



Original Contribution

Mutated glutathione-S-transferase reduced airway inflammation by limiting oxidative stress and Th2 response

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ABSTRACT

Oxidative stress is an important factor in the pathogenesis of asthma. Furthermore, antioxidants like GST are reduced in asthma patients. In the present study, the therapeutic effects of exogenous GST and mGST were evaluated in a mice model. GST mutated at residues 21/27 has reduced IgE binding with similar enzyme activity as that of GST. To evaluate the therapeutic effects of GST, BALB/c mice were immunized and challenged with ovalbumin. Mice were given GST, mGST, and α -lipoic acid by inhalation and sacrificed on Day 31 to evaluate inflammation and oxidative stress. Mice treated with mGST showed significantly reduced total cell count ($P < 0.01$) and eosinophils ($P < 0.01$) in BALF compared to GST- or PBS-treated groups. The lung inflammation score was lowest for the mGST-treated group along with reduced IL-4 ($P < 0.01$) and OVA-specific IgE than other groups. Oxidative stress as per the lipid peroxidation level in BALF of mGST-treated mice was reduced significantly in comparison to PBS- or GST-treated mice. In conclusion, inhalation of mGST reduced airway inflammation in mice. Mutated GST with reduced allergenicity has better therapeutic potential and can be explored as an adjunct therapy in asthma.

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Introduction

Airway inflammation and bronchial hyperresponsiveness are major factors in the pathogenesis of asthma. The inflammatory response is characterized by an increase of eosinophils and mast cells, mucus secretion, and activation of T cells through increased production of Th2 cytokines like IL-4, IL-5, and IL-13 [1–3]. Oxidative stress plays an important role in inflammation and tissue damage in asthmatics [4–6]. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated from macrophages and inflamed tissue to deplete antioxidants [4–6]. The inability to detoxify ROS perpetuates the inflammatory process in lung, activates a bronchoconstriction mechanism, and precipitates asthmatic symptoms [7]. Antioxidant levels are decreased both in pulmonary compartment and in peripheral blood in asthmatic patients [8]. This imbalance between oxidants and antioxidants favors skewing of the Th2 immune response while suppressing Th1 differentiation [9]. The intratracheal administration of a catalytic antioxidant was demonstrated to be effective in the treatment of

airway inflammation [10]. Aerosol delivery of glutathione reduced the oxidant–antioxidant imbalance in idiopathic pulmonary fibrosis [11].

Members of the glutathione-S-transferase (GST) superfamily are crucial for protecting cells from ROS [12]. They are known as phase II xenobiotic detoxifying enzymes that conjugate reactive intermediates with glutathione to produce less reactive water-soluble compounds and influence the synthesis of eicosenoid-like mediators via the modulation of ROS levels [12–15]. The oxidant scavenging and anti-inflammatory properties of catalytic antioxidants like GST can be exploited to modulate the innate immune response to achieve therapeutic benefits in inflammatory disorders. Previously, GST has been shown as a cross-reactive allergen among various fungi [16]. Allergenicity of an *Alternaria alternata* GST fragment (Alt a 13^{1–50}) was reduced by mutations at positions 21 and 27 [17]. The present study was aimed to investigate the effect of mutated glutathione S-transferase (mGST) in reducing the airway inflammation in a mice model.

Materials and methods

DNA constructs

A. alternata full-length GST was mutated at residues 21 and 27 to reduce IgE binding. Three-step PCR were performed to generate a double mutant (mGST) using *A. alternata* GST (Accession No. AY514673) as template. Initial PCR was carried out with an internal sequence-specific oligonucleotide GS1F as forward and GMR2 as reverse primers (Table 1). In another reaction, GMF3 and GS4R primers

Abbreviations: BALF, bronchoalveolar lavage fluid; CDNB, 1-chloro-2,4-dinitrobenzene; mGST, mutated glutathione S-transferase; OVA, ovalbumin; PAS, periodic acid Schiff; PBS, phosphate-buffered saline; ROS, reactive oxygen species; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TBA, thiobarbituric acid; TBARS, thiobarbituric acid-reactive substance.

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Table 1
Primers used for generation of plasmid mGST (Alt a13 T21F_N271)

Primers	Oligonucleotide sequence ^a
GS1F	5'GAATTCGATGTGACACAACCCCTCTGAA 3'.
GS4R	5'AAGCTTCCACCGCTCCAGAGCTACAACGA 3'.
GMF3	Fwd: 5' AAAGGCTTCGCCACGTCCACACACATTACCGTC 3'
GMR2	Rev: 5'GACGGTAATGTGTGTGGACGTGGCGAAGCCTTT 3'

^a EcoRI site-GAATTC, XhoI site-AAGCTT.

were used to obtain the PCR product. Finally, products of the above two steps were purified from agarose gel, mixed, and amplified using oligonucleotide GS1F as forward and GS4R as reverse primers leading to two point mutations threonine²¹ {ACA (T)} phenylalanine {TTT (F)} and asparagine²⁷ {AAC(N)} isoleucine {ATC (I)}. The product was cloned in vector pET 22b+ at the EcoRI/XhoI site and sequenced to confirm the mutations.

Protein expression and purification

GST and mGST were expressed and purified as per standard protocol using glutathione Sepharose-4B resin (GE, Healthcare)-based affinity chromatography [16]. Briefly, the plasmids containing the inserts were transformed into BL-21 *Escherichia coli* cells, induced with 1 mM IPTG for 4 h, and harvested. The pellet was suspended in 50 mM Tris-HCl (pH 7.5), sonicated, and centrifuged at 13,000 g for 30 min at 4°C. Supernatant was incubated with glutathione Sepharose-4B resin at 4°C. The unbound fraction was collected and washed 5 times and bound protein was eluted using 10 ml of buffer containing 15 mM reduced glutathione in 50 mM Tris-Cl (pH 8.0). The purified proteins were resolved on 10% SDS-PAGE and transferred to nitrocellulose membrane. IgE binding of electrotransferred proteins was evaluated using pooled patients' sera and the activity was assayed spectrophotometrically using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate [18].

IgE binding of GST and mGST

Sera were collected from 12 *A. alternata*-positive patients attending the outpatient department at the V. P. Chest Institute, Delhi. The patients selected for sera collection were suffering with allergic rhinitis, bronchial asthma, or both. Informed consent of patients was taken prior to sera collection. The human ethics committee of the Institute has approved the study protocol following the guidelines of Indian council of Medical Research, New Delhi. To determine specific IgE binding, ELISA plates (Nunc-ImmunoTm modules, Roskilde, Denmark) were coated with 100 ng of GST or mGST in 0.1 M carbonate buffer (pH 9.6) and incubated overnight at 4°C. The plates were washed, blocked with 3% defatted milk, and incubated with 12 patients' sera and 3 controls, individually (1:10) at 4°C overnight. The plates were washed with PBST (0.05% Tween 20) and PBS and incubated with anti-human IgE peroxidase (1:1000 PBS; Sigma Chemical Co., St. Louis, MO) for 3 h, and color was developed using orthophenylene diamine (OPD) and read at 492 nm [19].

Circular dichroism (CD) of GST and mGST

CD analysis was performed to compare the structure of GST and mGST (0.5 mg/ml in phosphate buffer) using far-UV range (190–300 nm) on a Jasco-715 spectropolarimeter (Jasco Inc., MD).

Animal study protocol

BALB/c mice aged 6–8 weeks were obtained from the National Institute of Virology (Pune, India). They were randomly divided in five groups of six mice each. Group 1 mice, used as control, were sen-

sitized, challenged, and treated with PBS. Four groups were given ip injection of 10 µg OVA with 1 mg alum in 100 µl of PBS on Days 1 and 14. The mice were subjected to airway challenge with OVA 4 µg/mice on Days 28, 29, and 30. After 1 h of the challenge, group 2 mice were treated with PBS and groups 3, 4, and 5 were given i.n. treatment with 2 µg GST/mGST/α-lipoic acid (SRL, India), respectively. Here α-lipoic acid (an antioxidant) has been used as a positive control to study the reduction in airway inflammation [20]. The study protocol was approved by the animal ethics committee of the Institute of Genomics and Integrative Biology, Delhi, following the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals, Chennai, India.

Collection of bronchoalveolar lavage fluid (BALF) and blood

After inhalation treatment, the mice were sacrificed with an overdose of sodium pentobarbital on Day 31 and a tracheotomy was performed. Ice-cold PBS (0.5 ml) was instilled into the lungs and bronchoalveolar lavage fluid was obtained by aspiration three times (total 1.5 ml) via tracheal cannulation. BALF was centrifuged, and supernatant collected and stored at -70°C before use. The total inflammatory cell number was assessed by a hemocytometer after excluding dead cells by staining with trypan blue. One hundred microliters of BALF was spread on the slide, fixed, and stained with Leishman for differential cell counts. Blood was collected, and sera were separated and used for analysis of serum immunoglobulins.

Determination of immunoglobulins and cytokines by ELISA

OVA-specific IgE, IgG1, and IgG2a were measured in serum by ELISA as described elsewhere [21]. Briefly, microtiter plates (Nunc, Denmark) were coated with 5 µg/ml of OVA. The plates were washed with PBS, blocked with 3% defatted milk, and incubated with mice sera for IgE (1:10), IgG1 (1:50), and IgG2a (1:50), individually. The plates were washed with PBST followed by PBS and incubated with IgG1-peroxidase and IgG2a-peroxidase (1:1000 PBS; BD Pharmingen, San Diego, CA). For IgE, biotinylated anti-mouse IgE (2 µg/ml, BD Pharmingen) was used, and plates were washed and incubated with streptavidin-peroxidase (1:1000; BD Pharmingen). Color was developed using orthophenylene diamine and absorbance read at 492 nm.

IL-4, IL-10, and IFN-γ levels were determined in BALF by ELISA using paired antibodies according to the manufacturer's instruction (BD Pharmingen). Briefly capture antibody (1:250 v/v) for each cytokine was coated separately on microtiter plates, incubated overnight, and blocked with 10% fetal calf serum (FCS). BALF samples (1:2 v/v) were added to wells and incubated for 2 h at 30°C. Biotinylated anti-IL-4 or anti-IL-10 or anti-IFN-γ was used to detect specific cytokine levels. The detection limit for IL-4 was 7.8 pg/ml and for IL-10 and IFN-γ it was 31.3 pg/ml.

Histopathology

Lungs were fixed with 10% neutral-buffered formalin and embedded in paraffin. The sections of 4 µm were cut and stained with hematoxylin and eosin (H and E) or periodic acid Schiff (PAS). Twelve slides were made for each type of staining (2 slides per mice) from every group and these were analyzed using light microscopy, for antigen-induced peribronchial and perivascular inflammation. Coded (blinded) samples of lung tissues were made and given to a pathologist. Semiquantification of inflammation score was performed by the pathologist on a scale of 0–4 for perivascular accumulation of inflammatory cells followed by statistical analysis [22]. Lung inflammation score was calculated in terms of eosinophil infiltration and mucus secretion. Score 0 (no or occasional inflammatory cells), score 1 (with a few inflammatory cells), score 2 (with scattered aggregates of inflammatory cells), score 3 (with thin layer of inflammatory cells sur-

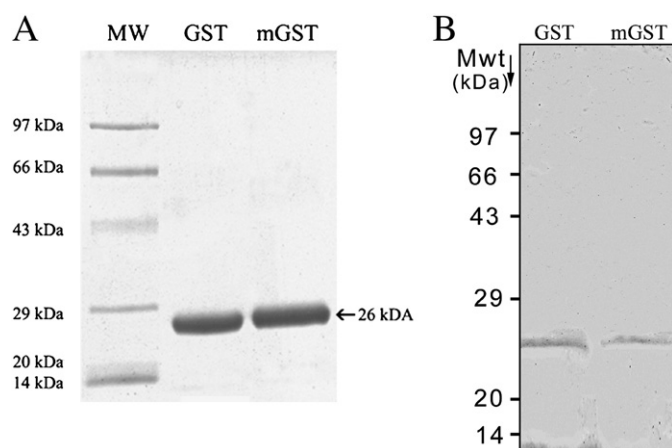


Fig. 1. (A) SDS-PAGE profile of GST and mGST: 10 μ g of protein was resolved on 12% SDS-PAGE and stained with CBB. (B) Immunoblot of GST and mGST: 10 μ g of each protein resolved by SDS-PAGE was transferred on nitrocellulose membrane, incubated with pooled sera (1:10) of *Alternaria alternata*-sensitive patients' sera, and detected using anti-human IgE peroxidase (1:1000).

rounding the airways and vessels), and score 4 (with a thick layer of inflammatory cells surrounding the airways and vessels).

Measurement of GST level and oxidative stress in BALF

GST activity in BALF was estimated by CDNB assay [18]. Oxidative stress was determined by measuring thiobarbituric acid-reactive substance (TBARS) concentration spectrophotometrically [23]. Malanodialdehyde (MDA) and thiobarbituric acid (TBA) react to form a product with maximum absorption at 532 nm. BALF (200 μ l) was mixed with 500 μ l of 10% w/v trichloroacetic acid to precipitate the protein. The precipitate was pelleted by centrifugation at 10,000 g/4°C/15 min and the collected supernatant was treated with 0.67% TBA in boiling water for 15 min. OD was measured at 532 nm after cooling and compared with standard malanodialdehyde.

Statistical analysis

Data were expressed as the mean $\pm$ standard deviation. The data were analyzed using GraphPad Prism 5.00 (Graphpad software Inc., USA). The sample size for each parameter tested was six animals. Statistical significance was calculated by paired *t* test to determine intragroup differences of means between untreated and treated mice. Probability of significance was assigned by nonparametric ANOVA

Table 2
Specific IgE binding with Individual patients' sera against GST and mGST

S.No.	Age/Sex	Clinical History ^a	Specific IgE values OD _{492nm}		
			GST	mGST	% reduction
1	60/F	A	0.399	0.280	30
2	26/M	A	0.464	0.161	65
3	15/M	AR	0.711	0.411	42
4	10/M	AR/C	0.577	0.319	45
5	19/M	A	0.428	0.132	69
6	33/F	AR	0.547	0.327	40
7	18/F	A	0.305	0.107	65
8	17/F	A	0.600	0.256	57
9	11/M	A	0.554	0.301	46
10	25/M	AR/U	0.372	0.137	63
11	20/M	A	0.451	0.241	46
12	12/M	A	0.536	0.315	41
C1			0.060	0.057	NS
C2			0.063	0.059	NS
C3			0.080	0.073	NS

^a A-Asthma, AR-Allergic rhinitis, C-Conjunctivitis, U-Urticaria.

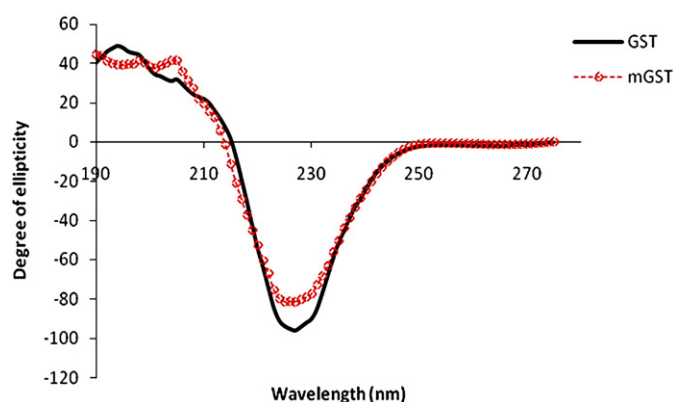


Fig. 2. CD analysis of purified GST and mGST: Proteins (1 mg/ml) were taken in 20 mM phosphate buffer and scanned from 190 to 270 nm. Minima at 225 nm showing α -helix predominance and the peaks are almost similar for both proteins.

using a Kruskal-Wallis test. Each parameter was compared in all the groups using Dunn's multiple comparison post test. $P < 0.05$ was considered significant in all cases.

Results

Protein purification and enzymatic activity

GST and mGST were expressed as soluble proteins and purified to homogeneity by glutathione Sepharose-4B resin. The yield of expressed proteins was approximately 1.5 and 1.0 mg/L for GST and mGST, respectively. They migrated as homogeneous proteins at a molecular weight of 26 kDa (Fig. 1A). Ten microgram of each GST and mGST proteins transferred on nitrocellulose membrane was recognized with *A. alternata*-sensitive patients' pooled sera. The intensity of the mGST band was less compared to GST (Fig. 1B). GST and mGST showed similar enzyme activity at 0.906 ± 0.021 /min/ μ g and 0.862 ± 0.034 /min/ μ g, respectively.

IgE binding with individual patients' sera

IgE binding of GST and mGST was determined by ELISA with allergic patients' sera ($n = 12$). Specific IgE binding for the GST ranged from OD 0.372–0.711 and mGST OD 0.137–0.411 whereas the mean value of controls was 0.068 ± 0.01 (Table 2). Specific IgE binding of mGST was reduced between 30 and 69% with 12 hypersensitive patients' sera compared to the GST.

Structural analysis of GST and mGST

CD spectra of GST and mGST showed that the secondary structure of the two proteins was similar with minima at 225 nm. Both proteins have predominantly α -helices (Fig. 2). Mutations at residues 21/27 have not affected the secondary structure.

Inflammatory cells in BALF

Analysis of BALF showed large number of total cells $\{(30.47 \pm 1.87) \times 10^4\}$ and eosinophils $\{(13.34 \pm 0.78) \times 10^4\}$ in OVA-immunized and OVA-challenged mice (Fig. 3). Administration of GST and mGST in OVA-immunized mice significantly decreased the ($P < 0.01$) cellular infiltration. The total cell number in mGST-treated group was nearly half $\{(13.70 \pm 2.52) \times 10^4\}$ of that of the GST administered group $\{(25.76 \pm 3.25) \times 10^4\}$. mGST caused significant reduction in total cells/eosinophil counts than GST ($P < 0.01$). However, the effect of mGST treatment was 70% of that of α -lipoic acid administration (Fig. 3). Here α -lipoic acid was taken as a standard, which showed

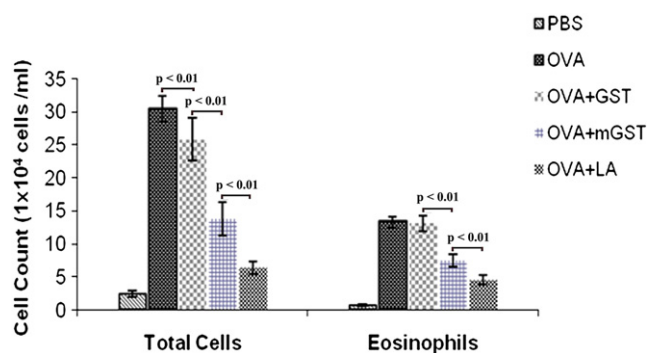


Fig. 3. Total cell count and eosinophil numbers in BALF on treatment of OVA-sensitized mice: Total cell count was determined using a hemocytometer. One hundred microliters of BALF was spread on the slide, fixed, and stained with Leishman for eosinophil count. Data are presented as mean $\pm$ SD. $n=6$ mice per group.

significant reduction ($P<0.01$) in the inflammatory cells compared to PBS-treated mice.

Serum immunoglobulins and cytokines in BALF

GST-specific IgE levels were higher in OVA-immunized and PBS-treated mice (Fig. 4). GST treatment showed significant reduction in IgE level ($P<0.01$) compared to OVA-immunized and PBS-treated mice. IgE levels decreased significantly by the administration of mGST ($P<0.01$) compared to GST. The reduction in IgE levels was maximum on treatment with α -lipoic acid. IgG1 levels, however, did not change on treatment with GST, mGST, or α -lipoic acid. Specific IgG2a levels were not detectable in any group.

IL-4 levels in the BALF of OVA-immunized and PBS-treated mice (120.33 ± 15.27 pg/ml) were more than 7 times higher than those of PBS control mice (16 ± 2.96 pg/ml). mGST treatment decreased IL-4 levels (71 ± 17.32 pg/ml) significantly compared to OVA-immunized and PBS-treated mice ($P<0.01$). GST treatment reduced the levels to 107.67 ± 20.82 pg/ml ($P<0.05$). α -Lipoic acid treatment showed maximum reduction in IL-4 levels (56 ± 10 pg/ml).

IL-10 levels were higher in OVA-immunized and PBS-treated mice compared to the PBS control. IL-10 level(s) however did not change on treatment with GST, mGST, or α -lipoic acid (Fig. 5). IFN- γ levels were also similar in all the treatment groups (Fig. 5). These data suggest that GST having intact allergen epitope was not able to change the cell

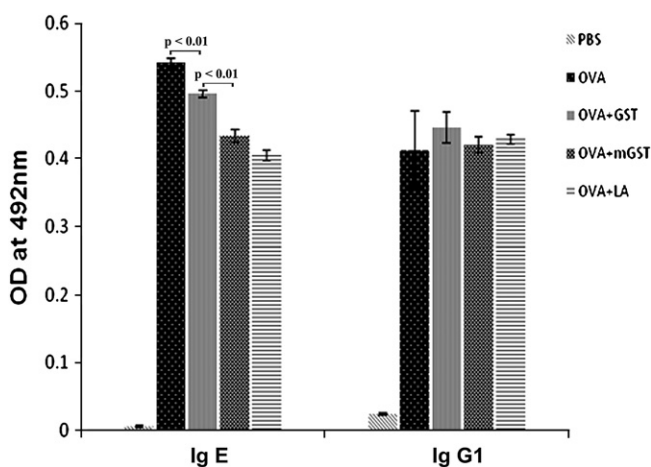


Fig. 4. Serum immunoglobulin levels after intranasal administration of GST, mGST, or α -lipoic acid. OVA-specific IgE, IgG1, and IgG2a were measured in serum by ELISA. Microtiter plates were coated with OVA, incubated with mice sera for IgE (1:10) and IgG1 (1:50) and detected using secondary antibodies, individually. Data are presented as mean $\pm$ SD. $n=6$ mice per group.

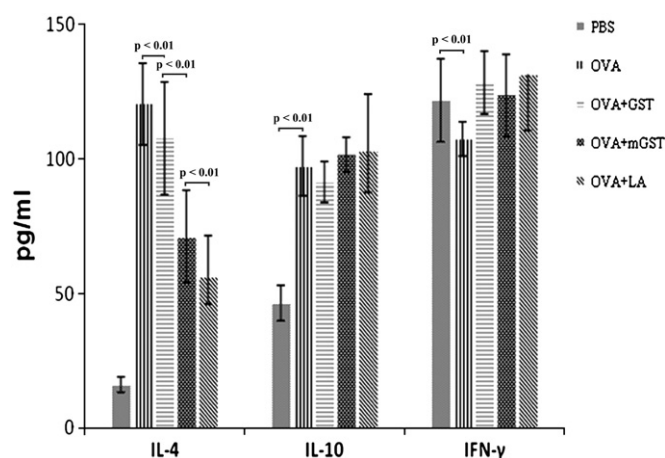


Fig. 5. Cytokine profile in BALF. IL-4, IL-10, and IFN- γ were determined by ELISA. Capture antibody (1:250 v/v) for each cytokine was coated on plates, blocked, incubated with BALF samples (1:2 v/v), and detected by biotinylated anti-IL-4 or anti-IL-10 or anti-IFN- γ . Data are presented as mean $\pm$ SD. $n=6$ mice per group.

milieu but mGST with reduced IgE binding has the potential to reduce Th2 cytokine level locally in the lungs.

Lung histology

Histopathological analysis of lung sections was performed to assess the anti-inflammatory or antiremodeling effect of GST or mGST. In the OVA-challenged PBS-treated group, numerous eosinophils were observed into the lung interstitium around airways and blood vessels along with narrowing of airway lumen. Mice treated for 3 days with GST or mGST showed improvement in the lung structure in terms of inflammation score compared to the OVA group (Fig. 6A). The recruitment of inflammatory cells in the airways was lowest in mGST-treated mice (Score 2) compared to OVA-immunized and PBS-treated mice (Score 4) or for GST-treated mice (Score 3). Mice treated with α -lipoic acid showed the lowest inflammation and eosinophil infiltration in lung tissue (Score 1).

In the PBS control group, a few PAS-stained epithelial cells were observed (Score 0). Contrary to this, epithelial cells were enlarged in OVA-immunized mice, along with airway narrowing and mucus plugs with increased goblet cell hyperplasia (Fig. 6B). The treatment with mGST has suppressed the mucus production and decreased the goblet cell hyperplasia.

Oxidative stress in lung tissues and BALF

Antioxidant levels in BALF and lung tissues were measured in terms of lipid peroxidation (TBARS). GST and mGST treatment both reduced the TBARS value compared to PBS treatment ($P<0.01$). α -Lipoic acid treatment also showed significant reduction ($P<0.01$) in TBARS value compared to PBS treatment (Fig. 7). However, the level of TBARS reduction was higher in the α -lipoic acid group than mGST and GST.

Discussion

Asthmatic patients have enhanced production of reactive oxygen species in blood monocytes, neutrophils, and eosinophils and the levels correlate with severity of the disease [24–27]. They have increased levels of oxidized glutathione in BALF and increased nitric oxide concentration in exhaled air [25]. Furthermore, concentrations of natural antioxidants, including GST, glutathione peroxidase, superoxide dismutase, glutathione, and vitamins C and E, are reduced in the blood cells, plasma, or BALF of asthmatic patients [28]. Individuals

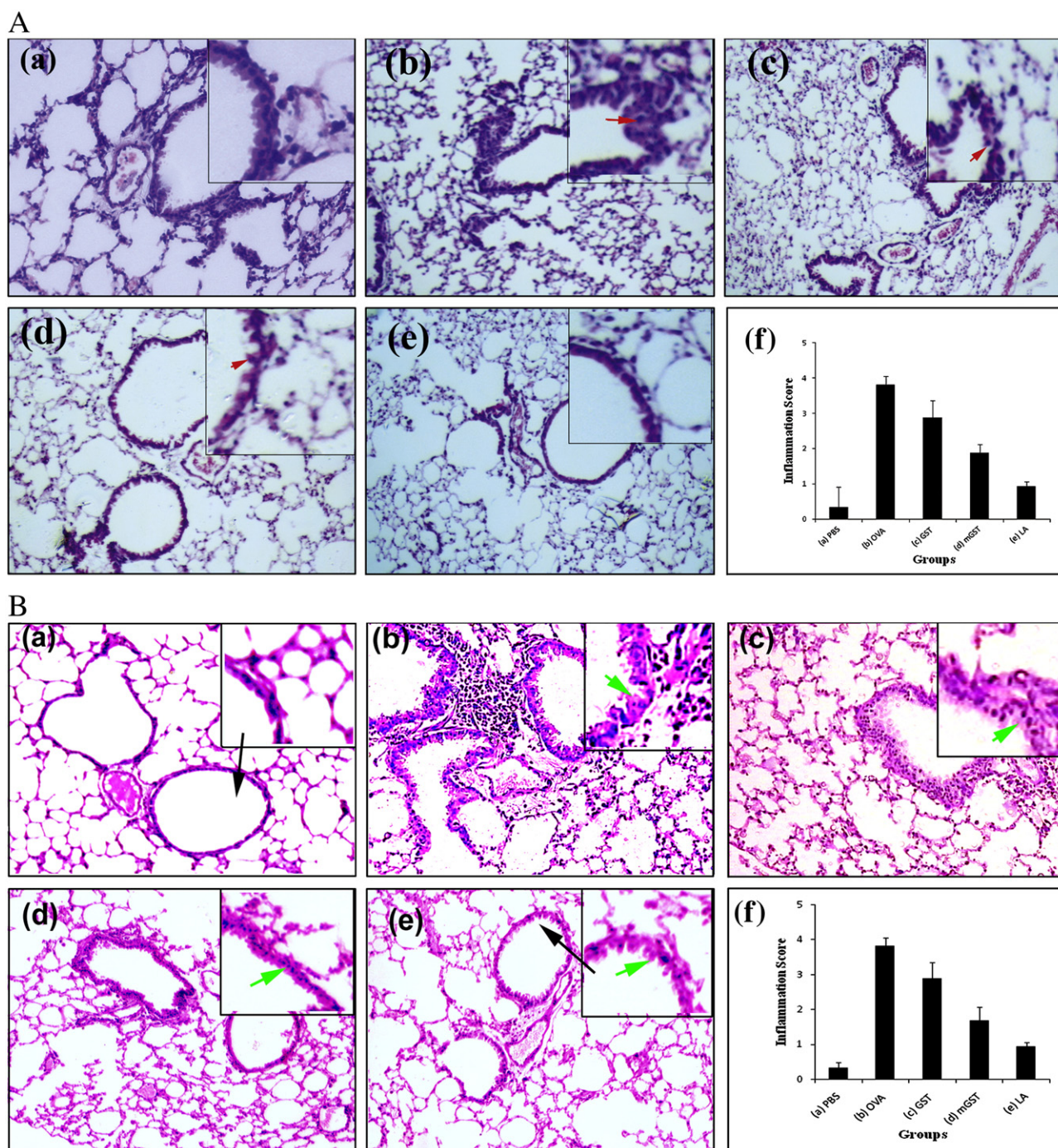


Fig. 6. (A) HE stained sections of the lungs showing eosinophil infiltration. Lungs were fixed with formalin and embedded in paraffin, and sections were cut and fixed on the slide for staining. (a) PBS control; (b) PBS treated; (c) GST treated; (d) mGST treated; (e) α-lipoic acid treated (f) inflammation score. Inflammatory cells are marked by arrow. (B) PAS staining of lung tissues showing goblet cell hyperplasia and mucus secretion. Lungs were fixed with formalin and embedded in paraffin, and sections were cut and fixed on the slide for staining. (a) PBS control; (b) PBS treated; (c) GST treated; (d) mGST treated; (e) α-lipoic acid treated (f) inflammation score. Mucus layer marked by green arrow.

having deficiency of GST are prone to asthma [29] and changes in its activity can have a repercussion on the disease. The administration of exogenous GST can help in the clearance of different reactive intermediates by GSH in asthmatics. Studies suggest a dietary benefit of antioxidant supplements in curbing allergic airway inflammation [20,30]. In the present study, localized treatment with antioxidant like GST and mGST was carried out to assess the effect on airway inflammation in a mice model.

In a previous study, GST (Alt a 13) was detected as a major allergenic protein of *A. alternata* [16]. Alt a 13^{1–50} was demonstrated to be an immunodominant fragment using patients' sera and mutations

reduced the IgE binding [17]. In the present study, mutations at 21 and 27 residues in full-length GST protein showed reduced IgE binding in the range of 30–69% with individual patients' sera. CD spectra revealed that mutations have not affected the secondary structure of the protein; therefore, no reduction was observed in enzymatic activity of mGST than GST. As there was no change in the secondary structure, the reduction in allergenic potential of mGST indicates the involvement of amino acid 21 and 27 residues in IgE binding.

The cytokines and chemokines released by Th2 cells regulate the activity of effector cells such as eosinophils and mast cells as well as induce IgE and other immunoglobulins [31–33]. In the present study,

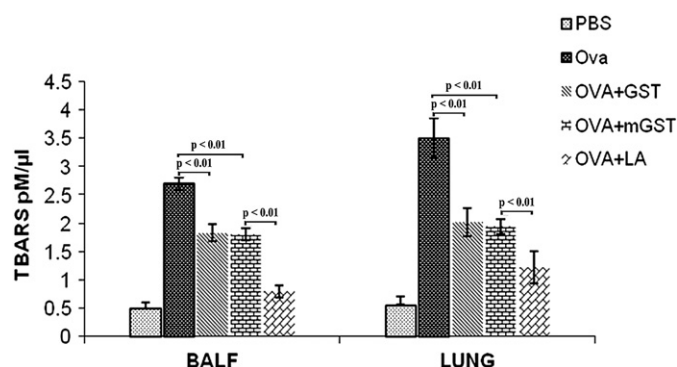


Fig. 7. Oxidative stress in BALF and lung tissues: Oxidative stress in BALF and lung tissues was determined by measuring thiobarbituric acid-reactive substance (TBARS) concentration spectrophotometrically. Data are presented as mean $\pm$ SD. $n=6$ mice per group.

GST or mGST treatment reduced serum IgE levels and release of IL-4 in BALF, thereby inhibiting the allergic inflammation and OVA-induced recruitment of inflammatory cells. GST induced lower effects than mGST that may be due to the higher allergenic potency of protein.

Eosinophil is a central effector cell in the inflamed asthmatic airways [34,35]. In the present study treatment with mGST significantly decreased the total number of inflammatory cells as well as eosinophil counts ($P<0.01$) in allergen-induced airway inflammation. Lung tissues of mGST-treated mice exhibited decreased mucus-secreting cells and were comparable to those of the α -lipoic acid-treated group ($P<0.01$) as visualized by PAS staining. There was a thick mucus layer due to goblet cell hyperplasia in GST- and PBS-treated groups. The difference in the therapeutic behavior of GST and mGST may be attributed to the allergenic potential.

ROS generation is enhanced in bronchioalveolar lavage cells of stable asthmatics [36] and increases after antigen challenge [37]. There are several markers of oxidative stress and a wide range of antioxidants that are altered in asthma [28,38,39]. OVA-immunized mice on treatment with mGST by inhalation showed reduction in the severity of airway inflammation accompanied with reduced oxidative stress. Despite a similar antioxidant response, mGST has demonstrated a better anti-inflammatory effect than GST and may be due to reduction in allergenicity. GST has properties of both an allergen and an antioxidant. It is essential that the allergenicity be reduced for use as a better therapeutic agent. The present study shows that reduction in allergenicity reduces the inflammatory response and therefore mGST will have a better potential as a therapeutic agent.

The inhalation route is preferred for the delivery of drug directly into the affected organ in asthma or other immune-inflammatory disorders. This causes fewer side effects, but the doses delivered are low compared to oral and intravenous routes. Standard antioxidants like α -lipoic acid are prescribed through the oral route. But, being a small molecule, it could be cleared easily from the system. mGST, a large molecule, will be retained in the system for a longer time. In addition, it has shown properties both as an antioxidant and as an immunomodulator in reducing the Th2 response. mGST may be modified further to enhance its half-life or block the protease cleavage site to prevent degradation.

In conclusion, inhalation of mGST reduced airway inflammation in mice as an antioxidant as well as an immunomodulator. Mutated GST with reduced allergenicity has better therapeutic potential than GST and can be explored for adjunct therapy in asthma.

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