Original Articles
Toxicology International, *May-Aug 2015 / Vol-22 / Issue-2, 88-96*
ISSN (Print) : 0971-6580
ISSN (Online) : 0976-5131
DOI: 10.22506/ti/2015/v22/i2/137668

Evaluation of Aldrin-Induced Oxidative Stress and Apoptosis in J774 Macrophages

Milindmitra K. Lonare*, Bhaskar Vemu, Amit K. Singh, Saloni Singla and Suresh K. Sharma

Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana - 141004, Punjab, India; milindmitra@gmail.com

Abstract

The present study was carried out to evaluate the aldrin-induced oxidative stress and apoptosis in murine macrophage (J774A.1) cells. Acute exposure of aldrin for 2 hrs was given to the J774A.1 cells cultured in DMEM with 10% FBS at 37oC and 5% CO2 in air with 95% relative humidity under in vitro system. Lethal concentration-50 (LC50) was calculated after exposure period as 7.24 µg/ml. Further, cells were exposed with three different concentration of aldrin (1.81, 3.65 and 7.24 µg/ml) and 0.1% DMSO was used as negative control. The antioxidant enzymes and non-enzymes were determined along with G6pDH, LDH and ALP enzymes in J774A.1 cells. Cells were monitored for cell morphology and apoptosis. Exposure of aldrin to J774A.1cells resulted in increase in lipid peroxidation and decrease in antioxidant enzyme/ non-enzymes system. Further it caused decrease in G6PDH enzymes activity and excess leakage of LDH and ALP enzymes. The aldrin-treated cells showed higher number of apoptotic cells with alteration in cell morphology indicating apoptotic and necrotic changes. These effects were noticed in dose dependant manner. In conclusion, the result of in vitro study suggests that the aldrin can induced the process of apoptosis and cell death through the generation of ROS and thereof oxidative insult in J774A.1 cells.

Keywords: Aldrin, Apoptosis, Antioxidant Enzymes, J774A.1 Cells, Oxidative Stress

Received: 27-07-2015 **Revised:** 10-08-2015 **Accepted:** 24-08-2015

1. Introduction

Pesticides are the group of chemicals widely used in the modern agricultural practices to enhance food production and to control vector born diseases and that contributes very large input of toxic chemicals in our environment.[1] Their usage has increased manifolds in recent years in disease control management without considering their potential harmful side effects on plants, animals and human beings. [2] The use of pesticides may accumulate into the food to a toxic level and affect the public health[3] contributing significantly to many diseased states such as cancer, neurodegeneration, autoimmune diseases, reproductive disorder and other chronic health effects.[4-8] Exposure to pesticides can also induce oxidative stress by increased production of free radicals. These radicals may alter antioxidant system and compromise detoxification mechanism and thereby increasing lipid peroxidation.[9] Oxidative stress seems to be play important role in the toxicity of various pesticides such as organophosphates, carbamates, organochlorines, pyrethroids and neonicotinoids and has been reported by many workers.[7,10] Apoptosis is an active process of cellular suicide that is triggered by a variety of physiological and chemical stress stimuli. Apoptosis is involve in the interaction between host cells and environmental toxicants, as toxicants appear to be capable of manipulating host cell apoptotic pathways.[11]

Organochlorine pesticides (OCPs) including aldrin, dieldrin, endosulphan, lindane and many more remain

* *Author for correspondence*

ubiquitous in the environment such as in food and other products due to their resistance to degradation and bioaccumulation along the food chain.[12,13] However, aldrin and dieldrin are chloroorganic insecticides which are recognised as endocrine disruptors.[14] Aldrin and other organochlorine compounds residue persist in reproductive organ of aquatic species indicating increasing risk of infertility.[3] Dieldrin and lindane induces neurotoxicity through the oxidative stress and mitochondrial dysfunction.[12] It has been cleared that subchronic DDT and lindane exposure causes immunotoxicity through lipid peroxidation and free radicals generation.[15]

Macrophages constitute the central cellular compartment of the mononuclear phagocytic system. They are unique among all other immune cells and they can enter any tissue and reside there as tissue macrophages, adapting and showing characteristics depending on the tissue where they populate such as Kupffer cells (liver), mesangial cells (kidney), microglial cells (brain), alveolar macrophages (lungs), and osteoclasts (bone). Macrophages scavenge for dead cells, as well as any foreign particles very promptly. The nature of the material phagocytosed would determine whether the macrophage would become activated or not. Activated macrophages produce inflammatory molecules that signal other cells of the innate and adaptive immune systems. Activated macrophages generate reactive oxygen species (ROS) in a phenomenon called "oxidative burst", which helps in the killing of ingested microbes.[16] However, excess exposure of toxicants or pesticides delivered *in vivo* by the systemic route or in a local compartment would undoubtedly be intercepted the mononuclear phagocytic system resulted into cell damage to them causing impairment in immune status may be responsible systemic toxic effect.[17]

However, no work has been done in this direction on aldrin. Therefore, the present *in vitro* study was undertaken to evaluate the interaction of aldrin with murine macrophage (J774A.1) cells, as well as to investigate the status of antioxidant system and apoptosis with aldrin toxicity.

2. Material and Methods

2.1 Chemicals and Plasticwares

Except where otherwise indicated, all the chemicals and media used for the *in vitro* study were purchased from HiMedia laboratories Pvt. Ltd. (India). Fetal bovine serum (FBS) and the sterile material used for culture (flasks and culture plates) were procured from MP Biomedicals (USA) and HiMedia laboratories Pvt. Ltd. (India), respectively.

2.2 Cell culture

J774A.1 cells were obtained from the National Centre for Cell Science, Pune, India and cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 1% l-glutamine, 1% Penicillin/Streptomycin solution and 10% Fetal Bovine Serum (FBS) at 37°C in a CO_2 incubator with 5% CO_2 and 95% relative humidity in air. When the cell culture reached more than 80% confluence, cells were dispersed with 0.025 M Trypsin-EDTA and reseeded in new flask. The culture medium was replaced every 72 h.

2.3 Neutral Red (NR) Uptake Assay

For lethal concentration-50 (LC_{50}) determination, J774A.1 cells were harvested from subconfluent monolayers after exposure to Trypsin-EDTA at 37°C. The resuspended cells were seeded in 96-well microplates, at an approximate initial density of 2 x 10^5 cells per well, on the same growth medium. When monolayers reached 80% confluency, these cells were treated with different concentrations of aldrin (Sigma, USA). Aldrin was dissolve in DMSO first and then DMEM medium supplemented with 10% of FBS to prepare different concentrations ranging from 0.25 to 250 µg/ml and were added to the cell cultures plate (after aspirating their medium) in a total volume of 200 µl/well. After two hours of incubation, cell viability was quantified by neutral Red (NR) uptake assay for LC_{50} as per the standard protocol.[18] The values of the percentages of cell viability were plotted against aldrin concentrations. Experiment was carried out in triplicate to determine LC_{50}. The DMSO (0.1%) was used as negative control. The calculated LC_{50} value of aldrin was 7.24µg/ml. Further, LC_{25} and $LC_{12.5}$ was calculated and used for further experiment.

2.4 Experimental Protocol

J774A.1 cells were treated with three different concentrations (1.81, 3.65 and 7.24 µg/ml) of aldrin based on LC_{50} value. Aldrin concentration was prepared in DMSO as describe earlier. J774A.1 cells were plated at a concentration of 2×10^5 cells/ml in a 24 well culture

plates and incubated at 37°C in a 5% CO_2 in incubator. Cells were exposed to aldrin for 2 h (short-term) after 80 % of confluency. Untreated cells were used as the control and DMSO (0.1%) was used as negative control for each experiment. After exposure period, culture medium was separated out and adherent cells were treated with 200 µl cell lysis buffer (0.5% Triton X100 with NET: 20 mM Tris, 100 mM NaCl and 1 mM EDTA, pH 7.5) to each well and supernatant was collected in microcentrifuge tube. Toxic effect on macrophage cells such as cell morphology and apoptosis were evaluated in adherent cells. Oxidative stress indicators and cytotoxicity markers were determined in culture medium and cell lysate.

2.5 Cellular morphology assay

Aldrin-induced morphological alterations in J774A.1 cells were examined under an inverted phase contrast microscope (Nikon ECLIPSE-Ti, Japan) after completion of exposure period. Following visual observations codes in the description of cell culture conditions were used. Numerical scoring of the cells were determined and documented as follows:

Note Code	Note Text
1	Normal Cell Morphology
2	Low Level of Cell Toxicity
3	Moderate Level of Cell Toxicity
4	High level of Cell Toxicity
1P	Normal Cell Morphology with Precipitate
2P	Low Level of Cell Toxicity with Precipitate
3P	Moderate Level of Cell Toxicity with Precipitate
4P	High level of Cell Toxicity with Precipitate
5P	Unable to View Cells Due to Precipitate

2.6 Apoptosis Assay

J774A.1 cells were harvested after treatment period and washed twice with PBS and gently mixed with a 2 µl mixture of acridine orange (AO) (1 µg/mL) and ethidium bromide (EB) (1 µg/mL) dye solution for 1-2 minute as per the standard protocol and observed under a fluorescence microscope (Nikon ECLIPSE-80i, Japan) to assess the apoptotic changes.[19]

2.7 Lactate Dehydrogenase and Alkaline Phosphatase Assay

After treatment period, the release of total amount of lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) in a medium was estimated as per the standard commercial kit (Ebra Mannheim, Germany). The absorbance was measured with a spectrophotometer. Cytotoxicity is expressed relative to the basal lactate dehydrogenase and alkaline phosphatase release by untreated control cells.

2.8 Glucose-6-Phosphate Dehydrogenase Activity Determination

Activity of glucose-6-phosphate dehydrogenase (G6PDH) was measured via the absorbance of NADPH generated in the reaction between G6PDH and its substrates glucose-6-phosphate (5 mM) and NADP (1 mM). The reaction mixture included Tris-Hepes 200 mM, pH 7.4 and 0.1 mM Na2EDTA. Absorbance was measured at 340 nm. G6PDH activity was expressed in nM NADPH/h/mg protein.[20]

3. Measurement of Antioxidant Enzymes/or Non-Enzymes

3.1 Catalase Activity Assays

Catalase (CAT) activity was determined spectrophotometrically as per the standard method.[21] Cell lysate was diluted with 50 mM phosphate buffer (pH 7.0). The exponential decrease of 10 mM hydrogen peroxide was measured at 240 nm in the presence of cell homogenate. Reaction mixtures without cell homogenate were used as tissue blanks.

3.2 Glutathione Peroxidase Activity Assay

Glutathione peroxidase (GPx) activity was determined by the method of Paglia and Valentine (1967).[22] Cell lysate was diluted with phosphate buffer (pH 7.6). Tert-butyl hydroperoxide was first reduced by glutathione peroxidase and then recycled by glutathione reductase coupled with nicotinamide adenine dinucleotide phosphate (NADPH) oxidation. The rate of decrease in NADPH was monitored spectrophotometrically at 340 nm as a measure of GPx activity.

3.3 Superoxide Dismutase Activity Assay

Superoxide dismutase (SOD) was estimated as per the method described by Madesh and Balasubramanian

(1998).[23] It involves generation of superoxide by pyrogallol auto-oxidation and the inhibition of superoxide dependent reduction of the tetrazolium dye MTT [3-(4-5 dimethyl thiazol 2-xl) 2,5-diphenyl tetrazolium bromide] to its formazan, measured at 570 nm. The reaction was terminated by the addition of dimethyl sulfoxide (DMSO), which helps to solubilize the formazan formed. The activity was expressed as SOD Units/mg protein (one unit of SOD is the amount (in mg) of protein required to inhibit the MTT reduction by 50 %).

3.4 Glutathione S-Transferase (GST) Activity

GST activity was determined by the method of Habig et al. (1974).[24] The reaction was initiated by adding 0.1 ml of GSH (30 mM) to the mixture. The change in absorbance was recorded at 340 nm at an interval of 30 sec for 5 min. The enzyme activity has been expressed as µM CDNB-GSH conjugate formed/min/mg protein, using a molar extinction coefficient of 9600 $M^{-1}cm^{-1}$.

3.5 Reduced Glutathione (GSH)

GSH was determined by estimating free-SH groups, using DTNB (dithiobis-nitro-benzoic-acid) method.[25] The absorbance of reactant mixture was read at 412 nm within 5 min. Reagent blank contained no sample and sample blank was without DNTB. The concentration of GSH was calculated using the extinction coefficient 13000/M/cm and the results were expressed as mM of GSH per gm protein.

3.6 Lipid Peroxidation

Lipid peroxidation (LPO) was measured in terms of malondialdehyde (MDA) production.[26] Lysate was incubated at 37 ± 0.5^{0}C for 2 h. To each sample, 250 µl of 10% trichloroacetic acid was added. The mixture was thoroughly mixed and centrifuged at 2000 rpm for 10 min. To 250 µl of supernatant, an equal volume of 0.67% thiobarbituric acid was added and kept in boiling water bath for 10 min, cooled and diluted with 250 µl distilled water. The absorbance was read at 535 nm. The amount of LPO was expressed as nanomoles of MDA formed per g protein.

3.7 Protein Determination

Protein levels were determined by the Lowry method with bovine serum albumin used as standard. This mixture was incubated at room temperature for 5 to 10 minutes and the optical density was measured at 750 nm.[27]

3.8 Statistical Analysis

Data obtained is represented as mean±standard deviation (SD) of at least three replications. Statistical significance was tested by one-way ANOVA and Tukey (HSD) and p-values inferior to 0.05 were considered significant.

4. Results

4.1 Effect of Aldrin on Cell Morphology

Cytotoxic effect of aldrin was assessed in primary cell cultures of murine macrophages (J774A.1 cells) by the NR Uptake cytotoxicity test and found that an exposure period of 2 h was sufficient to reduce cell viability. The LC_{50}, LC_{25} and $LC_{12.5}$ values were determined and were found to be 7.24, 3.65 and 1.81 µg/ml, respectively. The exposure of these concentration of aldrin to J774A.1 cells resulted in progressive alterations of cellular morphology such as cytoplasm vacuolization, shrinkage, formation of blebs on cell surface including reduction of nuclear sizes, which often displayed diverse degrees of chromatin condensation and detachment of cells (Figure 1: B, C, D). Some areas devoid of cells were also noticed in the same culture. In control cells, no alterations were observed (Figure 1: A). Numerical scoring of the cells of visual observations was documented (Table 1) in J774A.1 cells after completion of exposure period at different concentration of aldrin.

Table 1. Scoring of the cells morphology after visual observations of J774A.1 cells

Treatments	Note Codes (visual observations)	
Control	1	-
Vehicle control	1	1P
Aldrin-I (1.81 µg/ml)	2	2P
Aldrin-II (3.65 µg/ml)	3	3P
Aldrin-III (7.24 µg/ml)	4	4P

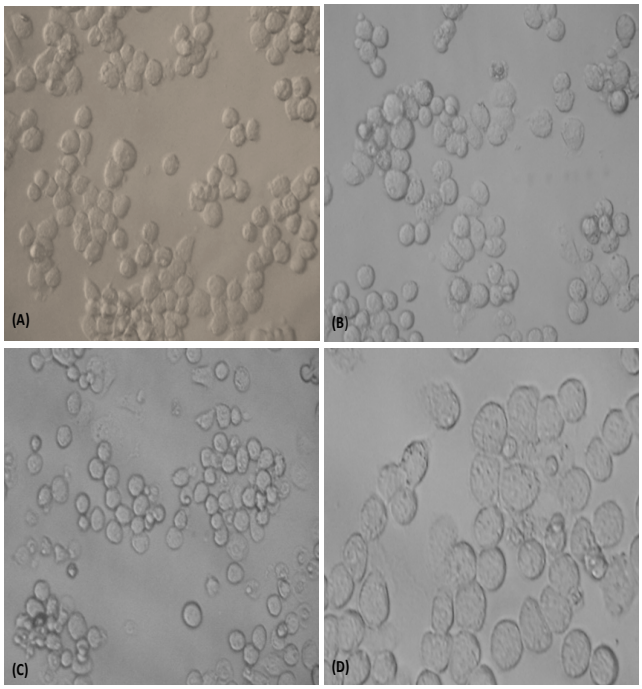


Figure 1. Representative photomicrographs under inverted microscope in control and aldrin exposed J774A.1 cells. (A) Showing normal cellular morphology of J774A.1 cells in the control group (20x). (B, C & D) J774A.1 cell showing varying degree of cell damage, shrinkage, chromatin condensation and the formation of blebs on the cell surface in aldrin treated cells (B & C: 20x; D: 40x).

4.2 Effect on Cell Apoptosis

In order to determine whether aldrin induces morphological alterations that could be attributed to an apoptotic mechanism, J774A.1 cells were treated with the aldrin for short periods and then stained with the nucleic acid-binding fluorochromes, AO and EB. Control untreated cells exhibited a higher number of green fluorescence due to exclusion of ethidium bromide but not of acridine orange. Viable cells showed a green nuclear fluorescence with intact structure (Figure 2: A). Aldrin treated cells showed typical features of apoptosis and necrosis. Apoptotic cells exhibited a bright green-orange nucleus showing condensation of chromatin (Figure 2: B, C). Some condensed nuclei were stained with orange fluorescent dye with ethidium bromide, indicating compromised membrane integrity, probably produced after the first stages of nuclear apoptotic condensation in treated cells. Late apoptotic cells have an orange nucleus showing condensation of chromatin and necrotic cells displayed as red fluorescent nucleus (Figure 2: C, D).

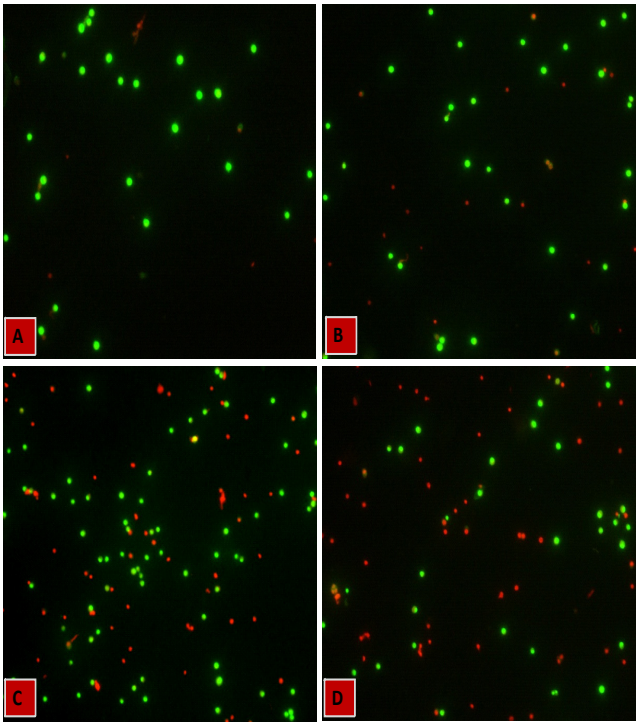


Figure 2. Representative fluorescent-micrographs showing cell apoptosis assayed by acridine orange and ethidium bromide staining. (A) Micrograph showing from lives cell population from control group (AO/EB staining: 20x). (B, C & D) Micrograph showing different degree of live, apoptotic and necrotic cell population after aldrin treatments (AO/EO staining: 20x). Viable cells showed green fluorescent nucleus; apoptotic cells with green-orange nucleus; non viable cells showed red fluorescence nuclei.

4.3 Effect on Antioxidant System

LPO in terms of malondialdehyde (MDA) was estimated in J774A.1 cells lyaste at end of exposure period and was found to be higher ($p < 0.05$) in aldrin treated cells, whereas, lower in control cells. Exposure of aldrin resulted in decrease ($p < 0.05$) in GSH level and CAT activities in all doses in dose dependant manner when compared with control group. A dose dependant decreased in SOD, GPx and GST activities in cells were observed in aldrin treatments (Table 2).

4.4 Effect on Cytotoxicity Indicator Enzymes

Exposure of aldrin to J774A.1 cells resulted in increase ($p < 0.05$) in ALP activity in dose dependent manner; while, the ALP activity was found to be lower in control group (Table 2). LDH activity was also increased ($p <$

Table 2. Status of cytotoxicity marker enzymes and antioxidant system in aldrin exposed J774A.1 cells

Treatment Groups	Parameters								
	ALP	LDH	G6PDH	LPO	GSH	CAT	SOD	GPx	GST
Control	2.50±	1.94±	22.07±	5.77±	0.099±	4.35±	0.268±	20.21±	0.0180±
	0.24	0.08	0.46	0.304	0.001	0.03	0.010	0.56	0.0002
Vehicle control	2.41±	1.99±	21.86±	5.99±	0.100±	4.54±	0.239±	20.23±	0.0186±
	0.05	0.11	0.82	0.28	0.003	0.07	0.007	0.51	0.0006
AD-I (1.81µg/ml)	4.52±	3.40±	16.19±	10.42±	0.070±	3.61±	0.188±	18.19±	0.0141±
	0.02	0.23*	0.16*	0.92*	0.001*	0.12*	0.001*	0.29*	0.0002*
AD-II (3.62 µg/ml)	5.87±	4.76±	14.57±	14.62±	0.059±	2.37±	0.158±	13.95±	0.0118±
	0.27*	0.31*	0.72*	1.38*	0.001*	0.11*	0.002*	0.36*	0.0005*
AD-III (7.23 µg/ml)	10.34±	8.26±	12.59±	24.54±	0.039±	1.40±	0.119±	10.75±	0.0059±
	0.38*	0.46*	0.63*	.50*	0.005*	0.04*	0.003*	0.24*	0.0004*

Each column represents mean ± SEM. * Superscript represents significant difference ($p < 0.05$) between groups when compared with control within a column. Assays were performed in 3 replicates from 3 independent experiments. ALP: alkaline phosphatase (KA Units/mL); LDH: lactate dehydrogenase (U/l); G6PDH: Glucose-6-phosphate dehydrogenase (U/l); LPO: lipid peroxidation (nmol of MDA/mg of protein); GSH: glutathione reduced (nmol of GSH/mg of protein); CAT: catalase (nM H2O2 utilized/min/mg protein); SOD: superoxide dismutase (Units/mg protein); GPx: glutathione peroxidise (µM NADPH oxidized to NADP/min/mg protein); GST: glutathione S-transferase (µmol/h/mg protein).

0.05) in aldrin treated cells as compared to the control. However, G6PDH activity was lowered ($p<0.05$) amongst the aldrin treated cells at end of exposure period in a dose dependant manner (Table 2).

5. Discussion

Oxidative stress in cell is a result of either an increase in ROS, an imbalance of intracellular antioxidant defense systems, or inability of the cell to repair oxidative damage. A number of recent studies reported the ability of organochlorine pesticides (e.g., endrin, lindane) to induce oxidative stress in different organ systems.[12,15, 28, 29]A cell defends itself against ROS by elaborate systems of biological defence. These systems include the inducible antioxidant enzymes, such as SOD, CAT and GPx, which suppress the formation of free radicals. Endosulfan caused oxidative stress in tissues, as demonstrated by lipid peroxidation and altered redox status.[30] Further, dieldrin and lindane work cooperatively to induce neurotoxicity through the induction of oxidative stress and mitochondrial dysfunction.[12] Organochlorine compounds (DDT and lindane) exposure resulted in lipid peroxidation, alteration in SOD activity and humoral immune suppression through generation of free radicals.[15] The purpose of our study was to clarify the nature of the aldrin on first defence system of body i.e. on macrophages taking into account the possible role of oxidative stress in an imbalance between ROS and antioxidant systems in the J774A.1 cells. We monitored the levels of endogenous enzymatic antioxidants GP_X, SOD, CAT, GST and non-enzymatic antioxidant GSH in aldrin-exposed J774A.1 cells. It was observed that acute exposure of aldrin to J774A.1 cells decrease the GP_X, SOD, CAT, GST enzyme levels and GSH content in addition to increase in lipid peroxidation. Similarly, other studies revealed decrease lung GP_X in in rat *in vivo* and fish adrenocortical cells *in vitro* in endosulfan.[31,32] Previous studies have reported that exposure to pesticides leads to oxidative stress.[33] Accumulation of oxygen free radicals in cells, leading to tissue damage as a result of oxidative binding of key intracellular molecules containing thiol groups like GSH and lipid peroxidation of biological membranes which might be of greatest importance in the cytotoxicity of pesticides and can be eventually responsible for cellular death.[34]

Increasing evidence provides support that lipid peroxidation and the resulting oxidative stress, play a pivotal role in apoptosis.[35,36] The published studies have demonstrated that ROS serve as a signal molecule for induction of apoptosis by activating an array of cell signalling molecules, such as caspases and kinases.[37] Pesticide/ toxicant responsible for the inhibition of GPx a particularly determines an increment and accumulation of intracellular damage, in that the cell dies or cannot complete its reproduction. Thus, pesticides are able to induce of apoptotic cell death.[29,38] In our study, the increase in apoptotic cells was observed with depressed enzymatic activity of G6PDH and increased level LDH along with ALP were detected in aldrin exposed cells. Here, G6PDH

is the key enzyme of the pentose phosphate pathway in carbohydrate metabolism, and it plays an important role in cell proliferation and antioxidant regulation within cells in various organs.[39] G6PDH activity is primary source of the reduced form of nicotinic adenine dinucleotide phosphate (NADPH) and that is an essential supplier of reducing equivalents. It is necessary for the adequate function of oxidative phoshorylation and antioxidative defense, including the conversion of glutathione from its oxidized form to its reduced form (GSH).[40] In present study clearly indicated that decrease in G6PDH activity responsible for depletion of GSH. GSH depletion reflects intracellular oxidation/peroxidation as a result of aldrin exposure in macrophages. Similarly, increase in level of LDH with use of alpha-cypermethrin pesticides has been reported in wistar rat, which indicate a shift towards anaerobiosis due to tissue cytotoxicity.[41] Decreased in the reduced GSH in rat erythrocytes has been reported with pyrethroids exposed animals.[42] Previous studies showed significant reduction in the GST activity in insecticides exposed workers together with a non-significant reduction in fungicides exposed workers.[43,44] This decrease in GST activity could be attributed to the direct binding of pesticide with GST.[43,45] Reduced glutathione (GSH) is the main non-enzymatic antioxidant defence within the cell, reducing different peroxides, hydroperoxides and radicals.[46]

In our study, a significant correlation was found between aldrin-induced oxidative stress production and aldrin-induced apoptosis. Similarly, maximum oxidative stress/ROS production and apoptosis induction was observed after incubation with DDT and its metabolites under *in vitro*.[47] Further, results have been reported with agents like dieldrin, endosulfan and DDT that are able to induce oxidative stress and apoptosis.[48-50] In conclusion, our data show an involvement of the preventive antioxidant enzymes/non-enzymes in defence against toxic effect of aldrin, suggesting that the aldrin can damage J774A.1 cells in the process of apoptosis through the generation of ROS and thereof oxidative stress. This is especially important since oxidative stress is thought to play a vital role in immunoinhibition by causing damage to metabolic and cellular enzymes of cells responsible for apoptosis and cell death in the presence of Aldrin and thus, inhibit the functions of macrophages.

6. References

1. Chaiyarat R, Sookjam C, Eiam-Ampai K, Damrongphol P. Organochlorine pesticide levels in the food web in rice paddies of Bueng Boraphet wetland, Thailand. Environ Monit Assess. 2015; 187(5):4469.
2. Badr A, Elkington TT. Antimitotic and chromo, toxic effects of isoproturon in A. cepa and H. vulgare. Environ Exp Bot. 1982; 22:265–70.
3. Gui D, Yu R, He X, Tu Q, Wu Y. Tissue distribution and fate of persistent organic pollutants in Indo-Pacific humpback dolphins from the Pearl River Estuary, China. Mar Pollut Bull. 2014; 86(1-2):266–73.
4. Iyer P. Developmental and reproductive toxicology of pesticides. In: Krieger R editor. Handbook of Pesticide Toxicology, Academic Press, San Diego, 2010; 1:375–420.
5. Parron T, Requena M, Hernández AF, Alarcon R. Association between environmental exposure to pesticides and neurodegenerative diseases. Toxicol Appl Pharmacol 2011; 256: 379–85.
6. Hernandez AF, Lacasana M, Gil F, Rodriguez-Barranco M, Pla A, Lopez-Guarnido O. Evaluation of pesticide-induced oxidative stress from a gene–environment interaction perspective. Toxicol. 2013; 307:95–102.
7. Lonare M, Kumar M, Raut S, Badgujar P, Doltade S, Telang A. Evaluation of imidacloprid-induced neurotoxicity in male rats: A protective effect of curcumin. Neurochem Inter. 2014; 78:122–9
8. Bedia C, Dalmau N, Jaumot J, Tauler R. Phenotypic malignant changes and untargeted lipidomic analysis of long-term exposed prostate cancer cells to endocrine disruptors. Environ Res. 2015; 140:18–31.
9. Abdollahi M, Ranjbar A, Shadnia S, Nikfar S, Rezaiee A. Pesticides and oxidative stress: a review. Med Sci Monit. 2004; 10:RA141–7.
10. Raina R, Verma PK, Pankaj NK, Prawez S. Induction of oxidative stress and lipid peroxidation in rats chronically exposed to cypermethrin through dermal application. J Vet Sci. 2009; 10:257–9.
11. Gao LY, Abu Kwaik Y. Hijacking of apoptotic pathways by bacterial athogens. Microb Infect. 2000; 2:1705–19.
12. Sharma H, Zhang P, Barber DS, Liu B. Organochlorine pesticides dieldrin and lindane induce cooperative toxicity in dopaminergic neurons: Role of oxidative stress NeuroToxicol. 2010; 31(2):215–22
13. Tobiszewski M, Orlowski A. Multicriteria decision analysis in ranking of analytical procedures for aldrin determination in water. J Chromatogr A. 2015; 1387:116–22.
14. Wrobel MH, Grzeszczyk M, Mlynarczuk J, Kotwica J. The adverse effects of aldrin and dieldrin on both myometrial contractions and the secretory functions of bovine ovaries and uterus in vitro. Toxicol Appl Pharmacol. 2015; 85(1):23–31.
15. Koner BC, Banerjee BD, Ray A. Organochlorine pesti-

cide-induced oxidative stress and immune suppression in rats. Indian J Exp Biol. 1998; 36(4):395–8.
16. Naqvi S, Samim M, Abdin MZ, Ahmed FJ, Maitra AN, Prashant CK, Dinda AK. Concentration-dependent toxicity of iron oxide nanoparticles mediated by increased oxidative stress; Inter J Nanomed. 2010; 5:983–9.
17. Mahajan S, Prashant CK, Koul V, Choudhary V, Dinda AK. Receptor specific macrophage targeting by mannose-conjugated gelatin nanoparticles: An in-vitro and in-vivo study. Curr Nanosci. 2010; 6(4):413–21.
18. Borenfreund E, Puerner J. Toxicity determined in vitro by morphological alterations and neutral red absorption. Toxicol Lett. 1985; 24:119–24.
19. Spector DL, Goldman RD, Leinwand LA. Morphological assessment of cell death. In: Spector DL, Goldman RD, Leinwand LA editors. Cells, a laboratory manual, in culture and biochemical analysis of cells. NY: Cold Spring Harbor Laboratory Press; 1997; 2:15.3–15.10.
20. Bergmeyer H. Methods of enzymatic analysis. New York: Academic Press. 1974.
21. Aebi H. Catalase in vitro. Methods Enzymol. 1984 ; 105:121–6.
22. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med. 1967; 70:158–69.
23. Madesh J, Balasubramanian KA. Microtitre plate assay for superoxide dismutase using MTT reduction by superoxide. Indian J Biochem Biophys 1998; 35: 184–8.
24. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem. 1974; 249:7130–9.
25. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem. 1968; 25:192–205.
26. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979; 95:351–8.
27. Lowry, OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin- Phenol reagent. J Biol Chem. 1951; 193:265–75.
28. Bachowski S, Xu Y, Stevenson DE, Walborg EF Jr, Klaunig JE. Role of oxidative stress in the selective toxicity of dieldrin in the mouse liver. Toxicol Appl Pharmacol. 1998; 150(2):301–9.
29. Kitazawa M, Anantharam V, Kanthasamy AG. Dieldrin-induced oxidative stress and neurochemical changes contribute to apoptotic cell death in dopaminergic cells. Free Radic Biol Med. 2001; 31:1473–85.
30. Hincal F, Gurbay A, Giray, B. Induction of lipid peroxidation and alteration of glutathione redox status by endosulfan. Biol Trace Elem Res. 1995; 47(1-3):321–6.
31. Dorval J, Leblond VS, Hontela A. Oxidative stress and loss of cortisol secretion in adrenocortical cells of rainbow trout (Oncorhynchus mykiss) exposed in vitro to endosulfan, an organochlorine pesticide. Aquat Toxicol. 2002; 63(3):229–41.
32. Pandey S, Ahmad I, Parvez S, Bin-Hafeez B, Haque R, Raisuddin S. Effect of endosulfan on antioxidants of freshwater fish Channa punctatus Bloch: 1. Protection against lipid peroxidation in liver by copper preexposure. Arch Environ Contam Toxicol. 2001; 41(3):345–52.
33. Lonare M, Kumar M, Raut S, More A, Doltade S, Badgujar P, Telang A. Evaluation of ameliorative effect of curcumin on imidacloprid-induced male reproductive toxicity in wistar rats. Environ Toxicol. 2015. Doi: 10.1002/tox.22132.
34. Lopez O, Hernandez AF, Rodrigo L et al. Changes in antioxidant enzymes in humans with long-term exposure to pesticides. Toxicol Lett. 2007; 171:146–53.
35. Kannan K, Jain SK. Oxidative stress and apoptosis. Pathophysiology. 2000; 7(3):153–63.
36. Simon HU, Haj-Yehia A, Levi-Schaffer F. Role of reactive oxygen species (ROS) in apoptosis induction. Apoptosis. 2000; 5:415–8.
37. Junn E, Mouradian MM. Apoptotic signaling in dopamine-induced cell death: the role of oxidative stress, p38 mitogen-activated protein kinase, cytochrome c and caspases. J Neurochem. 2001; 78:374–83.
38. Akbarsha MA, Sivasamy P. Apoptosis in male germinal line cells of rat in vivo: caused by phosphamidon. Cytobios. 1997; 91:33–44.
39. Tian WN, Braunstein LD, Pang J, Stuhlmeier KM, Xi QC, Tian X, Stanton RC. Importance of glucose-6-phosphate dehydrogenase activity for cell growth. J Biol Chem. 1998; 273:10609–17.
40. Cho SW, Joshi JG. Inactivation of glucose-6-phosphate 11. dehydrogenase isozymes from human and pig brain by aluminum. J Neurochem. 1989; 53:616–21.
41. Manna S, Bhattacharyya D, Mandal TK, Das S. Sub-chronic toxicity study of alfa-cypermethrin in rats. Iranian J Pharmacol Therapeutics. 2006; 5:163–6.
42. Kale M, Rathore N, John S, Bhatnagar D. Lipid peroxidative damage on pyrethroid exposure and alterations in antioxidant status in rat erythrocytes: A possible involvement of reactive oxygen species. Toxicol Lett. 1999; 105(3):197–205.
43. Ranjbar A, Pasalar P, Sedighi A, Abdollahi M. Induction of oxidative stress in paraquat formulating workers. Toxicol Lett. 2002; 131:191–4.
44. Ronald WT, Malcolm RS, John WL. Mechanism of action and fate of the fungicide chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile) in biological systems: I. Reactions with cells and subcellular components of Saccharomyces pastorianus. Pest Biochem Physiol. 2004; 3(2):160–7.
45. Kostaropoulos I, Papadopoulos AI, Metaxakis A et al. The role of glutathione S-transferases in the detoxification of some organophosphorus insecticides in larvae and pupae of the yellow mealworm, Tenebrio molitor (Coleoptera: Tenebrionidae). Pest Manag Sci. 2001; 57:501–8.
46. Vina J. Glutathione: Metabolism and Physiological Functions. CRC Press, Boston. 1990; 222–8.
47. Perez-Maldonado IN, Herrera C, Batres LE, Gonzalez-Amaroa R, Diaz-Barriga F, Yanez L. DDT-induced oxidative

damage in human blood mononuclear cells. Environ Res. 2005; 98:177–84.
48. Kannan K, Holcombe RF, Jain SK, Alvarez-Hernandez X, Chervenak R, Wolf RE, Glass J. Evidence for the induction of apoptosis by endosulfan in a human T-cell leukemic line. Mol Cell Biochem. 2000; 205:53–66.
49. Slim R, Toborek M, Robertson LW, Lehmler HJ, Henning B. Cellular glutathione status modulates polychlorinated biphenyl-induced stress response and apoptosis in vascular endothelial cells. Toxicol Appl Pharmacol. 2000; 166:36–42.
50. Kitizawa M, Anantharam V, Kanthasamy AG. Dieldrininducedoxidative stress and neurochemical changes contribute to apoptotic cell death in dopaminergic cells. Free Radical Biol. Med. 2001; 31:1473–85.