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ALLEVIATION OF ARSENIC INDUCED LUNG TOXICITY BY *OCIMUM SANCTUM* IN MURINE MODEL

Surbhi Kaushal, Vincy Garg, Aitizaz Ul Ahsan, Vijay Lakshmi Sharma and Mani Chopra*

Department of Zoology, Panjab University, Chandigarh - 160014, Punjab, India.

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Arsenic, Pleural toxicity, Oxidative stress, *Ocimum*

Correspondence to Author:
Dr. Mani Chopra

Assistant Professor,
Department of Zoology,
Panjab University, Chandigarh
- 160014, Punjab, India.

E-mail: zoologymani03@gmail.com

ABSTRACT: Arsenic is a well known pulmonary irritant and has been found associated with lung cancer in smelter workers exposed to arsenic via inhalation. The recent findings suggest the involvement of reactive oxygen species (ROS), generated during arsenic metabolism in inducing arsenic toxicity. Thus present work was designed to establish a correlation between arsenic induced oxidative stress, histopathological alterations and genotoxicity in pulmonary tissue of balb/c mice and assessing the therapeutic efficacy of *Ocimum sanctum* (200mg/kg b.wt., orally) against arsenic (1.57 mg/kg b.wt., 1/10th of LD_{50}) intoxicated mice. Sub-chronic arsenic exposure elicited significant elevation in lipid peroxidation, reduction in glutathione and antioxidant enzyme levels in lungs of mice. Histopathological observations revealed degeneration of bronchiolar epithelium, enhanced number of alveolar macrophage, reduction in alveolar space and alveolar hyperplasia, where as fragmented genomic DNA from the pulmonary tissue indicated oxidative stress mediated genotoxicity. *Ocimum* treatment abated the oxidative stress by commuting the antioxidants imbalance, thereby reducing as induced histological changes and genotoxicity. Thus, present study established the preventive efficacy of *Ocimum sanctum* leaf extract against arsenic mediated toxicity and other cellular toxic insults.

INTRODUCTION: In modern days, with deteriorating environmental quality, human life is exposed to numerous types of threats. The food for appeasing hunger, water for quenching thirst and air for breathing, all have become unsafe these days. There are number of environmental toxicants around us; ingestion, breathing or any kind of exposure to them can affect the human health tremendously. Arsenic, being a toxic element has made the human life miserable in the areas with a higher concentration of it in water, soil and air.

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ATSDR has ranked arsenic number one among the list of 20 hazardous substances [1]. It has been also classified as Group I human carcinogen by IARC and indicated its association with carcinogenesis of skin, lungs, bladder and many other organs [2]. Epidemiological findings suggest that arsenic toxicity plagues more than 137 million individuals in nearly 70 countries of which most affected regions includes the Ganga-Brahamputra-Meghna of India and Bangladesh, parts of China, Japan, Taiwan, Argentina and USA [3].

The maximum permissible limit (MPL) for arsenic intake through drinking water is 10µg/L as addressed by WHO. Nearly 26 million individuals in West Bengal are at risk of arsenic exposure, where its content is above the recommended MPL [4]. Recently, Central Ground Water Board has surveyed six districts of Punjab (Amritsar, Tarn

taran, Mansa, Kapurthala, Ropar, Fazilka) where arsenic contamination in groundwater has been found to be more than 0.05mg/L [5]. The most common ways of exposure are ingestion followed by inhalation and dermal absorption. Ingestion apart from contaminated water can also occur by contamination of soil by using pesticides containing arsenic, while inhalation can occur through contaminated fumes and mist [6]. The ingested as well as inhaled arsenic absorbed through gastrointestinal tract and lungs then enters blood stream where it binds with RBC's and further enters in major organs.

Lungs are one of the most sensitive organs for bioaccumulation [7] of arsenicals after it is metabolized in the liver and become available in the circulation. Arsenic exposure may lead to damage in the respiratory system, causing bronchitis, chronic obstructive pulmonary disease, and finally, malignancies [8]. Lung cancer rate is higher in workers exposed to arsenic trioxide and arsenical pesticides. In West Bengal and Bangladesh the prevalence rate of cough, shortness of breath and chest sound in lungs of both males and females were found to increase with increasing concentration of arsenic in water [9]. Approximately 32% to 55% of lung cancer cases were estimated to be associated with the combined effect of cigarette smoking and ingested arsenic, depending on the levels of both exposures [10].

Arsenic induces its toxic impact by producing excessive ROS like hydroxyl radicals (OH) and superoxide anions (O^{2-}) and disturbs pro / antioxidant balance results in enhanced cellular ROS burden and decreased antioxidant status. The imbalance between pro/antioxidant may alter multiple cellular pathways such as expression of growth factors, inhibition of DNA repair, altered DNA methylation, suppression of cell cycle checkpoint proteins [11]. Incessant consumption of arsenic causes different types of chronic diseases like the Blackfoot disease, pigmentation, keratosis, nausea and most importantly cancer in humans [12]. Considering this, arsenic toxicity has become a serious problem worldwide and there is still no specific, reliable and safe treatment. Recently, treatment with phytochemicals has become one of the best choice for overcoming metal induced toxification especially those which follow a free radical mediated mechanism.

The polyphenol extract of the phytochemicals was found to attenuate As-induced lung injury through free radical scavenging mechanism [13]. Great efforts have been made in an attempt to find safe and potent natural antioxidants from plant sources. *Ocimum sanctum* Linn. (Labiatae), commonly known as holy basil, is a well documented Indian traditional medicinal herb used for the treatment of various stress-related conditions and is distributed worldwide. *O. sanctum* is an ''elixir of life'' and has been demonstrated to possess diverse pharmacological properties like antioxidant [14], genoprotective, anti-tumourrgenic and anti-inflammatory potential [15]. Eugenol, methyleugenol, orientin, vicenin, β- elemene, β- caryophyllene, ursolic acid, rosmarinic acid, flavonoids, tannins, luteolin, apigenin and ocimumosides are major bioactive phytochemical constituents of *O. sanctum* [16]. Eugenol and ursolic acid from *O. sanctum* have been reported to induce protection against free radical induced cellular damage [14]. Keeping in view the pharmacological properties of *Ocimum*, present investigation has been undertaken to assess the preventive efficacy of leaf extract of *Ocimum sanctum* against arsenic induced oxidative stress mediated histopathological and genotoxic insult in pulmonary tissue of balb/C mice.

MATERIALS AND METHODS:

Experimental Animals: Adult female balb/C mice weighing 25-30 gm will be used in the present study. Animals were kept in temperature- and humidity-controlled conditions on a 12:12-hr light-dark cycle. The animals were left 7 days for acclimatization before the beginning of the experiment. All the animals were housed, cared and used experimentally in accordance with the 'Guide for the Care and Use of Experimental Animals' approved by Institutional Animal Ethics Committee, Panjab University, Chandigarh.

Chemicals:

- Sodium arsenite (Mol.Wt.129.9) was obtained from Himedia Lab Pvt. Ltd. Mumbai.
- All other chemicals of analytical grades specifications were obtained from Sisco Research Laboratory, Central Drug House and Merck millipore.

Estimation of LD_{50} and Dose Determination: LD_{50} for sodium meta-arsenite has been calculated which comes out to be 15.71 mg/kg b.wt. by drawing probit analysis graph and using SPSS software [17]. For calculating LD_{50} a range of toxicity was established by giving different doses of arsenic to different groups of six mice each. For the present investigations $1/10^{th}$ of LD_{50} of sodium meta arsenite was given to arsenic treated group.

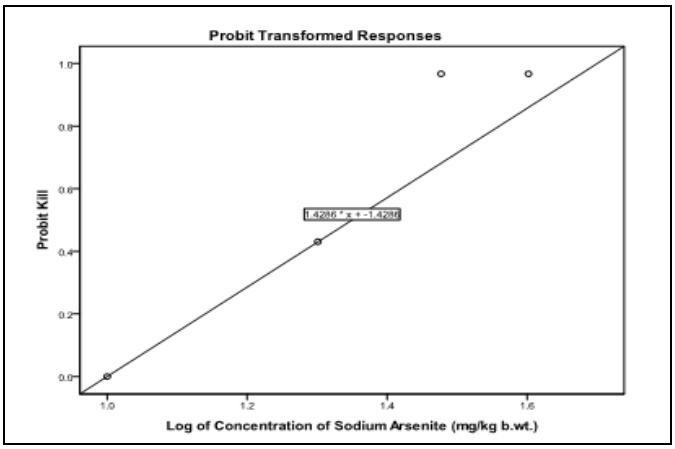

FIG. 1: PROBIT ANALYSIS CURVE OF LD_{50}

Preparation of Plant Extract: The leaves of *Ocimum sanctum* were collected in the month of September and October from and around the local areas of Panjab University, Chandigarh. The plant was identified by Mr. Gurudev (taxonomist), Department of Botany, Panjab University, Chandigarh and got voucher number 20520. The leaves were cleaned with distilled water and shade dried at room temperature. The shade dried leaves were powdered by using mixer grinder. The extract was prepared by using Soxhlet apparatus with 150 gm of powered leaves in 800 ml of absolute alcohol. The extract was filtered using Whatman filter paper and concentrated in rota evaporator at approximately 75 °C. A dose of 200 mg/kg b.wt. was dissolved in 0.05% DMSO and orally administered for 21 days.

Experimental Design:

Grouping of Animals: The animals were divided into following 5 groups of 5 animals each.

- **Group 1: Control Group:** Mice were given normal saline intraperitoneally for 10 days.
- **Group 2: Arsenic Treatment:** Mice were injected intraperitoneally with arsenic at a dose of 1.57 mg/kg b.wt. ($1/10^{th}$ of LD_{50}) for 10 days.
- **Group 3: DMSO Treatment:** 0.05% DMSO was given orally for 21 days.
- **Group 4: *Ocimum* Treatment:** Mice were given *Ocimum* (200 mg/kg b.wt.) for 21 days orally.
- **Group 5: *Ocimum* + As Treatment:** Mice were given *Ocimum* (200mg/kg b.wt.) orally for total 21 days, 11 days prior to arsenic exposure and for 10 days *Ocimum* and arsenic were given simultaneously.

At the end of each experiment, mice from each group were sacrificed. Lungs were removed and cleaned in normal saline. 10% homogenate was prepared in Tris - HCl buffer using homogenizer. The homogenates were used for spectrometric determination of lipid peroxidation (LPO), reduced glutathione (GSH) and protein concentration. The homogenates were centrifuged at 10000 rpm at 4 °C for 20 min and post mitochondrial supernatant was obtained for biochemical assays.

Phytohemical Screening: Presence of different phyto-constituents present in the extract was examined by phytochemical screening of the extract. Different chemical tests were performed using protocol of Harbone[18] for establishing the profile of ethanolic extract of *Ocimum sanctum.* Wagner's test was performed for detection of alkaloids, Molisch's test for presence of carbohydrates, Foam test for saponins, Alkaline reagent test for detection of flavonoids, Xanthoproteic test for proteins presence, Ninhydrin test for amino acid detection, ferric chloride test for presence of phenolic compounds, Salkowski test for detection of terpenoids, Magnesium and hydrogen chloride reduction test for glycosides and Gelatin test for presence of tannins.

Biochemical Parameters:

Oxidative Stress and Antioxidant Defence System: Lipid peroxidation, an index of oxidative stress, was analyzed in lungs of mice by method of Beuge and Aust [19]. Reduced glutathione was estimated in lungs according to the method of Beulter *et al.,* [20]. Assay of catalase and SOD activity in lungs were assessed in PMS by reported methods (Luck; Kono) [21, 22]. The activities of GST and GR in lungs were evaluated by methods of Habig *et al.* and Horn [23, 24]. Protein content was estimated by the method of Lowry *et al.,* [25].

Histopathological Studies: The histopathological alterations in lungs were evaluated by light microscopy (H and E staining) using method of Pearse [26]. Mice was dissected, pulmonary tissue taken immediately immersed in chilled saline, washed, cut into small pieces and fixed in bouin's fixative. The specimens were washed in tap water and then dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin. Sections of 5-6 µm thickness were cut by using a rotary microtone, stained with hematoxylin and eosin (H and E) dye and observed under light microscope.

Genotoxic Parameters:

DNA Fragmentation: Genomic DNA was extracted using standard Phenol: Chloroform: Isoamyl alcohol method by Sambrook *et al.*, [27]. DNA bands and fragments were observed in UV transilluminator and photographed on Gel-Doc system.

Statistical Analysis: All the values were expressed as Mean ± S.D. and statistical analysis was performed by one way ANOVA (analysis of variance) followed by Tukey's post hoc test. It is a parametric statistical analysis that compares between-and within-groups variance to measure differences between two or more groups. Value with $p \leq 0.05$ was considered as statistically significant, $p \leq 0.001$ very significant and $p \leq 0.0001$ extremely significant. The data analysis was done with SPSS version 21.

RESULTS:

Preliminary Phytochemical Analysis: Preliminary qualitative estimation of ethanolic extract of *Ocimum sanctum* (**Table 1**) revealed the presence of alkaloids, carbohydrayes, phenols, tannins, flavonoids, saponins, proteins, amino acids, phenols. The pharmacological properties of *Ocimum sanctum* are attributed to these phytochemicals.

TABLE 1: PHYTOCHEMICAL CONSTITUENTS OF ETHANOLIC EXTRACT OF *OCIMUM SANCTUM*

Phytochemical Constituents	Alkaloids	Carbohydrates	Phenols	Tannins	Flavonoids	Saponins	Proteins	Amino Acids	Terpenoids	Glycosides
Results	+	+	+	+	+	+	+	+	-	-

Biochemical Assays: The present study evaluated the protective efficacy of ethanolic extract against arsenic induced oxidative stress and imbalance of pro-and antioxidants. The results obtained from the study of subchronic exposure (10 days) of arsenic in balb/C mice and its successive modulation by *Ocimum sanctum* leaf extract treatment have been discussed with an intuition to correlate biochemical, histopathological and genomic changes.

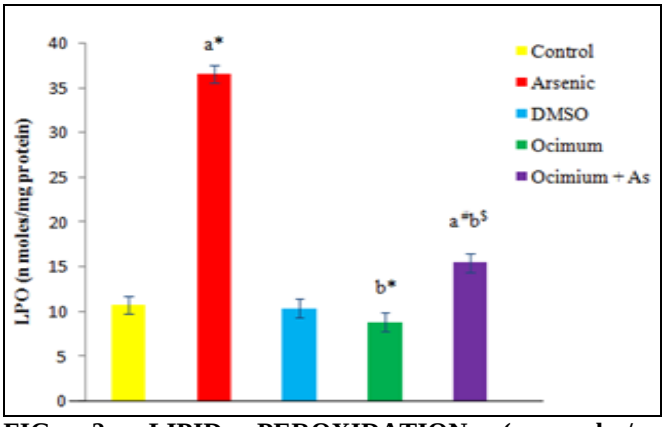

FIG. 2: LIPID PEROXIDATION (n moles/mg PROTEINS) IN LUNG OF CONTROL, AS, DMSO, OCIMUM AND OCIMUM + AS TREATED MICE

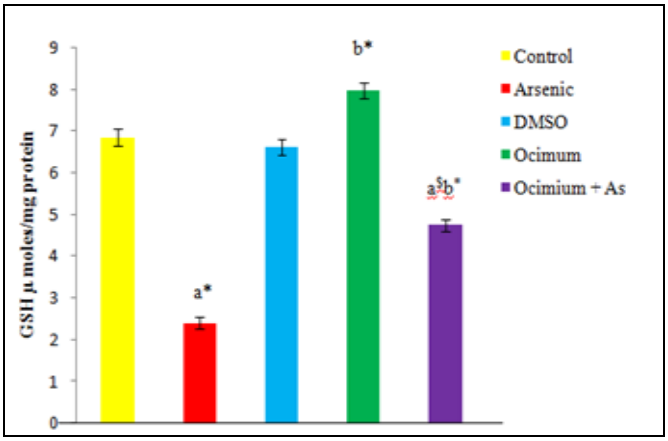

FIG. 3: LEVEL OF REDUCED GLUTATHIONE (µ moles/mg PROTEINS) IN LUNG OF CONTROL, AS, DMSO, OCIMUM AND OCIMUM + AS TREATED MICE

NOTE: Values are shown as Mean ± S.D. (n = 5)

ANOVA followed by tukey's honestly significant difference test

a: comparison with control group and b: comparison with arsenic treated group; **$**= $p \leq 0.05$ (statistically significant); **#**= ≤ 0.001 (very statistically significant); ***** = ≤ 0.0001 (extremely statistically significant).

Ten days arsenic intoxication in mice adversely affected redox status of lung tissue that led to extremely significant enhancement ($p \leq 0.001$) in

the levels of LPO in mice lungs and revealed two and half fold (240%) increase as compared to control group. Pre and concurrent supplementation of *Ocimum sanctum* (200mg/kg b.wt.) for 21 days significantly ($p \leq 0.001$) lowered the elevated pulmonary LPO levels by 196% ($a^{\#}$) as compared to control (**Fig. 2**). Administration of arsenic instigated a statistically significant decline (64% (a*) in levels of reduced glutathione in lung as compare to control group. Pre- and concurrent treatment of *Ocimum* (200mg/kg b.wt.) significantly ($p \leq 0.001$) raised the lowered levels of GSH by 34% ($b^{\#}$) as compared to arsenic treated mice (**Fig. 3**). A 53%(a*) and 60% (a*) decrease was observed in the levels of catalase and superoxide dismutase where as glutathione-S-transferase and glutathione reductase were lowered by 59%(a*) and 87% (a*) in lung tissue of arsenic administered mice as compared to control respectively. Prior and simultaneous treatment of *Ocimum sanctum* efficiently ($p \leq 0.001$) ameliorated the antioxidant alterations induced by arsenic and brought the decreased levels near control. *Ocimum* restored the pulmonary levels of CAT by 35% (b*), SOD by 33% (b*), GST by 65% ($b^{\$}$) and GR by 42% ($b^{\$}$) as compared to arsenic treated group. *Ocimum* treatment alone didn't show any significant alterations in the levels of all above assayed enzymes (**Table 2**).

TABLE 2: EFFECT OF ARSENIC TOXICITY ON ANTIOXIDANT DEFENSE SYSTEM AND ITS MODULATION BY *OCIMUM SANCTUM* IN LUNGS

Parameters / Groups	Catalase	Superoxide Dismutase	Glutathione-S-Transferase	Glutathione Reductase
Control	97.532±1.591	18.797±1.390	2.167±0.189	47.135±1.131
Arsenic	45.648±3.232a*	7.177±1.129a*	0.275±0.028a*	19.027±1.054a*
DMSO	97.592±3.417	18.727±0.619	2.145±0.217	47.247±1.294
Ocimum	97.837±1.873b*	18.892±2.318b*	2.182.169b*	47.139±1.103b*
Ocimum+ As	82.026±2.639$a^{\#}b$*	13.364±1.403$a^{\$}b$*	1.687±0.112$a^{\#}b^{\$}$	39.067±2.182$a^{\#}b^{\$}$

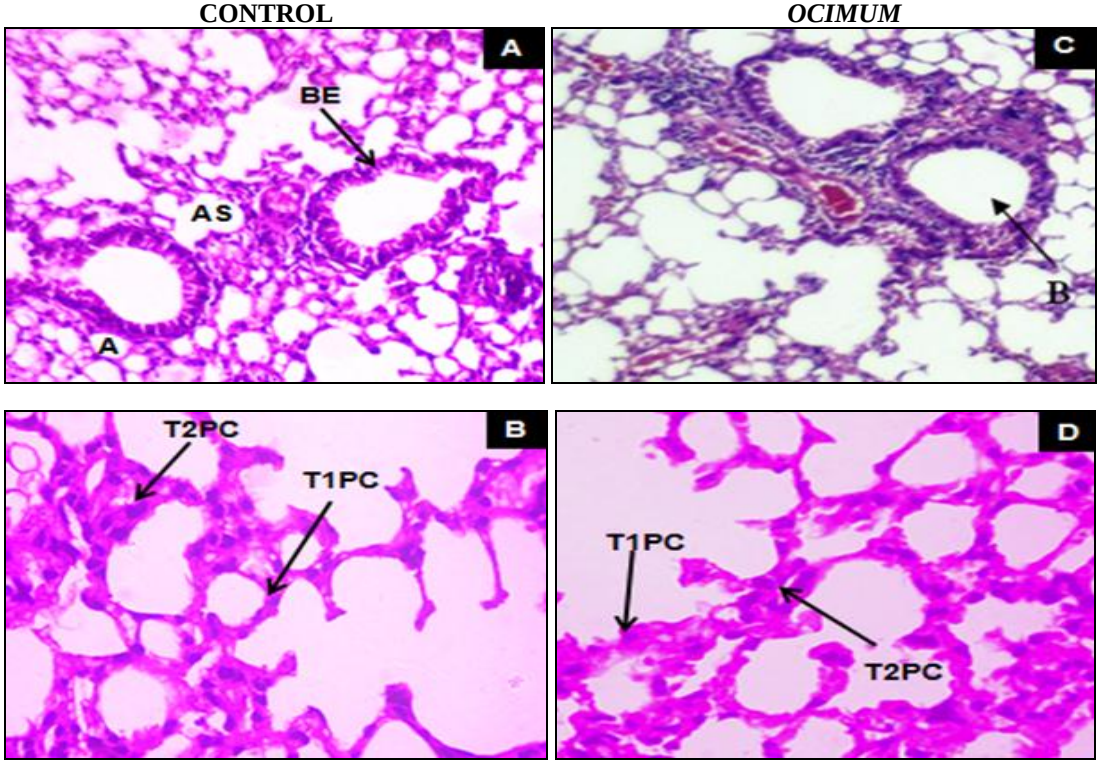

PLATE 1: LIGHT MICROGRAPHS SHOWING COMPARISON OF CONTROL AND *OCIMUM* TREATED MICE LUNG

Control group (A and B):- A: Terminal components of respiratory system showing normal alveolar sac and alveoli and normal cuboidal cells lining bronchiolar epithelium (100X). **B:** Normal type (T) 1 and 2 pneumocyte (400 X).

***Ocimum* group (C and D):- C:** Bronchioles with normal cuboidal epithelium (100X). **D:** Normal type 1 and 2 pneumocyte in alveolar wall (400X).

Abbreviations: A: Alveoli; AS: Alveolar sac; BE: Bronchiolar epithelium; PC: Pneumocyte; B: Bronchiole.

Histopathological Observations: The photo-micrographs from lung tissue of control group exhibited normal histo-architecture revealing squamous epithelium lined thin walled alveoli and capillaries, smaller bronchioles lined by simple cuboidal epithelium and thin layer of connective tissue (**Plate 1A**). The alveolar epithelium revealed two types of cells thin squamous cells called type I alveolar cells or type I pneumocytes interspersed with cuboidal cells called type II alveolar cells or type II pneumocyte (**Plate 1B**). Ten days arsenic administration instigated severe histological alterations in lung tissue revealing degeneration of bronchiolar epithelium, enhanced number of alveolar macrophage, reduction in alveolar space, conjunction of alveolar duct (**Plate 2A**) and alveolar hyperplasia of type II pneumocytes indicating involvement of inflammatory responses (**Plate 2B**). 11 days prior and 10 days simultaneous treatment of *Ocimum* prevented the As induced histological changes revealing normal alveolar space, alveolar duct, alveoli, well organized epithelia of bronchioles (**Plate 2C**) and reduced hyperplasia of pneumocytes (Type 1 and 2) in alveolar wall showing reduced inflammation (**Plate 2D**). *Ocimum* treatment preserved the normal histoarchitectutre of the lung tissue even after arsenic exposure. No remarkable histological changes were observed in DMSO and *Ocimum* treated pulmonary tissue of mice (**Plate 1C, D**).

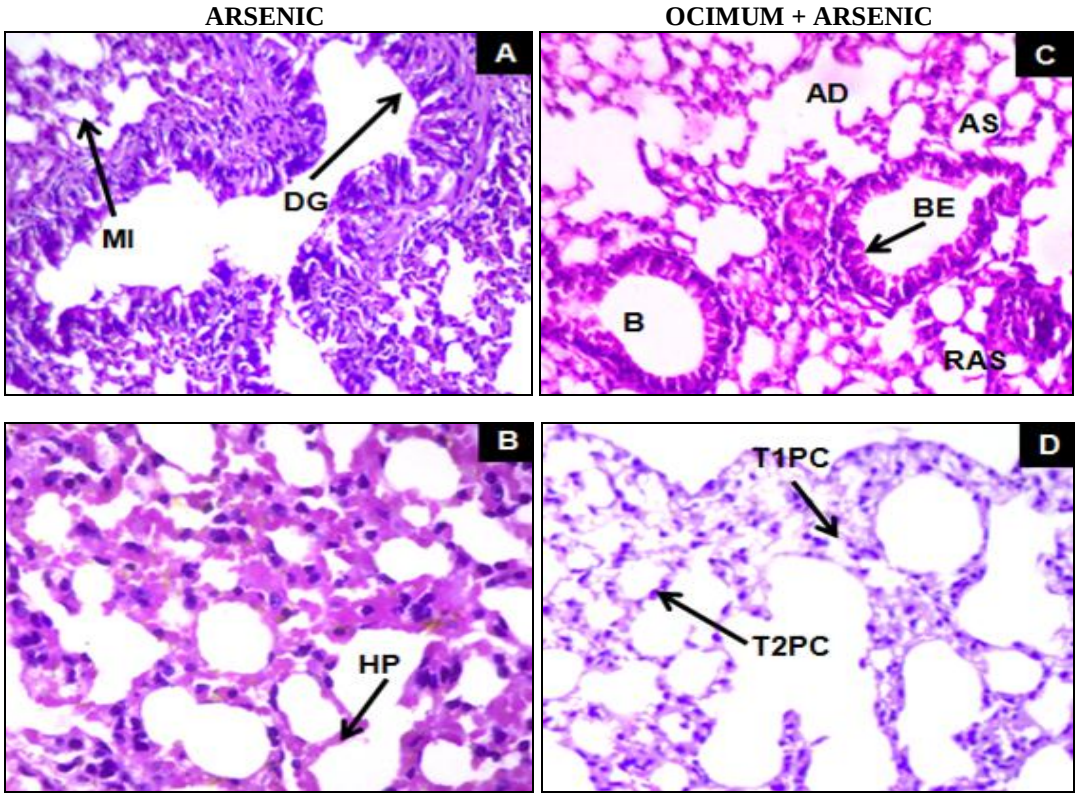

PLATE 2: LIGHT MICROGRAPHS SHOWING COMPARISON OF ARSENIC AND *OCIMUM* + ARSENIC TREATED MICE LUNG

Arsenic group (A and B):- A: Reduced alveolar space, degeneration of bronchiolar epithelium and macrophage infilteration (100X). **B:** Alveolar hyperplasia (HP) in type 2 alveolar cell (Pneumocyte) showing inflammatory response (400X).

***Ocimum* + Arsenic group (C and D): C:** Terminal components of respiratory system showing alveoli with reduced alveolar space, alveolar duct with well defined alveolar sac, bronchioles lined with well defined cuboidal epithelium (100X). **D:** Alveolar epithelium showing normal type 1 and 2 pneumocytes (400X).

Abbreviations: DG: Degeneration; HP: Hyperplasia; MI: Macrophage infilteration; RAS: Reduced alveolar space; AD: Alveolar duct; AS: Alveolar sac; B: Bronchiole; BE: Bronchiolar epithelium; PC: Pneumocyte.

Genotoxic Studies:

DNA Fragmentation: Arsenic exposure for 10 days produced large genomic DNA fragmentation in lungs revealing its genotoxic potential (Lane 3 and 4). Pre (11 days) and concurrent (10 days) administration of *Ocimum* in arsenic exposed animals revealed significant reduction in genomic damage in lungs (Lane 9 and 10) where as intact bands of DNA were observed in pulmonary tissue of control, DMSO and *Ocimum* treated mice (Lane

1 and 2, 5 and 6, 7 and 8). The present study indicates the genoprotective efficacy of *Ocimum* against free radical derived DNA damage.

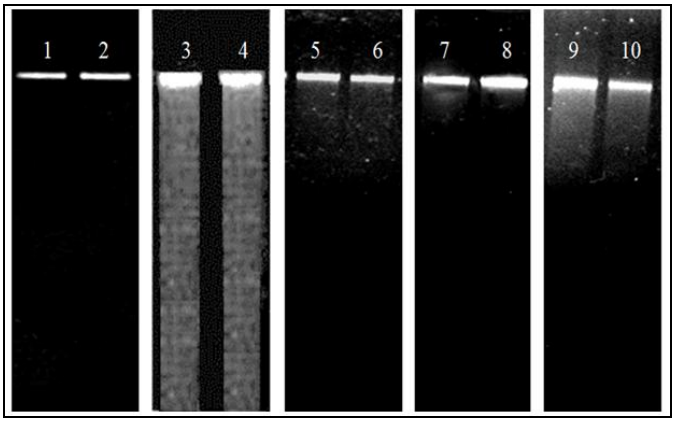

FIG. 3: STATUS OF DNA FRAGMENTATION IN LUNG TISSUE OF BALB/c MICE

Lane (1, 2)-Control; Lane (3, 4)-Arsenic; Lane (5, 6)-DMSO; Lane (7, 8)-Ocimum; Lane (9, 10)-Ocimum+As treated mice.

DISCUSSION: Ameliorating efficacy of *Ocimum sanctum* was evaluated against arsenic induced pleural toxicity. In the present study arsenic induced production of ROS caused marked elevation in LPO which is the most frequently documented and earliest consequence of arsenic toxicity [28]. These free radicals attack double bonds of polyunsaturated fatty acids and initiate a chain reaction that affect membrane integrity and cellular function which could cause peroxidation of membrane lipids, alter proxidant / antioxidants balance [29] and induce many pathophysiological alterations [30]. These observations are in conformity with the observation of Tandon *et al.*, who also evaluated the sodium arsenite induced cytological damage in rats [31].

Plausibly direct binding of arsenic to lipid membranes, due to its lipophilic nature might have enhanced the rate of lipid peroxidation. 11 days prior and 10 days (total 21 days) concurrent treatment of *Ocimum* showed a marked decline in the levels of lipid peroxidation in arsenic intoxicated mice. This protective potential of *Ocimum sanctum* could be attributed to its free radical scavenging ability due to presence of pharmacologically active constituents like eugenol, orientin and vicencin [32]. These active constituents of *Ocimum* might have chelated the metal ions and haulted the formation of free radicals by inhibiting step of the fenton reaction and possibly caused the lowering of LPO [33].

In the current study, a significant decline in GSH levels were observed in lung tissues of sodium arsenite intoxicated BALB/c mice. Antioxidant activity of GSH against ROS in cells has been attributed to its sulfahydryl group [34] that plays a crucial role in detoxification by forming conjugate with reactive metabolite or by donating proton to free radicals [35]. These observations agree well with previous studies of Nagashree *et al.*, who also documented arsenite induced low levels of GSH in albino rats [36]. GSH accounts for about 90% of the intracellular non-protein thiol content and participates in quenching of reactive oxygen species (ROS), xenobiotic detoxification and amino acid transport [37].

Ocimum treatment demonstrated marked elevations in the levels of GSH and antioxidant enzymes in arsenic intoxicated mice. Banu *et al*., demonstrated the therapeutic efficacy of *Ocimum sanctum* against arsenic induced toxicity in rats by enhancing both enzymatic and non enzymatic cellular antioxidants more or less to near normal levels and supported the present observation [38]. It has many bioactive components like eugenol, methyl eugenol luteolin, ursolic acid, b-caryophyllene, b-pinene rosmarinic acid, flavonoids and tannins. Thus alleviation in cellular antioxidant status is attributed to its free radical scavenging and metal chelating property of active components of *Ocimum.*

SOD and CAT are the major endogenous antioxidants enzymes that prevent oxidative cellular damage by scavenging ROS. SOD metabolizes the toxic superoxide radical into hydrogen peroxide [39] and catalase then decomposes hydrogen peroxide into water and oxygen [40]. A significant decline in the levels of both catalase and SOD was observed in pulmonary tissue of arsenic treated mice. This decline in their levels could be attributed to the excessive utilization of these enzymes in inactivating the free radicals generated due to arsenic intoxification [41]. Our results are well supported by Bashir *et al*., who have also documented arsenic induced decrease in the levels of SOD and CAT due to enhanced oxidative stress [42]. Glutathione-S-transferase plays a key role in cellular detoxification and conjugating reduced glutathione with a large number of electrophiles rendering them inactive [38]. Whereas Glutathione reductase is an integral component of cellular

antioxidant defense system which catalyzes the reduction of GSSG to the sulfahydryl reduced form glutathione by controlling the redox state of $NADP^+$/NADPH in tissues. In the present work, significant decrease in the levels of GST and GR was observed in lungs and these observations are in accordance with observations of Rodriguez *et al.*, and Messarah *et al.,* who documented decline in the levels of GST and GR in arsenic intoxicated mice [37, 43]. Arsenic mediated GSH depletion could have become responsible for observed decline in GST, GR levels.

Paralysed antioxidant and detoxifying system made the cell more susceptible to the attack of toxic compounds. The pre (11 days) and concurrent (10 days) treatment of *Ocimum* significantly restored the levels of antioxidant enzymes possibly by two ways, lowering the oxidative stress and decreasing the load on the endogenous defense system and furthermore, by strengthening the cellular detoxifying and antioxidant system. These observations lend support from few reports who documented the antioxidant and free radical scavenging potential of Eugenol and ursolic acid found in *Ocimum* extract and reported their protective efficacy against oxidative stress induced cellular damage [44].

Histopathological evaluation of pulmonary tissue from arsenic exposed mice vividly established arsenic as an effective pulmonary toxicant. The pulmonary histoarchitecture of mice revealed distorted and degenerated tissue histology. This could be strongly related to the high concentration of sulfhydryl compounds present in lung tissue which bound directly to arsenic and caused direct metal ion induced pathology in lungs. These observations are supported by NRC [45]. Moreover, decreased activity of endogenous enzymes and enhanced production of free radicals might have made the condition worst by enhancing lipid peroxidation and rendering no support against arsenic toxicity. *Ocimum* mediated improvement in lung histo-architecture could be due to direct chelation of metal ions reduced LPO, alleviated levels of endogenous antioxidants and reduced oxidative stress. Pharmacologically active components of *Ocimum* prevented arsenic induced structural changes by reducing oxidative stress by inhibiting fenton reaction derived formation of free radicals. A somewhat similar result was obtained by Baskaran *et al*. who documented the protective efficacy of *Ocimum* due to the presence of eugenol, in the extract that reduced the oxidative damage and preserved the normal histoarchitecture of the liver [46].

Present study established the genotoxic impact of arsenic by DNA fragmentation analysis and these observations are in close agreement with study of Dua *et al.,* who also demonstrated the arsenic induced genotoxicity in mice. The most plausible reason behind the genomic shearing could be the interaction between hydroxyl radicals and pyrimidine bases (principally thiamine) which resulted in the removal of hydrogen atom from methyl group and/or -C-H bond of deoxyribose that led to DNA breakage [47]. Biochemically superoxide radical is converted to hydrogen peroxide by superoxide dismutase, and if not broken down by catalase, then hydrogen peroxide could react with Fenton metal ions to produce hydroxyl radicals that might cause oxidative DNA damage [48]. The genoprotective potential of *Ocimum sanctum* could be attributed to its free radical scavenging activity that reduces oxidative stress and prevents metal induced genotoxicity. Presence of phenolic group in conjugation with double bond in orientin and vicenin might have promoted free radical scavenging activity and metal chelation property of *Ocimum* [49].

Thus present research work clearly demonstrated the alleviating potential of *Ocimum sanctum* leaf extract against arsenic induced biochemical, histopathological and genomic changes in pulmonary tissue in mice. It also established a strong correlation between arsenic induced imbalance of pro- and antioxidant status and oxidative insult generated histopathological and genotoxic alterations. *Ocimum sanctum* by re-establishing this balance could alleviate histoarchitectural and genotoxic changes.

CONCLUSION: Thus, this study establishes *Ocimum* as a potential antioxidant, pulmonary protective and genoprotective agent by demonstrating its pro- and antioxidant balancing property. Free radical scavenging, inhibition of fenton reaction, H-ion donation and reducing properties of many bioactive components of

Ocimum (eugenol, methyl eugenol, ursolic acid, b-pinene and rosmarinic acid) make it a strong candidate against metal induced toxicities.

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CONFLICT OF INTEREST: There is no conflict of interest.

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