ANTIFLAMMATORY EFFECT OF TARENNA ASIATICA (L) IN CARRAGEENAN INDUCED LUNG INFLAMMATION

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INTRODUCTION
Carrageenan is a high-molecular-weight sulphated poly-saccharide that is used in pharmacology to induce local inflammation (paw oedema and pleurisy). It is a pro-inflammatory polysaccharide useful to assess the contribution of mediators involved in vascular changes associated with acute inflammation. Acute lung inflammation is an important component of a number of pulmonary diseases.(1) The injection of carrageenan into the pleural space leads to pleurisy, characterized by an immediate neutrophils by carrageenan-containing macrophages and lung injury. In carrageenan-induced pleurisy, the initial phase of inflammation (oedema, 0-1 hr) has been attributed to the release of histamine, 5-hydroxytryptamine and bradykinin followed by a late phase (1-6 hr) mainly sustained by prostaglandin and pro-inflammatory cytokine release.(2) It appears that the onset of the carrageenan local inflammation is linked to neutrophil infiltration and the production of neutrophil-derived free radicals (Reactive Oxygen Species (ROS)), such as hydrogen peroxide, super-oxide and hydroxyl radicals, as well as to the release of other neutrophil-derived mediators(3). Neutrophil recruitment and activation result in parenchymatal lung damage and subsequent lung dysfunctions. The propose of the present study was to investigate the impact of age on the onset of carrageenan-induced lung inflammation. This was assessed by evaluating neutrophil infiltrate in the extravascular space, nitrotyrosine and lipid peroxidation, as signatures of lung damage, and the equilibrium between pro-and counter-inflammatory mediators in the pleural space. When compared with carrageenan-treated young rats, old rats exhibited a preponderance of pleural exudation and inflammatory cell infiltrate, which could be explained by a significant reduction in IL-10 production in old rats. We also demonstrated that this reduced IL-10 production was linked to a defective cAMP -responsive element binding protein (CREB)/ activating transcription factor (ATF) phosphorylation and CCAAT/enhancer-binding proteins (C/EBP), which mediate cAMP responsiveness by indirect mechanisms, indicating the crucial role of the CREB-dependent signal transduction pathway in IL-10 synthesis. Reduced IL-10 production may account for the delayed resolution of pulmonary infiltrates and the increased lung damage in old rats following carrageenan treatment.

The compact tree Tarenna asiatica (L) Kuntze ex Schumann belongs to family Rubiaceae. A large genus of shrubs and small trees occurring in plain lands and hilly regions with greyish brown bark, elliptic or oblong-lanceolate, coriaceous leaves, white fragrant flowers in cymes and black, multi-seeded berries. The species is used for its medicinal uses and timber wood obtained from it. A large evergreen shrub or a small tree occurring in the plain lands and hilly regions with greyish brown bark, elliptic or oblong-lanceolate, coriaceous leaves, white fragrant flowers in cymes and black, multi-seeded berries. The species is used for Suppurative in boils and Skin diseases. The young leaves of above mentioned plants were ground and made into paste and applied externally to affected portion on Sunday and Tuesdays for two to three months.

MATERIALS AND METHODS

Animals
Male albino mice weighing about 50-70g were obtained from the India Institute of science, Bangalore, Andra Pradesh. The animals were housed in poly propylene cages and maintained in controlled temperature with 12hours period of light dark and fed with standard mice feed and water were provided adlibitum.

Chemicals
Carrageenan, TBA, 2,4 DNPH reduced Glutathione were purchased from sigma chemicals Mumbai. All other reagents are analytical grade with high purity.

Plant materials and drug preparation
Dried Leaves of Tarenna asiatica were collected from Tamil University, Thanjaur, Thanjaur district, The tree Tarenna asiatica leaves were shade dried and finally powdered which was sieved through nice cloth and used as drug. The fine powder was dissolved in distilled water and before oral administration.

Induction and experimental procedure lung inflammation
Body weight of animals was recorded and they were divided into 3 groups of 6 animals each as follows.

Group I: Normal animal received with standard feed and water to allow adlibitum.

Group II: Mice received single intercoateal injection of Carrageenan.

Group III: Received oral administration of Tarenna asiatica powder (100 mg/kg) in adequor suspension before carrageenan induction.
Carrageenan-induced inflammation

Carrageenan-induced pleurisy was induced as previously described. Mice were anaesthetized with isoflurane and submitted to a skin incision at the level of the left sixth intercostals space. The underlying muscle was dissected and saline (0.2 ml) or saline containing 1% (w/v) λ-Carrageenan (0.2 ml) was injected into the pleural cavity. The skin incision was closed with a suture and the animals were allowed to recover. After injection injection of Carrageenan at 4 to 6hrs, the animals were killed by inhalation of CO₂. The chest was carefully opened and the pleural cavity rinsed with 2 ml of saline. Lung tissue was dissected and blood was collected.

The exudate and blood collected then serum separated were used for various biochemical analysis. Malondialdehyde was estimated by the thiobarbituric acid assay method (7). Reduced glutathione was estimated by method (7). Protein (8) Serum alpha-tocopherol (7). Estimation of Vitamin(C) and IgG (11) were quantitatively estimated Statistical deviation and student t test was calculated(13). Values set as lower than 0.001, .01 and 0.5 were considered as statistically significant.

RESULTS

In the present study, to evaluate the anti-inflammatory effect of the plant (Tarenna asiatica (L) Kuntze ex Schumann) the Carrageenan induced lung inflammation in mice model was selected. The following results were observed. The result changes were given as follows.

The level of exudate in lungcavity in experimental animals were represented in Table-1.

<table>
<thead>
<tr>
<th>Exudate volume (ml)</th>
<th>Normal group</th>
<th>Carrageenan group</th>
<th>Carrageenan + plant group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4 ml</td>
<td>0.9 ml</td>
<td>0.5 ml</td>
<td></td>
</tr>
</tbody>
</table>

The effect of plant Tarenna asiatica on the level of LPO and GSH in experimental animals (Table -2).

<table>
<thead>
<tr>
<th>LPO (μmole/ml)</th>
<th>Normal group</th>
<th>Carrageenan group</th>
<th>Carrageenan + Plant group</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.643 ±1.6</td>
<td>5.322 ± 0.04*</td>
<td>3.41 ± 0.81**</td>
<td></td>
</tr>
<tr>
<td>GSH (µg/l)</td>
<td>26 ± 1.3</td>
<td>9.2 ± 0.81</td>
<td>18.2 ± 0.91</td>
</tr>
</tbody>
</table>

* P<0.001- Significantly different from group – I
** P<0.01 Significantly different from group – II

The level of LPO was increased and GSH was decreased in Carrageenan group than normal but the administration of the plant crude extract minimized this changes by its antioxidant activity. The effect of the plant on vitamin-C and E in experimental mice (Table -3).

<table>
<thead>
<tr>
<th>Vit – (mg/dl)</th>
<th>Normal group</th>
<th>Carrageenan group</th>
<th>Carrageenan + Plant group</th>
</tr>
</thead>
<tbody>
<tr>
<td>41.2±7.6</td>
<td>14.2 ± 0.4*</td>
<td>34.2 ± 3.0**</td>
<td></td>
</tr>
<tr>
<td>Vit-E (mg/dl)</td>
<td>2.50± 0.02</td>
<td>1.12 ±0.06</td>
<td>1.63 ± 0.004</td>
</tr>
</tbody>
</table>

* P<0.001 Significantly different from group – I
** P<0.01 Significantly different from group – II

The level of Vit-C and E were decreased in Carrageenan group than normal group. Likewise the supplementation of the plant extract near normalized this level in treatment group. The effect of the level of total protein and albumin in normal and experimental animals (Table 4).

<table>
<thead>
<tr>
<th>Total protein g/dl</th>
<th>Normal group</th>
<th>Carrageenan group</th>
<th>Carrageenan + Plant group</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.1±0.03</td>
<td>5.53 ± 0.07*</td>
<td>6.7 ± 0.12**</td>
<td></td>
</tr>
<tr>
<td>Albumin g/dl</td>
<td>3.12±0.05</td>
<td>6.12 ±0.06</td>
<td>3.51 ± 0.15</td>
</tr>
</tbody>
</table>

*P<0.001 Significantly different from group – I
**P<0.01 Significantly different from group – II

The level of total protein was decreased in Carrageenan group than normal and the albumin level was decreased in control group than normal. This level changes were near normalized by the supplementation of the Tarenna asiatica plant extract. The effect of plant on IgE and leucocytes level in normal and experimental animals (Table 5).

<table>
<thead>
<tr>
<th>IgE IU/L</th>
<th>Normal group</th>
<th>Carrageenan group</th>
<th>Carrageenan + plant group</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.81±0.02</td>
<td>21.5±0.04*</td>
<td>14.2±0.10**</td>
<td></td>
</tr>
</tbody>
</table>

*P<0.001 Significantly different from group – I
**P<0.01 Significantly different from group – II

The level of IgE and WBC (leucocytes) level were increased in control group than normal group but the administration of the plant Tarenna asiatica minimized this level by its activity.

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DISCUSSION

Inflammation is a local response of living mammalian tissue to the injury. It is a body defence reaction in order to eliminate or limit the spread of injurious agents. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Acute lung inflammation is an important component of a number of pulmonary diseases. Injection of Carrageenan into the pleural space leads to pleurisy and lung injury. Generally the tissue damages are linked with lipid peroxidation and inflammation. This type of link was analyzed in this experiment by injection of carrageenan in lungs of mice.

The aim of the present study was a relationship between the inflammation and lipid peroxidation. The exudate volume was increased in carrageen an indicates the formation of oedema in lung cavity. Lipid peroxidation is free radical mediated process. It induces plethora of alteration in structure and function of cellular membrane (12). Reactive oxygen species generated endogenously by or exogenously are associated with the pathogenesis of various disease such as atherosclerosis, diabetes, cancer, arthritis and aging process(13,14). Inflammation is a complex process and ROS play an important role in the pathogenesis of inflammatory diseases (15,16). Lipid peroxidation has been implicated in the pathogenesis of various diseases including lung inflammation. It is well established that bioenzymes are very much susceptible to LPO, which is considered to be the starting point of many toxic as well as degenerative process(17).

Peroxidation brings about change in structure fluidity and permeability of membranes and inactivates a number of membrane bound enzymes and protein receptors (13). The enzyme peroxidise induces swelling, alteration of respiratory function and causes loss of SH group from the membrane bound protein (18,19). Injection of Carrageenan into the rats elicited an acute inflammatory responses characterized by accumulation of fluid containing a large number of neutrophils, subsequent lipid peroxidation and increased production of nitrite/nitrate (NOx), PGE2 tumor necrosis factor-X and 12-1/3 enhanced formation of NO by Nos may contribute to the inflammatory process(20,21). Generation of free radicals and nitric oxide by activated macrophages has also been implicated in causing oligo dendrocyte apoptosis (22). After tissue injury an animal will die as a spontaneous pain behavior and release various inflammatory mediators bradykinin, prostaglandins or cytokines which can activated and sensitize the peripheral nerve endings(22).

The increment of LPO in control group is due to the action of Carrageenan administration of the plant drug (T.A) reduced this level by its antilipid peroxidative effect (13). Glutathione is a ubiquitous thiol containing tripeptide, which plays a central role in cell biology. It is implicated in the cellular defence against xenobiotics and naturally occurring deleterious compounds such as free radicals and hydrogen peroxide. Gluthione status is a highly sensitive indicator of cell functionality and viability. Glutahione is the non-enzymatic antioxidant. Reduced glutathione under goes oxidation redirection through enzymatic control and in activities the free radicals. Due to the year utilization of GSH, its level was decreased in control group. But the plant drug treated groups redirected to near normal level, it may be due to its anti-inflammatory activity (20). From the observation of result concluded that the plant Tarenica asiatica has antioxidant and anti-inflammatory activity.

REFERENCES