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Hepatic Oxidative Stress and Lipid Dysregulation in Ovalbumin-Induced Asthma: Comparative Effects of Salbutamol, Montelukast, Prednisolone, and Hydrocortisone in Sprague Dawley Rats

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ABSTRACT

Background: This study assessed the hepatic oxidant–antioxidant status and serum lipid profile in ovalbumin (OVA)-induced asthma, comparing the effects of salbutamol, montelukast, prednisolone, and hydrocortisone in Sprague Dawley rats.
Methods: Forty-two (42) female Sprague–Dawley rats (150–200 g) were randomized into six groups (n = 7): negative control; OVA-asthma positive control; and OVA-asthma treated with salbutamol (2 mg/kg, oral, twice daily), montelukast (10 mg/kg, oral, daily), prednisolone (3 mg/kg, oral, daily), or hydrocortisone (5 mg/kg, i.p., daily). Asthma was induced by i.p. sensitization with OVA (1 mg) and aluminium hydroxide (20 mg) on days 1 and 7, followed by biweekly aerosol challenge with 1% OVA for 28 days. Treatments were administered for 28 days after asthma confirmation. Hepatic oxidative stress/antioxidant indices (total protein, SOD, CAT, GSH, GPx, MDA, H_2O_2, TAC) and serum lipids (total cholesterol, triglycerides, HDL, LDL) were quantified.
Results: Montelukast significantly increased hepatic SOD, CAT, and GPx activities compared with the OVA-positive control ($p<0.05$) and significantly reduced total cholesterol and LDL levels compared with the positive control ($p<0.05$). Prednisolone significantly increased total cholesterol and LDL levels compared to the negative control ($p<0.05$). Hydrocortisone significantly increased triglycerides and hepatic MDA, indicating heightened lipid peroxidation ($p<0.05$), and further reduced total protein compared with the positive control ($p<0.05$). Across treatment groups, hepatic TAC was significantly decreased compared to the negative control ($p<0.05$). At the same time, GSH was significantly reduced in all treated groups, with montelukast showing a further reduction compared to the positive control ($p<0.05$). Salbutamol produced no consistent improvement in oxidative or lipid indices.
Conclusion: In OVA-induced asthma, montelukast showed comparatively favorable antioxidant enzyme up-regulation and improved atherogenic lipid indices, whereas corticosteroids, particularly hydrocortisone, were associated with oxidative and/or metabolic worsening. Persistent TAC reduction across therapies suggests standard treatment may not fully normalize systemic redox imbalance.

Key words: asthma; ovalbumin; oxidative stress; liver; antioxidant enzymes

INTRODUCTION

Asthma is a chronic inflammatory airway disease characterised by variable airflow obstruction, hyperresponsiveness, and remodelling, triggered by allergens, pollution, or lifestyle factors. It remains incompletely understood but involves genetic predisposition (such as atopy and family history), environmental exposures, and dysregulated immune responses. Currently, global asthma prevalence exceeds 300 million individuals (1,2).

At the core of asthma pathophysiology is the Th2-mediated cascade, where allergens such as dust mites or pollen trigger the release of IL-4, IL-5, and IL-13, resulting in eosinophilic inflammation, mucus hypersecretion, and airway remodeling. Severe cases may involve Th1-mediated neutrophilia, which is triggered by lipopolysaccharide (LPS) via toll-like receptor 4 pathways (2–5).

Emerging evidence suggests a link between asthma-driven systemic inflammation and metabolic disturbances. A meta-analysis of over 32,000 participants showed higher levels of LDL and total cholesterol in individuals with asthma compared to controls (6). Other studies have found negative correlations between HDL-C and eosinophil counts, along with positive links to triglycerides in atopic asthma, suggesting an interaction between lipid inflammation (7,8). These findings are supported by a mechanistic review on how lipid metabolism influences immune regulation in asthma (9,10).

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Asthma-related oxidative stress disrupts the redox balance (e.g., reduced glutathione, SOD, catalase), with elevated reactive oxygen/nitrogen species in human and murine models (3,11). Such oxidative injury is implicated in extrapulmonary effects, including hepatic dysfunction.

The liver, a central metabolic organ, plays a crucial role in maintaining detoxification, protein synthesis, and lipid homeostasis. Oxidative stress can precipitate hepatic injury, fibrosis, and dysregulated lipid metabolism—linking pulmonary inflammation to systemic organ dysfunction (3,12),

OVA-induced asthma in rodents faithfully replicates human allergic inflammation, including airway hyperresponsiveness, eosinophilia, mucus production, and oxidative stress. It is widely used to study systemic effects, such as metabolic and hepatic changes (11,12).

Asthma treatments include short-acting β_2-agonists (e.g., salbutamol), corticosteroids (e.g., prednisolone, hydrocortisone), and leukotriene receptor antagonists (e.g., montelukast). While effective for airway symptoms, these drugs may also influence systemic oxidative stress, hepatic function, and lipid metabolism, although data are limited. This study aimed to investigate the effects of salbutamol, prednisolone, hydrocortisone, and montelukast on the liver's oxidant/antioxidant status and lipid profiles in female Sprague-Dawley rats with OVA-induced asthma.

MATERIALS AND METHODS

Experimental Animals

This study used forty-two (42) female Sprague–Dawley rats weighing 150–200 g. The animals were obtained from the University of Benin's animal house. Ethical approval was obtained from the College of Medical Sciences Ethics Committee, and all experimental procedures complied with international guidelines for the care and use of laboratory animals. Rats were housed in clean plastic cages in a controlled environment (12 h light/dark cycle) with free access to standard rat chow and water ad libitum.

Experimental Design and Duration

The experiment lasted six (6) weeks, comprising one week of acclimatisation, one week of sensitisation, and four weeks of challenge and drug treatment. Following acclimatisation, rats were randomly divided into six groups (n = 7 per group):

- **Group 1 (Negative control):** No induction, normal feed and water.
- **Group 2 (Positive control):** OVA-induced asthma, untreated.
- **Group 3:** OVA-induced asthma, treated with salbutamol.
- **Group 4:** OVA-induced asthma, treated with montelukast.
- **Group 5:** OVA-induced asthma, treated with prednisolone.
- **Group 6:** OVA-induced asthma, treated with hydrocortisone.

Asthma Induction Protocol

Asthma was induced following a modified protocol (13,14). On days 1 and 7, rats in Groups 2–6 were sensitised via intraperitoneal (i.p.) injection of 1 mg ovalbumen (OVA; Sigma-Aldrich) and 20 mg aluminium hydroxide dissolved in 0.9% saline. From day 14, animals were challenged biweekly for 28 days in a transparent chamber (50 × 40 × 30 cm) with aerosolised 1% w/v OVA in 0.9% saline using a compressor nebuliser (CNB 69009) that delivered≥0.25 mL/min of aerosol for 15 minutes.

Drug Administration

After asthma confirmation, treatment commenced for 28 days (4 weeks):

- Salbutamol: 2 mg/kg, oral, twice daily
- Montelukast: 10 mg/kg, oral, once daily.
- Prednisolone: 3 mg/kg, oral, once daily
- Hydrocortisone: 5 mg/kg, i.p., once daily.

Sacrifice and Sample Collection

At the end of the experimental period, animals were fasted overnight, weighed, and anaesthetised by inhalation of chloroform (20 mL on cotton wool in a closed chamber). Blood was collected by cardiac puncture and portal vein aspiration. Samples (5 mL and 1 mL) were transferred into plain and EDTA-coated tubes, respectively. Blood in plain tubes was left at room temperature for 30 minutes, then centrifuged at 5000 rpm for 15 minutes, and the serum was separated and stored at −4 °C for biochemical assays.

The liver and lungs were excised, rinsed in ice-cold saline, and sectioned. Portions were fixed in 10% formalin for histopathology, while others were stored at −4 °C for antioxidant and biochemical analyses.

Statistical Analysis

Data were expressed as mean ± standard error of mean (SEM). Differences among groups were analysed using one-way analysis of variance (ANOVA) followed by Tukey’s post hoc test. Statistical significance was set at $p < 0.05$. Analyses were performed using GraphPad Prism version 10.2.2 (GraphPad Software, San Diego, CA, USA).

RESULTS

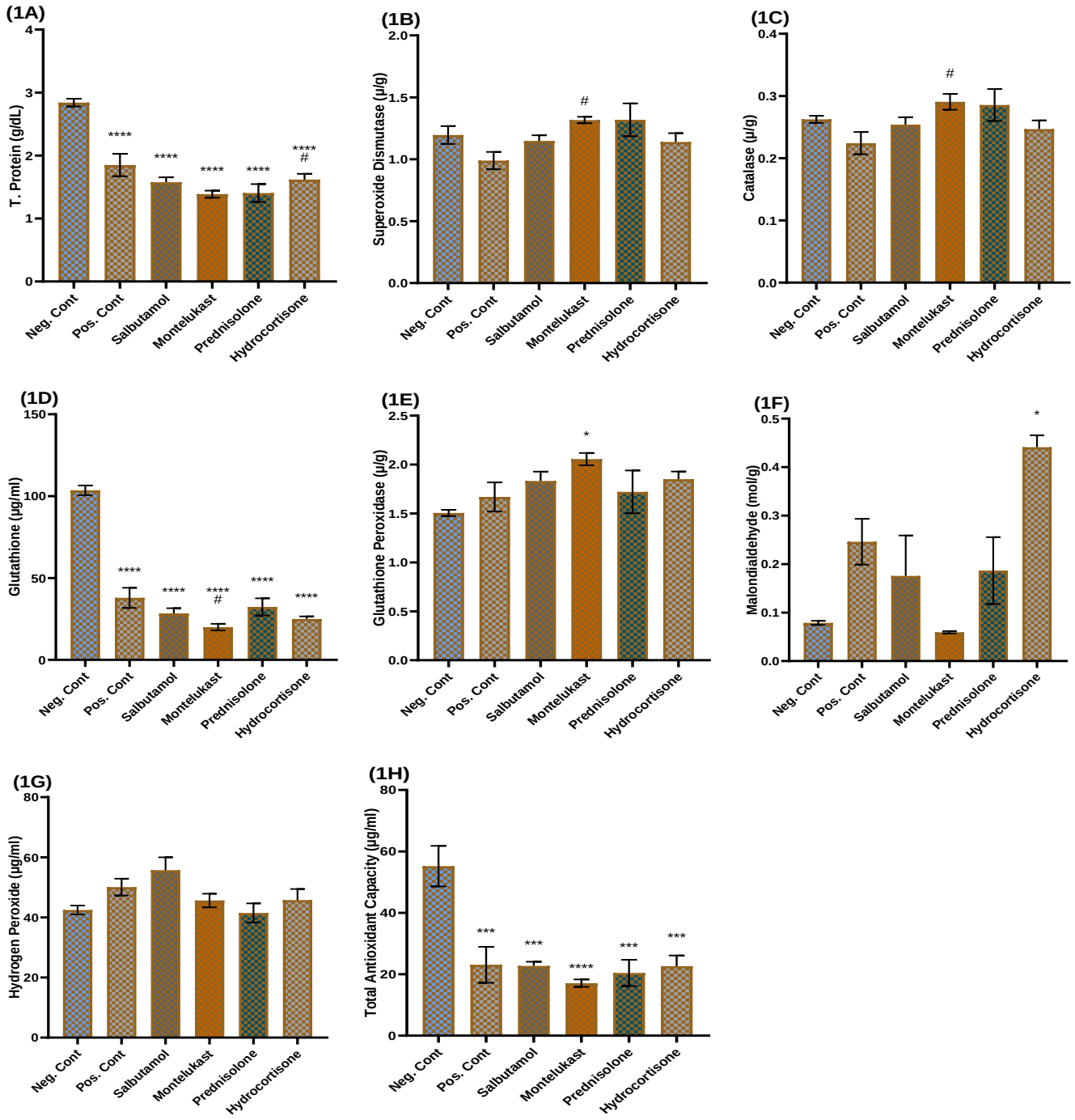


Figure 1. Effect of salbutamol, montelukast, prednisolone, and hydrocortisone on oxidative stress and antioxidant parameters in asthma-induced Sprague-Dawley rats. (A) Total protein, (B) superoxide dismutase (SOD), (C) catalase (CAT), (D) glutathione (GSH), (E) glutathione peroxidase (GPx), (F) malondialdehyde (MDA), (G) hydrogen peroxide (H_2O_2), and (H) total antioxidant capacity (TAC). Data are presented as mean ± SEM (n = 5 per group). *p < 0.05 compared with negative control; #p < 0.05 compared with positive control.

Total protein levels were significantly reduced in all treatment groups compared to the negative control, with an additional decrease observed in the hydrocortisone-treated group relative to the positive control (Fig. 1A). Superoxide dismutase activity showed no significant differences across groups when compared with the negative control; however, montelukast treatment produced a significant increase compared to the positive control (Fig. 1B). Similarly, catalase activity did not differ significantly from the negative control across groups, but was significantly elevated in the montelukast group

relative to the positive control (Fig. 1C). Glutathione levels were significantly reduced in all treatment groups compared to the negative control, with a further decrease in the montelukast-treated group compared to the positive control (Fig. 1D). Glutathione peroxidase activity was significantly increased only in the montelukast group, while no differences were observed in the other treatment groups compared to the negative control (Fig. 1E). In contrast, malondialdehyde levels were significantly elevated in the hydrocortisone group but remained unchanged in the other groups relative to the negative control (Fig. 1F). Hydrogen peroxide levels did not differ significantly among any of the groups compared to the negative control (Fig. 1G). Finally, total antioxidant capacity was significantly decreased in all treatment groups compared to the negative control (Fig. 1H).

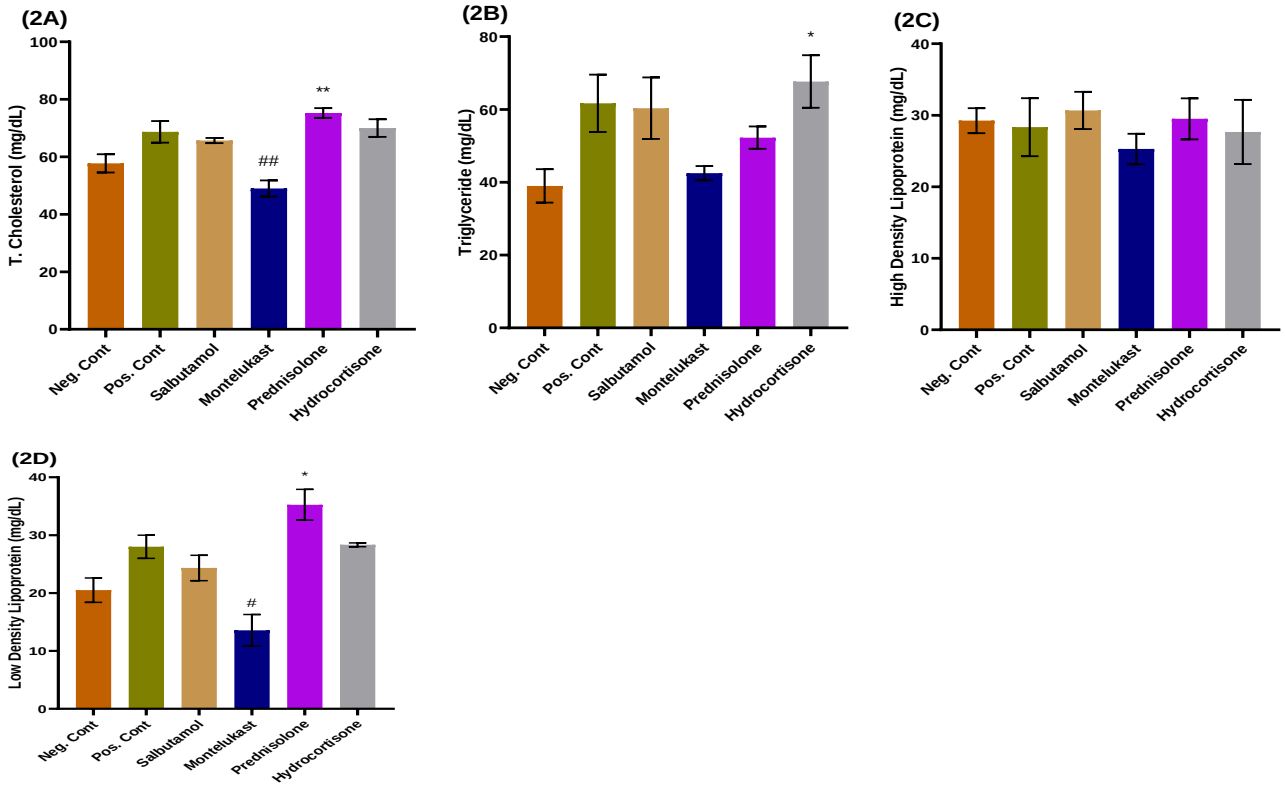


Figure 2. Effect of salbutamol, montelukast, prednisolone, and hydrocortisone on lipid profile in asthma-induced Sprague-Dawley rats. (A) Total cholesterol, (B) triglycerides, (C) high-density lipoprotein (HDL), and (D) low-density lipoprotein (LDL). Data are expressed as mean ± SEM (n = 7 per group). *p < 0.05 compared with negative control; #p < 0.05 compared with positive control.

The result demonstrates the effects of salbutamol, montelukast, prednisolone, and hydrocortisone on lipid profile parameters in asthma-induced Sprague-Dawley rats. Total cholesterol was significantly elevated in the prednisolone-treated group compared to the negative control. In contrast, montelukast significantly reduced cholesterol levels relative to the positive control (Fig. 2A). Triglyceride levels were markedly increased in the hydrocortisone-treated group, with no significant changes observed in the other treatment groups compared to the negative control (Fig. 2B). High-density lipoprotein (HDL) levels remained unchanged across all groups (Fig. 2C). In contrast, low-density lipoprotein (LDL) was significantly elevated in the prednisolone group compared to the negative control, while montelukast treatment resulted in a significant reduction compared to the positive control (Fig. 2D).

DISCUSSION

This study evaluated the effects of salbutamol, montelukast, prednisolone, and hydrocortisone on oxidative stress markers and lipid metabolism in asthma-induced Sprague-Dawley rats. The findings highlight distinct pharmacological impacts, with montelukast showing antioxidant benefits, corticosteroids contributing to oxidative and metabolic disturbances, and salbutamol exerting largely neutral effects.

Oxidative Stress Markers

A significant reduction in total protein and glutathione was observed across all treatment groups compared to the negative control, with hydrocortisone and montelukast showing the most pronounced reductions. Protein loss may reflect systemic catabolism or oxidative modification of proteins in asthma (15), while GSH depletion suggests heightened consumption of this key antioxidant in counteracting reactive oxygen

species (ROS) (16). These findings indicate that although pharmacological treatments target inflammation and airway tone, they do not restore intracellular thiol reserves.

Baseline SOD and CAT activities were not significantly altered compared to the negative control. However, montelukast produced a significant increase compared to the positive control, consistent with reports of its antioxidant properties (17). In contrast, salbutamol had no effect, which is expected given its primary bronchodilatory mechanism (18). Corticosteroids also did not significantly influence these enzymes, supporting evidence that their antioxidative action is largely indirect through inflammation suppression rather than enzyme upregulation (19).

Meanwhile, Montelukast significantly enhanced GPx activity, while other drugs showed no effect. This enzyme protects against lipid hydroperoxides and hydrogen peroxide, and its upregulation aligns with montelukast's reported ability to restore redox balance via leukotriene receptor blockade and improved glutathione utilization (20,21).

In addition, Hydrocortisone significantly increased MDA, a marker of lipid peroxidation, suggesting that this corticosteroid may exacerbate oxidative stress by impairing mitochondrial function and antioxidant defenses. Neither salbutamol nor montelukast elevated MDA, with montelukast showing protective effects consistent with reduced lipid peroxidation (22).

However, no significant changes in H_2O_2 were observed, indicating that this ROS may not be the primary mediator of oxidative damage in this model. However, TAC was significantly reduced across all treatment groups compared to the negative control. These finding highlights that while individual antioxidant enzymes may be modulated, none of the drugs fully restored global antioxidant capacity. Clinically, this suggests that adjunctive antioxidant supplementation (e.g., vitamins C and E, N-acetylcysteine) may be beneficial in asthma management (23).

Lipid Profile

Prednisolone significantly elevated cholesterol and LDL levels, consistent with glucocorticoid-induced dyslipidemia, which occurs via increased hepatic cholesterol synthesis and reduced LDL receptor expression (24,25). In contrast, montelukast lowered cholesterol and LDL relative to the positive control, suggesting a lipid-lowering benefit likely mediated by its anti-inflammatory action (26).

Hydrocortisone significantly increased triglycerides, consistent with corticosteroid-induced lipolysis and hepatic lipid synthesis (24,25). No significant effects on HDL were observed in any group, although literature suggests that prolonged corticosteroid use may impair HDL function despite transient increases (27).

Overall, montelukast demonstrated dual benefits by enhancing antioxidant enzyme activity (SOD, CAT, GPx) and improving lipid metabolism (lowering cholesterol and LDL). In contrast, prednisolone and hydrocortisone aggravated dyslipidemia, with hydrocortisone also elevating oxidative stress (MDA). Salbutamol, while effective for bronchodilation, had minimal impact on oxidative stress and lipid regulation.

Conclusion: These findings suggest that while standard asthma therapies control inflammation and bronchoconstriction, they exert differential effects on oxidative stress and lipid metabolism. Montelukast appears to provide added antioxidant and metabolic protection, whereas corticosteroids may worsen oxidative and metabolic disturbances. Salbutamol remains metabolically neutral but offers no antioxidant benefit. The consistent reduction in total antioxidant capacity across all treatments underscores the need for adjunct antioxidant therapy in asthma management.

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