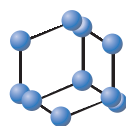


RESEARCH ARTICLE

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Montelukast Sodium Formulation Containing Green Tea Extract to Reduce the Oxidative Stress in Guinea Pig Model of Chronic Allergic Asthma

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Abstract: Aim and Background: The rationale of this study is that, treatment of asthmatic Guinea pig with combined administration of Montelukast sodium and Green Tea Extract (GTE) as a single capsule will mitigate the inflammatory injury in the airways and weaken the asthmatic response. Recent patents for the treatment of asthma researched a polyphenolic alternatives for antiasthmatic combination therapy, especially for those patients who remains unresponsive or poorly responsive for current asthma therapy (US7232585B2). Synergistic activity of green tea polyphenols and therapeutic, prophylactic agents are also reported in some recent patents (US20120172423A1, US20150320696A1). The present work is therefore aimed, to study the effect of Montelukast Sodium capsules coformulated with GTE on oxidative stress markers including Malondialdehyde (MDA), Glutathione (GSH) in different organs and Oxygen Radical Absorbance Capacity (ORAC) assay in plasma.

Materials and Methods: Guinea pigs were placed in histamine chamber and exposed to an aerosol challenge of 0.2% w/v histamine dihydrochloride in distilled water using pressurized air driven nebulizer at a pressure of 0.05 MPa-0.106 MPa for one week. After that, they were divided in to four groups of three each; control, asthmatic control, asthmatic treated with marketed preparation and asthmatic received developed capsules. After oral administration of formulations for three days, pigs were sacrificed and oxidative stress markers level including cytoarchitectural manifestation in tissues was studied.

Results: In comparison with the healthy control group, MDA level of the asthmatic animal liver and lung was found to be elevated as 0.059 ± 0.031 ($p < 0.002$) and 0.802 ± 0.310 ($p < 0.005$) respectively, whereas GSH level was declined as 13.223 ± 1.485 ($p < 0.0001$) in liver and 3.037 ± 0.282 ($p < 0.0004$) in lung tissues. TAC of asthmatic animal plasma was low as 2.132 ± 0.986 mM Trolox Eq/L ($p < 0.009$). The level of these biomarkers reverts back towards normal after treatment with marketed and developed formulation, although treatment with developed formulation was more efficacious since it was coformulated with GTE, which acts as an adjuvant for the management of inflammatory disease like asthma.

Conclusion: It is contemplated that, use of GTE as an adjuvant to anti leukotriene drug played a significant role in asthma management by reducing oxidant injury. Since, studies in animals do not directly translate to human biology, further multi-control studies with better sampled patient population and more number of patients are needed.

Keywords: Oxidative damage, green tea extract, asthma, malondialdehyde, glutathione, total antioxidant capacity.

1. INTRODUCTION

Montelukast Sodium (MS) is a selective orally active leukotriene receptor antagonist that inhibits the cysteinyl leukotriene CysLT₁ receptor. The drug is advised for the prophylaxis and chronic treatment of asthma in adults and pediatric patients, for the prevention of exercise-induced bronchoconstriction and relief of symptoms of seasonal allergic rhinitis [1]. MS, an alkaline stable drug decreases

bronchoconstriction, mucus production and inflammation in asthma. Asthma is a chronic inflammatory lung disease that causes airway obstruction, hyperreactivity and airway remodeling; it is also associated with decreased antioxidant defenses [2]. Studies of asthma pathogenesis postulated that airway mucosal inflammation is the prime cause underlying the clinical manifestations of asthma which include recurrent episodes of wheezing, chest tightness, cough and shortness of breath [3]. MS is recommended to be taken once daily in the evening for prevention of asthma. Many studies showed that cells involved in an asthmatic inflammatory process also participate in reactive oxygen species (ROS) production [3]. ROS present in asthmatic airways are derived from many

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sources, including exposure to environmental pro-oxidants, inflammatory cells infiltration in the airway, and decreased levels of cellular antioxidants [4]. Reactive species and antioxidants play a vital role in the immune system. An imbalance between pro-oxidant and antioxidant defense is known as oxidative stress, which can cause an increase in the airway and systemic inflammation subsequently, favoring asthma [5].

It is apparent from the increasing number of literature that treatment modalities targeting oxidative stress have a great potential for the treatment of asthma [2-6]. A number of studies reported the role of green tea as an antioxidant, polyphenols present in green tea made a major contribution to the antioxidant activity [7-9]. We prepared the capsules of MS together with green tea extract to prospect antioxidative agents that attenuate oxidative stress in combination with pharmacological treatment of asthma that acts by modulating leukotriene receptors. Antioxidant potential of GTE in attenuation of asthma was scrutinized by oxidative stress study, expecting that combined effect of MS and GT will lead to better management of asthma. Previous studies also demonstrated the treatment strategies using a combination of green tea extract and anticancer drugs [10], green tea extract in combination with Gentamicin [11], and green tea synergism with beta-lactams, tetracycline and fluroquinolones [12]. It has also been reported in patents that, GTE enhances the effect of the anti-cancer agents adriamycin and doxorubicin [13] and found to prevent and treat skin disorders [14].

This study was conducted to investigate the *in vivo* antioxidant potential of MS formulation containing GTE for the management of asthma. A guinea pig model of allergic asthma, chronically exposed to histamine aerosol was selected for the study. Oxidative stress parameters and histopathology of tissues of guinea pigs treated with developed capsules were elucidated and compared with marketed tablets to examine the adjuvant effect induced by the extract.

2. MATERIALS AND METHODS

2.1. Materials

Montelukast Sodium was obtained as a gift sample by Sanofi Aventis (Goa, India). The polymers Eudragit RSPO, RLPO and L100 were provided as a gift sample from Evonik Degussa India Pvt Ltd. Histamine was procured from Sigma Aldrich, India. Glutathione (GSH), Thiobarbituric acid (TBA), malondialdehyde (MDA), Bovine serum albumin and sodium dodecyl sulfate were obtained from Fluka Chemicals. All other chemicals and reagents were of analytical grade.

2.2. Development of Montelukast Sodium Capsules

The basic design of formulation is composed of a transparent capsule body filled with sustained release solid dispersion of MS and GTE powder. MS-GTE coformulated capsules were further coated to provide initial release lag time. This approach was consistent with the demand of drug during the early morning hour attack of asthma [15].

Solid dispersion was prepared by the solvent evaporation method. In this method, drug and polymer both were dis-

solved in ethanol. Afterward, the entire solvent was evaporated and obtained mass was dried, that make it suitable for heat labile drug MS having melting point 108°C [16]. Eudragit RSPO and RLPO polymers were used in different concentrations (1:1, 1:2, 2:1 and 2:2) with drug polymer proportions of 1:2, 1:2.5, 1:3, 1:3.5 and 1:4 %w/w. Different batches were prepared as per the Design expert software (Design expert version 10.0.0, Stat-Ease, Inc, Minneapolis, Minnesota, USA) to optimize the drug content and percent drug release using response surface methodology [17, 18]. Two independent variables, amount of Eudragit RSPO and RLPO were studied at three levels; low (-1), Medium (0) and high (+1). This gave 13 possible runs, drug content and percent cumulative drug release at predetermined times were seized as a dependent or response variables. All other formulations and processing variables were kept constant throughout the study [19]. Solid dispersions were physicochemically characterized by Powder X-Ray diffraction, IR Analysis, Differential Scanning Calorimetry and scanning electron microscopy [20].

Tea (*Camellia sinensis*) leaves were collected in the month of March from Kangra district of Himachal Pradesh, India. Previously reported methods with some modifications were adopted for; green tea leaves aqueous extract preparation and specifications [21-24], *in vitro* DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis,3-ethylbenzothiazoline-6-sulphonic acid) antioxidant assay [25, 26], total flavonoids by aluminum chloride method and total phenolic content determination by Folin Ciocalteu method [27, 28] and caffeine estimation [29].

Optimized solid dispersion (F7) and GTE were enclosed in transparent hard gelatin capsules and coated with Eudragit L100 solution. The resulting coated capsules were needed to be dissolved in pH 6.8 [30, 31]. Coating dispersion containing 10% w/v Eudragit L100, plastisized with 4% w/w (based on polymer concentration) triethyl citrate was used [32, 33]. Coating parameters (% weigh gain of capsules, coating film thickness, disintegration time) were scrutinized to get predetermined lag time or onset of drug release of 3.5-4 h [34].

Coated capsules were further subjected to dissolution study as per USP 27-NF 22 using Apparatus I, in acid and buffer stage (method A) and analyzed spectrophotometrically (UV-1800 Shimadzu, Japan) at 287.5 nm. MS standard solution scanned in UV spectrophotometer between 200 nm to 400 nm and showed two absorption maxima at 287.5 nm and 344 nm. The absorption at 287.5 nm was linear with almost similar r^2 to 344 nm. I used 287.5 nm as a measuring wavelength, meanwhile examined absorptivity of the GTE at a selected wavelength. The results show that the absorbance of GTE at selected λ_{max} 287.5 nm is negligible compared to MS absorption, which conveyed that there is no interference between GTE and MS at selected λ_{max} .

After *in vitro*, we assessed *in vivo* pharmacokinetic study in healthy male Guinea pigs to validate the developed capsules. The overnight fasted pigs of average weight 562 g were divided into two groups having three animals in each group. The first group received prepared capsule and the second group received marketed tablet (Montair10, by Cipla Ltd., India) as a single oral dose [35-37]. All the animals had ad libitum access to food and water after 4 h of drug dosing.

After a single dose, blood was collected in EDTA tubes at various time intervals to assess pharmacokinetic parameters [38]. Blood was immediately centrifuged (Remi CM-12 Plus, India) at 8000 rpm for 20 min at -4°C to separate plasma. Metoclopramide hydrochloride was used as Internal standard and MS was extracted from plasma sample by protein precipitation technique using saturated NaCl solution [39]. The Bioanalytical method was adopted as described earlier by Ranjan *et al.* 2013 and Shafaati *et al.* 2010, after performing partial method validation [40, 41]. Plasma samples were analysed by RP-HPLC, Cosmosil 5C18-MS-II (4.6ID $\times$ 250MM) column at wavelength 287 nm. Mobile phase consisting of a mixture of acetonitrile and sodium acetate buffer (20mM, pH adjusted to 5.0) in the ratio of 80:20 v/v was delivered at a flow rate of 1.5 ml/min, isocratically [42]. Pharmacokinetic parameters from plasma samples were calculated by one-compartment model, first order kinetics [43, 44].

Subsequently, we have examined the efficacy of developed capsules to reduce the oxidative stress in a Guinea pig model of chronic allergic asthma.

2.3. Animal Sensitization and Treatment

The study was conducted in accordance with the guidelines of the National Institutes of Health [45]. Overnight fasted male Durkin-Hartley Guinea pig of average weight of 510 g was placed in histamine chamber and exposed to an aerosol challenge of 0.2% w/v histamine dihydrochloride in distilled water using pressurized air driven nebulizer at a pressure of 0.05 MPa-0.106 MPa for one week [45-48]. The inhalation challenge was stopped immediately if the animal showed signs of respiratory distress [49]. On the first day of histamine aerosol challenge pre-convulsion time was noted as 128 ± 4 sec and on day seven it was measured as 40 ± 3 sec.

During the study, guinea pigs were kept under 12 h light/12h dark conditions at $20-23^{\circ}\text{C}$, with free access of food and water. After one week, guinea pigs were divided into three groups each containing three animals. These groups include 1) Sensitized animals, 2) sensitized animals received marketed tablets (Montair10; Mfg. by Cipla, India), 3) sensitized animals received prepared capsules (10 mg drug and 250 mg GTE per kg of body weight) and the fourth group contains three non sensitized healthy guinea pigs. Second and third groups were subjected to respective formulation once daily, orally for three days [46-48]. The first and fourth Group did not receive any treatment. The animals were fed after 4 h of drug dosing and had a free access of water throughout the study. On the fourth day, pigs were scarified by an overdose of sodium pentobarbital. Lungs and liver were removed intact for oxidative stress parameters and histopathological examination and blood was collected for assay of Total Antioxidant Capacity in serum [49].

2.4. Biochemical Study of Oxidative Stress Markers

The lung and liver tissue were washed with ice chilled saline followed by phosphate buffer pH 7.4 and cut into pieces. One piece was fixed in 10% buffered formaldehyde for histopathological examination and rest was used for Thiobarbituric Acid Reactive Substances (TBARS) assay,

Glutathione (GSH) level and total tissue protein estimation. TBARS and GSH are used as oxidative stress indicators in asthma. TBARS determinations were done in liver tissues according to Ohkawa *et al.* (1979) with modifications [50]. GSH levels were determined in liver homogenates using Ellmen's reagent [51-53]. Total tissue protein was estimated by Lowry assay proposed by Oliver H. Lowry in 1951 [54]. For lung tissue, perfuse and lavage the lung, Cut off the connective tissues and blotted using blotting paper to soak any hemolysed blood and proceeded as per liver tissues for TBARS and GSH estimation [53].

2.4.1. Estimation of MDA Level in Tissue Homogenate

The tissues were washed with cold saline, blotted and weighed. 5 ml of phosphate buffer pH 7.4 and 75 μl of 200 mM EDTA was added in minced tissue. Tissue suspension was homogenized at 2000 rpm for 10 min, followed by cold Centrifugation (Remi CM-12 Plus, India) at 6500 rpm for 10 min. 100 μl of 8.1% sodium dodecyl sulfate, 750 μl of 20% acetic acid, and 750 μl of 0.8% TBA were added in supernatant liquid and the volume was made up to 2 ml with distilled water. The liquid was heated over boiling water bath at 95°C for 60 minutes, took out and cooled in ice bath for 10 min. Subsequently, centrifuged again at 10,000 rpm for 10 min and analyzed spectrophotometrically (UV-1800 Shimadzu, Japan) at 532 nm keeping distilled water as a blank. MDA concentration in the samples was calculated by using linear regression $y = 0.0022x + 0.0005$, $r^2 = 0.999$ obtained from the standard curve [50].

2.4.2. Measurement of GSH Level in Tissue Homogenate

Tissue homogenate processed in the same way as described in section 2.4.1. For GSH estimation, 500 μl of tissue homogenate supernatant was taken and vortexed (Remi CM-101 Plus, India) with 500 μl of 5% ice-cold sulfosalicylic acid followed by centrifugation at 10,000 rpm for 10 min. 50 μl of supernatant was separated and shaken with 450 μl of phosphate buffer pH 7.4. Eventually, the resulting mixture was vortexed with 1500 μl of Ellman's reagent then allowed to stand for 5 min. Absorbance was read at 412 nm spectrophotometrically using phosphate buffer pH 7.4 as a blank [51-53]. GSH concentration was calculated from linear regression equation $y = 0.014x + 0.081$, $r^2 = 0.999$.

2.4.3. Total Tissue Protein Quantification

50 μl of tissue homogenate supernatant was taken and the volume was made up to 500 μl with distilled water. Lowry's solution (2.5 ml) was added to the liquid, vortexed and incubated for 10 min at 37°C . Later on, 250 μl of Folin's reagent was mixed and again incubated at 37°C for 30 min. Absorbance was read at 660 nm by UV Spectrophotometer keeping distilled water as a blank [42]. Standard solution of protein with a different concentration of Bovine serum albumin (200-1200 $\mu\text{g/ml}$) was prepared and treated in the similar manner to get regression equation $y = 0.074x + 0.008$, $r^2 = 0.981$.

2.4.4. TAC Estimation in Guinea Pig Plasma

Guinea pig blood sample was collected in EDTA coated sample tube and centrifuged at 8000 rpm for 20 min at -4°C , resultant plasma was separated and transferred in eppendorf tubes then immediately stored at $-20 \pm 2^{\circ}\text{C}$ until analysis [55].

Plasma samples stored at -20°C were first thawed at room temperature and shaken in vortex. Antioxidant assay was performed by using Zen-Bio ORAC kit (Oxygen Radical Absorbance Capacity kit, Life technologies, Delhi, India) and the procedure was adopted as per instruction manual. The assay evaluates the loss of fluorescein fluorescence over time due to peroxy-radical formation by the breakdown of AAPH (2,2'-azobis-2-methyl-propanimidamide, dihydrochloride) [55, 56]. Synergy HT fluorescence multiplate reader (Bio-Tek Instruments) was used for analysis. Trolox, a water soluble vitamin E analog, was used as a positive control inhibiting fluorescein decay in a dose dependent manner. ORAC value is the direct measure of the antioxidant capacity of the sample, higher ORAC value corresponds to higher antioxidant capacity [56, 57]. Total antioxidant capacity or ORAC value is expressed as mM Trolox Eq/L.

2.5. Histopathological Evaluation

Formalin-fixed tissues were prepared for histopath study. Tissues were dried and then embedded in paraffin wax to prepare paraffin block of tissue. Blocks were sectioned to make tissue slices of around $5\ \mu\text{m}$ thickness. Dewaxed tissues are then stained with hematoxylin-eosin dye and examined at 40X magnification [58].

Hepatic pathological changes including disruption in the radial orientation and necrosis of hepatocytes, inflammatory cells infiltration, and changes in the portal space were examined [49]. Inflammation and airway remodeling of the lung in guinea pigs were analyzed [50, 58-60].

2.6. Data and Statistical Analysis

The histopathological evaluation represents slides from three animals. All data were expressed as mean $\pm$ SEM of

three observations. The correlations between the studied parameters were statistically analyzed by GraphPad Prism Version 7.04 software with level of significance and confidence interval 5% and 95% respectively. The data of the asthmatic group were compared with the control group and the results of treated groups were also compared with asthmatic group using student t-test. Whereas, the data between two treated animal groups were compared by ANOVA.

3. RESULTS

3.1. Characterization of Developed Capsules

Table 1 summarizes 13 experimental runs obtained from response surface methodology. Drug content was obtained in the range of $88.81 \pm 7.60\%$ to $92.33\% \pm 7.66$ and it was maximum at drug-carrier ratio 1:2, formulation F7. Drug-carrier weight ratio 1:2 seems to be sufficient to entrap the drug moiety into the solid dispersion. Developed capsules were subjected for *in vitro* dissolution study and dissolution profile is shown in Fig. (1). Enteric coating of capsules prevents the release of drug in the acidic condition of the stomach.

GTE used in formulation had shown 95.13% antioxidant activity with DPPH and 93.76% when tested with ABTS. Total Phenolic and flavonoid content was estimated as 251.77 ± 6.59 mg gallic acid equivalent per g dry weight and 246.56 ± 2.58 mg Quercetin equivalent per g dry weight of extract respectively. Caffeine content was measured as $5.53 \pm 0.96\ \mu\text{g}/\text{mg}$ extract. Thus, the antioxidant activity of crude extract is not limited to phenolic compounds only, the presence of other components may contribute to the overall observed antioxidant potential. *In vivo* data supports the ability of developed capsules to halt the release for 4 h with mean C_{max} and t_{max} $515.29 \pm 1.67\ \text{ng}/\text{ml}$ and 6h, respectively.

Table 1. Composition of solid dispersion at Low (10mg), medium (15mg) and high level (20mg).

Formulation Code	Drug (mg)	Eudragit RSPO (mg)	Eudragit RLPO (mg)	Drug Content (%)	Cumulative Drug Release at 8 h (%)
F1	10	15	15	88.81 ± 7.60	85.92 ± 6.25
F2	10	10	20	89.10 ± 5.12	82.91 ± 6.58
F3	10	15	15	88.18 ± 1.95	85.01 ± 4.22
F4	10	15	15	89.52 ± 2.57	85.00 ± 6.48
F5	10	15	10	89.64 ± 1.82	92.70 ± 8.23
F6	10	10	15	90.11 ± 5.94	93.80 ± 8.12
F7	10	10	10	92.33 ± 7.66	95.90 ± 6.68
F8	10	15	20	90.00 ± 3.22	79.01 ± 5.81
F9	10	20	15	89.00 ± 2.94	78.82 ± 6.12
F10	10	15	15	90.90 ± 2.39	85.30 ± 8.20
F11	10	20	10	91.03 ± 6.21	88.02 ± 7.19
F12	10	20	20	90.87 ± 2.99	75.92 ± 6.67
F13	10	15	15	89.10 ± 4.78	85.11 ± 7.86

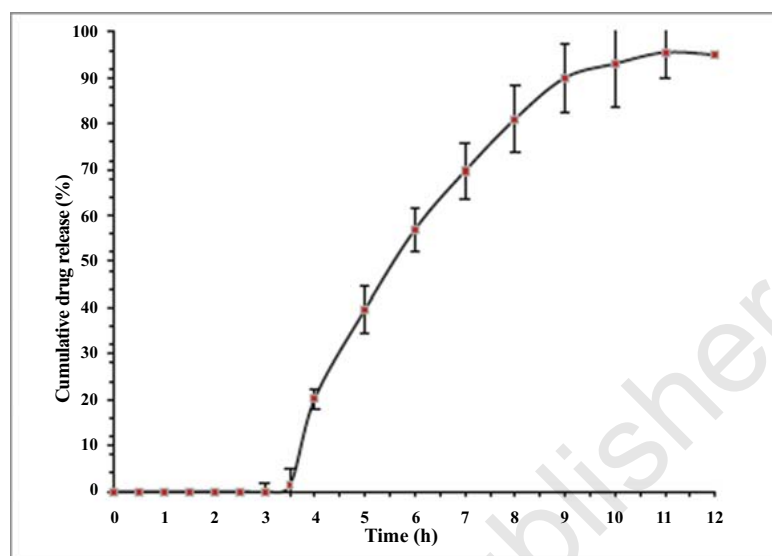


Fig. (1). Dissolution profile of developed capsules in pH 1.2 for initial 2 hours followed by pH 6.8.

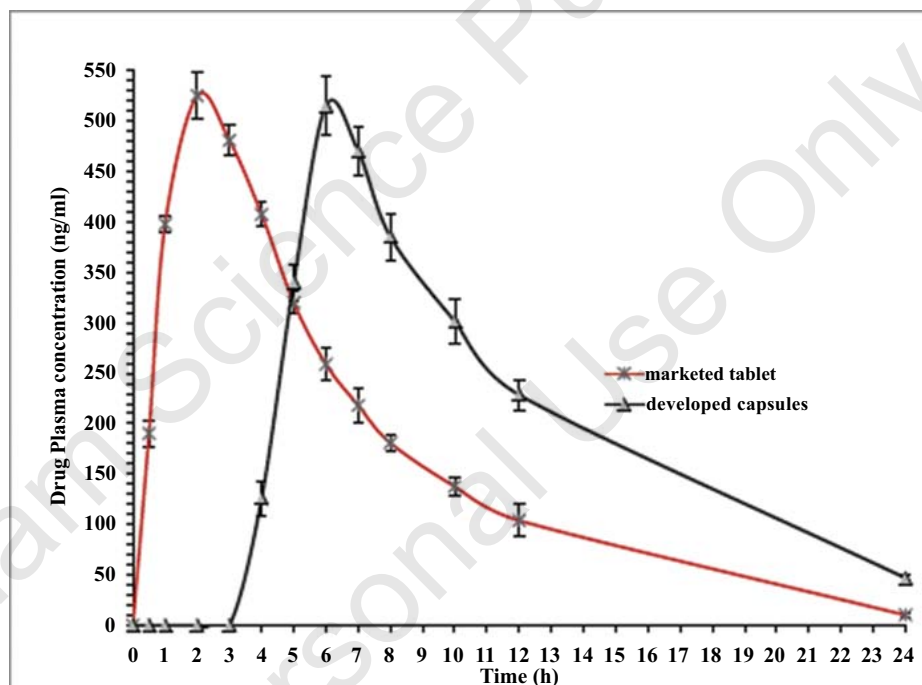


Fig. (2). Plot of time versus plasma concentration of Montelukast Sodium.

Other pharmacokinetic parameters like; $AUC_{0-\infty}$, $T_{1/2}$, elimination rate constant K_e , volume of distribution V_d and mean residence time MRT were reported as 5007.25 ± 38.27 ng h/ml, 7.52 ± 1.24 h, 0.09 ± 0.012 h⁻¹, 0.94 ± 0.08 L and 10.86 ± 1.35 h respectively. Pharmacokinetic profile of capsules was compared with that of marketed tablets at an equivalent dose and shown in Fig. (2).

3.2. Analysis of MDA and GSH Level in Tissue Homogenates

Biochemical estimation results show that GTE attenuates tissue lipid peroxidation which is measured as MDA level. Although significantly enhanced MDA level was noticed in organs (Liver and lung) of guinea pig sensitized by histamine

aerosol supporting to the manifestation of tissue damage, similar findings have also been suggested previously by Zemmouri *et al.* [61]. The effects of GTE on the therapeutic efficacy of antiasthmatic drug MS are listed in Table 2.

The percent change in MDA and GSH level in sensitized and treated animal groups as compared to control is calculated [62] and depicted in Figs. (3 and 4), respectively.

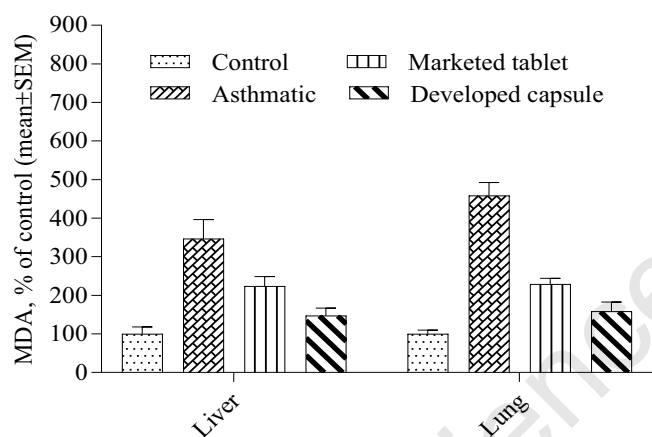
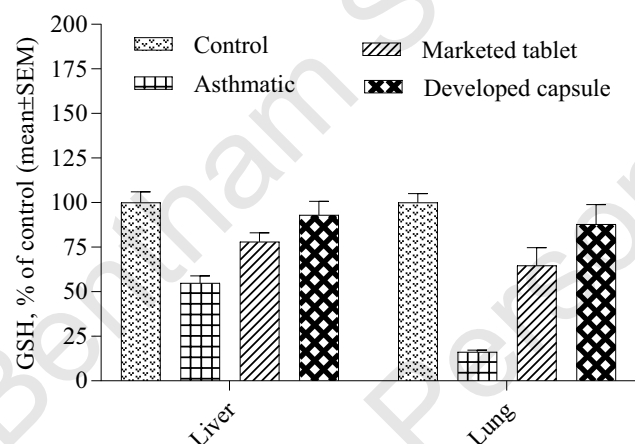
3.3. Total Antioxidant Capacity of Guinea Pig Plasma

The TAC value of healthy animal was calculated to be 6.971 ± 1.013 mM Trolox Eq/L; this value is relatively higher than the sensitized animal TAC which was 2.132 ± 0.986 mM Trolox Eq/L. Data presented as mean $\pm$ SEM. TAC values of

Table 2. Oxidative stress parameters in guinea pig tissue homogenate, values are mean $\pm$ SEM.

Parameters ($\mu\text{g}/\text{mg}$ Protein/g Wet Tissue)	Tissue	Control (Non Asthmatic)	Sensitized (Asthmatic)	Asthmatic Treated with	
				Marketed Tablets	Developed Capsules
MDA	Liver	0.017 $\pm$ 0.006	0.059 $\pm$ 0.031	0.038 $\pm$ 0.015*	0.021 $\pm$ 0.017*
	Lung	0.175 $\pm$ 0.061	0.802 $\pm$ 0.310	0.401 $\pm$ 0.120	0.278 $\pm$ 0.061
GSH	Liver	24.110 $\pm$ 1.082	13.223 $\pm$ 1.485	18.802 $\pm$ 0.663	22.403 $\pm$ 0.479
	Lung	18.742 $\pm$ 1.371	3.037 $\pm$ 0.282	12.150 $\pm$ 2.013	16.311 $\pm$ 0.615

* P>0.05 between two treated groups.

**Fig. (3).** Percent change in tissue MDA level as compared to control.**Fig. (4).** Percent change in tissue GSH level as compared to control.

sensitized animals treated with marketed tablets and developed capsules were reported as 3.780 ± 0.583 and 5.087 ± 0.958 mM Trolox Eq/L respectively with $p < 0.006$. TAC level shows a significant difference ($p < 0.009$) between control and sensitized animal groups which emphasizes the link between inflammation of asthma and oxidative stress. The sensitized group followed intake of developed capsules containing green tea extract along with drug and exhibited better improvement in TAC value which reflects the antioxidant potential of GTE [63].

3.4. Histopathological Images Analysis

The liver section of control animals showed a normal structure. While, in the sensitized group, hepatic enzyme activities were increased due to extensive tissue damages, infiltration of inflammatory cells and loss of cellular order towards the center as shown in Fig. (5A and B). These destructive effects were diminished in the treated groups. Treatment of sensitized animals with developed capsules has reduced the cell membrane damage caused by free radicals due to the protective effect of MS and antioxidant molecules found in GTE. However, marketed tablets also attenuate inflammatory cell infiltration in tissues but to a smaller extent. Comparison of the effect of marketed tablets and developed capsules in the liver injury of asthmatic guinea pig reveals that a combination of MS and GTE is more effective to normalize the tissue structure as seen in Fig. (6A and B).

Fig. (7) expressed normal lung structure of control animals. Lungs histopath examination of the sensitized group showed the area of chronic changes and cellular infiltration of eosinophils and lymphocytes in lung parenchyma with abnormally thickened bronchial wall. Bronchocontractive effect in lung tissues was shown due to inhaled histamine in Fig. (8). Enhanced blood vessel density and vascular area are identified as characteristics of remodeled airways in asthma [60]. Airway remodeling causes thickening of airway walls and as a result leads to airway narrowing. The improvement in lung tissue anatomy of asthmatic guinea pig after dosing was observed by a difference in the bronchial wall thickness, decrease or absence of cellular infiltrates, and regular cellular order. After dosing with marketed tablets, cellular infiltrates in lung reduced along with a decrease in bronchial wall thickness, Fig. (9). Lung of the animal group received developed capsules restoring its usual anatomy and appeared normal as shown in Fig. (10). It was observed from histopathology images of lungs that outcomes of the animal group treated with developed capsules were superior in comparison to groups that received the marketed tablet.

4. DISCUSSION

The lung is an important target organ of asthma and liver is an organ which is associated with many functions including metabolism and detoxification. Hence, we have assessed lung and liver function and histology. A significantly elevated level of MDA in sensitized animal's liver ($p < 0.002$) and lung ($p < 0.005$) is due to increased chemotaxis of leucocytes,

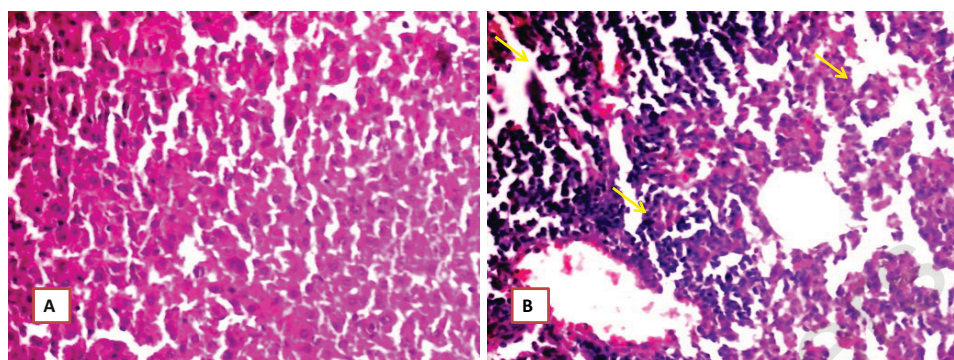


Fig. (5). Microscopic view of the liver (A) Control group with natural structure (B) Sensitized group; leucocytes and neutrophil infiltration, hepatic necrosis, hemorrhage and loss of cellular order can be seen (stain: hematoxylin-eosin, 40X magnification).

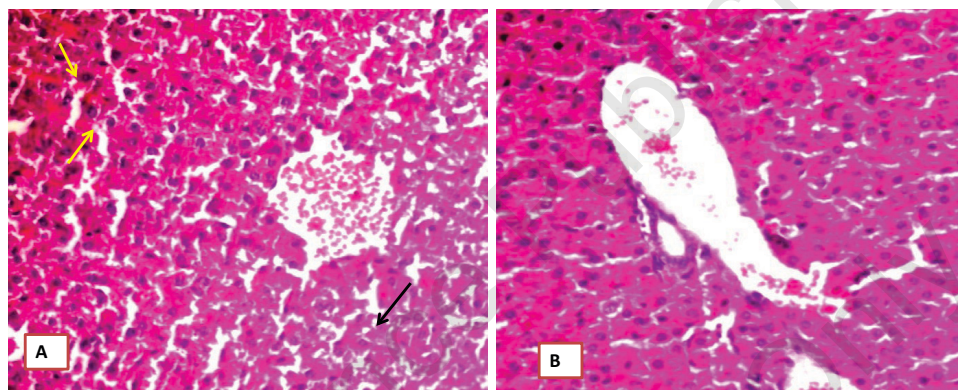


Fig. (6). Microphotograph of the liver in sensitized group (A) treated with marketed tablets with decreased infiltration of inflammatory cells, more regular cellular order and decreased congestion of sinusoids, (B) treated with developed capsules; tissues seem relatively healthy without any certain pathological changes (stain: hematoxylin-eosin, 40X magnification).

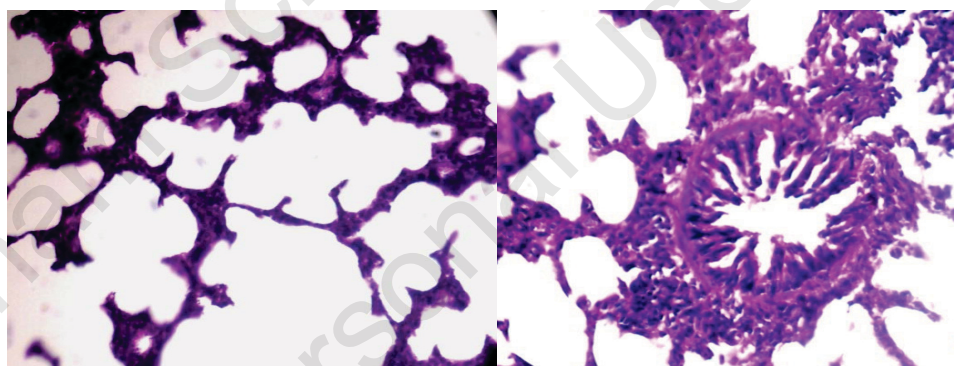


Fig. (7). Microscopic view of the lung in the control group with natural structure (stain: hematoxylin-eosin, 40X magnification).

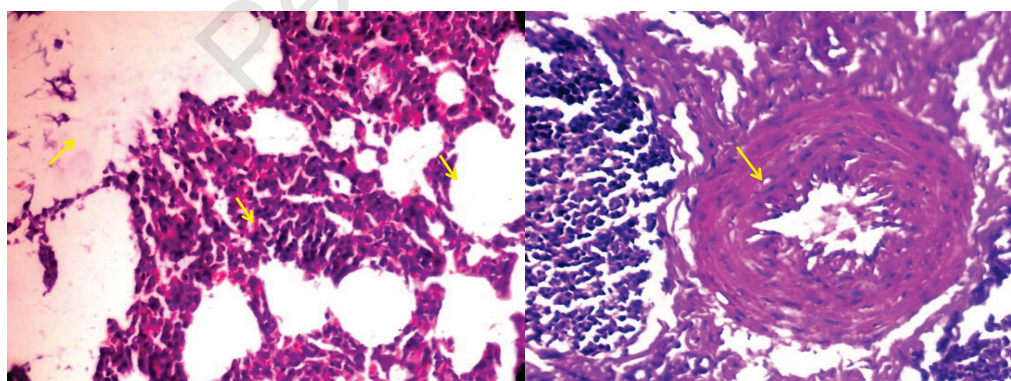


Fig. (8). Section from the lung of asthmatic guinea pig shows atelectasis, area of chronic changes and alveolar inflammation due to dense cellular infiltrates and, thickened bronchial wall (stain: hematoxylin-eosin, 40X magnification).

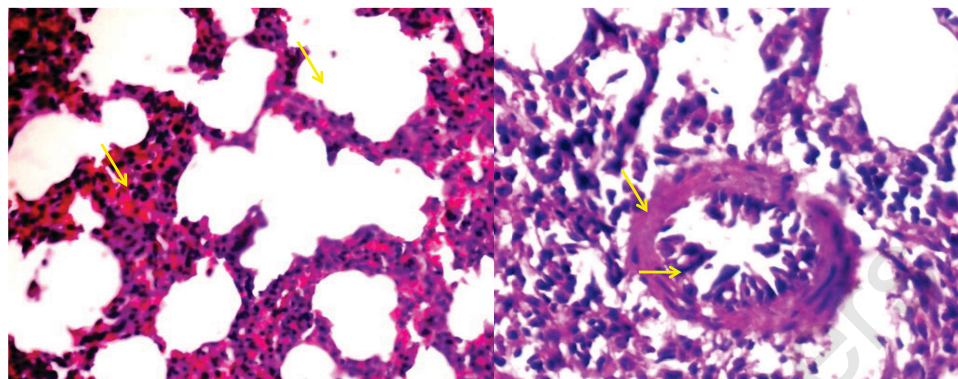


Fig. (9). Section from the lung of asthmatic guinea pig received marketed tablets shows decreased cellular infiltrates, decrease in thickness of bronchial wall and regular cellular order (stain: hematoxylin-eosin, 40X magnification).

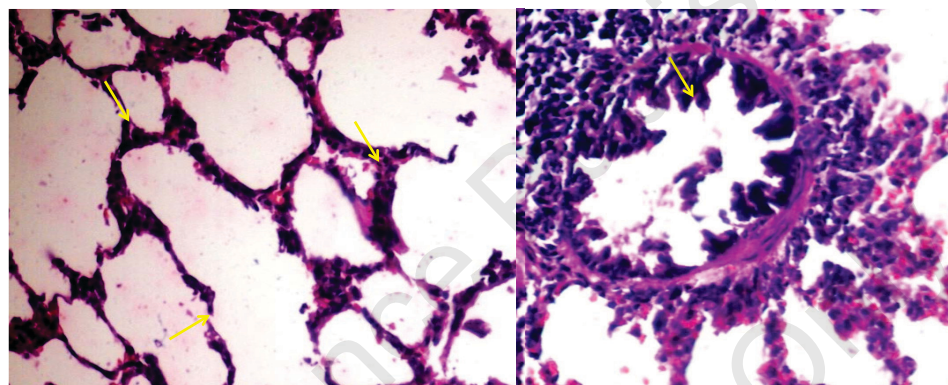


Fig. (10). Section from the lung of asthmatic guinea pig treated with developed capsules exhibits normal bronchial wall and relatively healthy tissue with more regular cellular order (stain: hematoxylin-eosin, 40X magnification).

infiltration of superoxide ions by activated inflammatory cells in the alveoli, which resultantly perpetuates inflammation [64]. Histamine sensitized guinea pigs treated with developed capsule reached MDA level more closely to control group as compared to marketed tablets. This may be due to the fact that antioxidants present in GTE help in reducing lipid peroxidation in case of asthma [65]. Similarly, a significant decrease in GSH level in the liver ($p < 0.0001$) and lung ($p < 0.0004$) was recorded in the sensitized animals as compared to control guinea pigs. Our outcomes were in agreement with Mak *et al.* in asthmatic patients [66]. Asthmatic animals treated with marketed tablets show significant improvement ($p < 0.001$) in oxidative status, although upon oral administration of developed capsules oxidative stress parameters were restored to near normal ($p < 0.0002$) as indicated by lipid peroxidase and GSH level. This reflects that treatment modalities focusing on coformulation of MS and GTE have great potential for the treatment of asthma over monotherapy of drug alone.

Plasma TAC evaluates the antioxidant response of the organism against the free radicals produced in a given disease. Plasma TAC was found to be decreased in the asthmatic group than healthy control group, owing to increased production of ROS which results in an imbalance between oxidative force and the antioxidant defense system [10].

Histopathological evaluation of tissues confirms its biochemical results. In asthmatic animals, there will be several histopathological changes in the cytoarchitecture of tissues

and same is contemplated in this study [67]. In asthmatic animals, increased activity of hepatic enzymes causes numerous types of inflammation, hepatocytes necrosis and congestion in sinusoids [49]. Asthmatic airway also revealed obvious inflammation including remodeling of lung tissues due to increased generation of ROS [60]. These effects declined in the treated groups. GTE has the capacity to modulate asthma induced oxidative stress and beneficial in opposing cytoarchitectural changes in lung and liver tissues.

The GTE used in this study was characterized for total Phenolic and flavonoid content, which are considered as the most powerful antioxidants of green tea. The extract dose that was administered in combination with MS is 250 mg/kg, which is equivalent to relatively low dose of phenolic and flavonoid content as 62.94 mg/kg and 61.64 mg/kg respectively. GTE in this dose was found to be effective in improving the antioxidant status indices, so it would be assumed that use of the whole extract instead of isolated compounds potentiates the antioxidant effect [68].

CONCLUSION

The outcomes revealed that combining Montelukast Sodium with green tea extract gives additional benefit in comparison with drug alone. We speculate that green tea antioxidants may prove to be beneficial in the prevention of oxidant injury in allergic inflammation, as an adjuvant to current pharmacological therapies. Since studies in animals do not directly translate to human biology, further multi-control

studies with better sampled patient population and more number of patients are needed.

CURRENT & FUTURE DEVELOPMENTS

It has been recognized that combined administration of Montelukast sodium and green tea extract as a single capsule improved the altered biochemical profile along with reversal of the histopathological manifestation towards normal in a Guinea pig model of chronic allergic asthma. It is hoped that antioxidant as an adjuvant to pharmacotherapy will be a better treatment option for the efficacious management of asthma instead of pharmacotherapy alone. Green tea extract synergism with Montelukast sodium will have the potential to address the unmet needs of allergic disease.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by CPCSEA (1044/PO/Re/S/07/CPCSEA) and Institutional animal ethical committee (Protocol no ITS/01/IAEC/2017) I.T.S College of Pharmacy, NH-34, Ghaziabad, UP, India.

HUMAN AND ANIMAL RIGHTS

No humans were used in this study. All the experiments conducted were according to CPCSEA guidelines (Committee for the Purpose of Control and Supervision of Experiments on Animal) India.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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