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Research Article

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

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ABSTRACT

The present study was attempt that the antiflammatory effect of *Tarenna asiatica* (L) in Carrageenan induced lung inflammation. The exudate and blood was collected from male albino mice. The serum was separated and used for various biochemical analysis. Malondialdehyde was estimated by the thiobarbituric acid assay method. Reduced glutathione, Protein, Alpha-tocopherol, Vitamin C and E , IgG was estimated. From the observations result, the level of LPO (Lipid Peroxidation) was increased and GSH (Reduced Glutothione) was decreased in carrageenan group than normal but the administration of the plant crude extract minimized this changes by its antioxidant activity. The level of Vit-C and E were decreased in Carrageenan group than normal group. The level of total protein was decreased in Carrageenan group than normal and the albumin level was decreased than normal.

Keywords: Antiflammatory effect, *Tarenna asiatica*, Carrageenan, Lung inflammation.

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 brady-kinin 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 mediatory[3]. Neutrophil recruitment and activation result in parenchymatal lung damage and subsequent lung dysfunctions.

The prupose 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-dependent signalling pathway in old rats. Recent reports have demonstrated that IL-10 transcription may involve SV40 promoter 1 (Spl) and signal transducer and activator of transcription 3 activation [4,5]. But also cAMP-responsive element binding protein (CREB)/ activating transcription factor (ATF) phosphorylation and CCAAT/enhancer-binding proteins (C/EBP),[5] which mediate cAMP responsiveness by indirect mechanisms, indicating the crucial role of the cAMP-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, multiseeded berries. The species is used for its medicinal uses and timber wood obtained from it. It is used for Suppuration 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.

Aim and scope of the present study

1. To assessment the anti-inflammatory activity of *Tarenna asiatica* (L) Kuntze ex Schumann in lung inflammation.
2. To estimate the amount of Lipid Peroxidation (LPO), Reduced Glutathione (GSH) Vitamin –E, C, Total protein, Albumin and IgE level in serum.

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, Thanjavur. Thanajvur district, The tree *Tarenna asiatica* leaves were shade dried and finally powdered which was sieved through nice cloth and used as drug [6] . 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 in 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 (0.2ml) 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 CO_2. 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[10] 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 1: Effect of the plant extract on exudates volume of treatment

Exudate volume (ml)	Normal group	Carrageenan group	Carrageenan +plant group
	0.4ml	0.9ml	0.5 ml

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

Table 2: Effect of plant extraction on LPO and GSH mice.

	Normal group	Carragenan group	Carrageenan + Plant group
LPO (µmole/ml	2.643 ±.16	5.322 ± 0.04*	3.41 ± 0.81**
GSH (µg/l)	26 ± 1.3	9.2 ± 0.81	18.2 ± 0.91

* P<0.001- Significantly different from group – I

** P<0.01Significantly 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 mices (Table -3).

Table 3: Effect of plant extract on vitamin C and Vitamin E in mice

	Normal group	Carrageenan group	Carrageenan + Plant group
Vit – (mg/dl)	41.2±7.6	14.2 ± 0.4*	34.2 ± 3.0**
Vit-E (mg/dl)	2.50± 0.02	1.12 ±0.06	1.63 ± 0.004

* P<0.001Significantly different from group – I

** P<0.01Significantly 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 4: Effect of the plant extract on total protein and albumin in mice

	Normal group	Carrageenan group	Carrageenan + Plant group
Total protein g/dl	7.1±0.03	5.51 ± 0.07*	6.7 ± 0.12**
Albumin g/dl	3.12±0.05	6.12 ±0.06	3.51 ± 0.15

*P<0.001Significantly different from group – I

**P<0.01Significantly 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 asciatica* plant extract. The effect of plant on IgE and leucocytes level in normal and experimental animals (Table -5).

Table 5: Effect of the plant extract on IgE and WBC in mice

	Normal group	Carragenan group	Carrageenan + plant group
IgE IU/L	9.81±0.02	21.5±0.04*	14.2±0.10**
W.B.C % (Leucocytes)	56%	76%	65%

*P<0.001Significantly different from group – I

**P<0.01Significantly 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.

DISCUSSION

Inflammation is a local response of living mammalian tissue to the injury. It is a body defiance 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 ling 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 excaudate 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 or exogenously are associated with the pathogenesis of various disease such as atherosclerosis, diabetes, cancer, arthritis and aging process[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), PGE_2 tumour necrosis factor-X and 12-1/3 enhanced formation of No by Nos may contribute to the inflammatory process[2,3,20,21]. 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 display spontaneous pain behaviour and releases various inflammatory mediators bradykinin, prostoglandins 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 it 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. Glutathione status is a highly sensitive indicator of cell functionality and viability. Glutathione 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 group as slightly near normalized this GSH level. Both these level alteration may be due to the antioxidative effect of these drugs.

Vitamin-E is thought to be an important chain breaking antioxidant [22,23]. It can directly scavenge reactive oxygen members and protect cellular membrane against lipid peroxidation. Vitamin-C is an important water soluble antioxidant in biological system and an essential micronutuient required for normal metabolic functioning of the body [14] . Vitamin-C helps to maintain the level of vit-E at optimum concentration. The reduced level of Vit-C and Vit-E in control group indicates that utilization of these for scavenging of free radicals which are produced from Carrageenan induction. The plant drug *Treana asiatica* as significantly increased the level of vit-C and vit-E in Carrageenan injected groups.

Proteins are easily attacked by ROS directly or indirectly through lipid peroxidation protein radicals can be rapidly transferred to other sites within the protein infra structure protein oxidative damage can result in the modification in structure, enzymes activity and signal pathways [3, 25].

Hyperalbuminemia is the Condition linked with inflammation. The decreased level of total protein and increased level of albumin were observed in Carrageenan group than others. Mast cells have a crucial rode in the development of many physiological changes during anaphylactic and allagic responses they can bind IgE antibodies to receptors on their surface. This leads to the rapid extracellular release of histamine, heparin, proteases and other mediators that are stored in the cell's cytoplasmic granules and synthesis and secretion of leukotrienes and prostoglandins, these produced result in broncho constriction, changes in blood vessel tone, increased vascular permeability and myraid other pro inflammatory effects[26].

The release of TNP-x and other mediators by mast cell can indeed confer either great benefit or harm [27] mast cell is a well known effector cell in allergic disease, mediators released from mast cells can cause bronchoconstriction, increased vascular permeability etc., and contribute to the pathophysiology of allergic disorders. The functions of mast cells can be manipulated for therapeutic ends by regulating their function with appropriate drugs. In this direction the *Tarena asciatica* is an attractive candidate in this present study. Increment of IgE level and WBC level were observed in control group. But the plant drug treated groups redirected to near normal level, it may be due to its anti-inflammatory activity [28]. From the observation of result concluded that the plant *Tarena asciatica* have antioxidative and anti-inflammatory activity.

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