Effect of Yoga Module on Pro-Inflammatory and Anti-Inflammatory Cytokines in Industrial Workers of Lonavla: A Randomized Controlled Trial

ABSTRACT
Introduction: Inflammatory markers play a very important role in the body’s defense mechanism. Pro-inflammatory markers and anti-inflammatory markers counterbalance each other. It is extremely essential for the body to maintain their balance for a good immune response.

Objectives: To study the effect of yoga practices on selected pro-inflammatory and anti-inflammatory cytokine among industrial workers.

Materials and Methods: Forty eight male study participants, aged 30-58 years, were randomly divided into experimental (n=24) & control (n=24) groups. Pro-inflammatory cytokine IL-1β and anti-inflammatory cytokine IL-10 were evaluated at the baseline and at the end of 12 wk of yoga training in both the groups. During the experimental study, all the study participants continued with their daily lifestyle and diet. Data were analysed using paired t-test and independent t-test.

Results: The result of within group comparison revealed that the yoga group showed a significant decrease in IL-1 β while significant increase in IL-10 (p < 0.05), whereas the control group revealed no change in IL-1 β (p > 0.05) and IL-10 (p > 0.05). Further, the results between the groups confirmed that the yoga group had significantly lower level of IL-1 β and increase in IL-10 as compared to control group (p < 0.05).

Conclusion: The present study has demonstrated that yoga practices could reduce pro-inflammatory cytokine and increase anti-inflammatory cytokine in industrial workers.

INTRODUCTION
Air pollution is a major environmental challenge in contemporary times. Increasing industrialization, in both developing and developed countries, exposes the common man to pollutants which adversely affect his health [1]. Particularly, industrial workers, who are in direct contact with the pollutants, are affected most due to ill effects of pollutants [2,3]. It is evident that Sulfur oxides (SOx), Nitrogen oxides (NOx), Hydrocarbons (HC), Carbon Monoxide (CO), Hydrogen sulfide (H2S), Ozone (O3), dust, and toxic chemicals are the common pollutants of Iron and steel industries [4,5]. These pollutants are responsible for serious health problems, associated with respiratory disorders and cardiovascular diseases, in workers of such industries [6,7]. Exposure to these pollutants leads to a systemic inflammatory response and an increase in pro-inflammatory cytokines [8-10]. Additionally, it has been observed that a range of inhaled pollutants stimulate alveolar macrophages to produce pro-inflammatory cytokines such as IL-1β, TNF-α, IL-6, IL-8, and Granulocyte macrophage colony stimulating factor (GM-CSF) [11-14].

Apart from pollution, drugs and pathogens, stress is also found to be a major contributing factor for inflammation. According to past studies, pro-inflammatory cytokines were found to be elevated in highly stressed individuals [15-19]. Physical inactivity and sedentary lifestyle were also found to be associated with increased risk of developing chronic low grade inflammatory states [20-21]. However, inflammation is body’s defense mechanism in response to illnesses, caused due to pathogens. A balance between pro-inflammatory cytokines and anti-inflammatory cytokines is maintained by the body. This balance is crucial for immediate immune response in acute and chronic conditions [22]. Nevertheless, recent studies showed anti-inflammatory effects of exercise [23-25]. Elevated levels of IL-10 and IL-1ra and decreased levels of pro-inflammatory cytokines like TNF-α, IL-1α, IL-1β and certain chemokines like IL-8 have been observed in individuals who exercised regularly [23]. It has been observed, in the past research studies that as anti-inflammatory cytokines increases, pro-inflammatory cytokines decreases with regular exercise [26-29]. Together the above findings suggest that regular physical activity plays an important role in decreasing pro-inflammatory cytokines and elevating anti-inflammatory cytokines. Therefore, interventions that aim to reduce systemic inflammation may not only help in the management of chronic disorders, but also in the prevention of these disorders in individuals having an increased risk. In this context, yoga intervention which combines healthy lifestyle and functioning, on the principles of psycho-neuro-immunology might be helpful for enhancing anti-inflammatory response. In fact, very few studies showed positive impact of yoga intervention on stress and markers of inflammation [30,31]. Keeping this in view, the authors designed this study to assess the efficacy of yoga training on pro-inflammatory and anti-inflammatory cytokines in industrial workers.

MATERIALS AND METHODS
The study was conducted in the Scientific Research Department, Kaivalyadhama, Lonavla. Of 60 male subjects in the age group of 30-58 y (41.5±5.2) from Nangargaon Industrial Estate, Lonavla, screened for this study, 48 were recruited for this research study. The study subjects were contacted through telephone and by making personal visits to Nangargaon Industrial Estate office. Prospective subjects were requested to fill up a registration form provided by
the Scientific Research Department. The registration form included the details of past medical history of the study participants. Medical examination of the study participants was also done by the resident medical officer of Kaivalyadhama.

The study participants suffering from cardiac disorders, diabetes, arthritis, physical disability etc., were excluded from the study. The participants addicted to alcohol and smoking, were also excluded from the study.

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The research study was approved by the Institutional Ethics Committee of Kaivalyadhama Yoga Institute, Lonavla. The Institutional Ethical Clearance number is Kdham/SRD/IEC-04/2013. Signed informed consent was obtained from all the participants after being informed about the study protocol at the outset. It was also mentioned to them that they were free to withdraw from the research study at any point of time, without giving any explanation.

Forty eight study participants were randomly assigned to either Experimental group (n=24) or the wait-listed control group (n=24) using chit method. However, one study participant in experimental group and two study participant in the control group did not undergo pre tests due to their absence during the baseline testing. There were 23 study participants in the experimental group and 22 study participants in the control group at the baseline testing. However, at the end of 12 wk, there were 19 study participants in the experimental group and 18 study participants in the control group due to 11 drop-outs [Table/Fig-1]. The reasons for drop-outs/exclusion were mainly disinterest, work pressure, lack of time, illnesses and absence during either pretesting or post testing. All the study participants were in good health and were fit to do yoga practices. Blood samples were collected at the baseline and after three months of yoga intervention to assess the effect of yoga on levels of inflammatory cytokines of industrial workers.

[Table/Fig-1]: Flow Diagram of Subject Enrolment and Attrition

- Excluded (n=12)
  - Not meeting inclusion criteria
  - Not fit to do yoga due to illness/diseases

- Randomized (n= 48)

- Allocated to 12 week yoga intervention (n=24)
  - Baseline assessment (n=23)

- Waitlist control (n=24)
  - Baseline assessment (n=22)
  - Did not undergo pretest due to work pressure (n=2)

- Post test (12 week) assessment (n=19)
  - Study dropout due to illness (n=2)
  - Lack of interest (n=3)

- Post test (12 week) assessment (n=18)
  - Study dropout due to absence during posttest (n=3)
  - Study dropout due to lack of interest (n=3)

- Analysed (n=19)

- Analysed (n=18)
Yoga Intervention
Yoga intervention was extended to the experimental group for a period of three months. Each yoga session was conducted for 45 min, six days a week, for 12 weeks, excluding weekly holidays. Yoga classes were conducted by a yoga expert, appointed by Kaivalyadhama Yoga Institute. Classes were conducted in the evening from 5.30-6.30 pm every day. Control group did not undergo any yoga training and continued with their daily schedule. The yoga training schedule is presented in [Table/Fig-2].

Data Collection
Biochemical testing
A 5 ml venous blood sample was collected at baseline, and after 12 wk of intervention, in the morning after 12 h of fasting. The blood sample was transported under refrigeration to the laboratory within 30 min, centrifuged for 10 min with the serum immediately separated following centrifugation. Two inflammatory markers i.e. IL-1β and IL-10 were measured using Diaclone (France) enzyme immune assay kit on an ELISA plate reader (Bio-Rad 680, Bio-Rad PW 40, USA), where the sensitivity limit was 0.2 mg/ml. Intra-assay coefficients of variation for IL-1β and IL-10 were 4.5% and 3.2% and inter-assay coefficients of variation for IL-1β and IL-10 were 8.7% and 7.3% respectively.

STATISTICAL ANALYSIS
Data analysis was done using statistical software (SPSS, Statistical Package for the Social Sciences, Version 20.0). Data was analysed using paired t-tests, independent t-test and descriptive statistical method. The mean values ± SD of pre and post variables are presented in [Table/Fig-3].

RESULTS
The demographic variables of subjects are presented in [Table/Fig-4] while the values of the outcome measures at pre and post testing are given in [Table/Fig-3]. The result of within group comparison revealed that the yoga group showed a significant decrease in IL-1β while significant increase was observed in IL-10 (t= 3.55 p<0.05; t= 2.4, p<0.05) [Table/Fig-3], whereas, the control group revealed no change in IL-1β (t= 1.1 p >0.05) and IL-10 (t= 2.2, p>0.05). This indicates yoga practice helps to increase anti-inflammatory cytokine and decrease pro-inflammatory cytokine. Further, the results between the groups confirmed that the yoga group had significantly lower level of IL-1β and increase in IL-10 as compared to control group (t=3.1 p < 0.05, t=2.38, p < 0.05).

DISCUSSION
The randomized control trial of 12 week yoga training could reduce IL-1β and increase IL-10 levels. The participants, in the study, were industrial workers working in direct contact with various pollutants emitted by industrial effluents. Previous studies have shown that people are more at risk with occupational exposure to ambient air pollutants than other means [32,33] that lead to a wide range of effects on human health, especially the cardiopulmonary system [34].

These air pollutants are thought to provoke airway inflammation via the release of mediators capable of exacerbating the lung disease in susceptible individuals [35]. The effluent fine and ultrafine particles directly stimulate macrophages and epithelial cells to produce inflammatory cytokines such as TNF-α, IL-6, IL-8 and IL-1β [36]. All of these cytokines are responsible for acute and chronic inflammation in the lung. However, IL-10 is a potent promoter of an anti-inflammatory state as it down regulates or completely inhibits the expression of several pro-inflammatory cytokines like IL-1β [29].

Recent findings demonstrate that physical activity induces an increase in the systemic levels of number of cytokines with anti-inflammatory properties [23,37]. According to another study, intense physical exercises induced a systemic anti-inflammatory response, evidenced by a marked increase in plasma IL-10 levels (peaked at

![Table/Fig-2]: Name and duration of various Pranayamas & Yogasanas included in yogic training

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Shavasana</td>
<td>2-5 minutes, adding 1 minute per week</td>
</tr>
<tr>
<td>2</td>
<td>Ardha-halasana (half plough Pose)</td>
<td>5 seconds initially, adding 5 seconds per week until fourty five seconds</td>
</tr>
<tr>
<td>3</td>
<td>Virasana (inverted pose)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Matsyasana (fish pose)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Naukasana (boat pose)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Setubandhasana (bridge pose)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Bhujangasana (cobra pose)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Ardha shalabhasana (half locust pose)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Shalabhasana (locust pose)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Dhanurasana (bow pose)</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Vakrasana (twisted pose)</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Gomukhasana (cow face pose)</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Paschimtanana (forward bending pose)</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Supta vajrasana (reclining adamanat pose)</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Ushtrasana (camel pose)</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Chakrasana (wheel pose)</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Utkatasana (chair pose)</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Vrikshasana (tree pose)</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Tadasana (mountain pose)</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Anulom- Vilom</td>
<td>5-10 mins per day</td>
</tr>
<tr>
<td>21</td>
<td>Bhamari</td>
<td>10 times per day</td>
</tr>
<tr>
<td>22</td>
<td>Ujjayi</td>
<td>11 times per day</td>
</tr>
<tr>
<td>23</td>
<td>Kapalabhati</td>
<td>5-10 mins per day</td>
</tr>
<tr>
<td>24</td>
<td>Om Chanting</td>
<td>Five minutes per day</td>
</tr>
</tbody>
</table>

![Table/Fig-3]: Pre test and post test values of selected variables after 12 weeks of yoga training

<table>
<thead>
<tr>
<th>Variable</th>
<th>Yoga Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre (M±SD)</td>
<td>Post (M±SD)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>0.75±0.30</td>
<td>0.55±0.16</td>
</tr>
<tr>
<td>IL-10</td>
<td>3.22±1.13</td>
<td>4.09±2.20</td>
</tr>
</tbody>
</table>

* p<0.05
1 hour post exercise) that was most likely mediated by increased plasma IL-6 levels (peaked immediately post exercise) [38]. It was also observed that exercise initially increases pro-inflammatory cytokines, which then activate anti-inflammatory cytokine [39-42]. Furthermore, very few studies, in relation to yoga and inflammatory markers, have shown positive impact of yoga practices. The findings of the present study are also in line with previous studies indicating reduction in pro-inflammatory cytokine and increase in anti-inflammatory cytokine. Yoga practices are found to be effective in reducing stress and improving physical, mental, and spiritual health. Reduction of stress and an empowered mind-body complex, in all probabilities, might have been instrumental in causing the above findings [31-43].

Finally, a reduction in the IL-1 beta and an increase in IL-10, is evident, even though lesser in magnitude, has been achieved through a very simple and inexpensive yoga intervention. This is of importance, because industrial workers are at higher risk of developing cardio-pulmonary disorders due to occupational hazards. Although, the study had a small sample, the results evident are very promising. Future studies, with larger population and with other pro-inflammatory and anti-inflammatory cytokines, are required to confirm the beneficial effects of yoga.

CONCLUSION

The present study has demonstrated that yoga practices could reduce pro-inflammatory cytokine and increase anti-inflammatory cytokine in industrial workers. Since, levels of inflammatory cytokines are important predictors of various cardio-pulmonary disorders; the current study implies that yoga practices seem to be instrumental in decreasing systemic inflammation. Further studies are needed to definitively establish the efficacy of yoga on various other pro- and anti-inflammatory cytokine levels in individuals exposed to occupational hazards.

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