Diseases caused by pesticide induce oxidative stress– A Review

[1]Rajdeep Kaur,[2]Yogeeta Thakur
[1]Research Scholar,[2]Assistant Professor
[1, 2] School of Agriculture Sciences and Technology
[1, 2]RIMT University, Mandi Gobindgarh, Punjab, India-147301

Abstract : Pesticides exposure is a serious issue causing various kinds of damage in DNA like oxidative damage, single strand breaks, double strands breaks, increased sister chromatin exchange etc. leading to various kinds of cancer like brain, skin, lymphoma, breast etc. In agriculture, high risk of exposure to hazardous chemicals is associated with the use of pesticides. Exposure to pesticides has been associated with an increase in the incidence of non-Hodgkin's lymphoma, multiple myeloma, soft tissue sarcoma, lung sarcoma, and cancer of the pancreas, stomach, liver, bladder and gall bladder, Parkinson disease, Alzheimer disease, and reproductive outcomes. Pesticides cause oxidative-stress, which cause DNA damage. There is inter-individual variability in the polymorphisms related to oxidative-stress in farmers. Some individuals or groups in the population have favorable polymorphisms are less likely to develop disease after exposure while others, those have unfavorablepolymorphisms are more likely to develop a particular disease. The requisite steps are needed to prevent foreseen health hazards like cancer and greater understanding of etiological mechanisms and opportunities for the development of new method of disease prevention. This can possibly be done by comparing and analyzing the DNA pattern of the persons in contact with the pesticides than the other ones.In this research work, the brief review related to the diseases caused by pesticide induced oxidative stress is carried out.

IndexTerms **-Pesticides, oxidative stress, organophosphates,reactive oxygen species (ROS).**

I. INTRODUCTION

A pesticide is generally characterized as a substance utilized against vermin including bugs, plant pathogens, weeds, molluscs, winged creatures, warm blooded animals, fish, nematodes (roundworms) and microorganisms that contend with people for sustenance, However the term pesticide has a more extensive meaning additionally incorporates herbicides, rodenticides, fumigants, nematocides, algaecides, ascaricides, molluscicides, disinfectants, defoliants and fungicides added substances (Hayes, 1991). The use of a wide range of chemicals to destroy pests and weeds is an important aspect of agricultural practice in both developed and developing countries. Undoubtedly, this has increased crop yield and reduced postharvest losses. However, the expanded use of such pesticides expectedly results in residues in foods, which has led to widespread concern over the potential adverse effects of these chemicals on human health. It is clear that the possibility for exposure to pesticides is greatest among farm workers (Ferroet al., 2012). Also, it is exceedingly plausible that less controlled and regulated uses of pesticides may offer the greatest opportunity for exposure to toxicologically significant quantities. Very limited epidemiological data are available for evaluation of the health effects of pesticides on humans. Only a small proportion of a population is likely to receive a pesticide dose high enough to cause acute severe effects; however, many more may be at risk of developing chronic effects (such as cancer, adverse reproductive outcome, and immunological effects) depending on the type of pesticide they are exposed to. The pesticides currently in use include a wide variety of chemicals with great differences in their mode of action, uptake by the body, metabolism, elimination from the body, and toxicity to humans (Al-Saleh IA, 1994). With pesticides that have a highly acute toxicity but are readily metabolized and eliminated, the main hazard lies in acute, short-term exposures. With others that have a lower acute toxicity but show a strong tendency to accumulate in the body, the main hazard is connected with long-term exposure, even to comparatively small doses. Other pesticides that are rapidly eliminated but induce persistent biological effects also present a hazard connected with long-term, low-dose exposures. Adverse effects may be caused not only by the active ingredients and the associated impurities, but also by solvents, carriers, emulsifiers, and other constituents of the formulated product. This review attempts to describe several aspects of the problem (Al-Saleh, 1994).

Pesticides exposure is a serious issue causing various kinds of damage in DNA like oxidative damage, single strand breaks, double strands breaks, sister chromatin exchange etc. leading to various kinds of cancer like brain, skin, lymphoma, breast etc. Pesticide formulations are complex mixtures which contain, besides the active ingredients, several other components, such as solvents, wetting and emulsifying agents, and additives (Hayes, 1991). Furthermore, it is typical of various agricultural uses of pesticides that different formulations are simultaneously used and that varying combinations are applied depending on the time of the growing season. This makes the exposures complex, and the biomonitoring of specific compounds for exposure evaluation may become difficult. The possible combined toxic effects of such complex exposures are not usually known. Thus, toxicity information concerning active ingredients or formulates alone is not sufficient to evaluate the risk of adverse health effects from pesticide exposure. As far as genotoxicity is concerned, the assessment of cytogenetic alterations in subjects occupationally exposed to pesticides may be used as a marker of early biological effects. Genotoxicological methods, such as cytogenetic assays, could therefore be used to get an overall picture of genotoxic exposure in work with pesticides (Scarpato et al., 1991).

Pesticides cause oxidative stress leading to the generation of free radicals and alterations in antioxidants or free oxygen radical scavenging enzyme systems (Almeida et al., 1997). Oxidative stress can be defined most simply as the imbalance between the production of free radicals capable of causing peroxidation of the lipid layer of cells and the body's antioxidant defence as shown in figure 1. Free radicals are defined as atoms or molecules that contain one or more unpaired electrons. The toxicity of many pesticides is associated with the production of free radicals, which are not only toxic themselves, but are also implicated in the pathophysiology of many diseases (Butterfield, 2002).

Exposure to pesticides can induce oxidative stress, by increased production of free radicals that further accumulate in the cell, by alteration in antioxidant defense mechanisms, including detoxification and scavenging enzymes, or by increasing lipid peroxidation as a result of the interaction between reactive oxygen species (ROS) and cellular or subcellular membranes (Abdollahi et al., 2004). Oxidative stress has been reported to play an important role in the toxicity of various pesticides, including organophosphates– OP (Possamai et al., 2007), N-methylcarbamates – NMC (Mansour et al., 2009), organochlorines – OC (Pal et al., 2009), pyrethroids – PYR (Raina et al., 2009), triazines (Singh et al., 2010), neonicotinoids (El-Gendy et al., 2010), dithiocarbamates and paraquat (Ahmad et al., 2010). Epidemiological studies in humans long-term exposed to a mixture of pesticides (OPs, synthetic PYRs and NMC) have reported stimulated antioxidant enzymes and lipid peroxidation in erythrocytes even in the absence of a decrease in acetylcholinesterase – AChE (Ogut et al., 2011). Experimental studies have shown that toxicant responsive genes, such as cytochrome P450s (CYP450) and glutathione S-transferases (GSTs) play a critical role in pesticide-induced toxicity. CYP450s play a pro-oxidant role while GSTs offer protection in pesticide-induced brain and lung toxicities (Ahmad et al,. 2010). Paraoxonase-1 (PON1), pseudocholinesterase (BChE) and carboxylesterases (CEs) also have a role by inactivating OPs and NMC through a catalytic (PON1) or noncatalytic hydrolysis (BChE, CEs).

II. TYPE OF POISONING

Pesticide exposure may result in either acute or chronic poisoning. Acute poisoning implies an incident where overt reactions follow closely upon exposure to an agent. In contrast, chronic poisoning refers to the situation where the toxic reactions appear gradually after prolonged exposure to the agent. In acute poisoning, the incriminating agent is more readily identifiable, but this is not always the case in chronic poisoning. It is much more difficult to assess the significance of the small doses that contaminate workers daily over long periods, because they do not cause clearly defined clinical symptoms. Both acute as well as chronic pesticide poisoning are health problems which need preventive intervention (Ellenhorn et al., 1997).Important routes of entry into the body are through the respiratory, oral or dermal routes. For most pesticides, dermal exposure and absorption are the most important routes of entry under occupational exposure situations. The oral route of absorption is extremely important in situations of accidental, suicidal or homicidal ingestion of pesticides. The important groups of pesticides which cause acute poisoning are the organochlorines, organophosphates, carbamates, pyrethroids and the nitro and chlorophenols. Increasingly, it is the organophosphates that are dominant in producing acute episodes of poisoning because of their extensive use as well as their toxicity. Table 1 shows Toxicological characteristics of pesticide poisoning (Ellenhorn et al., 1997; Lachance and Jeong 2001; Stohs 1995; Goodyear and Pierce, 2002; Fuchs et al., 2003).

Table 1 Toxicological characteristics of pesticide poisoning

Sr No	**Methoxychlor, lindane, toxaphene, chlordane**	**Neurotoxin, CNS, kidney, liver**
1	Diazinon, malathion, parathion, chlorpyrifos, Dichlorvos	Irreversible inhibition of red blood cell cholinesterase, acetyl cholinesterase, plasma Cholinesterase
2	Aldicarb, carbaryl, carbofuran	Reversible inhibition of red blood cell acetyl cholinesterase and plasma cholinesterase
3	Aluminum or zinc phosphide	Lungs, CNS, liver, kidney
4	2,4-D and 2,4,5-T	Skin, eyes, respiratory and GI tracts
5	Diquat, paraquat	Injury to epithelium, cornea, liver, kidney, and linings of GI and respiratory tract

The Occupational exposures predominated among the cases and could be identified with the following points (Gomes et al., 1999):

- Careless handling during preparation and application
- Lack of personal protective equipment or failure to use it due to heat-related discomfort
- Laxity of safekeeping of the chemicals
- Careless disposal of empty pesticide containers
- Consumption of food and beverages while working
- Lack of personal hygiene
- Deficiencies in safety training
- Weaknesses in occupational health legislation and regulations

III. REACTIVE OXYGEN SPECIES (ROS)

A free radical can be defined as any species capable of independent existence that contains one or more unpaired electrons. An unpaired electron is one that occupies an atomic or molecular orbital by itself; its presence usually cause free radicals to be attracted slightly to a magnetic field and makes them highly reactive, although the chemical reactivity of radicals varies over a wide spectrum. There are many free radicals in chemistry and biology. ROS is a collective term used to include not only the oxygen radicals, as superoxide and hydroxyl, but also some non-radical derivates of O_2. These include H_2O_2, hypochlorous acid (HOCl, an oxidizing and chlorinating agent produced by activated phagocytes). The main representatives ROS are O_2•-, OH•, and H_2O_2, which are able to interact at different levels and with different effectiveness in biologic systems (Droge 2002).Table 2 shows exogenous sources of free radicals (Ellenhorn et al., 1997).

Table 2Exogenous sources of free radicals (Ellenhorn et al., 1997)

Category	Compound
Drugs	Acetaminophen, clonazine, closapine, ciprofloxacin, cyclosporin, tricyclic antidepressants, nitrofuratoin, troglitazone, bleomycin, doxorubicin, aminotriazole, hyperbaric oxygen, 3,4-methylenedioxymethamphetamine (an illegal drug abused by some addicts)
Metal ions	Iron, copper, cadmium, nickel, chromium, mercury.
Radiation	Ultraviolet light, x-rays, gamma radiation.

The superoxide anionis considered the precursor of all radicals because, in most cases, is the first radical that is produced by cellular oxidase (Ardanaz and Pagano, 2006). It is formed by the reduction of oxygen for the transfer of a single electron, and can act both as reducing agent, yielding in turn an electron to an oxygen molecule, and as an oxidizing agent, with the formation of hydrogen peroxide. The superoxide anion is able to oxidize many biologically important compounds, such as catecholamines, polyphenols, leucoflavine, and to reduce cytochrome c, tetranitromethane and nitroblue tetrazolium (Fridovich, 1986).
The hydrogen peroxide, because of its small size and the lack of charge, is much more stable and has a higher diffusion when compared to superoxide anion. It is considered toxic for its ability to convert into hydroxyl radical, considered the most reactive and damaging between the oxygen radicals, through exposure to ultraviolet light or through interaction with metal ions (Liochev and Fridovich, 1994).
The hydroxyl radicalcan be produced, as well as through the two reactions mentioned above, also by the photochemical reaction of water, in which a molecule of water is cleaved in a homolytic mode with the formation of two free radical species (Pryor, 1986). OH• radicals are in fact responsible for a large part of the damage done to cellular DNA, proteins and lipids. Fig 1 shows sources of oxidative stress and damage induced by oxidative stress.

3.1Oxidative damage to protein

Proteins can be oxidatively modified in three ways: oxidative modification of specific amino acid, free radical mediated peptide cleavage, and formation of protein cross-linkage due to reaction with lipid per oxidation products. Protein containing amino acids such as methionine, cystein, arginine, and histidine seem to be the most vulnerable to oxidation. Free radical mediated protein modification increases susceptibility to enzyme proteolysis. Oxidative damage to protein products may affect the activity of enzymes, receptors, and membrane transport. Oxidatively damaged protein products may contain very reactive groups that may contribute to damage to membrane and many cellular functions. Peroxyl radical is usually considered to be free radical species for the oxidation of proteins. ROS can damage proteins and produce carbonyls and other amino acids modification including formation of methionine sulfoxide and protein carbonyls and other amino acids modification including formation of methionine sulfoxide and protein peroxide. Protein oxidation affects the alteration of signal transduction mechanism, enzyme activity, heat stability, and proteolysis susceptibility, which leads to aging (Freeman et al.,1984).

3.2Lipid per oxidation

Oxidative stress and oxidative modification of biomolecules are involved in a number of physiological and pathophysiological processes such as aging, artheroscleosis, inflammation and carcinogenesis, and drug toxicity. Lipid peroxidation is a free radical process involving a source of secondary free radical, which further can act as second messenger or can directly react with other biomolecule, enhancing biochemical lesions (Akhgari et al., 2003). Lipid peroxidation occurs on polysaturated fatty acid located on the cell membranes and it further proceeds with radical chain reaction. Hydroxyl radical is thought to initiate ROS and remove hydrogen atom, thus producing lipid radical and further converted into diene conjugate. Further, by addition of oxygen it forms peroxyl radical; this highly reactive radical attacks another fatty acid forming lipid hydroperoxide (LOOH) and a new radical. Thus lipid peroxidation is propagated. Due to lipid peroxidation, a number of compounds are formed, for example, alkanes, malanoaldehyde, and isoprotanes. These compounds are used as markers in lipid peroxidation assay and have been verified in many diseases such as neurogenerative diseases, ischemic reperfusion injury, and diabetes (Lovel et al.,1995).

3.3 Oxidative damage to DNA

Many experiments clearly provide evidences that DNA and RNA are susceptible to oxidative damage. It has been reported that especially in aging and cancer, DNA is considered as a major target (Woo et al., 1998). Oxidative nucleotide as glycol, dTG, and 8-hydroxy-2-deoxyguanosine is found to be increased during oxidative damage to DNA under UV radiation or free radical damage (Bagchi et al., 1995). It has been reported that mitochondrial DNA are more susceptible to oxidative damage that have role in many diseases including cancer. It has been suggested that 8-hydroxy-2-deoxyguanosine can be used as biological marker for oxidative stress (Hattori et al., 1997). Fig. 1 showssources of oxidative stress and damage induced by oxidative stress

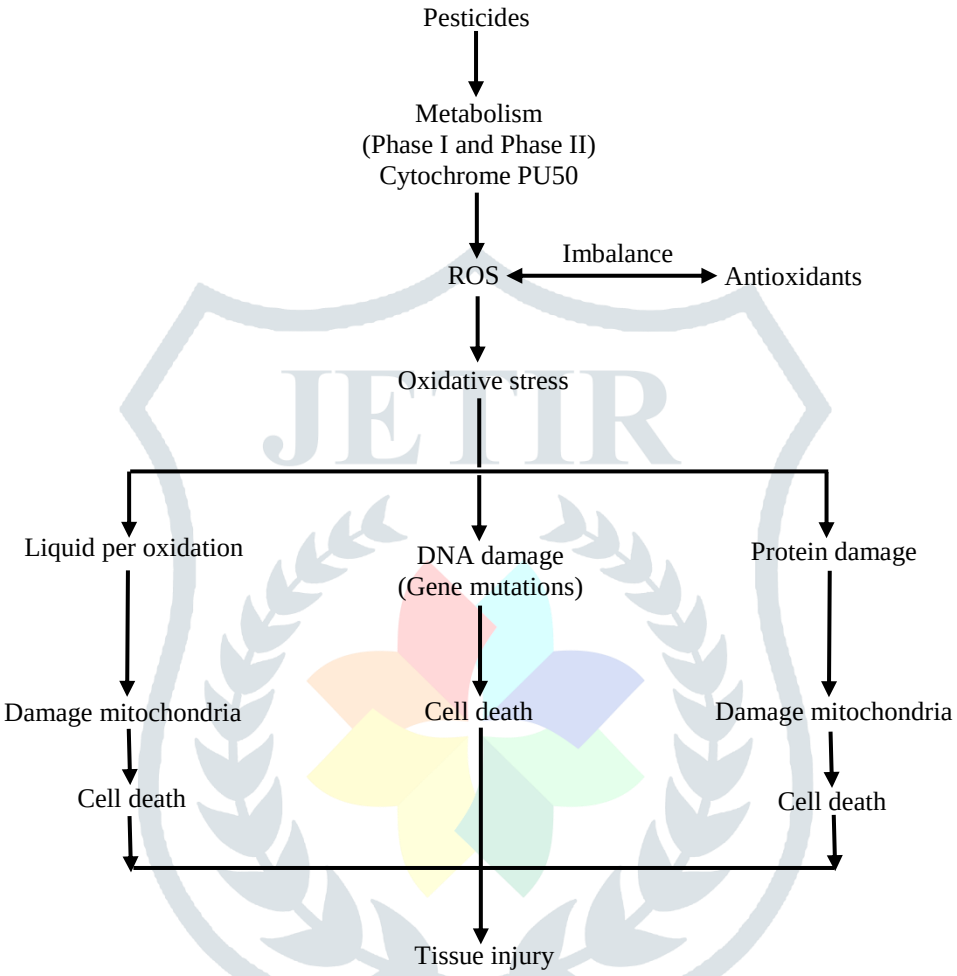

Figure 1 Sources of oxidative stress and damage induced by oxidative stress

3.4Oxidative stress and human diseases

A role of oxidative stress has been postulated in many conditions, including anthersclerosis, inflammatory condition, certain cancers, and the process of aging (Amr MM 1999). Oxidative stress is now thought to make a significant contribution to all inflammatory diseases (arthritis, vasculitis, glomerulonephritis, lupus erythematous, adult respiratory diseases syndrome), ischemic diseases (heart diseases, stroke, intestinal ischema), hemochromatosis, acquired immunodeficiency syndrome, emphysema, organ transplantation, gastric ulcers, hypertension and preeclampsia, neurological disorder (Alzheimer's disease, Parkinson's disease, muscular dystrophy), alcoholism, smoking-related diseases, and many others(Satefani et al.,1997). Table 3 showsDiseases in which oxidative stress is possibly involved in the pathophysiology (Ellenhorn et al., 1997; Lachance and Jeong 2001; Stohs 1995; Goodyear and Pierce, 2002; Fuchs et al., 2003).

Table 3Diseases in which oxidative stress is possibly involved in the pathophysiology

Disease category	Disease name
Autoimmune	Rheumatoid arthritis, immune-complex–mediated vasculitis, inflammatory bowel diseases
Eye	Cataract, age-related macular degeneration, retinopathy, cystic macular edema
GI tract	Hepatitis, pancreatitis, stomach, colitis

Kidney	Renal failure, renal interstitial fibrosis, nephropathy
Lung	Bronchial asthma, adult respiratory distress syndrome, cystic fibrosis, pneumonia, idiopathic pulmonary fibrosis, chronic obstructive pulmonary diseases
Neurodegenerative	Parkinson's, Huntington's, amyotrophic lateral sclerosis, progressive supranuclear palsy, Alzheimer's, multiple sclerosis, reflex sympathetic dystrophy, dementia, neuronal lipofuscinosis, Sickle cell disease, anemia, aging, glucose-6-phosphate dehydrogenase activity, fetal/neonatal hypoxia,
Red cells	Thalassemia, malaria infection
Skin	Contact dermatitis, atopic dermatitis, psoriasis, vitiligo
Vascular	Trauma, cancer, burns, inflammatory conditions, multiple organ dysfunction, toxicity of xenobiotics

3.5 Cardiovascular diseases

Heart diseases continue to be the biggest killer, responsible for about half of all the deaths. The oxidative events may affect cardiovascular diseases therefore; it has potential to provide enormous benefits to the health and lifespan. Poly unsaturated fatty acids occur as a major part of the low density lipoproteins (LDL) in blood and oxidation of these lipid components in LDL play a vital role in atherosclerosis(Esterbauer et al., 1991) The three most important cell types in the vessel wall are endothelial cells; smooth muscle cell and macrophage can release free radical, which affect lipid peroxidation with continued high level of oxidized lipids, blood vessel damage to the reaction process continues and can lead to generation of foam cells and plaque the symptoms of atherosclerosis. Oxidized LDL is antherogenic and is thought to be important in the formation of anthersclerosis plaques. Furthermore, oxidized LDL is cytotoxic and can directly damage endothelial cells (Neuzil et al.,1997).

3.6 Carcinogenesis

Reactive oxygen and nitrogen species, such as super oxide anion, hydrogen peroxide, hydroxyl radical, and nitric oxide and their biological metabolites also play an important role in carcinogenesis. ROS induce DNA damage, as the reaction of free radicals with DNA includes strand break base modification and DNA protein cross-links. Numerous investigators have proposed participation of free radicals in carcinogenesis, mutation, and transformation; it is clear that their presence in biosystem could lead to mutation, transformation, and ultimately cancer. Induction of mutagenesis, the best known of the biological effect of radiation, occurs mainly through damage of DNA by the HO. Radical and other species are produced by the radiolysis, and also by direct radiation effect on DNA, the reaction effects on DNA. The reaction of HO. Radicals is mainly addition to double bond of pyrimidine bases and abstraction of hydrogen from the sugar moiety resulting in chain reaction of DNA. These effects cause cell mutagenesis and carcinogenesis lipid peroxides are also responsible for the activation of carcinogens (Poppel et al., 1995).

3.7 Free radical and aging

The human body is in regular battle to keep from aging. Free radical damage to cells leads to the pathological changes associated with aging (Ashok et al.,1999). An rising number of diseases or disorders, as well as aging process itself, reveal link either directly or indirectly to these reactive and potentially destructive molecules (Sastre et al.,1996). The major mechanism of aging attributes to DNA or the accumulation of cellular and functional damage. Reduction of free radicals or decreasing their rate of production may delay aging. Some of the nutritional antioxidants will retard the aging process and prevent disease. Based on these studies, it appears that increased oxidative stress commonly occurs during the aging process, and antioxidant status may significantly influence the effects of oxidative damage associated with advancing age (Cantuti et al., 2000). Pesticides induce oxidative stress leading to the generation of free radicals and cause lipid peroxidation as molecular mechanisms involved in pesticide-induced toxicity. Increased lipid peroxidation and oxidative stress can affect the activities of protective enzymatic antioxidants that have been shown to be sensitive indicators of increased oxidative stress. (Agrawal et al., 1991; Khrer 1993; Almeidaet al., 1997). Exposure to pesticides may cause the net production of reactive oxygen species (ROS) in tissues when antioxidant defence mechanisms are overwhelmed. ROS are often free radicals (i.e, oxygen-containing species containing an unpaired electron, such as superoxide [O_2^-] and hydroxyl radical [OH]), which renders them highly unstable in a chemical sense. There are generally 4 mechanisms by which pesticides can increase the levels of ROS, such as superoxide (Fig. 1). However, regardless of the mechanism by which ROS are produced, a consequence of their overproduction is that they can cause extensive DNA and protein damage in cells (Green et al., 2008, Banerjee et al., 2001, Sherer et al., 2007, Choi et al., 2010).

It is generally accepted that ROS eventually cause DNA damage. In addition phase I pesticide detoxifying enzymes generate electrophilic compounds like carcinogenic epoxides, whereby insufficient cellular repair mechanisms may contribute to premature aging and apoptosis. Conversely, ROS-induced imbalances of the signalling pathways for metabolic protein turnover may also result in opposite effects to recruit malfunctioning aberrant proteins and compounds that trigger tumorigenic processes (Bertram and Hass 2008). Redox chemistry important for the generation of reactive oxygen species. Approximately 98% of the oxygen consumed in mitochondria is converted to water while 2% undergoes one electron reduction to superoxide. Consequently, DNA damage plays a role in the development of carcinogenesis, but is also associated with an aging process in cells and organisms (Bertram & Hass, 2008). Hence, additional actions of ROS must be important, possibly their effects on p53, cell proliferation, invasiveness and metastasis. Chronic inflammation predisposes to malignancy, but the role of ROS in this is likely to be complex because ROS can sometimes act as anti-inflammatory agents (Halliwell, 2007). The mutagenic origin of cancer

may be due, in some cases, to exposition to different carcinogens present in our environment (Claxton & Woodall, 2007). These mutations can affect genes of relevant xenobiotic metabolizing enzymes, produce polymorphisms leading to altered ligand affinity and activity, or influencing the expression of downstream target genes (Dong et al., 2008).

Organochlorine pesticides like DDT and dieldrin, are persistent organic pollutants that may accumulate in the environment and food. They are lipophilic and can be detected, after ingestion, in human breast milk and adipose tissue (Cok et al., 1997; Malarvannan et al., 2009). They are particularly harmful during pregnancy (these compounds cross the placenta and reach the fetus blood) and after birth (neonates are exposed through the breast milk) (Perera et al., 2005). These pesticides induce hepatic cell proliferation and are known as non genotoxic hepatocarcinogens (Stevenson et al., 1999).

IV. CONCLUSIONS

- Humans are exposed daily to a diversity of pesticides all the way through food, water and air, which are new to the cellular detoxification system. Health problems may occur upon exposure to pesticides.
- In last few tears frequency of oxidative stress-related diseases, such as cancers of breast and prostate and neurodegenerative diseases, has increased markedly all over the world. Pesticides involve both alterations in antioxidant defenses and accumulation of ROS leading to oxidative stress.
- All reported studies in humans or animals support the idea that pesticides induce oxidative stress as a mechanism of their toxic action in the body. These biochemical events mediate a number of redo dependent processes such as oxidative protein modifications, oxidative DNA damage, ER stress and alterations in mitochondrial function.

V. SCOPE OF FUTURE WORK

It is clear from current research that pesticide induced oxidative stress plays catastrophic role some diseases like non-Hodgkin's lymphoma, multiple myeloma, soft tissue sarcoma, lung sarcoma, and cancer of the pancreas, stomach, liver, bladder and gall bladder, Parkinson disease, Alzheimer disease, and reproductive outcomes. Thus, future research in the understanding pesticide-induced cytotoxicity and pesticideinduced cellular transformation is necessary for a complete understanding of the human health consequences to pesticide exposure, in order to establish improved usage regulations and reduction of exposure risk. Regarding the involvement of oxidative stress in the pathophysiology of many debilitating chronic diseases in human, more attention to the reduction of pesticide usage in the environment is suggested.

VI. ACKNOWLEDGEMENT

The author's very much thankful to RIMT University (Mandi Gobindgarh) for providing a facility to carry out the research work.

REFERENCES

References

1. Agrawal D, Sultana P, Gupta G. 1991Oxidative damage and changes in the glutathione redox system in erythrocytes from rats treated with hexachlorocyclohexane. Food and Chemical Toxicology ;29:459–462.
2. Akhgari M, Abdollahi M, Kebryaeezadeh (2003)A. Biochemical evidence for free radical-induced lipid peroxidation as a mechanism for subchronic toxicity of malathion in blood and liver of rats. Human & Experimental Toxicology. 22:205–211.
3. Almeida M, Fanini F, Davino SC, Aznar AE, Koch OR, Barros SBM. (1997) Pro and anti-oxidant parameters in rat liver after short-term exposure to hexachlorobenzene. Human & Experimental Toxicology.;16:257–261.
4. Al-Saleh IAJ . (1994) Pesticides: a review article. Journal of Environmental Pathology, Toxicology and Oncology ;13(3):151-61.
5. Amr MM.(1999) Pesticide monitoring and its health problems in Egypt, a Third World country. Search Results Toxicology Letters ,1999. 107:1–13.
6. Ardanaz N, Pagano PJ.(2006) Hydrogen peroxide as a paracrine vascular mediator: regulation and signalling leading to dysfunction. Advances in Experimental Medicine and Biology , 231, 237-251.
7. Ashok BT, Ali R.(1999) The aging paradox: Free radical theory of aging. Experimental *Gerontology*.;34:293–303.
8. Bagchi D, Bagchi M, Hassoun EA, Stohs SJ. (1995). In vitro and in vivo ngeneration of reactive oxygen species, DNA damage and lactate dehydrogenase leakage by selected pesticides. Toxicology, 104(1–3):129–140
9. Banerjee BD, Seth V, Ahmed RS. (2001);Pesticideinduced oxidative stress: perspectives and trends. Reviews on Environmental Health. 16:1-40.
10. Butterfield DA(2002);: Amyloid beta-peptide (1–42)-induced oxidative stress and neurotoxicity: implications for neurodegeneration in Alzheimer's disease brain. A review. *Free Radical Research*, 36:1307–13.
11. Cantuti-Castelvetri I, Shukitt-Hale B, Joseph JA. (2000);Neurobehavioral aspects of antioxidants in aging. International Journal of Developmental Neuroscience. 18:367–81.
12. Choi YJ, Seelbach MJ, Pu H. 2010.Polychlorinated biphenyls disrupt intestinal integrity via NADPH oxidase-induced alterations of tight junction protein expression. Environmental Health Perspectives;118:976-981.
13. Dröge W. 2002Free radicals in the physiological control of cell function. Physiological Reviews., 82, 47–95.
14. Ellenhorn MJ, Schonwald S, Ordog G, Wasserberger J: Ellenhorn's 1997Medical Toxicology: Diagnosis and Treatment of Human Poisoning, Williams & Wilkins, Maryland,; 1614–63

15. Esterbauer H, Pubi H, Dieber-Rothender M. 1991 Effect of antioxidants on oxidative modification of LDL. Annals of *Medicine*;23:573–81.
16. Freeman BA, Crapo JD. 1982. Biology of disease: Free radicals and tissue injury. *Laboratory Investigation*.;47:412–26.
17. Fridovich I1986. Biological effects of the superoxide radical. Archives of *Biochemistry* and *Biophysics*., 246, 245-256.
18. Fuchs J, Zollner TM, Kaufmann R, Podda M: 2001Redox-modulated pathways in infl ammatory skin diseases. Free Radic Biol Med,;30: 337–53 82.
19. Kashihara N, Sugiyama H, Makino H: 2003Implication of apoptosis in progression of renal diseases. Contributions to Nephrology,;139: 156–72
20. Glatthaar BE, Horing DH, Moser U. 1986The role of ascorbic acid in carcinogenesis. Experimental *Medicine* and *Biology*.; 206:357–77.
21. Gomes, J., Lloyd, O.L., Revitt, D.M., 1999. The influence of personal protection, environmental hygiene and exposure to pesticide on the health of immigrant farm workers in a desert country. International Archives of *Occupational* and *Environmental Health* 72, 40–45.
22. Goodyear-Bruch C, Pierce JD: 2002Oxidative stress in critically ill patients. American Journal of Critical Care,; 11: 543–51
23. Green RM, Hodges NJ, Chipman JK, O'Donovan MR, Graham M. 2008Reactive oxygen species from the uncoupling of human cytochrome P450 1B1 may contribute to the carcinogenicity of dioxin-like polychlorinated biphenyls. Mutagenesis.; 23:457-463.
24. Hattori Y, Nishigori C, Tanaka T, Ushida K, Nikaido O, Osawa T. 1997. Hydroxy-2-deoxyguanosine is increased in epidermal cells of hairless mice after chronic ultraviolet B exposure. Journal of Investigative Dermatology,.;89:10405–9.
25. Hayes WJ, Laws ER. 1991Dosage and other factors influencing toxicity. Handbook of pesticides toxicology, Academic Press, San Diego.;1:39–105.
26. Hernandez A, O lopez, L Rodrege, F Gil, G Pena, J L Serrano, T Parron, J C Alvarez, J A Lorante, APla , 2013 Changes inerthrocytes enzymes in human long term exposed to pesticides influence of several markers of individual susceptibility. Toxico letc 159, pp13 -21.
27. Khrer JP. 1993Free radicals as mediator of tissue injury and disease. *Critical* Reviews in *Toxicology*.;23:21–48.
28. Lachance PA, Nakat Z, Jeong WS2001,: Antioxidants: an integrative approach. Nutrition 17: 835–38
29. Liochev SI, Fridovich I. 1994, The role of O2•, in the production of •OH: in vitro and in vivo. Free Radical Biology and Medicine. 16, 29-33.
30. Lovell MA, Ehmann WD, Buffer BM, Markesberry WR. 1995Elevated thiobarbituric acid reactive substances and antioxidant enzyme activity in the brain in Alzemers disease. Neurology. ;45:1594–601.
31. Neuzil J, Thomas SR, Stocker R. 1997, Requirement for promotion, or inhibition of α- tocopherol of radical induced initiation of plasma lipoprotein lipid peroxidation. Free Free Radical *Biology* and *Medicine*. ;22:57–71.
32. Poppel GV, Golddbohm RA. 1995, Epidemiologic evidence for β – carotene and cancer prevention. American Journal of Clinical Nutrition. ;62:1393–5.
33. Pryor WA. 1986, Oxyradicals and related species: their formation, lifetimes and reactions. Annual Review of Physiology., 48, 657-667.
34. Sastre J, Pellardo FV, Vina J. 1996 Glutathione, oxidative stress and aging. Age.;19:129–39.
35. Scarpato L, Migliore G, Angotzi A, Fedi L, Miligi N, Loprieno1996. Cytogenetic monitoring of a group of Italian floriculturists: no evidence of DNA damage related to pesticide exposure. *Mutation* Research.:73–82.
36. Sherer TB, Richardson JR, Testa CM. 2007, Mechanism of toxicity of pesticides acting at complex I: relevance to environmental etiologies of Parkinson's disease. Journal of Neurochemistry.;100:1469-1479.
37. Silva J, Moraes, Heuser VD, Andrade VM, Silva FR, Katia K, Emmel V, Rohr P, Bordin D, Andreazza A, Salvador M, Joao AP. Henriques, Erdtmann B. 2008.Evaluation of genetic damage in a Brazilian population occupationally exposed to pesticides and its correlation with polymorphisms in metabolizing genes. Mutagenesis.;23(5):415–422.
38. Sokol RJ. 1988; Vitamin E deficiency and neurologic diseases. Annual Review of Nutrition. 8:351–73.
39. Stefanis L, Burke RE, Greene LA. 1997 Apoptosis in neurodegenerative disorders. Current Opinion in Neurology.1997;10:299–305.
40. Stohs SJ: 1995 The role of free radicals in toxicity and disease. Journal of Basic Clinical Physiology Pharmacology; 6: 205–28
41. Woo RA, Melure KG, Lee PW. 1998DNA dependent protein kinase acts upstream of p53 in response to DNA damage. Nature. ;394:700–4.