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ORIGINAL ARTICLE

Zinc and iron complexes of oleanolic acid, (OA) attenuate allergic airway inflammation in rats

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Abstract

Oleanolic acid (OA) is a hydroxyl pentacyclic triterpene acid (HTAs) used in various ailments. Inflammatory diseases may be profoundly influenced by iron (Fe) and zinc (Zn) status. We studied the anti-asthmatic effects of two metal complexes (Fe and Zn) of OA in the ovalbumin (OVA)-induced rat model. Delayed type hypersensitivity (DTH) was measured. Total and differential leucocyte count was done in blood as well as bronchoalveolar lavage fluid (BALF). The mRNA expression levels of pro-inflammatory cytokines were measured in lung tissue by reverse transcription polymerase chain reaction. The levels of cyclooxygenase-2 (COX-2), immunoglobulin E (IgE) and 5-lipoxygenase (5-LOX) were estimated by enzyme linked immunosorbent assay. Splenocyte proliferation was performed through BrdU uptake method and nitric oxide levels were measured by colorimetric assay kit. The acute toxicity study was also done for the complexes. The asthmatic group developed allergic airway inflammation shown by increased DTH and inflammatory markers in blood and BALF. OA + Fe and OA + Zn displayed significant decrease in DTH, NO, expression of IL-4, 5, 13, 17, toll-like receptor-2, nuclear factor-kappa B and tumor necrosis factor-α; serum IgE, COX-2, and 5-LOX. The metal complexes also attenuated OVA-stimulated splenocyte proliferation. While no hepatotoxic or nephrotoxic potential was shown by OA + Fe and OA + Zn. Our findings indicate that both OA + Fe and OA + Zn possess significant anti-asthmatic effect which may be ascribed to its immunomodulatory and anti-inflammatory features.

Keywords Allergic asthma (AA) · Oleanolic acid (OA) · Zinc (Zn) · Iron (Fe) · Bronchoalveolar lavage fluid (BALF) · Immunoglobulin E (IgE) · Cyclooxygenase-2 (COX-2) · 5-Lipoxygenase (5-LOX)

Introduction

World Health Organization (WHO) statistical report shared that till 2020, asthma, along with other chronic obstructive pulmonary diseases will be considered among top three foremost reasons of mortality. A projected figure of 0.3 billion individuals worldwide at present suffer from asthma and there may be an additional 0.1 billion asthmatics by 2025 (Bijanzadeh et al. 2011).

Asthma is a long-lasting inflammation of the airways involving, mastocytes, eosinophils, neutrocytes and T-cells. A network of communication amongst inflammatory cells and substances is seen (Mali and Dhake 2011). This process is linked to raised inflammatory cells and Th2 derived airway eosinophilia and BALF populated cytokines, along with lung contains increased level of COX-2 and raised mucus secretion (El Mezayen et al. 2006). LOX acts on arachidonic acid (AA) to generate lipoxins which are linked to inflammatory presentation of asthma (Kazani et al. 2014).

A considerable number of asthmatics experience refractoriness to the effect of high-dose inhalational as well as to systemic steroid therapy. Additionally, several of unwanted effects are linked to corticosteroids, including dysphonia, oropharyngeal candidiasis, adrenal suppression, glucose intolerance, cataract, glaucoma, susceptibility to infection, osteoporosis. Consequently, various attempts have been made to explore alternative, innovative, precise and with least toxic remedies in asthma (Shahzad et al. 2009).

Oleanolic acid (OA, 3β-hydroxyolean-12-en-28-oic acid) is a bioactive pentacyclic triterpenoid. OA is ubiquitous in *P. ginseng Olea europaea*, *Arctostaphyllos uva-ursi*, *Solanum*

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incanum (Shanmugam et al. 2014). It has a variety of pharmacological actions underlying its effects as a cytotoxic, anti-inflammatory, anti-asthmatic, antioxidant and hepatoprotective agent (Kim et al. 2014a, b). OA and its derivatives a range of biological agent, supporting their pharmaceutical uses for multiple diseases (Lin et al. 2016).

The scientific accomplishment of inorganic medicines, for instance platinum (II) chemotherapy agents and gold comprising anti-arthritic therapy, has led significant advancement in the practice of transition metals in medical field. Various transition metals can accept an extensive collection of matching numbers, geometrical, oxidation positions and ligand binding attractions which can be engaged in the development of newly developed beneficial agents (Heffern et al. 2013). Iron is critically important in normal immune function, while iron deficiency can be associated with an increased risk of infection (Baker and Ghio 2009). Zinc has been used both for the treatment and prevention of diarrhea in infants and children. It is also effective in treatment of Wilson's disease, cardiac conditions, neurodegeneration, cancer, immunologic conditions and itself the aging process (Prasad 2014). Keeping in view the effective pharmacological features of OA, it was taken into consideration that addition of OA moiety with zinc and iron metals might result in the improved intrinsic activity.

Materials and methods

Study settings

The study was conducted at University of Health Sciences, Lahore and. The metal complexes were synthesized at Riphah Institute of Pharmaceutical Sciences (RIPS) Islamabad.

Preparation of experimental animals

Female Sprague–Dawley rats, 6–8 weeks old, weighing 220–240 g were procured from University of Health sciences (UHS), Lahore and National Institute of Health (NIH), Islamabad. The animals were kept in standard environmental conditions of animal house i.e. dark/light cycles 12 h, humidity (60–70%) and temperature (25 °C). The animals were acclimatized for a period of 7 days before the commencement of experiments and they had free access to water and food ad libitum. The study was approved by ethical review committee of University of Health Sciences, Lahore. The ethical review committee approval number is UHS/1120.

Animal groups

Animals were randomly divided into seven groups, each group included 6 rats. All the groups underwent intraperitoneal (i.p.) sensitization and intranasal (INS) challenge with ovalbumin (OVA) except the negative control group which was sham-sensitized and challenged with PBS only.

Group-I (Negative Control): Sham-sensitization and challenge with PBS with no treatment.

Group-II (Asthmatic): Induction of airway inflammation with no treatment given.

Group-III: Induction of airway inflammation along with 3 mg/kg b.w of Fe treatment given orally.

Group-IV: Induction of airway inflammation along with OA-Fe complex treatment @ 2 mg/kg b.w. given orally.

Group-V: Induction of airway inflammation along with 1 mg/kg b.w. Zn treatment given orally.

Group-VI: Induction of airway inflammation along with OA-Zn complex treatment @ 2 mg/kg b.w given orally.

Group-VII: Induction of airway inflammation along with 2 mg/kg b.w. dexamethasone (Dex) given orally.

Metal complexes preparation

Melting points of Fe and Zn complexes are 218 °C and 275 °C respectively. Preparation of Zn and Fe complexes of OA was carried out by using standard methods at RIPS, Islamabad (Batool et al. 2015).

Drugs and dosage

- Dex 2 mg/kg b.w (Hirshfeld et al. 2000), Fe 3 mg/kg b.w (Hirshfeld et al. 2000), Zn 1 mg/kg b.w (Hirshfeld et al. 2000), Fe-OA complex 2 mg/kg b.w (Kim et al. 2013), Zn-OA complex 2 mg/kg b.w (Kim et al. 2013).

Induction of asthma

Allergic asthma was induced by i.p. sensitization and intranasal challenge with ovalbumin (OVA). All experimental rats except group I were exposed to OVA sensitization on day zero by injecting (i.p.) 1 mg OVA emulsified in 50 mg aluminum hydroxide (alum) (AppliChem, Chemica Synthesis Services, Germany) in 1 ml (PBS) volume. Two-weeks after first sensitization, the rats were subjected to second sensitization in the same manner. After 21-days rats were exposed to intranasal challenge with OVA (1 mg/ml PBS) once a day for 1 week till day 28. The animals in the group I (control group) were sham-sensitized and challenged with PBS (Shahzad et al. 2009).

Table 1 Primers sequences

Marker	Fwd/Rev	Sequence	Amplified band base pair
TLR2 (Shabbir et al. 2014)	Forward	5′-CCA GAT GGC CAG AGG ACT CA-3′	212
	Reverse	5′-TGT GAG TCC CGA GGG AAT AGA-3′	
NF-kB (Shabbir et al. 2014)	Forward	5′-CAA GGA AGA GGA TGT GGG GTT-3′	207
	Reverse	5′-AGC TGA GCA TGA AGG TGG ATG-3′	
TNF-α (Shabbir et al. 2014)	Forward	5′-CCT CTT CTC ATT CCT GCT CGT-3′	266
	Reverse	5′-TGA GAT CCA TGC CAT TGG CC-3′	
IL-13 (Fang et al. 2015)	Forward	5′-TCTCGCTTGCCTTGGTGG-3′	232
	Reverse	5′-CATTCAATATCCTCTGGGTCCTGT-3′	
IL-4 (Shahzad et al. 2009)	Forward	5′-TGC TGT CAC CCT GTT CTG C-3′	395
	Reverse	5′-ATT TCC CTC GTA GGA TGC TTT-3′	
IL-5 (Shahzad et al. 2009)	Forward	5′-TGC TTC TGT GCT TGA ACG TTC TAA C-3′	298
	Reverse	5′-TTC TCT TTT TGT CCG TCA ATG TAT TTC-3′	
IL-17 (Shabbir et al. 2014)	Forward	5′-AGG CCC TCA GAC TAC CTC AA-3′	200
	Reverse	5′-CTC TCA GGC TCC CTC TTC AG-3′	

Blood sample collection

On day 29 after the last challenge all rats were anesthetized using light ether vapors and were immediately sacrificed after aspirating blood through intra-cardiac puncture. Partial blood samples were collected in EDTA vacutainers for haematological assays. Rest of the blood samples were collected in gel containing vacutainers for serum separation. (Okoko and Yama 2011; Kusemiju et al. 2012; Khan et al. 2015).

Delayed type hypersensitivity (DTH)

Delayed type hypersensitivity (DTH) was performed to confirm the in vivo inflammatory response. Ovalbumin was injected 24 h before sacrificing the rats into pinna of right ear (to observe inflammatory response) and phosphate buffer solution in the pinna of left ear (as control) of all rats. Immediately after sacrificing the rats, the left and right ears were dissected apart from body and weighed. The difference in the weight of both ears then represented the value of DTH (Ashraf et al. 2015).

Inflammatory cells in blood and BALF

Lavage of lungs was performed by instilling and withdrawing 2 ml ice cold PBS through trachea and collection of BALF was performed. For total leucocyte count in blood and BALF, automated hemocytometer (Sysmex XT-1800i) was used while for differential leucocyte count, including eosinophils, lymphocytes and neutrophils, after cytocentrifugation preparations were fixed and stained with Giemsa Wright to count cells under light microscope (Ashraf et al. 2015).

Determination of mRNA expression of IL-4, IL-5, IL-13, IL-17, TNF-α, NF-κB, TLR2

Total RNA from lung tissue was extracted by total RNA isolation kit (Favorgen, Ping-Tung 908, Taiwan). The purity and yield of the RNA was determined by Nanodrop 2000C (Thermo Scientific, USA). RNA was converted to cDNA using RevertAid First Strand cDNA Synthesis kit (Thermo Scientific, USA). Absolute quantification of the proinflammatory cytokines (Table 1) was performed by real time-PCR. The PCR reaction was subjected to ABI Prism 7000 sequence detection system (Applied Biosystems USA) according to the recommended thermal cycling conditions of SYBR green manufacturer.

Enzyme-linked immunosorbent assay for IgE, COX-2 and LOX

Serum IgE, COX-2 and 5-LOX was measured by ELISA according to kit manufacturer's protocol (Elabscience Biotechnology Co., Ltd, USA). Briefly, samples were added in pre-coated wells of a 96 well microtiter plate. IgE, COX-2 and 5-LOX antibody was added, followed by streptavidin–horseradish peroxidase. Then chromogen solution was added subsequently. An incubation period of 10 min was allowed at 37 °C away from light. After this, stop solution was added and optical density was measured at 450 nm.

Ovalbumin-specific splenocyte proliferation

Spleens were thoroughly washed with Hank's balanced salt (HBS) solution, augmented with antibiotics. The tissue was crushed fine in 5 ml HBS solution and filtered through 45 µm nylon membrane syringe filter. Lysis of the RBCs was done using equal volume of NH_4Cl solution. The final cell mix was centrifuged at 1000 rpm for 10 min. The cells were layered in 96 well flat bottom culture plate (Corning, USA) along with culture media; 100 uL of RPMI-1640 supplemented with 10% heat inactivated FBS and 2% antibiotics (Invitrogen, USA). Cell proliferation was triggered by adding different concentrations of OVA to the wells containing the cells and culture media. The plate was incubated in water jacketed incubator (SHELAB, USA) at 37 °C with 5% CO_2 for 72 h. The splenocyte proliferation was measured by BrdU cell proliferation assay kit (Exalpha Biologicals Inc., USA) and the absorbance was measured at 450 nm by ELISA plate reader.

Estimation of nitric oxide

Detection of NO in serum and BALF was performed using NO Colorimetric Assay Kit (BioVision Incorporated USA) and the absorbance was measured at 540 nm.

Biochemical and hematological investigations

To determine the toxic effects of the metal complexes with OA, serum levels of liver enzymes (ALT, ALP), serum creatinine, serum urea, Hb level using commercially available kits (Analyticon Biotechnologies AG, Germany) (Humalyzer 3500). RBC and platelet counts were determined (Sysmex XT-1800i).

Statistical analysis

Data was analyzed by using GraphPad Prism (v. 6). Quantitative variables were compared and differences among groups were analyzed by using one-way ANOVA followed by Post hoc Tukey's test or student's *t* test where applicable. Data was presented as mean ± SEM and the *P* value ≤ 0.05 was considered as statistically significant.

Results

Effect of zinc and iron complexes of OA on blood TLC, eosinophils, neutrophils and lymphocytes

Our findings indicate a significant rise in blood TLC of asthmatic group as compared with negative control group. Significant reduction was seen in treatment with OA + Fe, Zn, OA + Zn and Dex as compared to asthmatic group while non-significant reduction was observed when Fe group compared with asthmatic group (Fig. 1a). Similarly, there was a significant rise in blood eosinophils, neutrophils and lymphocytes of asthmatic group as compared with negative control group respectively. Significant reduction was seen in treatment with OA + Fe, Zn, OA + Zn and Dex respectively as compared to asthmatic group, while non-significant reduction was observed in Fe group compared to asthmatic group (Fig. 1b–d).

Effect of zinc and iron complexes of OA on BALF TLC, eosinophils, neutrophils and lymphocytes

Our findings indicate significant increase in BALF TLC of asthmatic group as compared to negative control group. Significant reduction of BALF TLC was seen in treatment with Fe, OA + Fe, Zn, OA + Zn and Dex respectively as compared to asthmatic group (Fig. 2a). Our findings indicate a significant rise in BALF eosinophils, neutrophils and lymphocytes of asthmatic group as compared to negative control group respectively. Significant reduction was seen in treatment with Fe, OA + Fe Zn, OA + Zn and Dex respectively as compared to asthmatic group (Fig. 2b–d).

Effect of zinc and iron complexes of OA on serum and BALF Nitric oxide (NO)

There was significant increase in both serum and BALF NO of asthmatic group as compared with negative control respectively. Significant reduction was seen in treatment with OA + Fe, Zn, OA + Zn and Dex respectively as compared to asthmatic group, while non-significant reduction was observed when Fe group was compared to asthmatic group (Fig. 3a, b).

Effect of zinc and iron complexes of OA on serum IgE, COX-2 and LOX

There was significant increase in IgE, COX-2 and LOX in asthmatic group as compared to negative control. Significant reduction was seen in treatment with OA + Fe, Zn, OA + Zn and Dex respectively as compared to asthmatic group. Fe group showed non-significant reduction in IgE and COX-2, whereas significant reduction in LOX was seen when compared to asthmatic group (Fig. 4a–c).

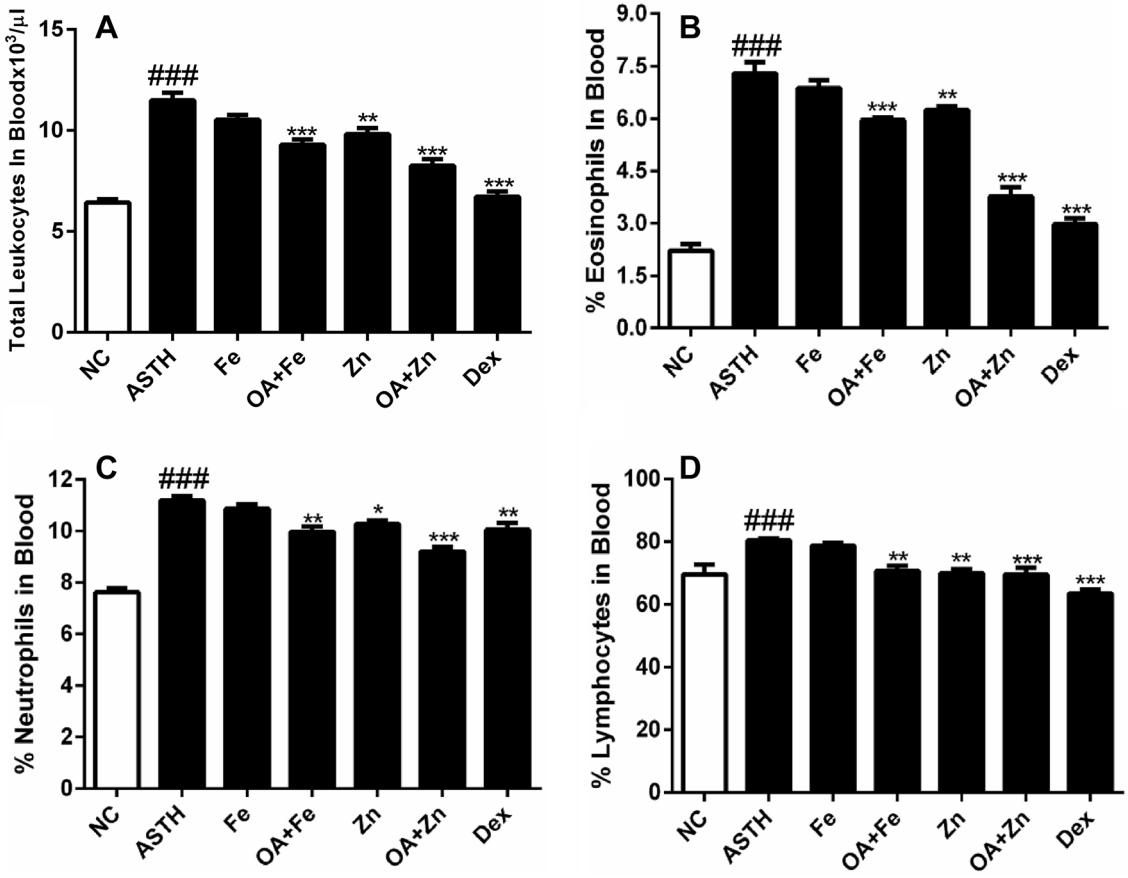


Fig. 1 Zn, OA + Fe, OA + Zn, Dex significantly decreased blood **a** TLC, **b** eosinophils, **c** neutrophils and **d** lymphocytes respectively. Findings are represented as Mean ± SEM for 6 rats. *, **, ***$P < 0.05$, $P < 0.01$, $P < 0.001$ respectively when drug-treated groups compared to asthmatic group while $^{\#\#, \#\#\#}P < 0.01$, $P < 0.001$ in a respective manner when asthmatic group compared to negative control group

Effect of zinc and iron complexes of OA on IL-4, IL-5, IL-13, IL-17, TNF-α, NFκB and TLR2

Results indicate significant increase in expression of IL-4, IL-5, IL-13, IL-17, TNF-α, NFκB and TLR2 of asthmatic group as compared to negative control group respectively. Significant reduction was seen in treatment with Fe, OA + Fe, Zn, OA + Zn and Dex respectively as compared to asthmatic group, with exception to IL-13 which in case Fe group showed non-significant result Fig. 5(i) a–d, Fig. 5(ii) e–g.

Effect of zinc and iron complexes of OA on BrdU incorporation

Our findings indicate a significant increase in BrdU uptake of asthmatic group as compared to negative control group. Significant reduction was seen in treatment with Fe, OA + Fe, Zn, OA + Zn and Dex as compared to asthmatic group (Fig. 6a).

Effect of zinc and iron complexes of OA on delayed type hypersensitivity

Our findings indicate a significant increase in DTH of asthmatic group as compared to control. Significant reduction was seen in treatment with OA + Fe, Zn, OA + Zn and Dex as compared to asthmatic group while non-significant reduction was observed when Fe group was compared to asthmatic group (Fig. 6b).

Effect of zinc and iron complexes of OA on hematological parameters

OA + Fe and OA + Zn did not exert any significant effect on the RBC count, Hb and platelet count (Fig. 7a–c).

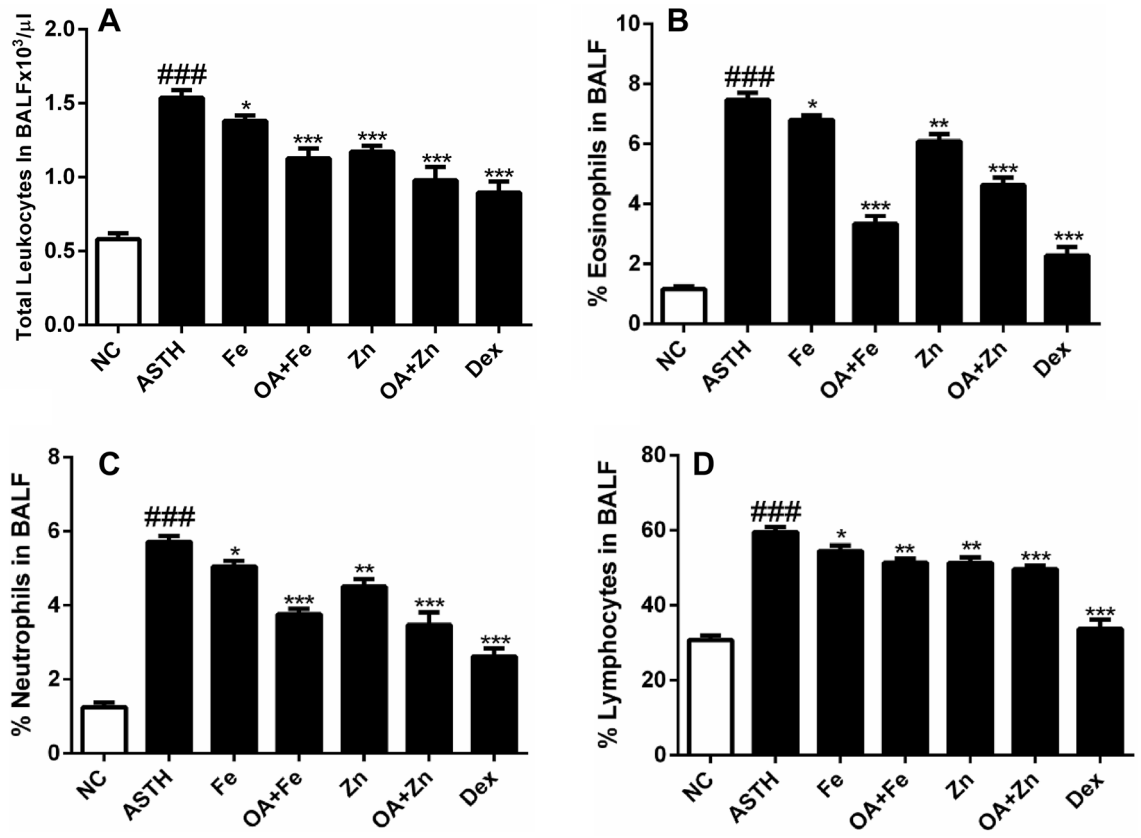


Fig. 2 Fe, Zn, OA + Fe, OA + Zn, Dex significantly decreased BALF **a** TLC, **b** eosinophils, **c** neutrophils and **d** lymphocytes respectively. Findings are represented as Mean ± SEM for 6 rats. *, **, ***$P < 0.05$, $P < 0.01$, $P < 0.001$ respectively when drug-treated groups compared to asthmatic group, while $^{###}P < 0.001$ when asthmatic group compared to negative control group

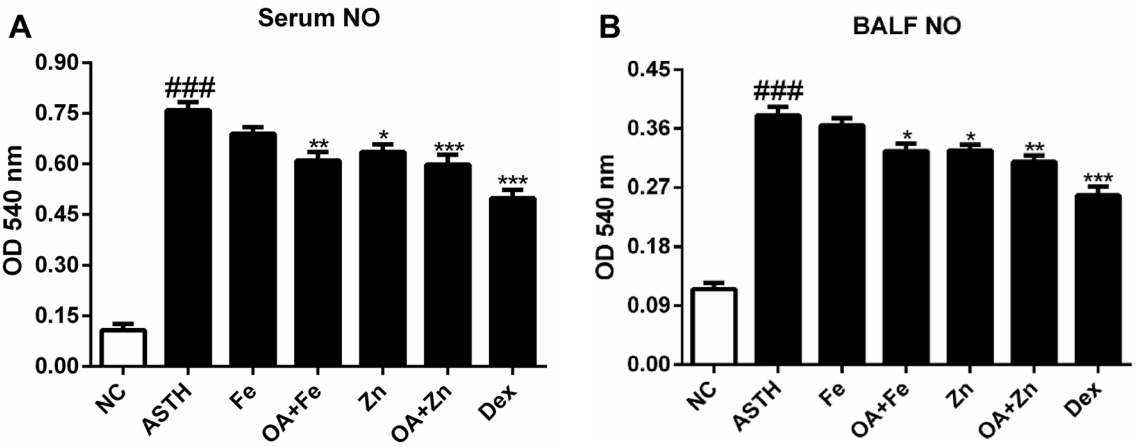


Fig. 3 Zn, OA + Fe, OA + Zn, Dex significantly decreased **a** serum NO, **b** BALF NO respectively. Findings represented as Mean ± SEM for 6 rats. *, **, ***$P < 0.05$, $P < 0.01$, $P < 0.001$ respectively when drug-treated groups compared to asthmatic group while $^{###}P$ value < 0.001 when asthmatic group compared to negative control group

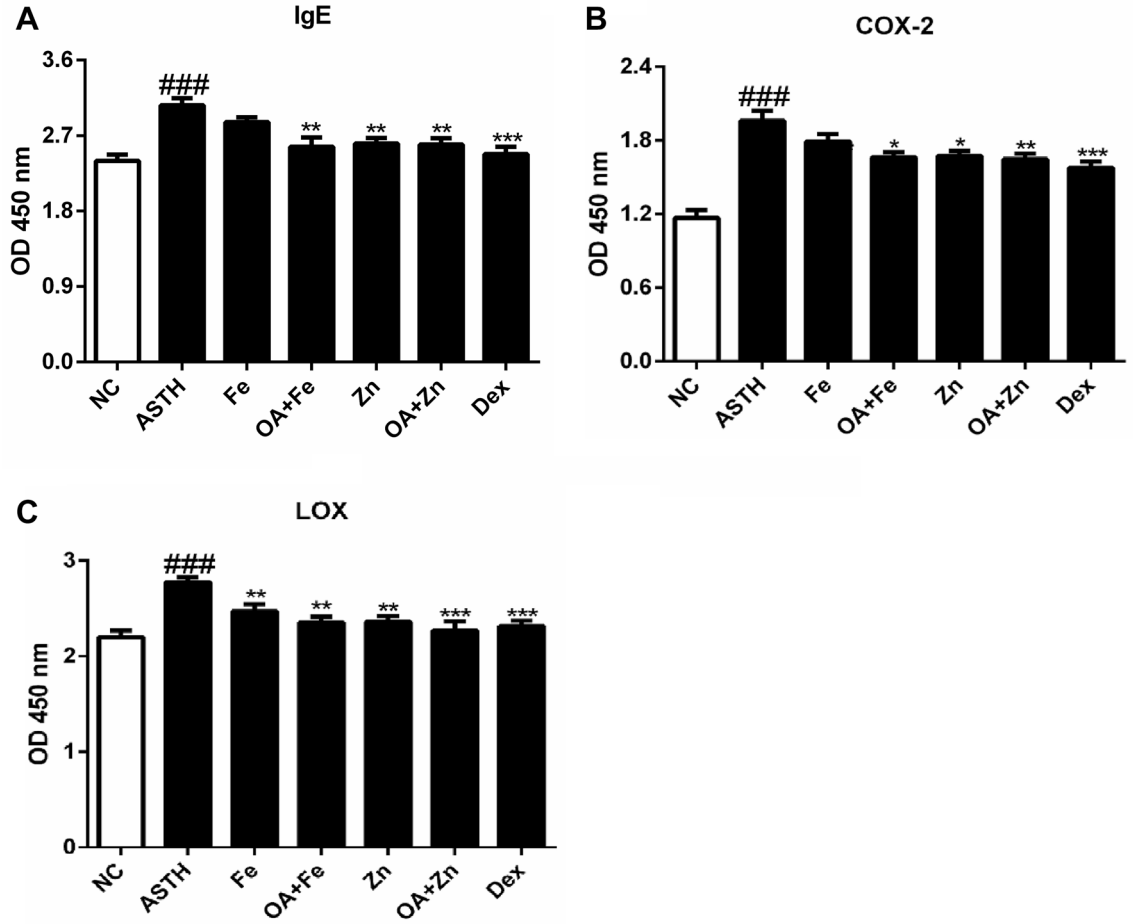


Fig. 4 Zn, OA + Fe, OA + Zn, Dex significantly reduced serum **a** IgE, **b** COX-2 and Fe exceptionally reduced significantly **c** LOX along with other drug-treated groups. Results are indicated as Mean ± SEM for 6 rats. *, **, ***$P < 0.05$, $P < 0.01$, $P < 0.001$ respectively when drug-treated groups compared to asthmatic group, while $^{###}P < 0.001$ when asthmatic group compared to negative control group

Effect of zinc and iron complexes of OA on ALT, ALP, serum urea and creatinine

No hepatotoxic or nephrotoxic potential was shown by Zn and Fe complexes of OA (Fig. 8a–d).

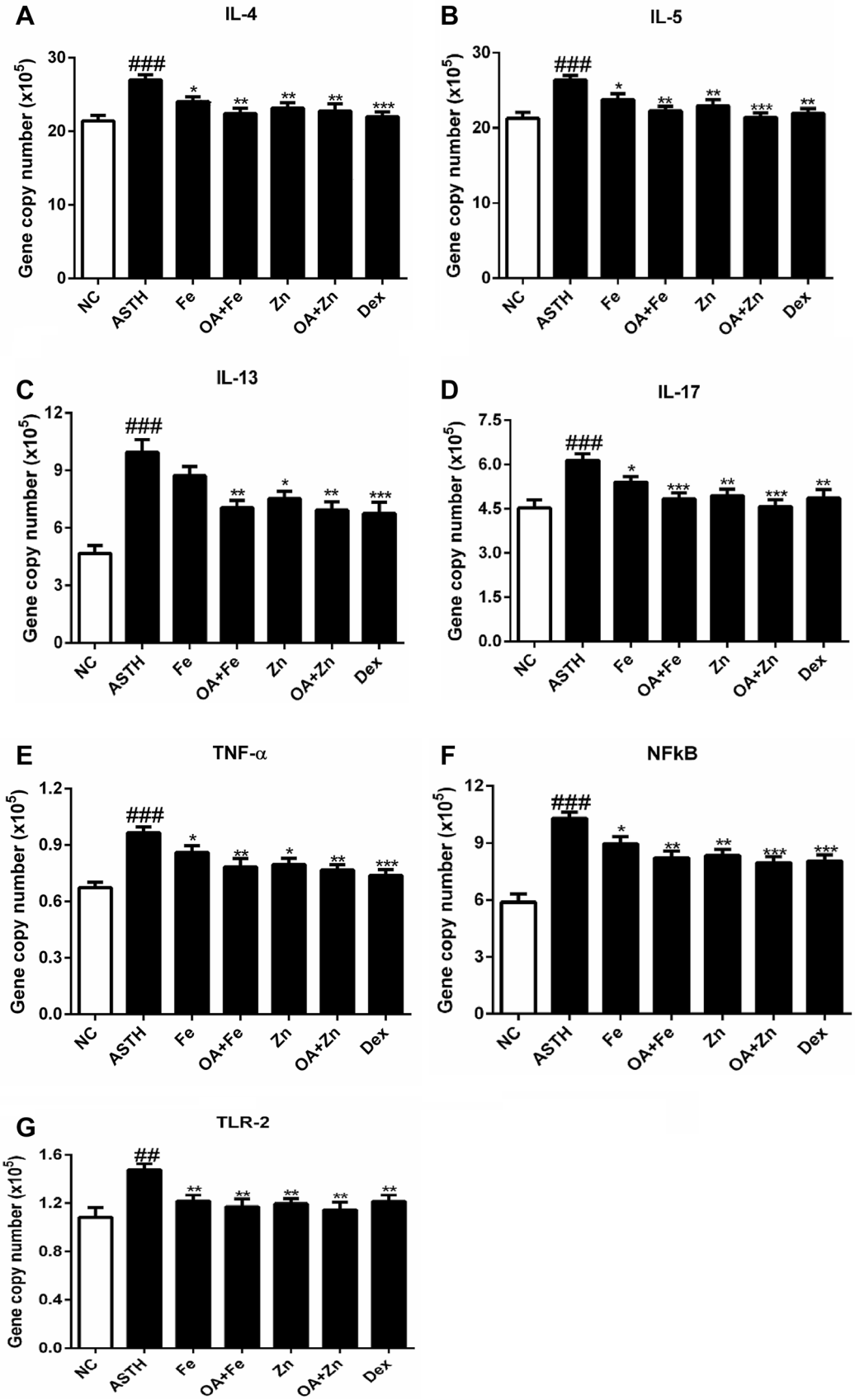
Acute physiopathologic effects

Acute physiopathologic effects of novel compounds was tested according to the guidelines of OECD. Six female rats were divided into 02 groups, (OA + Fe, OA + Zn complexes) with 03 animals in each group. They were administered 1000 fold higher than the therapeutic dose of the novel metal complexes of triterpene acids for 14 days and observed for the signs including; change in body weight, change of behavior, food and water intake, mortality rate. No remarkable finding was observed in any of the set features (Diallo et al. 2010). Our results suggest that OA + Fe, OA + Zn are relatively safe when administered orally in rats.

Discussion

In the current study, anti-inflammatory and immunomodulatory activities of Zn and Fe complexes of OA were evaluated by using OVA-induced rat asthmatic model. OA is an important compound that is identified for presenting useful properties in asthma and inflammatory status (Lee et al. 2010). Allergic asthma is decreased by Fe administration in an animal model (Maazi et al. 2011). Zinc is vital for function of cells in immune system displaying its modulation (Yousef and Elmorsy 2017).

Antigen-induced IgE causes activation of antigen-presenting cells (APCs), that successively prime T-cell reactions (Palaniyandi et al. 2015). Present study demonstrated that Zn, OA + Fe, OA + Zn attenuated IgE. These findings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◂**Fig. 5** i and ii. OA + Fe, Zn, OA + Zn, Dex significantly reduced **a** IL-4, **b** IL-5, **c** IL-13, **d** IL-17, **e** TNF-α, **f** NFκB and **g** TLR-2. Fe with exception to IL-13, significantly reduced expression of all above genes. Results are indicated as Mean ± SEM for 6 rats. *, **, ***$P < 0.05$, $P < 0.01$, $P < 0.001$ respectively when drug-treated groups compared to asthmatic group and $^{\#\#}, ^{\#\#\#}P < 0.01$, $P < 0.001$ respectively when asthmatic group compared to negative control group

are consistent with prior experimental (Kim et al. 2011, 2013, 2014a, b).

Expression of COX-2 gene is elevated in asthmatics, suggesting the involvement of COX-2 byproducts in airway inflammation (Park and Christman 2006). Effect of Fe, Zn, OA + Zn, OA + Fe and Dex on COX-2 were determined. The data showed that Zn, OA + Fe, OA + Zn significantly suppressed COX-2. Our findings are consistent with to the earlier studies (Shin et al. 2015; Cheng et al. 2016). Stimulation of mast cells in the airways is mediated through IgE-receptor which causes oxygenation of AA by 5-LO and lipoxins formation (Laitinen et al. 1993). CysLTs entire formation path has predominant expression in various forms of cells engaged in inflammation and gets activated during airway allergic inflammatory condition (Montuschi 2010).

Present study demonstrated that Fe, Zn, OA + Zn, OA + Fe significantly attenuated LOX. Our findings are in line with prior experimental work (Masid-de-Brito et al. 2014; Zaman et al. 2006; Montuschi 2010; Chauhan and Ducharme 2012).

Different TLRs have various effects in response to certain agonists with TLR2 plays major part in stimulating (AAD) (Thorburn et al. 2016). TLR2 is a major contributor to the maintenance of the adaptive Th2-cytokine-driven inflammatory disorder (Li et al. 2014). TLR2 ligands have been shown to promote Th2 responses (Agrawal et al. 2003) and it has been shown that these TLR2 ligand-expressing pathogens are associated with acute exacerbations of asthma (Johnston and Martin 2005). NF-kB is the leading controller for pro-inflammatory genes and is linked to asthma both in animal models and in clinical studies (Toledo et al. 2013). In prior studies it has been proposed that TLR2/MyD88/NFκB pathway mediates OVA-induced airway inflammation. In current study, we detected increased TLR2 and NF-κB expression in the lung tissue of asthmatic group, whereas treatment with Fe, Zn, OA + Fe, OA + Zn significantly decreased the expression. The results were compared with Dex which did reduce both TLR2 and NF-κB expression significantly. In case of TLR2 our findings are consistent with previous studies (Redecke et al. 2004; Shishodia et al. 2003; Thorburn et al. 2016). In case of NF-kB our findings are consistent with earlier experimental work (Keen et al. 2001; King et al. 2009; Kim et al. 2017; Liang et al. 2013).

Most important mastocytes-derived cytokine is TNF-α and its impression on inflammatory airway is widely accepted. TNF-α production was moreover established in the BALF from asthmatics and from allergen-challenged animals. TNF-α receptor inhibitors prove to inhibit BHR in an AA model (Trezena et al. 2003). TNF-α in BALF and TNF-α protein mRNA expression level in bronchial biopsy samples were raised in severe asthmatics equated to milder cases (Nam et al. 2009). Present study revealed that Fe, Zn, OA + Zn, OA + Fe declined TNF-α expression in a significant manner that could be the outcome of reduced TLR2 and NF-κB expression. Our findings are consistent with the prior studies (Abbas et al. 2005; Hutchison et al. 2008).

IL-4 performs a key part in Th2 cell proliferation and cytokine formation and IgE production, whereas IL-13 performs an essential role in the pathophysiological characteristics. Clinical experiments investigating the intrinsic activity of antagonizing IL-4/IL-13 applying biologic methodologies indicate potential (Gour and Wills-Karp 2015). Our results indicate that Fe, Zn, OA + Zn, OA + Fe significantly reduced IL-4 whereas Zn, OA + Zn, OA + Fe significantly reduced IL-13. These findings are in line with the prior experimental work (Steinke and Borish 2001; Gour and Wills-Karp 2015; Finkelman et al. 2010).

IL-5 is a precise stimulant of eosinophilic triggering factor which gets its production by mast cells, eosinophils and Th2 lymphocytes (Lee et al. 2009). Concentrations of IL-5 link to the clinical findings and mRNA of IL-5 is up-regulated in the mucosal wall of bronchi after allergen triggered asthma and declined after effective management with long term controllers (Menzies-Gow et al. 2007). Our data showed that Fe, Zn, OA + Zn, OA + Fe significantly reduced IL-5. Our findings are consistent with the earlier experimental works (Cho et al. 2004; Takatsu 2011; Leckie et al. 2000; Kim et al. 2013; Roa et al. 2007; Chen et al. 2009; Jee et al. 2005).

It has been established that IL-17 was amplified in BALF and blood in asthmatics (Molet et al. 2001; Chakir et al. 2003). Lung and blood showed high concentrations of IL-17 in AA and related to intensity of asthma (Hashimoto et al. 2005). IL-17 furthermore induces synthesis of additional proinflammatory cytokines, comprising TNF-α and by aiming and iNOS and COX-2, activates an increase in PGE_2 and NO in different cell forms (Trajkovic et al. 2001; LeGrand et al. 2001). Stimulation with IL-17 also induces the production of GM-CSF (Laan et al. 2003). Present study displayed that Fe, Zn, OA + Fe, OA + Zn significantly attenuated IL-17. Our findings are consistent with earlier studies (Kim et al. 2013, 2014a, b).

Increased amounts of NO formed by iNOS results in vasodilation, plasma extravasation, increased mucus production and stimulation by Th2 cells indirectly in addition to play part in asthma pathophysiology. NO donors or iNOS

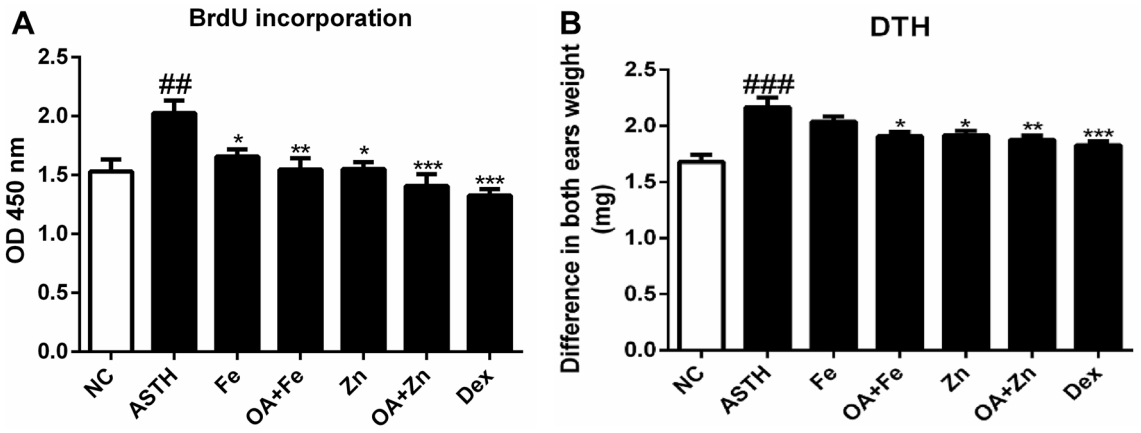

Fig. 6 Fe, Zn, OA + Fe, OA + Zn, Dex significantly inhibited **a** Ova-stimulated splenocyte proliferation. Zn, OA + Fe, OA + Zn, Dex significantly inhibited **b** delayed-type hypersensitivity (DTH). Results are presented as Mean ± SEM for 6 rats. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ respectively when drug-treated groups compared to asthmatic group, while $^{\#\#, \#\#\#}P < 0.01$, $P < 0.001$ respectively when asthmatic group compared to negative control group

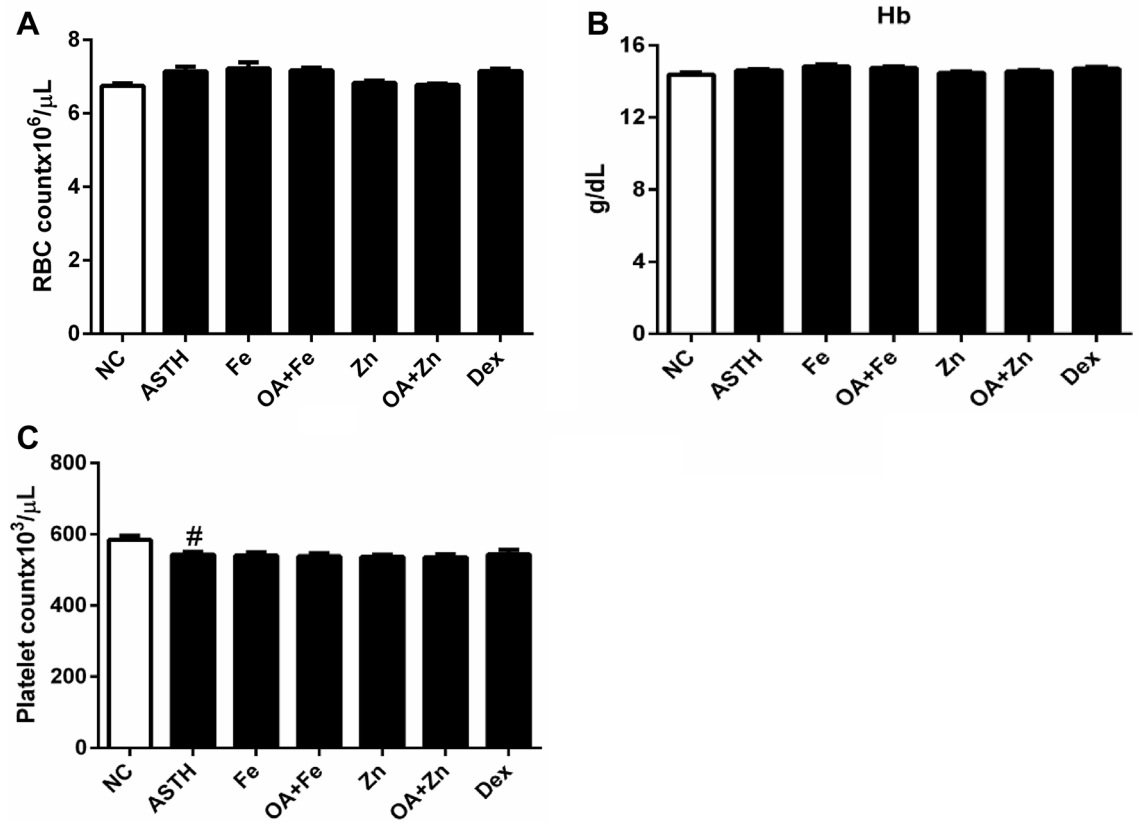

Fig. 7 Non-significant difference was observed in all groups when drug-treated groups compared to negative group for the determination of **a** RBC count, **b** Hb respectively

blockers might have therapeutic capability in asthma management (Prado et al. 2011). Present study displayed that Zn, OA + Fe, OA + Zn significantly attenuated the levels of NO both in serum and BALF. Our findings are in consistent with earlier experimental works (Shahzad et al. 2009; Jeon et al. 2015; Ghosh and Erzurum 2012) proving that NO pathways

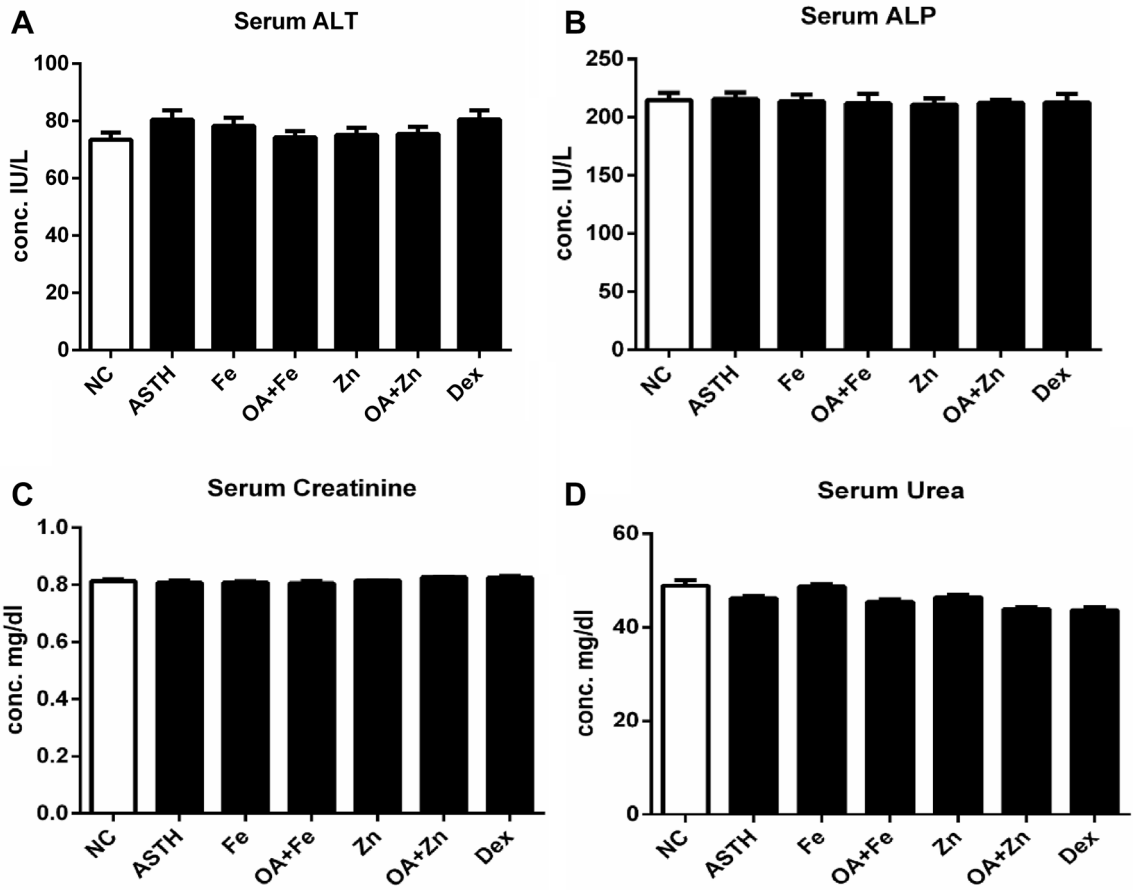

Fig. 8 Non-significant difference was observed in all groups when drug-treated groups compared to negative control group for the determination of serum **a** ALT, **b** ALP, **c** creatinine, **d** urea

play modulatory role in the pathogenesis of asthma, leads open gateway for the therapeutic approaches.

We determined the effect of Fe, Zn, OA + Fe, OA + Zn on splenocyte proliferation and DTH. The data showed that Fe, Zn, OA + Fe and OA + Zn significantly reduced splenocyte proliferation and DTH. Our findings are consistent with earlier experimental work (Ashraf et al. 2015; Shahzad et al. 2009).

The results displayed non-significant findings of Hb, RBC and significant results in case of platelet count when asthmatic group was compared to the drug-treated groups. Our study is not line with earlier experimental work (Shabbir et al. 2014). Our findings also exhibited significant rise in TLC, eosinophils, neutrophils and lymphocytes both in the blood as well as in BALF in asthmatic group as compared to Fe, OA + Fe, Zn, OA + Zn in which values were normalized. Our study is in line with the earlier experimental work (Shahzad et al. 2009; Tian et al. 2015; Neamati et al. 2009). To explore the safety profile of OA + Fe, OA + Zn we performed serum ALT, ALP, creatinine and urea assays which showed that OA + Fe, OA + Zn have no significant hepatotoxic and nephrotoxic properties.

Conclusion

Iron and zinc complexes of OA ameliorated allergic airway inflammation in rats. Iron and zinc complexes of OA inhibited NF-kB pathway which then switched off gene expression of Th2-cytokines. Decreased expression of pro-inflammatory cytokines may contribute in reduction of eosinophilia in blood and in airways. Effects of OA + Fe, OA + Zn displayed potentiation as compared to Fe and Zn alone. In general OA + Fe, OA + Zn exhibited results comparable to standard drug dexamethasone.

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Compliance with ethical standards

Conflict of interest All authors declare no conflict of interest.

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