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Induction of immunotoxicity and oxidative stress by imidazole on immune cells

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ABSTRACT

Imidazole is a known irritant to rabbit eye and possesses moderate oral toxicity. However Imidazole toxicity to immune cells if any, is unclear therefore needs to be scientifically evaluated. Aim of the present study was to evaluate the immuno-toxicity properties, if any, of imidazole parent compound. The study included assessment of the expression of immune modulatory cytokine mediators using real time PCR from the total RNA, isolated from mixed and T lymphocytes. Nitrosative stress was assessed by Nitric oxide formation. Apoptotic potential of imidazole on T lymphocytes using Annexin V assay kit was carried out using flow cytometer. Higher concentration of imidazole (10mg/ml) showed a significant increase in pro inflammatory cytokines like interleukin-1, monocyte chemo attractant protein-1, tumour necrosis factor beta, whereas drastic reduction in anti inflammatory cytokine, interleukin -10 was noted. This study also demonstrated that Imidazole (10 mg/ml) induces mixed and T lymphocyte proliferation by DNA synthesis significantly. The mRNA expressions of mitogen-activated protein kinases 14 gene, inducible Nitric oxide synthase and Bcl-2–associated X protein were also significantly increased ($p<0.01$). However, lower concentrations of imidazole did not exhibit such effect on both types of cells. Continuous exposure of workers to imidazole used in industries could suppress the body's immune system, especially cellular immunity thereby triggering the inflammatory pathway and targeting the p38 MAPK signal transduction pathway. These observations are of interest in view of workers in pesticide, pharmaceutical industries. ! 2015 Trade Science Inc. - INDIA

KEYWORDS

Imidazole;
Splenocytes;
T lymphocytes;
Cytokines;
Oxidative stress;
Apoptosis.

INTRODUCTION

Humans and ecosystems are continually exposed to a very complex mixture of chemicals the composition of which is always changing. Various physiological and environmental factors are known to modulate chemical-induced immune toxicity[1]. Some chemicals can illicit an enhanced immune response resulting in tissue damage and immune mediated disease. Enhancement of the immune system can lead to chemical hypersensitivity and autoimmune disease[2]. Imidazoles are present in many antifungal,

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anti protozoa and anti helmintic medications. It is part of the theophylline molecule, found in tea leaves and coffee beans which stimulate the central nervous system. It is present in the anticancer drug mercaptopurine which combats leukemia by interfering with DNA synthesis. It is also present in the structure of many natural or synthetic drug molecules, i.e., cimetidine, azomycine, metronidazole, clonidine, losartan and phenytoin[3]. Imidazole possesses moderate oral toxicity in a scientifically valid study. LD50 in rats was determined to be 960-970 mg/kg body weight. 80% Imidazole is corrosive to skin under occlusive conditions. Imidazole is a known irritant to rabbit eye when tested according to OECD TG 405. Imidazole is used in the chemical industry as an intermediate in the production of pharmaceuticals, pesticides, and dye intermediates, auxiliaries for textile dyeing and finishing, photographic chemicals and corrosion inhibitors[4].

Safety assessment of a Chemical or material intended to be used in its manufacture is guided by toxicological studies recommended by the Biological Evaluation of Medical Devices technical committee of the International Organization for Standardization (ISO) documents 10993.13 The need for immunotoxicology testing has been outlined in ISO 10993-20: 'Tests for immunotoxicology'[5].

The immuntoxicological response with respect to irritation, allergy etc of imidazole especially on immune cells like its effect on inflammatory cytokines, apoptosis, and oxidative/nitrosative stress has not been studied yet although its derivatives are been used as antimicrobial, anti-fungal agent, anticancer agents etc.

The lymphocytes particularly T lymphocytes are correlated with the delayed type hypersensitivity which in turn leads to oxidative stress and underlying parameters such as cytokine/chemokine release[6, 7]. Some reports suggested that Imidazole may induce oxidative stress by elevating the intracellular content of reactive oxygen species (ROS) and this mechanism is postulated to be the main cause of irritation[7, 8].

Further studies showed that imidazole derivatives i.e. levamisole and parent molecule imidazole augment phytohemagglutinin-induced proliferation of human peripheral blood lymphocytes, mouse thymocytes and T-cell enriched splenocytes. Both agents increase cyclic GMP levels of T-cell enriched mouse spleen cells with a dose-response curve which closely parallels their effects on proliferation and lower cyclic AMP levels in these cells[9,10].

Nitric oxide (NO) is an inflammatory mediator, which acts as a cytotoxic agent and modulates immune responses and inflammation. p38 mitogen-activated protein kinase (MAPK) signal transduction pathway is activated by chemical and physical stress and regulates immune responses. Studies have shown that p38 MAPK pathway regulates NO production induced by inflammatory stimuli[11]. Lee et al in 1994 reported that a class of pyridinyl imidazoles inhibits the MAP kinase homologue, termed reactivating kinase (RK).

With this back ground knowledge and it becomes necessary to assess the affecting dose of imidazole at which toxic effects are observed on the immune system which is not been addressed yet. This preliminary study was focused on the dose dependent effect of imidazole on proliferation of T lymphocytes, mRNA expressions of Forkhead box 4 gene to assess the regulatory T lymphocytes (T_{reg}), certain inflammatory cytokines involved in T lymphocyte mediated immunity and on the oxidative/nitrosative stress i,e nitric oxide synthase activity. The study was also aimed to throw light on the imidazole induced apoptosis and p38 MAPK cell signaling pathway with the help of mRNA expressions of apoptotic factors and MAPK 14 gene respectively.

Sodium Dodecyl Sulphate (SDS) was used as the positive control for the study. From several studies it was found that the skin is sensitive to aqueous solution of the surfactant mainly sodium dodecyl sulfate (SDS), a well-known model skin irritant, SDS penetrates into the skin and disrupts skin barrier. It is well established, both in vitro and in vivo, that the SDS skin penetration is dose-dependent, and that it increases with an increase in the total SDS concentration above the critical micelle concentration (CMC) of SDS[13,14].

METHODS

Reagents

Imidazole was procured from Merck Chemical Co.,

Mumbai, India; Sodium dodecyl sulphate (sodium lauryl sulphate) from Sigma Chemical Co., St Louis, Missouri, USA; RNase, Ethanol, Bromophenol Blue, Ethidium Bromide and Taq Polymerase from Fermantas, USA; Mouse Oligonucleotide Primers for Interleukin-1, Interleukin-10, Tumour necrosis factor β (TNF β), Bax, MAPK 14, iNOS, Monocytes Chemo attractant Protein-1 (MCP-1), Forkhead Box 4 and Gylceraldehyde Phosphate Dehydrogenase GAPDH were procured from Eurogentec, Belgium; RPMI-1640 from Himedia, Mumbai, India; RT^2 SYBR Green ROX Q PCR Master Mix, Rneasy Lipid Tissue Mini Kit, Qiazol lysis Reagent and RT2 First Strands Kit from Qiagen, Hilden, Germany. Mouse T cell selection kit from Stem Cell Technologies Inc, Canada; ^{3}H Thymidine from BRIT, India. All the other chemicals used were of analytical grade and were purchased from qualified vendors.

Animals

Swiss albino mice were selected for this study. The animals were procured from the Division of Laboratory Animal Sciences of Biomedical Technology Wing (BMT), Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST), Thiruvananthapuram, Kerala, India with prior approval from Institutional Animal Ethics Committee and in accordance with approved Institutional protocol. Animals were maintained in a 12 h light and dark cycle at controlled environmental conditions of temperature (22 ± 3ºc) and humidity (50–70%). They were fed with standard pellet diet and provided water *ad libitum*.

All animals were with care, without causing pain or distress. The care and management of the animals comply with the regulations of The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India.

EXPERIMENTAL DESIGN

Healthy Swiss Albino mice were sacrificed by cervical dislocation. Spleen were dissected out immediately and washed with sterile phosphate buffered saline (PBS). The Splenocytes single cell suspension was collected by disrupting the isolated spleen in PBS this was further used for various evaluations.

Splenocytes single cell suspension was treated with various concentrations of imidazole (10mg/ml, 1 mg/ml and 0.1 mg/ml) and positive control SDS (5mg/ml) were carried out individually. Since not much literature was available on imidazole for LD 50 in mice the doses were selected according to the LD_{50} in rats (960-970 mg/kg body weight)[4] criteria. Briefly, splenocytes single cell suspension was divided in to several groups; each group contained 1 ml of the splenocytes single cell suspension in triplicate. First group was kept as normal control, treated with 1mg/ml of Physiological Saline; group II were treated with 5mg/ml SDS as positive control; group III were treated with 10 mg/ml of imidazole; groups IV were treated with 1 mg/ml of imidazole, groups V were treated with 0.1 mg/ml of imidazole. All groups were used to determine various biochemical and molecular parameters.

Tritiated (^{3}H) thymidine incorporation assay using isolated splenocytes (mixed lymphocytes) and enriched T lymphocytes

Isolated spleen from mice (as mentioned in experimental design) were used for the isolation of mixed lymphocytes and enriched T lymphocytes to study the cell proliferation by tritiated thymidine incorporation assay in cell culture. Viability of mixed lymphocytes and enriched T lymphocytes were assessed using trypan blue dye exclusion method[15]. Single cell splenocytes suspension was used to isolate enriched T lymphocytes according to the kit pack insert in an automated cell separator (ROBOSEP). Mixed lymphocytes and enriched T lymphocytes were cultured at a density of 2 X10^{5}and 1x10^4 cells / ml respectively in a 96 well plate with RPMI-1640 medium supplemented with 10% FBS, streptomycin (100 µg/ml) and penicillin (100 Units/ml) for 24 h at 37°C in a CO_2 incubator. After 24 h of incubation the cultured wells were treated with1mg/ml physiological saline (normal control), 5mg/ml SDS alone (positive control) and various concentrations of imidazole (as mentioned in experimental design) were added in the respective 96 well plate. After 48 h at 37°C of incubation, the cultured treated wells labelled with ^{3}H-thymidine at a concentration of 1µci/ml and incubated further for 24 h at 37ºC. Cells were harvested after

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72 h and radioactivity in terms of counts per minute (cpm) were measured by Liquid Scintillation Counter (Triathler, Hidex).

Real time PCR analysis for determining m-RNA expression of specific cytokines

Single Cell splenocytes suspension and isolated enriched T lymphocytes were further cultured separately at a density of $2x10^5$and $1x10^4$ cells / ml respectively in a $35cm^2$ culture dish with RPMI-1640 medium supplemented with 10% FBS, streptomycin (100 µg/ml) and penicillin (100 Units/ml) for 24 h at 37°C in a CO_2 incubator for the isolation of total RNA. After 24 h of incubation 5mg/ml SDS alone (positive control) and various concentrations of imidazole (as mentioned in Experimental Design) were added in the respective culture dish. Cells treated with physiological saline served as normal control. After 48 h of incubation, total RNA was isolated from cultured mixed lymphocytes and T lymphocytes using trizol reagent (Sigma, USA). Following the manufacture's protocol quantity and purity was analyzed spectrophotometrically.100 ng of mRNA were used for cDNA synthesis of IL-10, IL- 1, MCP-1, Bcl 2,MAPK 14, Forkhead Box 4, iNOS, TNFβ and GAPDH in a reaction volume of 20µl using RT^2 first strand kit (Qiagen, Germany). The synthesis was carried out in Eppendrof master cycler, Germany.

The mouse oligo nucleotide forward and reverse primer sequence used to determine specific mRNA gene expressions were F-TAAGGCTGGCCACACTTGAG and R-GTTTTCAGGGATGAAGCGGC for Interleukin 10 (IL-10) [Accession No. NM 010548.2]; F-CTCTCCCCAGCTTTTCCAGG and R-TCTCTGGGCTTGACTGCTTG for Interleukin 1 (IL-1) [Accession No. NM 001177975.1]; F-AGATGCAGTTAACGCCCCAC and R-GACCCATTCCTTCTTGGGGT for Monocyte Chemoattractant protein 1 (MCP-1) [Accession No. NM 011333.3]; F-AGGCTCACGTCACCAAGTCCC and R-TGGTCTCGAAAGCTACGTGGGAGG for for Mitogen-activated protein kinase 14 (MAPK14) [Accession No. NM 001168514.1], F-TTGCTCTCTAGGAGCGGTCT and R-CCAGGACCTTAGGGATGGGA for Forkhead Box 4 (foxp4) [Accession No. NM 018789.2], F-CCAGGATGCGTCCACCAAGA-3′ and R-GGTGAGGACTCCAGCCACAA-3′ for BCL2-associated X protein (Bax) [Accession No. NM 007527.3], F-TGCCAGCTCCAGGATTTCAG and R-CTCAGCCCTCACTTGACCTG for Tumour Necrosis Factor beta (TNFβ) [Accession No. NM 011610.3] and F-GCGTGGGGACAGCCGCATCTT and R-ATCGGCAGAAGGGGCGGAGA for Glyceraldehyde Phosphate Dehydrogenase (GAPDH) [Accession No. BC 023196.1]. The real time PCR reaction was carried out with RT^2 SYBR Green ROX Q PCR master mix of total reaction volume of 25 ml; real time PCR amplifications were done using a Chromo 4 System, Bio- Rad (MJ Research, CA) for 40 cycles as per manufacture's protocol. Glyceraldehyde Phosphate Dehydrogenase (GAPDH) was used as the house keeping gene. The level of gene expression is reported as the ratio between the mRNA level of the target gene and the GAPDH, a reference gene using the comparative $2^{\delta\delta ct}$ method[16].

Live/dead/apoptotic assay using isolated splenocytes and enriched T lymphocytes

Apoptosis assay was carried out to evaluate the cytotoxicity and apoptosis induced by imidazole on mixed lymphocytes and enriched T lymphocytes using Vybrant Apoptosis Assay Kit (Molecular Probes). After 72 h of in vitro culture of splenocytes and enriched T lymphocytes, cells were harvested and washed with PBS later stained with Annexin V – Alexa flour 488 and PI according to manufacturer's protocol. Analysis was carried out by Flow cytometry and percentages of live, apoptotic and dead cells were estimated using BD FACS Diva software.

Statistical analysis

All the samples were run in triplicates; statistical comparison was done with three or more groups using one-way Analysis of Variance (ANOVA) followed by Dunnetts' test. P Values<0.001 and <0.05 were considered significant.

RESULTS

Effect of imidazole on lymphocytes on iNOS mRNA expression

As shown in Figure 1, *in vitro* imidazole treatment

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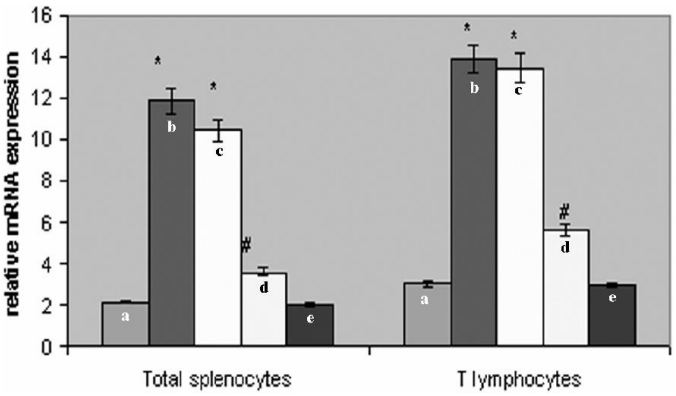

Figure 1 : Effect of imidazole on iNOS expression on isolated total splenocytes and T lymphocytes from mouse. a- Normal control, b- Positive control SDS (5mg/ml), c- imidazole (10mg/ml), d- imidazole (1mg/ml), e- imidazole (0.1mg/ml), Values are mean ± S.D; *P < 0.001, #P < 0.005 (compared to respective normal control).

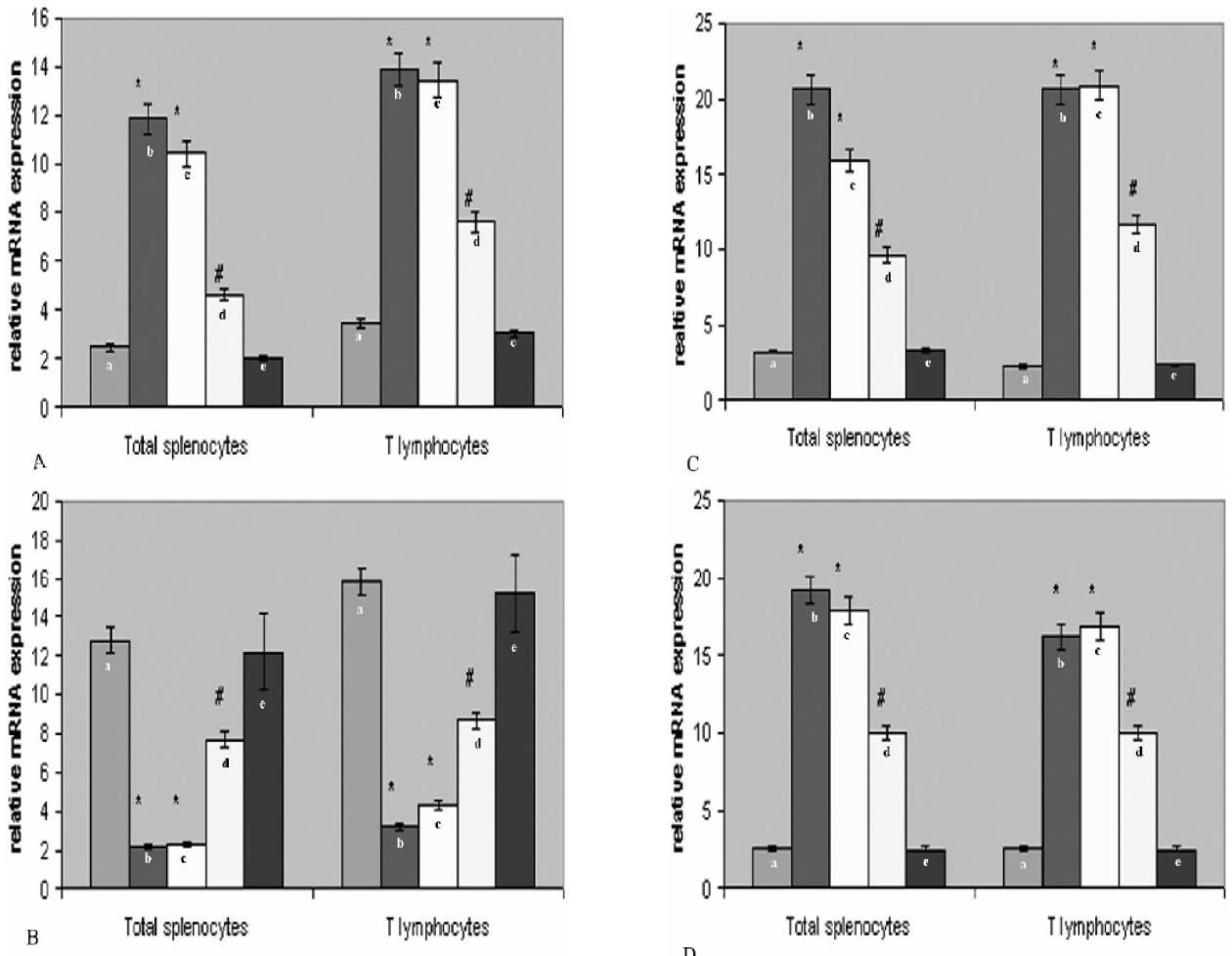

Figure 2 : a) Effect of imidazole on IL-1 expression on isolated total splenocytes and T lymphocytes from mouse. a- Normal control, b- Positive control SDS (5mg/ml), c- imidazole (10mg/ml), d- imidazole (1mg/ml), e- imidazole (0.1mg/ml), Values are mean ± S.D; *P < 0.001, #P < 0.005 (compared to respective normal control); c) Effect of imidazole on IL-10 expression on isolated total splenocytes and T lymphocytes from mouse. a- Normal control, b- Positive control SDS (5mg/ml), c- imidazole (10mg/ml), d- imidazole (1mg/ml), e- imidazole (0.1mg/ml), Values are mean ± S.D; *P < 0.001, #P < 0.005 (compared to respective normal control); c) Effect of imidazole on MCP-1 expression on isolated total splenocytes and T lymphocytes from mouse. a- Normal control, b- Positive control SDS (5mg/ml), c- imidazole (10mg/ml), d- imidazole (1mg/ml), e- imidazole (0.1mg/ml), Values are mean ± S.D; *P < 0.001, #P < 0.005 (compared to respective normal control); d) Effect of imidazole on TNFβ expression on isolated total splenocytes and T lymphocytes from mouse. a- Normal control, b- Positive control SDS (5mg/ml), c- imidazole (10mg/ml), d- imidazole (1mg/ml), e- imidazole (0.1mg/ml), Values are mean ± S.D; *P < 0.001, #P < 0.005 (compared to respective normal control).

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resulted in a drastic increase in the levels iNOS of both mixed and T lymphocytes at higher concentration (10mg/ml) similar to that of positive control (SDS 5mg/ml). However, lower concentration of imidazole (0.1 mg/kg) did not show any significant change in iNOS expression when compared with control.

Alterations in the mRNA levels of inflammatory cytokines - IL-1, IL-10, MCP-1 and TNFβ by imidazole on lymphocytes

As shown in Figure 2 A, lymphocytes cultured cells treated with higher concentration of imidazole i,e 10 mg/ml resulted in a remarkable 6 fold decrease in the levels of IL-10 mRNA expression. Whereas, IL-1 mRNA and MCP-1 expressions was increased by 9 fold and 3 fold respectively (Figure 2 B and 2 C). The positive control also showed a 4 fold increase in MCP-1 expression. Interestingly, from Figure 2D it was observed that TNFβ mRNA expressions were increased by 6 fold in higher concentration imidazole treated lymphocyte culture cells. Positive control showed a similar pattern of increase to that of imidazole (10mg/ml). However, at a low concentration (0.1mg/ml) imidazole did not induce any changes in the m-RNA levels compared to control in lymphocyte culture cells.

Effect of imidazole on ^{3}H-Thymidine incorporation into mixed and T lymphocytes

Imidazole (10mg/ml) treatment increased ^{3}H-Thymidine incorporation in both mixed and T lymphocytes by 2 fold and 3 fold respectively similar results were observed with the positive control. The lower concentrations did not induce such decrease in proliferation rate when compared with control (Figure 3).

Effect of imidazole on cell viability and apoptosis and other apoptotic factors

From the Figure 4 it was noted that imidazole increased the Bax expression in lymphocyte cultured cells. mRNA expression of Bax increased by 4 folds with respect to control. Whereas, the lower concentrations of imidazole, showed no significant effect on Bax expression when compared with control.

The FACS analysis for apoptosis with Annexin V assay revealed a similar pattern. Wherein, apoptotic cells in T lymphocytes increased with the increase in imidazole concentration significantly (Figure 5 A, B, C).

Effect of imidazole on Treg cells (Forkhead box 4)

The results of the (Figure 6) indicated that imidazole at a higher concentration (10 mg/ml) increased the

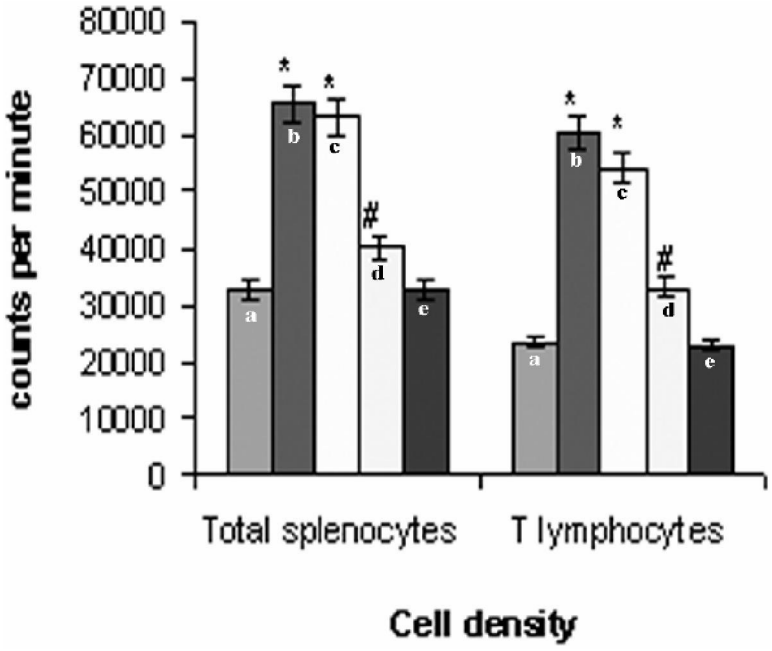

Figure 3 : Effect of imidazole on ^{3}H thymidine incorporation on isolated total splenocytes and T lymphocytes from mouse. a- Normal control, b- Positive control SDS (5mg/ml), c- imidazole (10mg/ml), d- imidazole (1mg/ml), e- imidazole (0.1mg/ml), Values are mean ± S.D; *P < 0.001, #P < 0.005 (compared to respective normal control).

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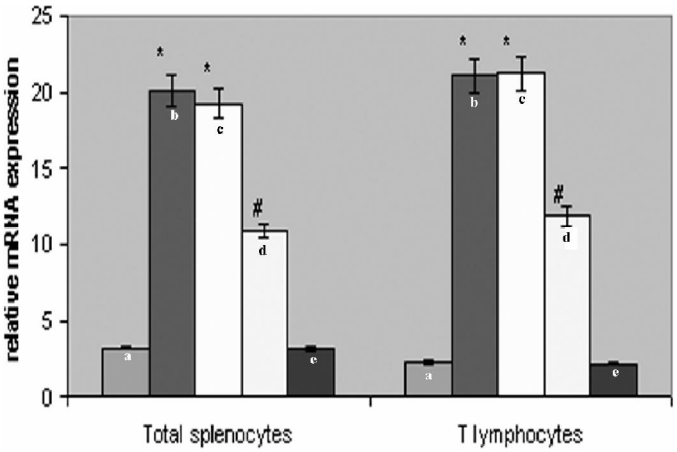

Figure 4 : Effect of imidazole on Bax expression on isolated total splenocytes and T lymphocytes from mouse. a- Normal control, b- Positive control SDS (5mg/ml), c- imidazole (10mg/ml), d- imidazole (1mg/ml), e- imidazole (0.1mg/ml), Values are mean ± S.D; *P < 0.001, #P < 0.005 (compared to respective normal control)

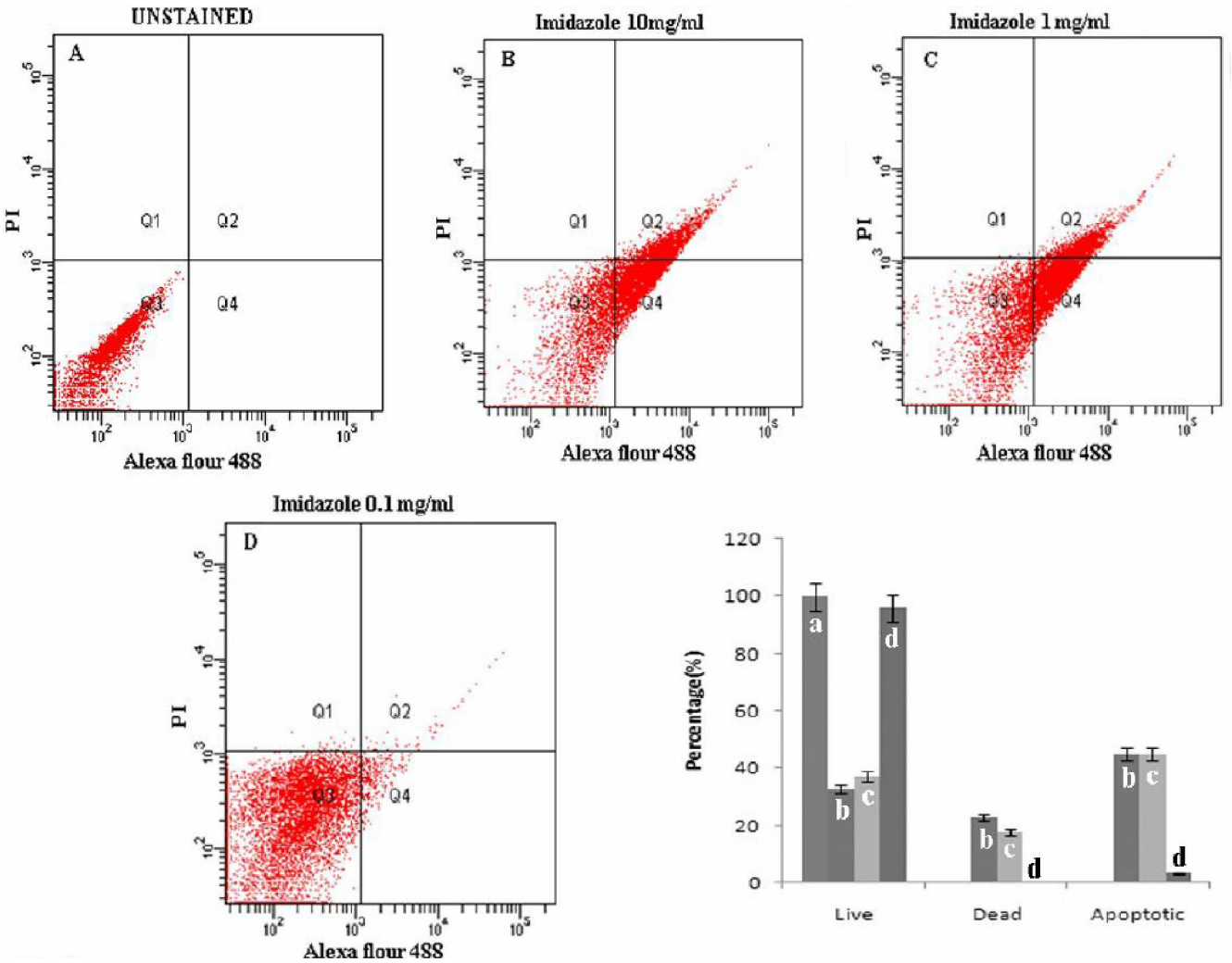

Figure 5 : Effect of imidazole on induction of Apoptosis on isolated T lymphocytes from mouse by Annexin V assay. A. Un-stained T lymphocytes control; B. imidazole (10mg/ml) ; C. imidazole (1mg/ml); D. imdazole (1mg/ml) ; imidazole (0.1 mg/ml); E. Graphical representation of quantitative data (*n* = 4). a- Unstained T lymphocytes control, b- imidazole (10mg/ml) c- imidazole (1mg/ml), d- imidazole (0.1mg/ml)

Fox04 expression. mRNA expression of Forkhead box 04 increased by 2 fold similar to the positive control. Whereas the lower concentrations of imidazole did not show any such effect, which were similar with control.

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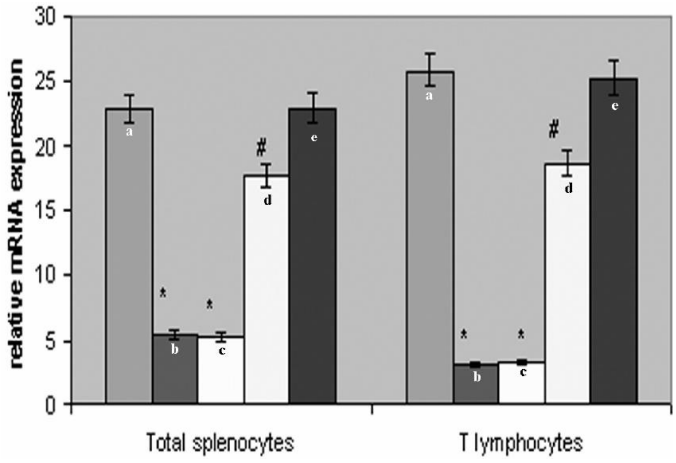

Figure 6 : Effect of imidazole on Forkhead box 04 expression on isolated total splenocytes and T lymphocytes from mouse. a- Normal control, b- Positive control SDS (5mg/ml), c- imidazole (10mg/ml), d- imidazole (1mg/ml), e- imidazole (0.1mg/ml), Values are mean ± S.D; *P < 0.001, #P < 0.005 (compared to respective normal control)

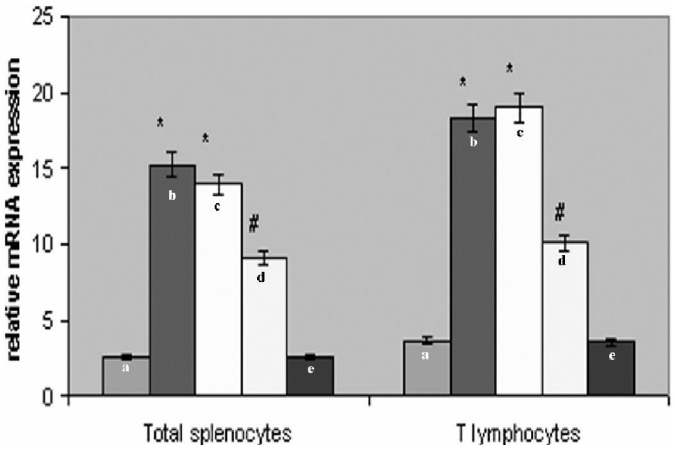

Figure 7 : Effect of imidazole on MAPK 14 expression on isolated total splenocytes and T lymphocytes from mouse. a- Normal control, b- Positive control SDS (5mg/ml), c- imidazole (10mg/ml), d- imidazole (1mg/ml), e- imidazole (0.1mg/ml), Values are mean ± S.D; *P < 0.001, #P < 0.005 (compared to respective normal control)

Effect of imidazole on MAPK 14 mRNA expression

Figure 7 suggests that imidazole at a higher concentration (10 mg/ml) increased the MAPK14 expression. mRNA expression of MAPK 14 remarkably increased by3 fold similar to the positive control. The lower concentrations of imidazole showed similar pattern as that of control.

DISCUSSION

The present preliminary study shows that parent imidazole enhances immune response in vitro by initiating irritation process as an inflammatory mediator to modulating immune responses and inflammation by p38 mitogen-activated protein kinase (MAPK) signal transduction pathway which in turn regulates oxidative stress induced immune responses.

Earlier studies suggested that levamisole and imidazole augment phytohemagglutinin-induced proliferation of human peripheral blood lymphocytes, mouse thymocytes and T-cell enriched splenocytes[10]. Isoprinosine has direct effects upon the *in vitro* proliferation of lymphocytes by increasing cyclic adenosine 3',5'-monophosphate and cyclic guanosine 3',5'-monophosphate[10].

Our observations state that imidazole increases proliferation of mixed and T lymphocytes (without mitogen) in a concentration dependent manner in lieu with other studies. Our finding from the study highlights the fact that imidazole accelerates the immune system by increasing the cell proliferation both total and T lymphocytes.

Derivatives of imidazole i,e Benzimidazole and

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Clotrimazole showed immunosuppressive action and inhibition of lymphocyte activation by [3H]- TdR incorporation study[17]. This may be due to masking of functional groups in the derivatives of imidazole which are combined with other compounds. Such a masking was absent in parent imidazole were the functional groups are exposed and are able to regulate the immune cells. Interestingly our study showed that parent imidazole was able to modulate the Treg cell in both mixed and T lymphocytes by attenuating expression of fox P4 mRNA in a concentration dependent manner which emphasis the fact that imidazole plays pivotal role in immune responses and autoimmune disease. Immunosuppressive nature of imidazole derivative drugs Mizoribine which was contradictory to the parent imidazole findings as an immune enhancer was demonstrated to inhibit T lymphocyte proliferation by depleting these cells of guanine ribonucleotides as a consequence of inhibiting the enzyme inosine monophosphate (IMP) dehydrogenase. Also, immunosuppressive agents' azathioprine and 6-mercaptopurine (6MP) inhibited T cell proliferative responses after T cell activation[18].

Bax and Bak play an essential role in the control of T cell proliferation by modulating ER Ca(2+) release. Imidazole induces apoptosis in HL-60 cells associated with intracellular acidification, caspase-3 activation and DFF-45 cleavage[19]. Here in our study we found that parent imidazole exhibited apoptosis in normal cultured T lymphocytes in a concentration dependent manner. Which was evident by the increased expression of Bax a pro apoptotic factor by imidazole in both mixed and T lymphocyte cultures. Among all of the cytokines analysed, IL-1, TNF β was found to be most abundantly expressed in both mixed and T lymphocytes. IL-1, TNF β, is the cytokines specific for T lymphocytes and is produced during inflammation. It is a known fact that TNF- β is a potent mediator of inflammatory and immune responses. TNF- β is produced by activated T and B lymphocytes.

We also demonstrated that the expression of Bax pro apoptotic factor increased with the increase in imidazole concentration in both mixed and T lymphocytes. Therefore our study provides information regarding the imidazole mediated inflammatory response together with the initiation of apoptosis in both mixed and T lymphocytes.

Azathioprine, an immune suppressive imidazole derivative drug suppressed the activation of MAPK, NF-kb and bcl-x [L][20,21]. Also, p38 activation is required for heat induced apoptosis in RAW 264.7 cells and stimulation of p38 leads to cell death[22]. Interestingly, our study showed that parent imidazole increasing the MAPK 14 mRNA expression specific for immune cells suggesting an oxidative stress related p38 signal transduction pathway activation. MAPK p38 is a major pathway adopted by immune cells and oxidative stress inducers. Several studies have showed that Monocyte chemotactic protein-1 (MCP-1) enhances the recruitment of inflammatory cells following pulmonary infection with C. neoformans[23]. Elevated MCP-1 mRNA levels were detected primarily in the non-T cell populations of the synovial fluidand synovial tissue samples[24]. Moreover, increase in MCP-1 expression suggests the fact that infiltration of monocytes/macrophages to the site of inflammation.

Also, increased expressions of both MCP-1 and MAPK 14 demonstrate mechanism of imidazole action through p38 MAPK associated JNK signalling pathways evident from our results. Immune stimulating cytokines or bacterial pathogens activate iNOS and generate high concentrations of nitric oxide (NO) through the activation of inducible nuclear factors, including NFkB[25]. p38 MAPK has been reported to have either up-regulatory role, down-regulatory role or no role in iNOS expression. JNK is an important post-transcriptional regulator of LPS-induced iNOS expression and NO production[11].

In our current study parent imidazole was found to increase the iNOS levels in a concentration dependent manner. Therefore study highlights the fact that parent imidazole increased the iNOS expression in a concentration dependent manner suggesting a state nitrosative stress. Anti-inûammatory interleukin-10 (IL-10), and not pro inûammatory IL-6 and IL-23 Cytokine signalling endowed Treg cells with the ability to suppress pathogenic Th17 cell responses[26].

CONCLUSION

Together with the results on the over expression of inflammatory cytokines, increasing iNOS activity, modulating Treg cells and induction of apoptosis this study suggests that parent imidazole acts as an immune enhancer and an agent triggering inflammation and

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nitrosative stress in both mixed and T lymphocytes in vitro. Further in vivo studies are in progress to understand the effect of parent imidazole on lymphocyte generating sites as well as on other organs involved in immune system.

Continuous exposure of workers to imidazole used in industries engaged in the production of pharmaceuticals, pesticides, dye intermediates, auxiliaries for textile dyeing and finishing, photographic chemicals and corrosion inhibitors could suppress the bodies immune system is predominantly the T lymphocyte mediated cellular immunity triggering the inflammation pathway as evident by the expression of cytokines and targeting the p38 MAPK signal transduction pathway to a prominent extends. These results provide useful information for proper management of imidazole associated immuno toxicity. Further in vivo studies are in progress to understand the effect of parent imidazole on lymphocyte generating sites as well as on other organs involved in immune system.

ABBREVIATIONS

IL-1, interleukin 1; IL10, interluekin 10; IL6, interleukin 6; MCP-1, monocyte chemo attractant protein-1; TNFβ, Tumour Necrosis Factor beta; MAPK 14, mitogen-activated protein kinases 14 gene; iNOS, cytokine-inducible Nitric oxide synthase; Bax, Bcl-2–associated X protein

Conflict of interest

The authors declare that they have no competing interests and financial assistance for this work.

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