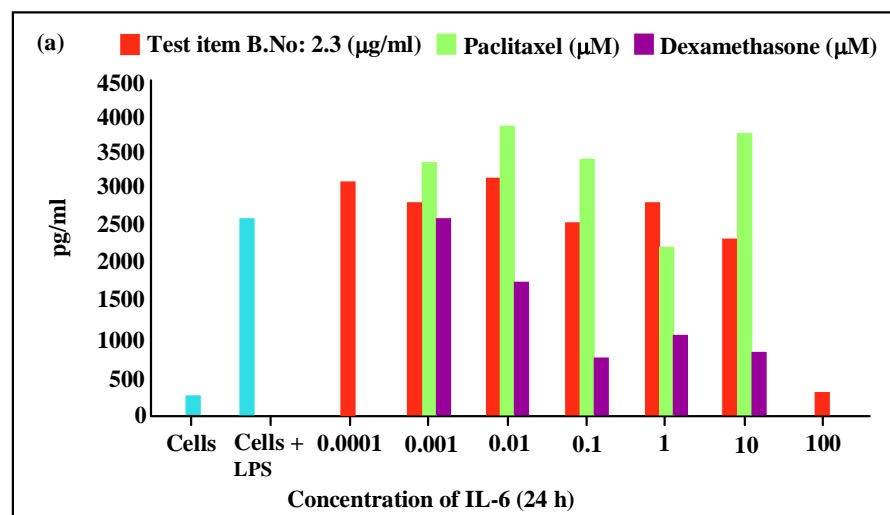


Figure 3(a): Bar chart showing concentration of test compound and controls, dexamethasone and paclitaxel, against the concentration of IL-6 at 24 h. after induction with LPS, determined using ELISA.

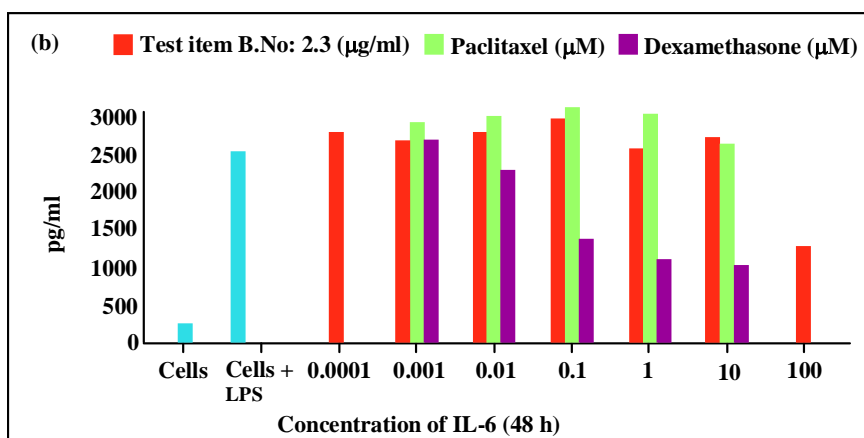


Figure 3(b): Bar chart showing concentration of test compound and controls, dexamethasone and paclitaxel, against the concentration of IL-6 at 48 h. after induction with LPS, determined using ELISA.

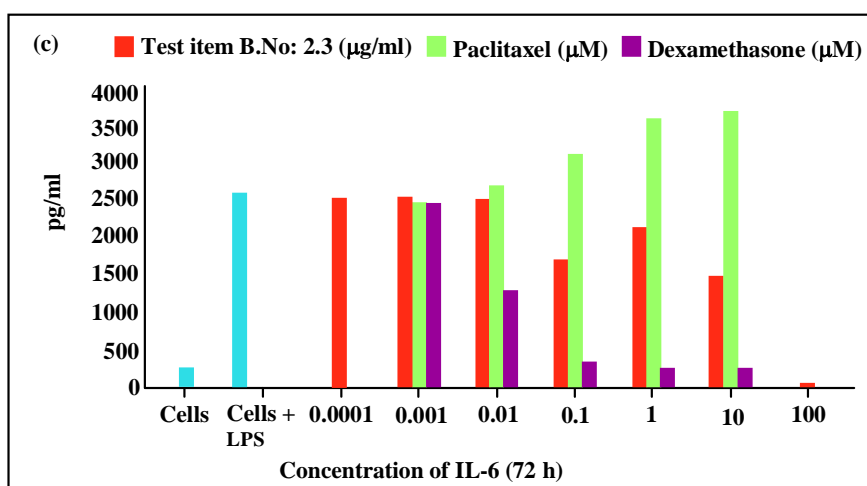


Figure 3(c): Bar chart showing concentration of test compound and controls, dexamethasone and paclitaxel, against the concentration of IL-6 at 72 h. after induction with LPS, determined using ELISA.

3.3 Lipoxygenase inhibition assay results

Positive controls indomethacin and aspirin showed an inhibition of 93% and 60%, respectively of lipoxygenase activity. Test Compound B2.3 shows 58% inhibition compared to 30% inhibition shown by dimethyl sulfoxide (Figure 4).

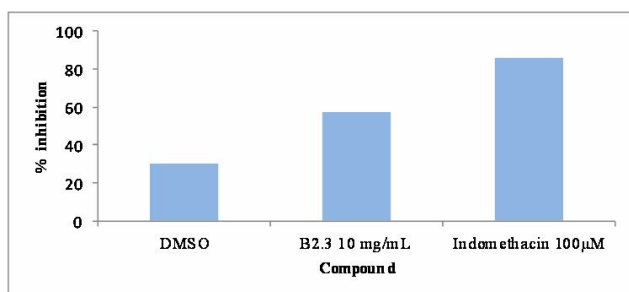


Figure 4: Bar chart showing lipoxygenase inhibition assay with DMSO, test compound B2.3 (10 mg/ml) and indomethacin (100µM). Data analysis and plotting was performed using Microsoft Excel software.

3.4 MIC results

A significant MIC of 32 µg/ml was observed against ATCC 49619 and ATCC 700904 *Streptococcus pneumoniae*. As per the standard guidelines, any extract having MIC less than 100 µg/ml is considered significant for antimicrobial activity and can be further evaluated for drug development process (Table 1).

S.No.	Test compounds	ATCC49619 <i>Streptococcus pneumoniae</i>	ATCC 700904 <i>Streptococcus pneumoniae</i>
1	Test compound B2.3	32 µg/ml	64 µg/ml
2.	Ciprofloxacin	1 µg/ml	2 µg/ml
3.	Vancomycin	0.25 µg/ml	0.25 µg/ml

Table 1: Minimum inhibitory concentration for Panchatulasi oil variants, Panchatulasi drops, marketed compound, ciprofloxacin and vancomycin against *Streptococcus pneumoniae* strains

3.5 Organ burden of bacteria

The organ burden of bacteria was found to be 6.87 ± 0.46 for 75 mg/kg bw concentration for test compound B2.3 against 5.32 ± 0.62 of amoxicillin, respectively. A higher dose of 150 mg/kg showed bacterial reduction as significant as 6.45 ± 0.55 . As per the literature, any antibacterial synthetic compound showing more than 2 \log_{10} reduction (Choi *et al.*, 2012) in organ burden model(s) considered as significant effective dose 50 (ED_{50}). The reduction of the bacteria was found to be statistically significant at $p < 0.05$. Hence, in *in vitro* lung infection, the test compound B2.3 of the Panchatulasi drops proved to be effective in reducing *Streptococcus pneumoniae* growth (Table 2).

Animal groups (mg/kg)	Log ₁₀ value
Untreated 0 h.	5.22 ± 0.25
Untreated 24 h. post treatment	7.55 ± 0.45
B.No:2.3 (150)	6.45 ± 0.55
B.No:2.3 (75)	6.87 ± 0.46
Ref compound amoxicillin (10)	5.32 ± 0.62

Table 2: Log₁₀ bacterial load in lung tissue samples for the untreated sample (control), test compound sample at 75 and 150 mg/kg, and the reference compound amoxicillin at 10 mg/kg.

4. Discussion

The modulation of the immune system has been studied extensively to understand the role of cytokines and immunomodulatory agents in disease pathophysiology. One of the key inflammatory molecules is tumor necrosis factor-alpha (TNF- α), a proinflammatory cytokine that is considered to be a master regulator of cellular cascades. TNF- α has been implicated as a cofactor for mucosal Th1 responses (Plevy *et al.*, 1997), and TNF- α inhibitors have been developed against several autoimmune diseases. However, during the early stages of infection, TNF- α shields against bacterial and viral infection by targeting neutrophil migration and accrual towards the infective site (Malaviya and Abraham, 2000). Its increase in concentration is essential for death of bacteria, fungi and viral infections, oligodendrocytes, endothelial and epithelial cells (Lim and Constantinescu, 2010). TNF- α acts as an initial inflammatory cytokine, however, subsequently regulates neutrophil and eosinophil recruitment into the lung and airspace. Its absence or inhibition has demonstrated increased susceptibility to infection in rheumatoid arthritis, prolonged infection and inability to eliminate bacteria from the middle ear in otitis media and increased risk of acquiring a serious infection in inflammatory bowel disease (Wine and Alper, 2012; Deepak *et al.*, 2013). The test compound B2.3 showed increase in the concentration of TNF- α till non-cytotoxic concentrations of 10 μ g/ml, suggesting that the formulation may assist in clearance of bacterial and viral infections. Although there was a dose dependent increase in TNF- α levels at each time point, the overall concentration decreased between 24 and 72 h. The test compound could, therefore, impact innate signaling of TNF- α by modulating the extent of the cytokine release.

An increase in TNF- α is usually accompanied by increased IL-6 levels, and is associated with the development of an inflammatory

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response. However, in the present study IL-6 concentration was decreased at 24, 48 and 72 h. This is in fact similar to the trend observed in another study using a mouse model, where pretreatment of *E. coli* challenged mice with anti-IL-6 monoclonal antibodies led to an increase in bioactivity of serum TNF- α (Starnes *et al.*, 1990). IL-6 is known to cause activation of T cells *via* increased CCR7 expression along with differentiation of cytotoxic cells and is involved in bacterial sepsis in adults (Buck *et al.*, 1994). Treatment of infections *via* IL-6 inhibition may, therefore, be more effective and safer than TNF- α inhibition. This was demonstrated in a Phase III clinical study where IL-6 inhibition provided sustained relief in patients suffering from rheumatoid arthritis that did not respond well to TNF- α inhibition (Emery *et al.*, 2008).

The formulation exhibited lipoxigenase inhibition almost equivalent to aspirin. In addition to inhibition of prostaglandin synthetase, aspirin and indomethacin both inhibit arachidonic acid metabolism *via* the lipoxigenase pathway. 5-LOX and 15-LOX (Arachidonate 5-lipoxigenase and Arachidonate 15-lipoxigenase) are involved in the metabolic pathway for synthesis of pro-inflammatory leukotriene lipid mediators (Eicosanoid mediators) in cells such as neutrophils and eosinophils (Serhan *et al.*, 2008). In rhinoviral infection, there is an increased expression of 5 lipoxigenase enzyme, and eicosanoids have been implicated in bronchoconstriction, mucus hyper secretion, vasodilation, airway edema and leukocyte chemotaxis (Seymour *et al.*, 2002). The essential oils of basil contain α -linolenic acid, metabolized by the body to eicosapentaenoic acid, which competitively inhibits the formation of prostaglandins and leukotrienes (Singh, *et al.*, 2008). The test compound B2.3 could, therefore, alleviate respiratory infections and other autoimmune related disease by inhibiting the production of inflammatory lipids *via* the lipoxigenase pathway. (Liu *et al.*, 2010).

Streptococcus pneumoniae (the pneumococcus) is the most commonly observed pathogen in community acquired pneumonia, responsible for the development of septic shock, bacterial meningitis and acute respiratory distress syndrome (ARDS) (Schuchat *et al.*, 1997). Amongst the family of micro-organisms, *Streptococcus pneumoniae* has shown resistance towards different antimicrobial therapies and is a common causative pathogen in community-acquired respiratory tract infections (RTIs), including acute otitis media, acute bacterial exacerbations of chronic bronchitis and acute bacterial sinusitis (Jenkins *et al.*, 2008). The antimicrobial activity demonstrated by the test compound on this bacterial strain could support its use in treating infections, especially upper respiratory tract infections that are also associated with cytokine mediated immune responses.

The results obtained can be attributed to the constituents, primarily linolenic acid and eugenol which inhibit lipoxigenase, TNF- α and IL-1 β ; β -Caryophyllene inhibits lipoxigenase, IL-6 and TNF- α ; Carvacrol inhibits IL-6 and IL-1 β ; Linalool inhibits IL-6 (Naidu, 1995; Miguel, 2010). The test compound could, therefore, act by stimulating the humoral immune response *via* T cell and subsequent B cell activation (Vaghasiya *et al.*, 2010) *via* regulation of various cytokines as described above. The graphic in Figure 5 depicts the immunomodulatory effects of different species of basil at various levels of the immune response.

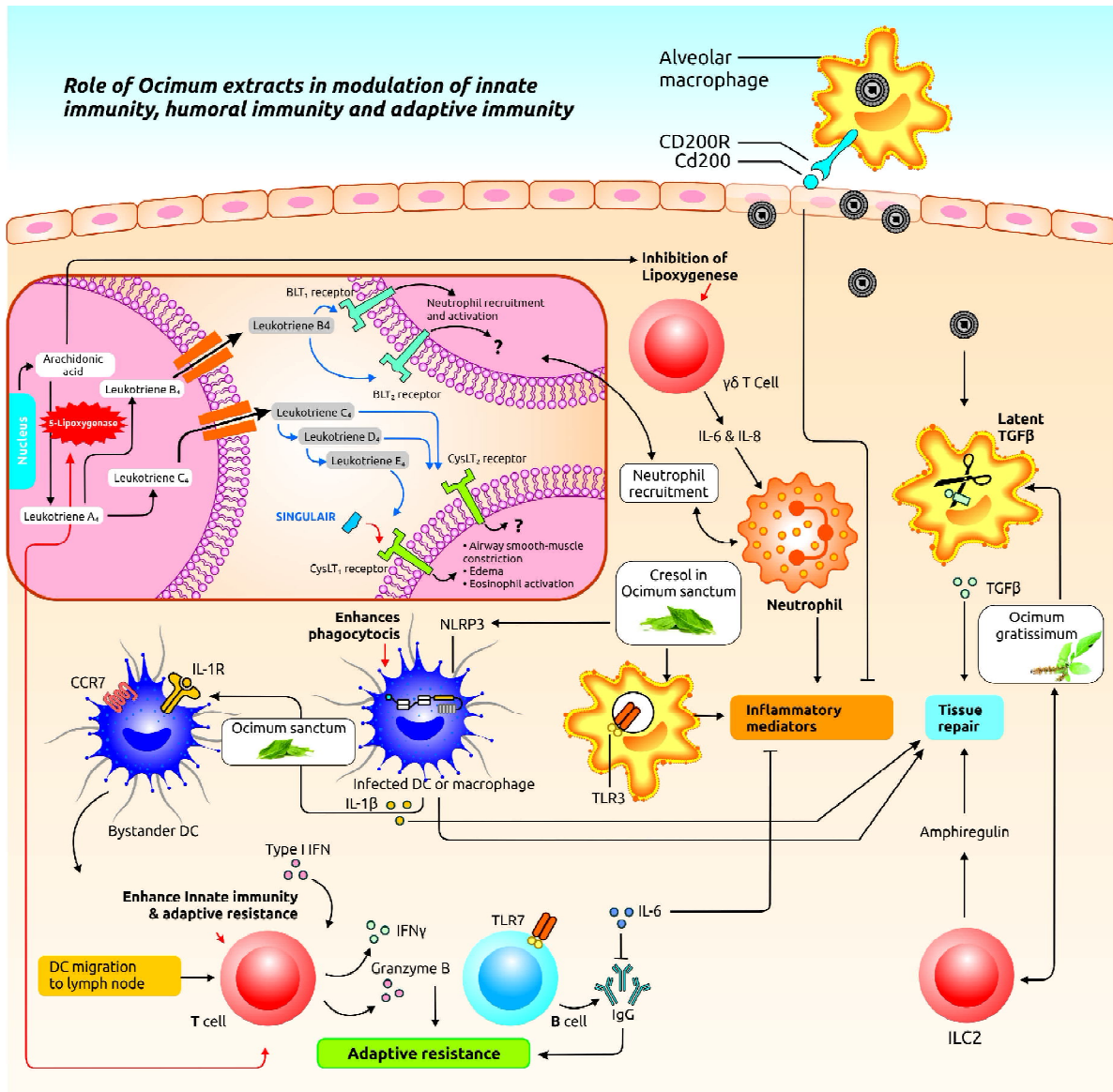


Figure 5: Diagram depicting the immunomodulatory effects of different species of basil at various levels of the immune response.

FIGURE EXPLANATION : DCs and macrophages that are infected with bacteria release interleukin-6 (IL-6), which enables bystander DCs to upregulate CC-chemokine receptor 7 (CCR7) expression and migrate to the draining lymph nodes to stimulate T cells. NLRP3 inflammasomes also increase disease tolerance by promoting tissue repair, NLRP3 inflammasomes are regulated by cresol and quercetin present in *Ocimum sanctum* (<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4179937/>). The IFN, significantly increases due to *Ocimum sanctum*, stimulating T Lymphocytes to produce IFN- γ , consequently enhancing the adaptive immunity (<http://www.ncbi.nlm.nih.gov/pubmed/?term=ifn+gamma+ocimum+sanctum>). The lipoxigenase activity of T- Lymphocytes affects arachidonic acid pathway and consequently inhibits leukotriene B₄ due to the essential oils of basil (<http://nopr.niscair.res.in/handle/123456789/4500>), whereas B cells secrete antibodies to mediate adaptive immune protection of the host (<http://www.kejapub.com/ijpbr/docs/IJPBR10-01-01-05.pdf>). Inflammatory cytokines (such as IL-6 and tumour necrosis factor - TNF) that are induced as a result of innate signalling can also lead to pathology when the duration and extent of cytokine release is increased. Negative regulators of cytokines and inflammatory cells - such as IL-10, IL-6 and CD200-CD200R-suppress inflammatory consequences, while positive regulators offer tissue repair-such as amphiregulin which produced due to release of ILC2 and TGF-Beta stimulated by *Ocimum gratissimum* (<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3246787/>)-promote a return to homeostasis. The balance between these negative and positive regulators determines whether the host succumbs to disease or can enter a state of tolerance (right).

5. Conclusion

The *in vitro* and *ex vivo* preclinical studies described, support immunomodulatory, anti-inflammatory and antimicrobial properties of basil that have been well established in Indian Rasayana Ayurvedic literature. The formulation, Panchatulasi drops could, therefore, be investigated further as an immunomodulator and anti-bacterial for treatment of respiratory infections and possibly in prevention of other infections caused by *Streptococcus pneumoniae*. The anti-inflammatory properties could be further investigated similar to such concentrations.

Conflict of interest

We declare that we have no conflict of interest.

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