Group Design
6.1. Group 1: Control

Guinea pigs (n=11) were injected with an equal volume of normal saline as the antigen adjuvant solution at the same predetermined sites. After 28\textsuperscript{th} day, the animals were anesthetized and basal airway mechanics were recorded. Afferent activity from either RAR or SAR was recorded. After recording the activities for 10 breaths (control period), the animals were given an inhalation of normal saline for 1 min. The changes in afferent activity and pulmonary mechanics were recorded for subsequent 100 breaths. After recovery, histamine inhalation was given starting with the dose of 0.04 mg/ml for 1 min. The changes in afferent activity and pulmonary mechanics were recorded for another 100 breaths. After recovery, increasing doses of histamine inhalation were given in doubling concentrations until the airway resistance increased by 50\%. After each inhalation, the changes in afferent activity and airway mechanics were recorded for the subsequent 100 breaths. The maximum concentration of histamine administered was never more than 5 mg/ml. An interval of 15 min was given between the histamine inhalations to allow the recovery of afferent activity and pulmonary mechanics and to avoid tachyphylaxis.

At the end, the animals were euthanized by an over dosage of anesthesia (Urethane – 5 g/kg) for collection of blood for biochemical assays and lung tissue for histopathological examinations.

6.2. Group 2: Ovalbumin Sensitized and Challenged

(a) Early Asthmatic Response

These guinea pigs (n=11) were sensitized with ovalbumin as described previously. Four weeks after sensitization, the animals were anesthetized and basal airway mechanics were measured. Afferent activity from RAR or SAR was recorded. After recording the values for 10 breaths (control period), the animals were challenged with ovalbumin for 1 min and RAR/SAR activity was recorded for subsequent 100
breaths. The early asthmatic response was defined as an increase in airway resistance by 50%. After recovery, histamine inhalation was given starting with the dose of 0.04 mg/ml for 1 min. The changes in afferent activity and pulmonary mechanics were recorded for another 100 breaths. The responses to increasing doses of histamine inhalation were determined in the same way as described in Group 1 (control) until the airway resistance increased by 50%.

At the end, the animals were euthanized by an over dosage of anesthesia (Urethane – 5 g/kg) for collection of blood and lung tissue for biochemical assays and histopathological examinations.

(b) Late Asthmatic Response

The guinea pigs (n=11) were sensitized with ovalbumin. Four weeks after sensitization, the animals were challenged with ovalbumin in the guinea pig body box in the conscious state. After 24 hours of challenge, the animals were anesthetized and basal airway mechanics was measured. Afferent activity from RAR or SAR was recorded. After recording the values for 10 breaths (control period), histamine inhalation was given starting with the dose of 0.04 mg/ml for 1 min. The changes in afferent activity and pulmonary mechanics were recorded for another 100 breaths. The responses to increasing doses of histamine inhalation were determined in the same way as described in Group 1 (control) until the airway resistance increased by 50%.

At the end, the animals were euthanized by an over dosage of anesthesia (Urethane – 5 g/kg) for collection of blood and lung tissue for biochemical assays and histopathological examinations.

6.3. Group 3: In vivo Generation of Reactive Oxygen Species (ROS)

The guinea pigs (n=11) were anesthetized and basal airway mechanics were measured. Afferent activity from either RAR or SAR was recorded. After recording the values for 10 breaths (control period), the animals were given an inhalation of xanthine (0.1%) for 1 min followed by an inhalation of xanthine oxidase (1U/ml) for 1 min. The changes in afferent activity were recorded for another 100 breaths. After recovery, histamine inhalation was given starting with the dose of 0.04 mg/ml for 1 min. The
changes in afferent activity and pulmonary mechanics were recorded for another 100 breaths. The responses to increasing doses of histamine inhalation were determined in the same way as described in Group 1 (control) until the airway resistance increased by 50%.

At the end, the animals were euthanized by an over dosage of anesthesia (Urethane – 5 g/kg) for collection of blood for biochemical assays and lung tissue for histopathological examinations.

6.4. Group 4: Ovalbumin Sensitized and Challenged - Antioxidant Supplementation

(a) Early Asthmatic Response after Antioxidant Supplementation

The interventions in this Group (n= 11) were the same as in Group 2a excepting that the guinea pigs were fed on the standard diet supplemented with vitamin C (2 mg/kg body weight) and vitamin E (7 mg/kg body weight) for a period of four weeks. The protocol followed for the data collection was the same as described in Group 2a.

(b) Late Asthmatic Response after Antioxidant Supplementation

The interventions in this Group (n= 11) were the same as in Group 2b excepting that the guinea pigs were fed on the standard diet supplemented with vitamin C (2 mg/kg body weight) and vitamin E (7 mg/kg body weight) for a period of four weeks. The protocol followed for the data collection was the same as described in Group 2b.