Original Article

In Vivo and in Vitro Study of the Effect of the Anti-Asthmatic Drug Montelukast on DNA and Activity of Free Radical Scavenging Enzymes

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Abstract

This work aimed to investigate the potentiality of Montelukast to alter DNA and the activity of free radical scavenging enzymes (FRSE) in mammalian blood serum and mice liver tissues. DNA was extracted from Balb/c mice pretreated with different concentrations of Montelukast (1.25, 2.5 and 5.0 mg/kg body weight (BW)) and human blood treated with (2.5, 5.0 and 10.0) µg/ml solution to examine the effects of Montelukast in vivo and in vitro, respectively. DNA damage was assessed using gel electrophoresis of S1-nuclease digested DNA to detect the presence of single strand breaks. To determine the effect of Montelukast on FRSE, the activity of glutathione s-transferase (GST), superoxide dismutase (SOD) and catalase (CAT) was measured in liver tissue and serum from Balb/c mice and in human blood treated with Montelukast as described above. Our investigation has shown that Montelukast may possess an indirect effect on DNA that is associated with decreased FRSE activity; the treatment was not associated with increased numbers of ss-breaks (single-strand breaks) in DNA in vivo or in vitro. However, GST, SOD, and CAT activity was significantly decreased compared to the untreated control ($p < 0.05$) in liver tissue in vivo from mice treated with 2.5 and 5.0 mg/kg BW Montelukast and in human tissue treated with 5 and 10.0 µg/ml Singular. Meanwhile, the enzymatic activity in vitro was significantly decreased ($p < 0.05$) in GST, SOD and CAT either mouse or human blood serum with increasing the concentration of Montelukast in liver tissue.

Keywords: Montelukast, DNA, Free Radical Scavenging Enzymes, Serum, Liver

Introduction

Asthma is a common respiratory disease that is caused by the interaction of genetic susceptibility and exposure to various environmental factors. This disease can be categorized as hypersensitivity type I [1] and worldwide affects approximately 10% and 5% of children and adults, respectively [2]. Asthma is characterized by several physiological signs such as airway wall thickening, bronchoconstriction that limits airflow, wheezing and cough. The airways of asthma patients are usually inflamed and show signs of edema and inflammatory cell infiltration, principally by eosinophil, granulocytes, mast cells and lymphocytes [3].

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Increased levels of total IgE are frequently observed in asthma patients [4] Cysteinyl leukotriene's (CysLTs) are mediators of asthma and can promote bronchial smooth muscle contraction and mucus secretion, as well as increased microvascular permeability and proliferation of fibroblasts and smooth muscle cells [5]. Montelukast is used as anti-asthmatic agent that has Montelukast sodium (MK) as the active ingredient [6]. Chemically, Montelukast can be described as [1-{[(1R)–(3(2-(7-chloro-2-quinolinyl)-(E)-ethenyl-phenyl)-3-(2-(1-hydroxy-1-methylethyl) phenyl) propyl) thio) methyl} cyclopropylacetic acid-sodium salt. Patients typically take Montelukast once orally at bedtime [7] at a dose of 4 mg or 5 mg for children and 10 mg for adults depending on age [8]. Treatment with Montelukast reduced a variety of asthma symptoms, including changes in lung function and structure [9]. Neonatal hyperoxia in rats impairs lung development and causes permanent functional and structural changes of the lungs. Free oxygen radicals play a direct role in hyperoxia-induced neonatal lung injury, while oxygen metabolites activate phospholipase and cause AA release and metabolism in lung tissues. Levels of leukotriene (LT) B4 or cysteinyl LTs (cys-LT C4, D4, and E4) are increased in the Broncho alveolar lavage fluid from infants with bronchopulmonary dysplasia [10]. MK inhibits the effects of leukotrienes by acting as a specific antagonist of cysteinyl leukotriene receptors (CysLT-receptor) [5]. In children that are allergic to house dust mites, treatment with 5 mg MK provided significant improvement in allergen-induced cutaneous late phase-phase responses [11]. In cystic fibrosis (CF) patients, inflammatory processes contribute to progressive lung tissue damage while sputum from CF patients contains sufficient amounts of CysLTs to have biological effects. MK therapy provided clinical benefit to CF patients through decreases in coughing and wheezing [12]. MK also induced apoptosis of peripheral blood and lung T-lymphocytes in a rat model of asthma, suggesting that the ability of MK to induce lymphocyte apoptosis could contribute to its anti-inflammatory effects [12]. Moreover, MK treatment ameliorated lung symptoms subsequent to respiratory syncytial virus bronchiolitis [13]. To investigate the effect of CysLT-receptor inhibition by Montelukast acting as a receptor antagonist, [9] exposed rats to hyperoxic (50% O2) conditions for 14 days after birth. The animals were then treated with MK and somatic growth and morphometric parameters were assessed on day 15. The animals exposed to hyperoxia showed significant increases in CysLT levels in bronchoalveolar lavage fluid. Furthermore, the hyperoxic animals had decreases in the specific internal surface area of lung tissue and non-significant increases in mean chord length and mean alveolar diameter as well as a decrease in lung volume. Inhibition of arachidonic-acid (AA) production by MK did not provide substantial protection against these oxygen-induced changes [9]. In a study involving 46-year-old asthma patients, treatment with Montelukast was associated with elevated eosinophil count in peripheral blood and glomerulonephritis, suggesting that the treatment had promoted a severe inflammatory reaction [14]. The ability of Montelukast to inhibit the action of leukotriene in inflammatory cascades could also be related to observations that Montelukast treatment facilitated myofibroblast relaxation that in turn ameliorates capsular contracture [15]. The liver is often the first organ to be challenged by contaminants and is the main organ for xenobiotic metabolism, both in terms of detoxification and bioactivation. Generally, responses of detoxification enzymes in the liver such as glutathione S-transferase (GST), superoxide dismutase (SOD), and catalase (CAT) to toxicant exposure are sensitive indicators that precede cellular damage [16]. GST is a major family of phase II detoxification enzymes found in most organisms [17]. SOD metalloenzymes are essential for defending organisms against oxidation products such as superoxide anions ($O\cdot 2^{-}$) that are produced in cells by enzymatic and non-enzymatic metabolic processes [18]. The SOD enzymes contain transition metals such as Fe, Mn, or Cu/Zn at their active sites [19]. CAT can decompose hydrogen peroxide (H2O2) without producing free radicals and has long been recognized as a defense against oxidative stress [20]. Liver, erythrocytes and kidney have high levels of catalase, whereas other tissues such as the brain and pancreas as well has the serum have low catalase levels [21]. In cells, catalase mainly localizes to peroxisomes [22]. Montelukast is used as an anti-asthmatic agent [23] suggested that MK could reverse synthetic inflammatory reaction in several organ tissues- liver, lung, heart and kidney- of sepsis induced rats. Alterations assessed in the cytokines (IL-6 and TNF alpha) as well as the oxidative stress parameters SOD, total glutathione (GSH), myeloperoxidase (MPO), and lipid peroxidation (LPO) were reversed post MK administration in vivo. Besides, toxicity induced in 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (DXN) pretreated rats was attenuated after MK gavage administration. DXN treatment for 60 days caused significant reduction in the oxidative stress parameters; GSH, CAT, and SOD in rat`s livers and cytokines, whereas 10 mg/kg/day of MK reversed the toxic effects [23]. Moreover, [24] reported that MK administration post doxorubicin (DXO) was found to increase CAT, SOD, and GSH in liver homogenate compared to untreated control and to MK pretreated DOX groups. Suggesting that MK can treat the oxidative stress damage in kidney induced by DXO. Multiple studies have focused on its clinical effects and pharmacokinetics. In particular, the effect of Montelukast on liver function as measured by assays of cytochrome activity P450-2C8 was examined [25]. However, to our knowledge, no studies have assessed whether Montelukast affects free radical scavenging enzymes that could in turn alter DNA stability in the blood serum and liver tissue of healthy human or mice model. In this investigation, we examined the effects of Montelukast on DNA stability as well as on the activity of key enzymes, namely glutathione S-transferase, superoxide dismutase and catalase that are involved in free radical scavenging mechanisms in mammalian systems both in vivo and in vitro. Results from this study will be important for understanding

potential clinical side effects of Montelukast use.

Materials And Methods

Chemicals

The following chemicals were used: Montelukast Sodium (Singular) [26]. 1-chloro-2,4-dinitro-benzene (CDNB) and reduced glutathione GSH (Aldrich Chemical Co. Inc. ,USA), guanidine hydrochloride and agarose (Across Organic, New Jersey), sodium acetate and boric acid (BDH Chemicals Ltd., England), hydrogen peroxide H2O2 (C.B.H. Lab Chemicals Co., UK), potassium chloride KCl (Fluka-Garantie, Switzerland), chloroform (Gaindand chemical Co., UK), EDTA and isoamylalcohol (J.T. Baker Chemical Co., USA), isopropanol (Koch-light Laboratories Ltd, England), ammonium acetate (Merck Chemical Co., USA), potassium phosphate (Park Scientific Limited, Northampton, U.K), pyrogallol, succinic acid, Tris-base and sucrose (Sigma Chemical Co., USA), lambda Hind III DNA marker (λ Hind III) and S1 nuclease purchased from (Promega Chemical Co., USA).

Experimental Animals

All investigations were carried out on 7-8 week old male Balb/c mice weighing 20-30 grams. The mice were housed in cages (6 per cage) under regulated conditions of 20-22°C temperature, 60-80% relative humidity, and a 12-hour light-dark cycle. The animals were fed and given water as needed. The rats were obtained from the vivarium at Amman's Isra University's Faculty of Pharmacy. The study's protocol (SREC/22/09/054) was approved by the Ethical Committee of Isra University's Faculty of Allied Medical Sciences and Pharmacy. Rats of both sexes were housed in a vivarium on a regular diet with free access to food and water at a temperature of 22-23°C, following the "day-night" retention regimen. All animals were housed in plastic cages with bedding as needed [27], with animals of each sex maintained apart. Before each trial, the animals were transported to the laboratory and quarantined for 15 days under similar conditions [28].

Human samples

For in vitro studies, human blood was obtained from apparently healthy volunteer, 3 males and 4 females, 20-25 years old. The protocol (SREC/22/09/055) of the study was approved by the Ethical Committee of the Faculty of allied medical sciences and pharmacy, Isra University.

In vitro assay of Montelukast effect on single strand break in mice and human DNA from whole blood

The method of the purification of DNA from blood [29] was used to isolate DNA from human blood throughout this investigation. DNA samples were treated with Montelukast at different concentrations, namely, 2.5, 5, and 10µg /ml and 1.25, 2.5, and 5 mg/kg in mice and human blood samples respectively. The mixture was incubated overnight at 37°C[30] 0.62 and 1.24 U of S1 nuclease was added to 0.15µg of DNA in a standard buffer solution (30 mM sodium acetate, 15 mM NaCl, 1mM ZnSO4, pH 4.6.). The reaction mixture was incubated at 37°C for 30 min [31]. Finally, ethanol was added to precipitate DNA, dried and dissolved in TE buffer for agarose gel electrophoresis. Electrophoresis was used for the detection of DNA single strand breaks (ss-breaks). The DNA samples from mice pretreated with Montelukast and from human were electrophoresed after S1-nuclease action. If the drug does not induce single strand breaks, the S1-nuclease does not act and the DNA remains intact. Otherwise, the DNA after agarose gel electrophoresis will show several too many bands due to its fragmentation by the enzyme [32].

In vivo assay of Montelukast effect on single strand break in mice DNA from whole blood

The animals were divided into 4 groups, 12 mice each. (Total 48 mice/experiment). One group served as a control, injected with 0.4ml D.W, the other three groups were treated with Montelukast at the following doses: 5mg, 2.5mg and 1.25mg/kg. The drug was dissolved in distilled water and the solution administered intraperitoneally at a volume of 0.4 ml/mouse, daily for 15days [33]. At the end of the injection period, blood was collected by cervical dislocation and the DNA was isolated. DNA was prepared from whole blood, treated with S1-nuclease and examined for ss-breaks, using gel electrophoresis as previously described for human DNA.

Free radical scavenging enzymes study

The animals were divided into (9 mice/experiment), blood and the liver tissue were collected, serum were isolated, and liver was extracted, then were treated with Montelukast at the following doses: 10µg/ml, 5µg/ml and 2.5µg/ml. The drug desired concentration was prepared in distilled water, then mixed with sera or liver extracts samples and incubated for 10 minutes before enzymatic assay. Control groups were treated with D.W. [34].

Preparation of the liver tissue extract

The liver was collected from the dissected mice and perfused with 1.15% KCl. 20% (w/v) homogenate was prepared after the addition of ice-cold 1.15% KCl. The homogenate was centrifuged at 15000g for 30 min. The separated supernatant was used directly for enzymatic assay or stored at _70 oC until required [35].

Preparation of the sera

Blood was collected from the animal in clean plastic or glass tubes. Clotted blood was removed from walls of the clotting tube by means of a stick. This step is so important to free the clot from the walls so maximum shrinkage of clot occurs. Ringed blood was stored at 4°C for 18-24 hr to get maximum volume of serum. The blood was centrifuged at x1000 g for

20-30 min at 4°C [36].

Enzyme assays

Glutathione-S-Transferase (GST), Catalase (CAT) and Superoxide dismutase (SOD) were estimated by known standard methods as mentioned below:

Glutathione-S-Transferase

The enzyme activity was measured by increasing in absorbance at 340 nm of CDNB-GSH conjugate generated as a result of GST catalysis between reduced glutathione (GSH) and 1-chloro-2, 4-dinitro-benzene (CDNB) [34]. The enzyme GST catalyzes the following reaction

$$\text{CDNB + GSH} \xrightarrow{\text{GST}} \text{CDNB-GSH (conjugate)}$$

The assay mixture contains 2.9 ml of 0.1M potassium phosphate buffer pH 6.5, 50 µl of 0.1M GSH and 30 µl of 0.1 M CDNB in a total volume of 3 ml mixed thoroughly, and 50 µl of serum liver tissues homogenate sample. The absorbance was recorded for 3 minutes using UNICAM-Heylos spectrophotometer. The activity of the enzyme was calculated on the basis of molar extinction coefficient of (9.6 mM-1 cm-1). One unit of activity of the enzyme was defined as 1 µmol of CDNB conjugated to GSH per minute.

Catalase

The enzyme activity was measured by the decrease in absorbance at 240 nm. The decomposition of hydrogen peroxide (H2O2) was followed by the loss of its absorbance at 240 nm [37; 38] The enzyme catalase catalyzes the following reaction

$$2\ H2O2 \xrightarrow{Catalase} 2H2O + O2$$

The assay mixture containing 0.03 M of hydrogen peroxide was prepared in 0.05 M potassium phosphate buffer pH 7.0 in a total volume of 3 ml and 10 µl of serum or liver tissues homogenate sample. The absorbance was recorded for 3 minutes by UNICAM-Heylos spectrophotometer. The activity of the enzyme was calculated on the basis of molar extinction coefficient of (43.6 M-1 cm-1). One unit of activity of the enzyme was defined as decomposition of 1.0 µmol H2O2 per minute.

Superoxide dismutase

The enzyme activity was measured by the increase in absorbance at 420 [39]. The procedure depends upon autoxidation of pyrogallol:

$$\text{Pyrogallol} + O2 \xrightarrow{autoxidation} \text{oxidation products} + O2$$

$$2O.2 + 2H+ \xrightarrow{SOD} O2 + H2O2$$

The assay mixture contained 2.85 ml 0.05 M tris-succinate buffer pH8.2, 50 µl of serum or liver tissues homogenate. After mixing, the reaction was started by adding 100 µl of 8 mM pyrogallol solution. The absorbance was recorded for 3 minutes by UNICAM-Heylos spectrophotometer.

A reference set consisting of 50 µl of distilled water instead of the sample solution.

$$\text{Unit of SOD activity} = \frac{(\Delta A/\min ref - \Delta A/\min sample) \times 30}{\Delta A/\min ref / 2 \times 0.05}$$

Where:

ΔA/min ref = Change of absorbance per min. in reference set.

ΔA/min sample = Change of absorbance per min. in sample.

One unit of activity of the enzyme was defined as the amount of enzyme which caused 50% inhibition of pyrogallol autoxidation under assay conditions.

Statistical Analysis

The results were the mean SEM; *=p<0.05 indicates a significant difference of all parallel measurements. All statistical comparisons and reliability were determined using the Student's criterion at a level of significance of 95%. Statistical examination of the chemical experiment outcomes" (2015). GraphPad Prism software, version 8, was used to assess significant differences between experimental groups using one-way ANOVA, followed by Tukey's post hoc test for multiple comparisons. A p0.05 difference was judged statistically significant.

Results And Discussion

Montelukast effect on single strand break formation

In vivo assessment of Montelukast role in single strand break formation showed that daily injection for 15 days at 5mg, 2.5mg, and 1.25mg /kg, followed by 0.62 and 1.24U of S1-nuclease, and electrophoresis, the DNA was found to exhibit one genomic DNA band, indicating that the DNA treated with different concentrations of the drug did not exhibit ss-breaks. Similar results were obtained in the in vitro studies. So, electrophoresis of isolated mice and human DNA pre-treated with Montelukast at: 10µg/ml, 5µg/ml, 2.5µg/ml, and 1.25, 2.5, and 5 mg/kg respectively followed with S1-nuclease digestion showed one genomic DNA band, hence did not induce ss-breaks.

Montelukast effect on the Free radical scavenging enzymes activity in vivo

Various biochemical analysis of the antioxidant enzymes was

investigated in liver tissues collected from mice pretreated in vivo with1.25, 2.5, and 5 mg/kg Montelukast solution for 15

days Table (1). The results indicated that the activity of GST significantly decreased ($p < 0.05$) with increased the concentration of Montelukast in all groups. A similar trend was observed when the activities of SOD and CAT were followed. Besides, enzymatic analysis of serum presented in Table (2) revealed that the activity of GST significantly decreased ($p < 0.05$) with increased the concentrations of Montelukast in all groups. Likewise, the activity of SOD and CAT progressively decreased as Montelukast doses increased.

Montelukast effect on the Free radical scavenging enzymes activity in vitro

Different doses of Montelukast ranging from 2.5 to 10 µg/ml were used to investigate its effect on liver enzymes in vitro, as presented in Table (3). The activity of GST, SOD, and CAT significantly decreased ($p < 0.05$) with the increased concentration of Montelukast in all groups. Here again, the enzyme activity exhibited an inverse relation with Montelukast concentration as reported in vivo. A significant decrease in GST, SOD and CAT activities ($p < 0.05$) at 2.5, 5 mg/kg BW was observed. However, the decrease in GAT, SOD and CAT activity at the dose of 1.25mg/kg was not significant. In vitro studies showed a significantly decreased ($p < 0.05$) in the activities in GST, SOD and CAT in all the groups. A significant decrease in GST, SOD and CAT at the doses of 5 and 10µg/ml ($p < 0.05$). However, no significant decrease in the activities of GAT, SOD and CAT was observed at the dose of 2.5µg/ml. Montelukast effect on the serum enzymes; GST, SOD and CAT are presented in table (4). In all groups, results indicate that the activities of the circulating enzymes: GST, SOD and CAT are significantly decreased ($p < 0.05$) with increased Montelukast concentration.

Table 1: The in vivo effect of various doses of Montelukast on the hepatic enzymes: GST, SOD and CAT.

Groups	*GST(U) ± S.D.*	*SOD(U) ± S.D.*	*CAT(U) ± S.D.*
Control	1168.3 ± 280.6	924.5 ± 57.0	793041.3 ± 158157
1.25mg/kg	1047.14 ± 245.7	853.9 ± 28.27	571077.2 ± 164836
2.5mg/kg	887.85 ± 186.1	753.6 ± 28.87	524085 ± 165822
5mg/kg	605.61 ± 120.8*	457.5 ± 58.1*	287333.8 ± 139440*

Data are presented as mean SEM; *=p<0.05 indicates a significant difference between several dosages. Each value represents a mean of 24 measurements.

Table 2: The in vivo effect of various doses of Montelukast on the serum enzymes: GST, SOD and CAT.

Groups	*GST(U) ± S.D.*	*SOD(U) ± S.D.*	*CAT(U) ± S.D.*
Control	250.7 ± 41.3	857.4 ± 6.4	359264.7 ± 302564
1.25mg/kg	147.2 ± 18.4*	664.1 ± 9.5	346018.3 ± 207858
2.5 mg/kg	69.79 ± 2.08*	432.3 ± 5.2*	275970.0 ± 74310
5 mg/kg	47.9 ± 6.34 *	379.5 ± 7.1*	253657 ± 26060

Data are presented as mean SEM; *=p<0.05 indicates a significant difference between several dosages. Each value represents a mean of 12 measurements.

Table 3: The in vitro effect of various doses of Montelukast on the hepatic enzymes: GST, SOD and CAT

Groups	*GST(U) ± S.D.*	*SOD(U) ± S.D.*	*CAT(U) ± S.D.*
Control	680.9 ± 113.4	896.67 ± 44.5	937963.9 ± 239318
2.5µg/ml	550 ± 98.8	868.6 ± 37.8	648336.1 ± 168767
5µg/ml	273 ± 36.5*	752.6 ± 28.65	507334.4 ± 193471*
10µg/ml	201.97 ± 50.1*	415.1 ± 47.3*	418077.2 ± 178941*

Data are presented as mean SEM; *=p<0.05 indicates a significant difference between several dosages. Each value represents a mean of 18 measurements.

Table (4): The in vitro effect of various doses of Montelukast on the serum enzymes: GST, SOD and CAT.

Groups	*GST(U)*	*SOD(U)*	*CAT(U)*
Control	173.0 ± 9.26	806.1 ± 4.8	342151.5 ±19105.5
2.5µg/ml	117.7 ± 3.8	671.0 ± 3.6	313005.6 ± 13109
5µg/ml	94.4 ± 3.58	420.2 ± 2.4*	274432.0 ± 15198
10µg/ml	43.75 ± 5.51*	352.0 ± 8.7*	187563.4 ± 53445*

Data are presented as mean SEM; *=p<0.05 indicates a significant difference between several dosages. Each value represents a mean of 9 measurements.

The interaction of DNA with drugs used to treat various diseases is an important aspect of studies on the biological effects of these agents. Drugs and other compounds can interact with DNA in three principal ways: (i) Interaction between the drug and proteins that bind DNA; (ii) Promotion of RNA binding to DNA to form triple helical structures; and (iii) Binding of aromatic ligands to the DNA double helix. Such interactions between drugs and DNA are mediated by electrostatic interactions, intercalation between base pairs as can occur with organic molecules containing aromatic rings, and interactions with the major and/or minor grooves of the DNA double helix. Binding of agents with the minor groove of DNA involves numerous hydrogen bonding and electrostatic interaction with the bases and phosphate back bone. Binding to the major groove of DNA also involves hydrogen bonding and subsequent formation of DNA triple helices [40]. Several reports studied DNA stability by investigating mechanism of Drug-DNA interaction [41]. Corticosteroids can affect chromatin structure and repress gene expression through deacetylation of histones that increases their affinity for DNA [42]. Mitomycin-C causes DNA breakage, particularly in telomere repeat sequences [43]. Cisplatin has been used to treat ovarian, bladder and lung cancer and other types of cancer. Cisplatin inhibits replication, transcription, translation and DNA repair via binding to purine bases of DNA, although at high doses, cisplatin exhibits hepatotoxicity. Antioxidative defense mechanisms against free-radical mediated organ damage and genotoxicity are also affected by cisplatin and free radicals generated in response to cisplatin treatment can promote DNA fragmentation [44]. Interestingly, a study by [45] reported that immunoprecipitation of a DNA binding histone acetyltransferase (HAT) after MK treatment showed that MK changed HAT associated factor KappaB p65 activity in phorbol myristate acetate pre-induced U937 cell, hence TNF-alpha-IL8 mRNA expression was blocked. A study of the oxidative stress parameters –total oxidant status (TOS), total antioxidant status (TAS)- and DNA damage based alkaline comet assay in the plasma of pediatric asthma patients pretreated with MK for 6 months showed that MK cause minor non-significant TOS, TAS and DNA improvement compared to control group [46]. To the best of our knowledge, our study is the first to examine whether Montelukast affects DNA stability through ss-breaks formation in vitro and in vivo. We observed no single strand breaks in DNA in our assays, indicating that Montelukast treatment does not affect DNA stability. Accordingly, S1-nuclease could not cleave DNA, which remained intact (genomic DNA). If a drug promotes ss-DNA breaks, S1-nuclease can hydrolyze phosphodiester bonds in the complementary strand leading to DNA fragmentation. We also investigated the effect of Montelukast on the activity of free radical scavenging enzymes. The activity of the free radical scavenging enzymes GST, SOD and CAT significantly decreased ($p < 0.05$) in the liver of mice treated with 2.5 and 5 mg/kg Montelukast compared to untreated mice. Serum exposed to 5 and 10 µg/ml Montelukast also showed decreased activity of GST, SOD and CAT, although these decreases were not significant. These decreased enzymatic activities following Montelukast treatment could occur via several mechanisms: (i) non-competitive inhibition; (ii) changes in gene expression of these enzymes; and/or (iii) a direct reaction between Montelukast and free radicals that decreases the incidence of free radicals that in turn down regulates expression of these enzymes. GST, SOD and CAT activity decreased gradually from low doses to high doses of Montelukast relative to the untreated control. Significant changes were observed with higher doses in the liver tissue, which is consistent with the high level of these scavenging enzymes in the liver relative to that in the serum [47].

Data Availability

All the data used to support the findings of the study can be obtained from the corresponding author upon request.

Conflicts Of Interest

The authors declare that they have no conflicts of interest.

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