PESTICIDE INDUCED OXIDATIVE STRESS AND THE ROLE OF ANTIOXIDANT DEFENSE SYSTEM IN ANIMAL BODY

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Abstract

Pesticides are widely used to control the pest. Toxicities of pesticides occur in the animal body in different ways including oxidative stress. During the pesticide metabolism reactive oxygen species (ROS) are produced which can irreversibly oxidize the major biological molecules leading to the oxidative stress. Antioxidants inhibit oxidative damage to neutralize free radicals by donating electrons. They catalyze the breakdown or conversion of ROS into more stable components by various biochemical pathways. The antioxidant defense system comprises of various enzymatic and non-enzymatic antioxidants. The major enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione *S*-transferease (GST) and xanthine oxidase (XOD) whereas glutathione, vitamins (vitamin C and E), β-carotene, uric acid, melatonin are most abundant among the non-enzymatic antioxidants. During the detoxification processes an alteration in the cellular ROS and enzymatic and non-enzymatic antioxidant components may occur in the animal body which indicates the unambiguous oxidative stress biomarkers in the pesticide toxicity.

Key words: pesticides, ROS, oxidative stress, enzymatic antioxidants, non-enzymatic antioxidants, biomarkers

Introduction

Synthetic chemical pesticides are widely used to control pests and prevent various plant diseases. In spite of numerous benefits, the use of pesticides brings also substantial hazard to the public health and environment. Pesticides can be classified on the basis of their chemical structure like organophosphates, organochlorines, carbamates, synthetic pyrethroids *etc* [1]. Pesticide toxicities occur due to inhibition of acetylcholinesterase, block of sodium and potassium channels, oxidative stress and dysfunction in the cellular physiology resulting in alterations in metabolic and vital functions of the cells and ultimately the cell death includes cellular necrosis and apoptosis.

The oxidative stress develops when there is an imbalance between prooxidants and antioxidants ratio, leading to the production of reactive oxygen species (ROS) [2, 3]. During the course of pesticide metabolism, the oxidative stress is mainly attributed to the production of ROS in the animal body [4]. The toxicity of many pesticides is associated with the production of ROS, which are not only toxic themselves, but are also implicated in the pathophysiology of many diseases. Increased ROS production enhances the activity of the antioxidant defense system that degrades the excess ROS and help in the detoxification process and lessens the potential damages caused by the oxidative stress due to toxicity of the environmental pollutants [5].

So, the objectives of the present review are to describe the pesticide induced oxidative stress due to the production of ROS and the role of ROS scavenging antioxidant defense system to alleviate such stress in the animal body.

Reactive Oxygen Species

Reactive oxygen species (ROS) is a term which encompasses all highly reactive, oxygen-containing molecules, including free radicals. The major types of ROS include hydroxyl radical ($HO^{\bullet}$), superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), nitric oxide radical ($NO^{\bullet}$), hypochlorite radical (ClO^-), and various lipid peroxides (ROOH) [6]. All are capable of irreversible oxidation of fundamental biological molecules like proteins, membrane lipids, nucleic acids, carbohydrate and other small essential molecules, resulting in cellular damage [7, 8].

ROS are generated by a number of pathways. Most of the oxidants produced by cells occur as the consequence of normal aerobic metabolism in the mitochondrial electron transport system, oxidative burst from phagocytes, and the xenobiotic metabolism, *i.e.*, detoxification of toxic substances. Consequently, things like vigorous exercise, which accelerates cellular metabolism; chronic inflammation, infections, and other illnesses; exposure to allergens; and exposure to drugs or toxins such as cigarette smoke, alcohol, pollution, pesticides, and herbicides may all contribute to an increase level of ROS in the cell [9].

Oxidative Stress

Oxidative stress occurs when the balance between antioxidants and ROS are disrupted because of either depletion of antioxidants or accumulation of ROS. Increased oxidative stress at the cellular level can come about as a consequence of many factors, including exposure to alcohol, medications, trauma, cold, infections, poor diet, toxins, radiation, or strenuous physical activity. Oxidative stress due to higher production of ROS in the body may change DNA structure, result in modification of proteins and lipids, activation of several stress-induced transcription factors, and production of pro inflammatory and anti-inflammatory cytokines. When oxidative stress occurs, cells attempt to counteract the oxidant effects by activation or silencing of genes encoding various defensive enzymes, transcription factors, and structural proteins [10, 11]. Protection against all of these processes is dependent upon the adequacy of various antioxidant substances that are derived either directly or indirectly from the diet.

Antioxidant Defense System

There are several mechanisms to counteract the damage caused by the oxidative stress in the animal body. The basic and the most prominent protective mechanisms of the body are the antioxidant defense systems. The term antioxidant has been defined as any substance that delays or inhibits oxidative damage to a target molecule. These molecules are stable enough to neutralize free radicals by donating electrons [12]. Antioxidants catalyze the breakdown or conversion of ROS into more stable components by various biochemical pathways. The antioxidant defense system comprises of various enzymatic and both water and lipid soluble non-enzymatic antioxidants [13-15]. If the antioxidant system is unable to eliminate or neutralize the excess ROS, there is an increased risk of oxidative damage [16-18].

Enzymatic Antioxidants

The antioxidant enzymes provide the first line of cellular defense to oxidative damage due to ROS. In the animal body the main antioxidant enzymes which can play a crucial role in the ROS detoxification process include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione *S*-transferease (GST) and xanthine oxidase (XOD) *etc*. [19-21]. As with other antioxidant metabolites, the contributions of these enzymes to antioxidant defenses can be hard to separate from one another.

Superoxide dismutase (SOD)

Superoxide dismutases (EC 1.15.1.1) are a class of closely related enzymes that catalyze the breakdown of the superoxide anion into oxygen and hydrogen peroxide [22, 23]. The SODs remove $O_2^{\bullet-}$ by catalyzing its dismutation, one $O_2^{\bullet-}$ being reduced to H_2O_2 and another oxidized to O_2 . It removes $O_2^{\bullet-}$ and hence decreases the risk of $OH^{\bullet}$ formation *via* the metal catalyzed Haber-Weiss reaction [24]. SOD enzymes are present in almost all aerobic cells and in extracellular fluids [25]. These enzymes contain metal ion cofactors that, depending on the isozyme, can be copper, zinc, manganese or iron. In humans, the copper/zinc SOD is present in the cytosol, while manganese SOD is present in the mitochondrion [26]. There also exists a third form of SOD in extracellular fluids, which contains copper and zinc in its active sites [27]. The mitochondrial isozyme seems to be the most biologically important of these three. The prokaryotic Mn-SOD and Fe-SOD, and the eukaryotic Cu/Zn-SOD enzymes are dimers, whereas Mn-SODs of mitochondria are tetramers.

Catalases (CAT)

Catalase (EC 1.11.1.6) is a common enzyme found in nearly all living organisms exposed to oxygen such as bacteria, plants and animals. It is a very important enzyme in protecting the cell from oxidative damage by catalyzing the decomposition of hydrogen peroxide to water and oxygen [28]. Catalase has one of the highest turnover numbers of all enzymes *i.e.*one catalase molecule can convert millions of hydrogen peroxide molecules to water and oxygen each second [29]. Catalase is a tetramer of four polypeptide chains, each over 500 amino acids long [30]. It contains four porphyrin heme (iron) groups that allow the enzyme to react with the hydrogen peroxide. The optimum pH for human catalase is approximately 7 [31] . The pH optimum for other catalases varies between 4 and 11 depending on the species [32]. The optimum temperature also varies by species [33].

Glutathione reductase (GR)

Glutathione reductase (EC 1.8.1.7) also known as glutathione-disulfide reductase (GSR) is an enzyme that in humans is encoded by the GSR gene. It is a potential enzyme of the ascorbate-glutathione (ASH-GSH) cycle and plays an essential role in defense system against ROS by sustaining the reduced status of GSH. Glutathione reductase catalyzes the reduction of glutathione disulfide (GSSG) to the sulfhydryl form glutathione (GSH), which is a critical molecule in resisting oxidative stress and maintaining the reducing environment of the cell [34-36]. Glutathione reductase functions as dimeric disulfide oxido-reductase and utilizes an FAD prosthetic group and NADPH to reduce one molar equivalent of GSSG to two molar equivalents of GSH. The glutathione reductase is conserved between all kingdoms. In bacteria, yeasts, and animals, one glutathione reductase gene is found; however, in plant genomes, two GR genes are encoded. Drosophila and Trypanosomes do not have any GR at all [37]. In these organisms, glutathione reduction is performed by either the thioredoxin or the trypanothione system, respectively [37, 38]. GR is involved in defence against oxidative stress, whereas, GSH plays an important role within the cell system, which includes participation in the ASH-GSH cycle, maintenance of the sulfhydryl (-SH) group and a substrate for GSTs [39].

Glutathione S-transferases (GST)

Glutathione *S*-transferases (EC 2.5.1.18), previously known as ligandins, comprise a family of eukaryotic and prokaryotic phase II metabolic isozymes. GSTs can constitute up to 10% of cytosolic protein in some mammalian organs [40, 41]. GSTs catalyze the conjugation of the reduced form of glutathione (GSH) *via* a sulfhydryl group on a wide variety of substrates in order to make the compounds more water-soluble [42, 43]. This activity detoxifies endogenous compounds such as peroxidised lipids and enables the breakdown of xenobiotics. GSTs may also bind toxins and function as transport proteins, which gave rise to the early term for GSTs, ligandin [44, 45].

Glutathione peroxidase (GP_X)

Glutathione peroxidase (EC 1.11.1.9) is a selenium-containing enzyme that protects the organism from oxidative damage. The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water. Several isozymes are encoded by different genes, which vary in cellular location and substrate specificity. Glutathione peroxidase 1 (GPx1) is the most abundant version, found in the cytoplasm of nearly all mammalian tissues and is a very efficient scavenger of hydrogen peroxide, while glutathione peroxidase 4 (GPx4) is most active with lipid hydroperoxides. It is expressed in nearly every mammalian cell, though at much lower levels. Glutathione peroxidase 2 (GPx2) is an intestinal and extracellular enzyme, while glutathione peroxidase 3 (GPx3) is extracellular, especially abundant in plasma [46]. So far, eight different isoforms of glutathione peroxidase (GPx1-8) have been identified in humans.

Xanthine oxidase (XOD)

Xanthine oxidase (XOD, EC 1.17.3.2) is an essential enzyme that converts hypoxanthine to xanthine, subsequent to uric acid. These enzymes, contain FAD, molybdenum and Iron, are exclusively found in liver, intestine and little amount in other tissues of animals [47]. Xanthine oxidase plays a vital role in transformation of toxic ammonia into nontoxic uric acid. It produces hydrogen peroxide which is very dangerous to the animal, and then it

converts into HO and O_2. Further, the uric acid may act as an antioxidant and free radical scavenger protects the cells from oxidative damage [48, 49].

Nonenzymatic Antioxidants

The major nonenzymatic antioxidants comprise of various low-molecular-weight compounds, such as thiol (sulfhydryl) group containing glutathione, vitamins (vitamins C and E), β-carotene, uric acid, melatonin *etc*.

Glutathione

Glutathione is a cysteine-containing peptide found in most forms of aerobic life [50]. It is not required in the diet and is instead synthesized in cells from its constituent amino acids [51]. Glutathione has antioxidant properties since the thiol group in its cysteine moiety is a reducing agent and can be reversibly oxidized and reduced. It can act as a scavenger for hydroxyl radicals, singlet oxygen, and various electrophiles. Reduced glutathione reduces the oxidized form of the enzyme glutathione peroxidase, which in turn reduces hydrogen peroxide (H_2O_2), a dangerously reactive species within the cell. Reduced glutathione also donates protons to membrane lipids and protects them from oxidant attacks [52]. In addition, it plays a key role in the metabolism and clearance of xenobiotics, acts as a cofactor in certain detoxifying enzymes, participates in transport, and regenerates antioxidants such and Vitamins E and C to their reactive forms. In cells, glutathione is maintained in the reduced form by the enzyme glutathione reductase [53]. The ratio of GSSG/GSH present in the cell is a key factor in properly maintaining the oxidative balance of the cell. In some organisms glutathione is replaced by other thiols, such as by mycothiol in the Actinomycetes, bacillithiol in some Gram-positive bacteria, [54, 55] or by trypanothione in the Kinetoplastids [56, 57].

Vitamin C

Ascorbic acid or "vitamin C" is a monosaccharide oxidation-reduction (redox) catalyst found in both animals and plants. It is, maintained in the cells, in its reduced form by reaction with glutathione, which can be catalysed by protein disulfide isomerase and glutaredoxins [58, 59]. Due to its redox catalyst property, it can reduce, and thereby neutralize, reactive oxygen species such as hydrogen peroxide [60]. It converts vitamin E free radicals back to vitamin E. Its plasma levels have been shown to decrease with age [61, 62].

Vitamin E

Vitamin E is the collective name for a set of eight related tocopherols and tocotrienols, which are fat-soluble vitamins with antioxidant properties [63, 64]. Of these, the α-tocopherol form is the most important lipid-soluble antioxidant, and it protects membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction [63, 65]. This removes the free radical intermediates and prevents the propagation reaction from continuing. This reaction produces oxidised α-tocopheroxyl radicals that can be recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate, retinol or ubiquinol [66]. It is evident that α-tocopherol efficiently protects glutathione peroxidase 4 (GPX4)-deficient cells from cell death [67]. Vitamin E triggers apoptosis of cancer cells and inhibits free radical formations [68].

Carotenoids (β-Carotene)

Carotenoids are structurally and functionally a very diverse group of natural organic pigments of the isoprenoid type [69]. They occur ubiquitously in plants, phototropic bacteria and cyanobacteria [70]. Although not synthesized by humans and animals, they are also present in their blood and tissues. Fruits and vegetables constitute the major sources of carotenoid in the animal body and are important precursors of retinol (vitamin A). Carotenoids are known to be very efficient physical and chemical quenchers of singlet oxygen ($^1O_2$), as well as potent scavengers of other reactive oxygen species (ROS) [71-73]. Among the members of the carotenoids, primarily β-carotene has been found to react with peroxyl (ROO^-), hydroxyl (OH^-), and superoxide (O_2^-) radicals.96 Carotenoids show their antioxidant effects in low oxygen partial pressure but may have pro-oxidant effects at higher oxygen concentrations [74]. Carotenoids may also affect the apoptosis of cells [75].

Uric acid

Uric acid is a powerful antioxidant and is a scavenger of singlet oxygen and hydroxyl radicals. At physiological concentrations, urate reduces the oxo-heme oxidant formed by peroxide reaction with hemoglobin, protects erythrocyte ghosts against lipid peroxidation, and protects erythrocytes from peroxidative damage leading to lysis [76]. Uric acid's antioxidant activities are also complex, given that it does not react with some oxidants, such as superoxide, but does act against peroxynitrite,[77] peroxides, and hypochlorous acid [78]. The plasma urate level in humans (about 300μM/L) is considerably higher than other antioxidant level, making it one of the major antioxidants in humans.

Melatonin

Melatonin, a pineal hormone, has been implicated in oxidative damage and aging process [79, 80]. It has a potent antioxidant activity and is a potentially promising candidate for the control of aging and other ROS-mediated pathogenesis [81]. Unlike other antioxidants, melatonin does not undergo redox cycling. Thus once oxidized, melatonin cannot be reduced to its former state because it forms several stable end-products upon reacting with free radicals. Therefore, it has been referred to as a terminal (or suicidal) antioxidant [82]. Melatonin declines significantly with the increase in age [80]. This decline in melatonin coincides with the increased oxidative damage and pathogenesis.

Effect of Pesticide Induced Oxidative Stress on Antioxidant Defense System

Many pesticides have been shown to be associated with the induction of oxidative stress *via* formation of ROS and alterations in antioxidant or free oxygen radical scavenging enzyme systems [83-87].

Many scientific works have been reported the effect of pesticide induced oxidative damages on the antioxidant defense system of the various aquatic animals, laboratory experimental animals, in-vitro cell culture and even pesticide affected human being also. Amal *et al.* [88] have studied the effects of acute organophosphorus toxicity on the biomarkers of oxidative stress and apoptosis of the cell. They found significant decrease in the levels of reduced glutathione and catalase and increase in the level of malonyldialdehyde (MDA) (end product of lipid peroxidation) in acute organophosphate affected patients. Decreased GPx activity in gills, muscle, liver and brain of parathion treated goby fishes were reported [89]. In different

tissues of diazinon exposed *Cyprinus carpio*, a decreased GPx activity was also observed [90]. Box *et al.* [91] showed that organophosphate pesticide and exposure to environmental pollutants caused a significant reduction in CAT activities in different tissues of the brown bullhead fish, *Ictalurus nebulosus* and the mussel, *Mytilus galloprovincialis*, respectively. Whereas, Isik and Celik [92] reported in rainbow trout exposed to diazinon and methyl parathion a decrease in SOD activities in liver, gills and muscle tissues separately. Banaee *et al.* [19] found that the levels of total antioxidant capacity in hepatocytes of fishes exposed to diazinon were significantly decreased. They also observed the increased SOD and CAT activities due to over production of superoxide radicals and H_2O_2 in hepatocytes of diazinon exposed fishes. Following 2-chlorophenol exposure, alterations in SOD and CAT activities in fish, *Carassius auratus* were reported [93]. Naveed and Janaiah [94] observed the reduction in XOD activity in liver of fish, *Channa punctatus* exposed to an organophosphate insecticide, triazophos leads to increase in cellular oxidative damage.

Elisi *et al*. [95] have shown that carbamate exposure resulted in depletion of intracellular reduced glutathione (GSH) content and a decrease in GSH/GSSG ratio in the mammalian CHO-K1 cells accompanied by the induction of GR and GPx activities. A drastic increase in the activities of CAT and SOD and decrease in the GST activity were observed in RBC´s membrane of Wistar rats exposed to a single sub-acute dose of a carbamate pesticide, carbofuran (CF). The erythrocytes fragility as well as oxidative stress induced by pesticides got recovered near to normal by vitamin C treatment [96]. Recently, generation of ROS in rat brain and liver due to chronic oral administration of carbofuran has been reported [97]. The results demonstrated that carbofuran treatment caused significant increase in lipid peroxidation (LPO) and significantly induced activities of antioxidant enzymes, SOD and CAT in rat brain.

In another study, endosulfan has been reported to induce oxidative stress in rat's heart as there was significant rise in the activities of SOD, GPx and CAT which could be prevented by use of vitamin E as an antioxidant [98]. Some workers [99, 100] have demonstrated that acetofenate, an organochlorine insecticide treatment can cause macrophage apoptosis by inducing oxidative stress on mouse macrophage cell line RAW264.7. In the rat cerebral hemisphere, the effects of oral administration of hexachlorocyclohexane (HCH, lindane) on the extent of LPO and levels of antioxidant enzymes have been evaluated [101]. They reported elevated level of LPX after 7 days of treatment in crude homogenate and decreased activities of cerebral CAT and GPx activity (both selenium-dependent and –independent isoenzymes) throughout the treatment period. The involvement of the antioxidant enzymes (CAT and GPx) in defense against the genotoxicity induced by phosphamidon and dieldrin has been demonstrated in primary mouse lung fibroblast cultures [102]. These two pesticides damaged DNA through the generation of ROS and therefore produce oxidative stress. The CAT activity decreased only in the damage induced by phosphamidon, while GPx protected against damage induced by both phosphamidon and dieldrin.

A survey of literature indicates that not much work has been conducted on evaluation of pyrethrin induced oxidative stress. However, some reports indicate that certain pyrethrins have the potential to generate oxidative stress in various tissues of some mammalian systems [103]. Exposure of deltamethrin has been shown to induce oxidative stress and cause perturbations in various biochemical parameters including LPO, antioxidant and neurotransmission enzymes; the toxicity however, has been shown to be reduced by treatment with vitamin E [104]. In rats brain and liver, cypermethrin induced oxidative stress has been observed which got ameliorated by treatment with vitamin E or allopurinol [105]. In another

study, the use of vitamin E with selenium has been reported to protect the rats from cypermethrin induced oxidative stress [106]. Gabbianelli *et al*. [107] have reported that cypermethrin treatment in rats induced a significant increase in the lipid peroxidation and a significant decrease in the acitivity of GPx.

Conclusion

The indiscriminate application of the chemical pesticides has caused various environmental hazards and health related issues. All reported studies in humans or animals support that pesticides induce oxidative stress due to the production of ROS leading to development of different pathophysiological conditions of many diseases. Although the cells are equipped with antioxidant defense mechanisms to detoxify the harmful effects of ROS, cellular damage occurs when there is production of excess ROS or when the antioxidant defense system is not properly functioning. A great deal of research has also established that the induction of the cellular antioxidant machinery is important for protection against the oxidative damages due to ROS. During the pesticide metabolism or the detoxification process, a biochemical alteration in ROS and variations in enzymatic and non-enzymatic antioxidant levels must occur, which indicate a stressful condition of the cell.

So, in the animal body, cellular ROS and the ROS scavenging antioxidant molecules could be the potential biomarkers of the oxidative stress due to pesticide toxicity. Further research of the antioxidant defense system and ROS in the molecular and genetic level is warranted not only to deal with the oxidative damages but also to cure various pathophysiological disorders.

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References

1. Gerkowicz, M., Wroblewska, E., and Turski, W., 2002, “The use of the radioisotope method in studies of pesticide penetration into the eyeball.” Ann. Agric. Environ. Med., 9, pp. 29-31.
2. Ahmad, I., Hamid, T., Fatima, M., Chand, H.S., Jain, S.K., Athar, M, and Raisudin, S., 2000, “Induction of hepatic antioxidants in freshwater catfish (Channa punctatus Bloch) is a biomarker of paper mill effluent exposure.” BiochimBiophysActa., 1523, pp. 37-48.
3. Li, W., Yin, D., Zhou, Y., Hu, S., ang, L., 2003, “3, 4-Dichloroaniline-induced oxidative stress in liver of crucial carp (Carassius auratus).” Ecotoxicol. Environ. Saf., 56, pp. 251–255.
4. Agrawal, A., and Sharma, B., 2010, “Pesticides induced oxidative stress in mammalian systems.” Int. J. Biol. Med. Res., 1, pp. 90-104.

5. Banaee, M., 2013, Insecticides - Development of Safer and More Effective Technologies, InTech, Chap. 4.
6. Yoshikawa, T., and Naito, Y., 2002, "What Is Oxidative Stress?" Japan Medical Association *Journal*, 45(7), pp. 271–276.
7. Stadtman, E. R., and Levine, R. L., 2000, "Protein oxidation." Ann. N. Y. Acad. Sci., USA 90, pp. 191–208.
8. Richter, C., Park, J. W., Ames, B. N., 1988, "Normal oxidative damage to mitochondrial and nuclear DNA is extensive." Proc. Natl. Acad. Sci., USA, 85, pp. 6465–6467.
9. Percival, M., 1996, Antioxidants, Clinical Nutrition Insights, Advanced Nutrition Publications, Inc.
10. Dalton, T.P., Shertzer, H.G., and Puga, A., 1999, "Regulation of gene expression by reactive oxygen," Ann. Rev. Pharmacol Toxicol., 39, pp. 67–101.
11. Scandalios, J.G., 2004, "Encyclopedia of Molecular Cell Biology and Molecular Medicine," Wiley-VCH, Weinheim, Germany, pp. 489–512.
12. Abdollahi, M., Ranjbar, A., Shadnia, S., Nikfar, S., and Rezaie, A., 2004, "Pesticides and oxidative stress: a review," Med Sci Monit, 10(6), pp. 141-147.
13. Gill, S.S., Khan, N.A., Anjum, N.A., and Tuteja, N., "Amelioration of cadmium stress in crop plants by nutrients management: Morphological, physiological and biochemical aspects," Plant Stress, in press.
14. Mittler, R., Vanderauwera, S., Gollery, M., and Breusegem, F. V., 2004, "Reactive oxygen gene network of plants," Trends Plant Sci., 9, pp. 490-498.
15. Singh, S., Anjum, N.A., Khan, N.A., and Nazar, R., 2008, "Abiotic Stress and Plant Responses," IK International, New Delhi, pp. 159-189.
16. Üner, N., Oruç, E. Ö., Sevgiler, Y., Sahin, N., Durmaz, H., Usta, D., 2006, "Effects of diazinon on acetylcholinesterase activity and lipid peroxidation in the brain of *Oreochromis niloticus*," Environmental Toxicology and Pharmacology, 21, pp. 241-245.
17. Oruç, E. Ö., Usta, D., 2007, "Evaluation of oxidative stress responses and neurotoxicity potential of diazinon in different tissues of *Cyprinus carpio*," Environmental Toxicology and Pharmacology, 23, pp. 48-55.
18. Isik, I., Celik, I., 2008, "Acute effects of methyl parathion and diazinon as inducers for oxidative stress on certain biomarkers in various tissues of rainbow trout (*Oncorhynchus mykiss*)." Pesticide Biochemistry and Physiology, 92, pp. 38-42.
19. Banaee, M., Sureda, A., Mirvagefei, A. R., and Ahmadi, K., 2013, "Biochemical and histological changes in the liver tissue of Rainbow trout (*Oncorhynchus mykiss*) exposed to sub-lethal concentrations of diazinon," Fish Physiol Biochem, 39(3), pp. 489-501.
20. Davies, K . J., 1995,"Oxidative stress: the paradox of aerobic life". Biochemical Society Symposium, 61, pp. 1–31. doi:10.1042/bss0610001. PMID 8660387.
21. Sies, H., 1997, "Oxidative stress: oxidants and antioxidants". Experimental Physiology, 82 (2), pp. 291–295. doi:10.1113/expphysiol.1997.sp004024. PMID 9129943.
22. Zelko, I. N., Mariani, T. J., Folz, R. J., 2002, "Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression," Free Radical Biology & Medicine, 33(3), pp. 337–349. doi:10.1016/S0891-5849(02)00905-X. PMID 12126755.
23. Bannister, J. V., Bannister, W. H., Rotilio, G., 1987, "Aspects of the structure, function, and applications of superoxide dismutase". CRC Critical Reviews in

Biochemistry, 22(2), pp. 111–180. doi:10.3109/10409238709083738. PMID 3315461.

24. Gill, S. S., Tuteja, N., 2010, "Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants," Plant Physiology and Biochemistry, 48, pp. 909-930.
25. Johnson, F., Giulivi, C., 2005, "Superoxide dismutases and their impact upon human health," Molecular Aspects of Medicine, 26(4–5), pp. 340–352. doi:10.1016/j.mam.2005.07.006. PMID 16099495.
26. Bannister, J. V., Bannister, W. H., Rotilio, G., 1987, "Aspects of the structure, function, and applications of superoxide dismutase," CRC Critical Reviews in Biochemistry, 22(2), pp. 111–180. doi:10.3109/10409238709083738. PMID 3315461.
27. Nozik-Grayck, E., Suliman, H. B., Piantadosi, C. A., 2005, "Extracellular superoxide dismutase," The International Journal of Biochemistry & Cell Biology, 37(12), pp. 2466–2471. doi:10.1016/j.biocel.2005.06.012. PMID 16087389.
28. Chelikani, P., Fita, I., Loewen, P. C., 2004, "Diversity of structures and properties among catalases," Cellular and Molecular Life Sciences, 61(2), pp. 192–208. doi:10.1007/s00018-003-3206-5. PMID 14745498.
29. Goodsell, D. S., 2004, "Catalase," Molecule of the Month, RCSB Protein Data Bank.
30. Boon, E. M., Downs, A., Marcey, D., 2007, "Catalase: H_2O_2: H_2O_2Oxidoreductase," Catalase Structural Tutorial Text.
31. Maehly, A. C., Chance, B., 1954, "The assay of catalases and peroxidises," Methods of Biochemical Analysis, 1, pp. 357–424. doi:10.1002/9780470110171.ch14.
32. "EC 1.11.1.6 - catalase". BRENDA: The Comprehensive Enzyme Information System. Department of Bioinformatics and Biochemistry, Technische Universität Braunschweig. Retrieved 2009-05-26.
33. Toner, K., Sojka, G., Ellis, R., "A Quantitative Enzyme Study; CATALASE", bucknell.edu. Archived from the original on 2000-06-12. Retrieved 2007-02-11.
34. Deponte, M., 2013, "Glutathione catalysis and the reaction mechanisms of glutathione-dependent enzymes," Biochim. Biophys. Acta., 1830(5), pp. 3217–66. doi:10.1016/j.bbagen.2012.09.018. PMID 23036594.
35. Meister, A., 1988, "Glutathione metabolism and its selective modification," J. Biol. Chem., 263(33), pp. 17205–17208. PMID 3053703.
36. Mannervik, B., 1987, "The enzymes of glutathione metabolism: an overview," Biochem. Soc. Trans., 15(4), pp. 717–718. PMID 3315772.
37. Kanzok, S. M., Fechner, A., Bauer, H., Ulschmid, J. K., Müller, H. M., Botella-Munoz, J., Schneuwly, S., Schirmer, R., Becker, K., 2001, "Substitution of the thioredoxin system for glutathione reductase in *Drosophila melanogaster*," Science, 291(5504), pp. 643–646. doi:10.1126/science.291.5504.643. PMID 11158675.
38. Krauth-Siegel, R. L., Comini, M. A., 2008, "Redox control in trypanosomatids, parasitic protozoa with trypanothione-based thiol metabolism," Biochim. Biophys. Acta., 1780(11), pp. 1236–48. doi:10.1016/j.bbagen.2008.03.006. PMID 18395526.
39. Reddy, A. R., Raghavendra, A. S., 2006,"Photooxidative stress," Physiology and Molecular Biology of Stress Tolerance in Plants, Springer, The Netherlands, pp. 157-186.
40. Boyer, T. D., 1989, "The glutathione S-transferases: an update," Hepatology, 9(3), pp.486–96. doi:10.1002/hep.1840090324. PMID 2646197.
41. Mukanganyama, S., Bezabih, M., Robert, M., 2011, "The evaluation of novel natural products as inhibitors of human glutathione transferase P1-1". J Enzyme Inhib Med Chem., 26(4), pp. 460–467. doi:10.3109/14756366.2010.526769. PMID 21028940.

42. Douglas, K. T., 1987, "Mechanism of action of glutathione-dependent enzymes," Adv. Enzymol. Relat. Areas Mol. Biol., 59, pp. 103–167. PMID 2880477.
43. Oakley, A., 2011, "Glutathione transferases: a structural perspective," Drug Metab. Rev., 43 (2), pp. 138–151. doi:10.3109/03602532.2011.558093. PMID 21428697.
44. Leaver, M. J., George, S.G., 1998, "A piscine glutathione S-transferase which efficiently conjugates the end-products of lipid peroxidation," Marine Environmental Research, 46 (1–5), pp. 71–74. doi:10.1016/S0141-1136(97)00071-8.
45. Litwack, G., Ketterer, B., Arias, I.M., 1971, "Ligandin: a hepatic protein which binds steroids, bilirubin, carcinogens and a number of exogenous organic anions," Nature, 234(5330), pp. 466–467. doi:10.1038/234466a0. PMID 4944188.
46. Muller, F. L., Lustgarten, M. S., Jang, Y., Richardson, A., Van Remmen, H., 2007, "Trends in oxidative aging theories," Free Radical Biology & Medicine, 43(4), pp. 477–503. doi:10.1016/j.freeradbiomed.2007.03.034. PMID 17640558.
47. Sathyanarayana, U., 2005, Biochemistry, Books and Allied (P). Ltd., Kolkata, India.
48. Sheehan, D., 2001, "Structure, function and evolution of glutathione transferase: implications for classification of non-mammalian members of an ancient enzyme superfamily," J. Biochem., 360, pp. 1-16.
49. Guskov, E.P., 2002, "Atlantion as a free radical scavenger," J. Dokl. Biochem. Biophys., 383, pp. 105-107.
50. Meister, A., Anderson, M. E., 1983, "Glutathione," Annual Review of Biochemistry, 52, pp. 711–60. doi:10.1146/annurev.bi.52.070183. 003431. PMID 6137189.
51. Meister, A., 1988, "Glutathione metabolism and its selective modification," The Journal of Biological Chemistry, 263(33), pp.17205–17208. PMID 3053703.
52. Curello, S., Ceconi, C., Bigoli, C., Ferrari, R., Albertini, A., Guarnieri, C., 1985, "Changes in the cardiac glutathione status after ischemia and reperfusion," Experientia, 41, pp. 42–43.
53. Meister, A., 1994, "Glutathione-ascorbic acid antioxidant system in animals," The Journal of Biological Chemistry, 269(13), pp. 9397–400. PMID 8144521.
54. Gaballa, A., Newton, G. L., Antelmann, H., Parsonage, D., Upton, H., Rawat, M., Claiborne, A., Fahey, R. C., Helmann, J. D., 2010, "Biosynthesis and functions of bacillithiol, a major low-molecular-weight thiol in Bacilli," Proceedings of the National Academy of Sciences of the United States of America, 107(14), pp. 6482–6486. Bibcode:2010PNAS..107.6482G. doi:10.1073/pnas.1000928107. PMC 2851989 . PMID 20308541.
55. Newton, G. L., Rawat, M., LaClair, J. J., Jothivasan, V. K., Budiarto, T., Hamilton, C. J., Claiborne, A., Helmann, J. D., Fahey, R. C., 2009, "Bacillithiol is an antioxidant thiol produced in Bacilli", Nature Chemical Biology, 5(9), pp. 625–627. doi:10.1038/nchembio.189. PMC 3510479 . PMID 19578333.
56. Fahey, R. C., 2001, "Novel thiols of prokaryotes," Annual Review of Microbiology, 55, pp. 333–356. doi:10.1146/annurev.micro.55.1.333. PMID 11544359.
57. Fairlamb, A. H., Cerami, A., 1992, "Metabolism and functions of trypanothione in the Kinetoplastida," Annual Review of Microbiology, 46, pp. 695–729. doi:10.1146/annurev.mi.46.100192.003403. PMID 1444271.
58. Meister, A., 1994, "Glutathione-ascorbic acid antioxidant system in animals", The Journal of Biological Chemistry, 269(13), pp. 9397–9400. PMID 8144521.
59. Wells, W. W., Xu, D. P., Yang, Y. F., Rocque, P. A., 1990, "Mammalian thioltransferase (glutaredoxin) and protein disulfide isomerase have dehydroascorbate reductase activity," The Journal of Biological Chemistry, 265(26), pp. 15361–15364. PMID 2394726.

60. Padayatty, S. J., Katz, A., Wang, Y., Eck, P., Kwon, O., Lee, J. H., Chen, S., Corpe, C., Dutta, A., Dutta, S. K., Levine, M., 2003, "Vitamin C as an antioxidant: evaluation of its role in disease prevention," Journal of the American College of Nutrition, 22(1), pp. 18–35. doi:10.1080/07315724.2003.10719272. PMID 12569111.
61. Bunker, V. W., 1992, "Free radicals, antioxidants and ageing," Med Lab Sci., 49, pp. 299–312.
62. Mezzetti, A., Lapenna, D., Romano, F., Costantini, F., Pierdomenico, S. D., 1996, "Systemic oxidative stress and its relationship with age and illness," J. Am Geriatr Soc., 44, pp. 823–827.
63. Herrera, E., Barbas, C., 2001, "Vitamin E: action, metabolism and perspectives," Journal of Physiology and Biochemistry, 57(2), pp. 43–56. doi:10.1007/BF03179812. PMID 11579997.
64. Packer, L., Weber, S. U., Rimbach, G., 2001, "Molecular aspects of alpha-tocotrienol antioxidant action and cell signalling," The Journal of Nutrition, 131(2), pp. 369S–73S. PMID 11160563.
65. Traber, M. G., Atkinson, J., 2007, "Vitamin E, antioxidant and nothing more," Free Radical Biology & Medicine, 43(1), pp. 4–15. doi:10.1016/j.freeradbiomed.2007.03.024.PMC 2040110.PMID 17561088.
66. Wang, X., Quinn, P. J., 1999, "Vitamin E and its function in membranes," Progress in Lipid Research. 38(4), pp. 309–336. doi:10.1016/S0163-7827(99)00008-9. PMID 10793887.
67. Seiler, A., Schneider, M., Förster, H., Roth, S., Wirth, E. K., Culmsee, C., Plesnila, N., Kremmer, E., Rådmark, O., Wurst, W., Bornkamm, G. W., Schweizer, U., Conrad, M., 2008, "Glutathione peroxidase 4 senses and translates oxidative stress into 12/15-lipoxygenase dependent- and AIF-mediated cell death," Cell Metabolism, 8(3), pp. 237–248. doi:10.1016/j.cmet.2008.07.005. PMID 18762024.
68. White, E., Shannon, J. S., Patterson, R. E., 1997, "Relationship between vitamin and calcium supplement use and colon cancer," Cancer Epidemiol Biomarkers Prev., 6, pp. 769–774.
69. Landrum, J.T., 2010, "Carotenoids: Physical, Chemical, and Biological Functions and Properties," CRC Press: Boca Raton, FL, USA.
70. Scheer, H., 2003, "The Pigments. In Light-Harvesting Antennas in Photosynthesis," Green, B.R., Parson, W.W., Eds.. Kluwer Academic Publishers, Dordrecht, the Netherlands, pp. 29–81.
71. Fiedor, J., Fiedor, L., Haessner, R., Scheer, H., 2005, "Cyclic ednoperoxides of β-carotene, potential pro-oxidants, as products of chemical quenching of singlet oxygen," Biochim. Biophys. Acta, 1709, pp. 1–4.
72. Edge, R., Truscott, T.G., 2010, "Properties of Carotenoid Radicals and Excited States and Their Potential Role in Biological Systems," In Carotenoids: Physical, Chemical, and Biological Functions and Properties; Landrum, J.T., Ed.; CRC Press: Boca Raton, FL, USA, pp. 283–308.
73. Cvetkovic, D., Fiedor, L., Fiedor, J., Wiśniewska-Becker, A., Markovic, D., 2013, "Molecular Base for Carotenoids Antioxidant Activity in Model and Biological Systems: The Health-Related Effects," In Carotenoids: Food Sources, Production and Health Benefits; Yamaguchi, M., Ed., Nova Science Publishers: Hauppauge, NY, USA, pp. 93–126.
74. Rice-Evans, C. A., Sampson, J., Bramley, P. M., Holloway, D. E., 1997, "Why do we expect carotenoids to be antioxidants in vivo?" Free Radic Res., 26, pp. 381–398.
75. Birben, E., Sahiner, U. M., Sackesen, C., Erzurum, S., Kalayci, O., 2012, "Oxidative Stress and Antioxidant Defense," WAO Journal, 5, pp. 9–19.

76. Ames, B. N., Cathcart, R., Schwiers, E., and Hochstein, P., 1981, "Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: A hypothesis," Proc. NatL Acad. Sci. USA, Biochemistry, 78(11), pp. 6858-6862.
77. Sautin, Y. Y., Johnson, R. J., 2008, "Uric acid: the oxidant-antioxidant paradox," Nucleosides, Nucleotides & Nucleic Acids, 27(6), pp. 608–619. doi:10.1080/15257770802138558. PMC 2895915 . PMID 18600514.
78. Enomoto, A., Endou, H., 2005, "Roles of organic anion transporters (OATs) and a urate transporter (URAT1) in the pathophysiology of human disease," Clinical and Experimental Nephrology, 9(3), pp. 195–205. doi:10.1007/s10157-005-0368-5. PMID 16189627.
79. Reiter, R. J., 1997, "Aging and oxygen toxicity: relation to changes in melatonin," Age, 20, pp. 201–213.
80. *Reiter RJ, Guerrero JM, Garcia JJ, Acuna-Castroviejo D., 1998, "Reactive oxygen intermediates, molecular damage, and aging," Ann NY Acad Sci, 854, pp. 410-424.*
81. Bandyopadhyay, U., Das, D., and Banerjee, R. K., 1999, "Reactive oxygen species: Oxidative damage and pathogenesis," Current Science, 77(5), pp. 658-666.
82. Tan, D. X., Manchester, L. C., Reiter, R. J., Qi, W.B., Karbownik, M., and Calvo, J. R., 2000, "Significance of melatonin in antioxidative defense system: reactions and products," Biological Signals and Receptors, 9 (3–4), pp. 137–159. doi:10.1159/000014635. PMID 10899700.
83. Uner, N., Oruc, E. O., Sevgiler, Y., Sahin, N., Durmaz, H., Usta, D., 2006, "Effects of diazinon on acetylcholinesterase activity and lipid peroxidation in the brain of *Oreochromis niloticus*," Environmental Toxicology and Pharmacology, 21, pp. 241–245.
84. Slaninova, A., Smutna, M., Modra, H., and Svobodova, Z., 2009, "A review: Oxidative stress in fish induced by pesticides," Neuroendocrinology Letters, 30, pp. 2–12.
85. Lushchak, V. I., 2011, "Environmentally induced oxidative stress in aquatic animals," Aquatic Toxicology, 101, pp.13–30.
86. Stara, A., Machova, J., Velisek, J., 2012, "Effect of chronic exposure to simazine on oxidative stress and antioxidant response in common carp (*Cyprinus carpio* L.)," Environmental Toxicology and Pharmacology, 33, pp. 334–343.
87. Stara, A., Kristan, J., Zuskova, E., and Velisek, J., 2013b, "Effect of chronic exposure to prometryne on oxidative stress and antioxidant response in common carp (*Cyprinus carpio* L.)," Pesticide Biochemistry and Physiology, 105, pp. 18–23.
88. Amal, A., Mashali, Howaida, A., Nounou, Gihan, M. S., Manal, H., and Abdel, A., 2005, "Role of oxidative stress and apoptosis into acute organophosphorus intoxicated patients," J. Med. Res. Inst., 26, pp. 255-263.
89. Monteiro, M., Quintaneiro, C., Pastorinho, M., Pereira, M. L., Morgado, F., Guilhermino, L., and Soares, A. M. V. M., 2006, "Acute effects of 3,4-dichloroaniline on biomarkers and spleen histology of the common goby, *Pomatoschistus microps*," Chemosphere, 62(8), pp. 1333-1339.
90. Oruç, E. Ö., and Usta, D., 2007, "Evaluation of oxidative stress responses and neurotoxicity potential of diazinon in different tissues of *Cyprinus carpio,"* Environmental Toxicology and Pharmacology, 23(1), pp. 48-55.
91. Box, A., Sureda, A., Galgani, Pons, A., Deudero, S., 2007, "Assessment of environmental pollution at Balearic Islands applying oxidative stress biomarkers in the mussel *Mytilus galloprovincialis*," Comparative Biochemistry and Physiology, Part C: Toxicology & Pharmacology, 146(4), pp. 531-539.

92. Isik, I., and Celik, I., 2008, "Acute effects of methyl parathion and diazinon as inducers for oxidative stress on certain biomarkers in various tissues of rainbow trout (*Oncorhynchus mykiss*)," Pesticide Biochemistry and Physiology, 92, pp.38–42. doi:10.1016/j.pestbp.2008.06.001
93. Luo, Y., Su, Y., Lin, R., Shi, H., Wang, X., 2006, "2-Chlorophenol induced ROS generation in fish *Carassius auratus* based on the EPR method," Chemosphere, 65, pp. 1064-1073.
94. Naveed, A., Janaiah, C., 2011, "Effect of Triazophos on protein metabolism in the fish, *Channa punctatus,"* Curr Res J Biol Sci., 3, pp. 124-128.
95. Elisi, M, Monica, F., Pierluigi, B., Gu, U. F., and Maria, J. R., 2009, "Effects of four carbamate compounds on antioxidant parameters," Ecotoxicol Environ Safety, 72, pp. 922-930.
96. Rai, D. K., Rai, P. K., Rizvi, S. I.,Watal, G., and Sharma, B., *2009, "*Carbofuran-induced toxicity in rats: protective role of vitamin C," *Experimental and Toxicologic* Pathology., 61(6), pp.531–535. doi: 10.1016/j.etp.2008.11.003.
97. Tulok, I., Matkovics, A., 1997, "Antioxidansok a megelozesben es a gyogyításban," Hazio Tvk Szle., 2, pp. 446-450.
98. Jalili, Sh., Ilkhanipour, M., Heydari, R., Farshid, A. A., Salehi, S., 2007, "The effects of Vitamin E on endosulfan induced oxidative stress in rat heart.," Pakistan Journal of Nutrition, 6, pp. 375-380.
99. Selen, O., Misra, H., 2006, "Pesticides induced oxidative stress in thymocytes," Mol Cell Biochem., 290, pp. 137-144.
100. Zhao, M., Zhang, Y., Wang, C., Fu, Z., Liu, W., and Gan, J., 2009, "Induction of macrophage apoptosis by an organochlorine insecticide acetofenate," Chem Res Toxicol., 22, pp. 504-510.
101. Sahoo, A., Samanta, L., Chainy, G. B. N., 2000, "Mediation of oxidative stress in HCH induced neurotoxicity in rat," Archiv Environ Contam Toxicol., 39, pp. 7-12.
102. Rosadele, C., Gabriella, A., 2003, "The role of oxidative stress in the in vitro induction of micronuclei by pesticides in mouse lung fibroblasts," Mutagenesis, 18, pp. 127-132.
103. Abdollahi,, M., Ranjbar, A., Shadnia, S., Nikfar, S., and Rezaie, A., 2004, "Pesticide and oxidative stress: A review," Med Sci Monitor, 10, pp. 144-147.
104. Yousef, M. I., Awad, T. I., and Mohamed, E. H., 2007, "Deltamethrin-induced oxidative damage and biochemical alterations in rat and its attenuation by Vitamine," Toxicol., 227, pp. 240-247.
105. Giray, B., Gürbay, A., and Hincal, F., 2001, "Cypermethininduced oxidative stress in rat brain and liver is prevented by Vitamin C or allopurinol," Toxicol Lett., 118, pp. 139-146.
106. Atessahin, A., Yilmaz, S., Karahan, U., Purun, C. Ü. U., and Tasdemir, B., 2005, "The Effects of Vitamin E and Selenium on Cypermethrin-Induced Oxidative Stress in Rats," Turk J Vet Anim Sci., 29, pp. 385-391.
107. Gabbianelli, R., Falcioni, G., Nasuti, C., and Cantalamessa, F., 2005, "Cypermethrin-induced plasma membrane perturbation on erythrocytes from rats: reduction of fluidity in the hydrophobic core and in glutathione peroxidase activity," Toxicol. , 175, pp. 91-101.