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LAMBDA CYHALOTHRIN INDUCED TOXICITY ON ANTIOXIDANT ENZYMES IN SELECTED TISSUES OF ALBINO MICE

Gokul K[1], Dr. Suseela M[2] , Dr. Venkata Chandrudu M[3]*

[1]Research Scholar, Department of Zoology, Rayalseema University, Kurnool, AP, India. Email: gokulkanchi07@gmail.com
[1]Assistant Professor, Department of Zoology, Vikrama Simhapuri University PG Centre, Kavali, Nellore, AP, India. Email: drsuseelameesala@gmail.com
**Corresponding Author: Assistant Professor (MTS), Department of Zoology, University College of Science, Rayalseema University, Kurnool, AP, India. Email: chandramidde4@gmail.com*

ABSTRACT

Lambda-cyhalothrin is a synthetic type II pyrethroid, insecticide used worldwide in agriculture, home pest control, protection of food stuff and disease vector control. The present study was under taken to evaluate the influence of long term intoxication of mice with sub toxic doses of Lambda cyhalothrin on the activity of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and Lipid peroxidation in selected tissue Liver and Kidney of Albino mice. The mice were exposed to Lambda-cyhalothrin, during 10, 20 and 30 consequent days. This exposure caused significant decrease in antioxidants enzyme activities and reduced glutathione content was observed in Lambda cyhalothrin group compared to controls. The activity of catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase were decreased, due to consumption of these enzymes to neutralize free radicals generated by Lambda.

Key Words: Antioxidant enzymes, Lambda cyhalothrin, Pyrethroid, SOD, CAT, GR, GST

INTRODUCTION:

Pollutants can act synergistically to cause uncertain long-term effects on biodiversity. Much of the recent work examining the effects of pesticides has concentrated on the newer generations of pesticides such as pyrethroids, carbamates and organophosphates, although there has been a resurgence of interest in the older organochlorine insecticides. Synthetic pyrethroids represent one quarter of the insecticides used in the agriculture all over the world. This belongs to diverse class of potent, broad spectrum insecticides used to control insect pests in animals, agriculture, households, and stored products. Synthetic pyrethroids of the second and third generations are photostable and highly effective against broad spectrum of insects.

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Oxygen is the primary oxidant in metabolic reactions designed to obtain energy from the oxidation of a variety of organic molecules. Oxidative stress results from the metabolic reactions that use oxygen, and it has been defined as a disturbance in the equilibrium status of pro-oxidant/anti-oxidant systems in intact cells. This definition of oxidative stress implies that cells have intact pro-oxidant/anti-oxidant systems that continuously generate and detoxify oxidants during normal aerobic metabolism. When additional oxidative events occur, the pro-oxidant systems outbalance the anti-oxidant, potentially producing oxidative damage to lipids, proteins, carbohydrates, and nucleic acids, ultimately leading to cell death in severe oxidative stress. Mild, chronic oxidative stress may alter the anti-oxidant systems by inducing or repressing proteins that participate in these systems, and by depleting cellular stores of anti-oxidant materials such as glutathione and vitamin E.

Free radicals are highly reactive molecules generated by biochemical redox reactions that occur as a part of normal cell metabolism. The human body has an inherent synergistic and multilevel defense mechanism, which comprise of two major classes of cellular protection against ROS. Free radical scavenger enzymes namely SOD, CAT and GPx represent the enzymatic part. The non-enzymatic part includes a large number of natural and synthetic antioxidant compounds (GSH and vitamins) that have the ability to inhibit oxidative stress by scavenging the highly destructive free radicals (Halliwell, 1996). The deleterious effects of the free radicals are kept under check by a delicate balance between the rate of their production and the rate of their elimination by these defense systems. When there is an excessive addition of free radicals from exogenous sources added to the endogenous production, the available tissue defense system becomes overwhelmed resulting in oxidative damage to the tissues. The extent of ROS-induced oxidative damage can be exacerbated by a decreased efficiency of antioxidant defense mechanisms.

Lipid peroxides exert their toxic effects through two general mechanisms. Since lipids are responsible for maintaining the integrity of cellular membranes, extensive peroxidation of lipids alters the assembly, structure, and dynamics of lipid membranes Lipid peroxides are produced in many cellular contexts and can serve as signaling molecules through post-translational modification of proteins. Malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acids peroxidation in the cells. An increase in free radicals causes over production of MDA. Malondialdehyde level is commonly known as a marker of oxidative stress and the antioxidant status. This aldehyde is a highly toxic molecule and should be considered as more than just a

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marker of lipid peroxidation. MDA readily combines with several functional groups on molecules including DNA, proteins, and lipoprotein.

The present study was under taken to evaluate the influence of long term intoxication of mice with subtoxic doses of lambda cyhalothrin on the activity of catalase, glutathione S-tranferase, SOD, glutathione reductase, glutathione peroxidase and glutathione-S-transferase (GST) in different tissue of mice.

MATERIALS AND METHODS:

Chemicals

Lambda cyhalothrin with 95% purity was used as the test chemical for the present study.

Animals

Healthy and sexually matured animals of the same age group of 60 days and weight 30±5 grams were taken from parental stock obtained from Veterinary College, Bangalore and maintained a colony and they were kept in well cleaned and sterilized cages. Prior to experimentation the animals were acclimatized according to the instructions given by Behringer (1973).

Experimental design

As the acute Oral LD_{50} value of Lambda cyhalothrin was 24 mg/kg body weight, five fold lower ($1/5^{th}$) concentration (4.8 mg/kg body weight) was selected as sub lethal dose to study the effect of Lambda cyhalothrin. Healthy adult animal's weight (30±5g) was divided into four groups having ten animals each. The second, third and fourth groups of animals were termed as experimental animals. To the second group of animal's multiple doses of Lambda cyhalothrin for l0 days with one day interval (i.e. on 1st, 3rd, 5th, 7th, 9th day etc.) was administered orally with the help of a gavage. Similarly doses were given to the third and fourth group of animals for 20 and 30 days respectively. The first group of animals was considered as control.

Isolation of tissues

The control and experimental animals after a stipulated period (*i.e.* on 11th, 21st and 31st day) were sacrificed and the tissues were quickly isolated, cleaned in physiological saline and processed immediately for microscopic analysis under ice-cold conditions. The tissues were also quickly isolated and were kept in deep freezer at -80°C and used for biochemical analysis.

Biochemical analysis:

Superoxide dismutase activity: Superoxide dismutase activity was measured as the inhibition of photo reduction of nitro blue tetrazolium (NBT) by an enzyme as per the method of Beauchamp and Fridovich (1971).

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Catalase activity: Catalase activity was measured by the following method of Aebi (1984).

Glutathione Peroxidase: Se-Dependent Glutathione Peroxidase was determined by a modified version Flohe and Gunzler (1984) at 37˚C

Glutathione Reductase (GR): GR activity was determined by a slightly modified method of Carlberg and Mannervik (1985)

Statistical analysis:

All data are expressed as mean ± standard deviation. Data were analyzed by the analysis of variance test, using the SPSS version 13.0 for Windows (SPSS, Inc., Chicago, Illinois, USA) statistical program, and the individual comparisons of groups were obtained by using Scheffe's post-hoc analysis at $P < 0.001$ and $P < 0.05$.

RESULTS AND DISCUSSION

In this present investigation oral exposure of albino mice to Lambda cyhalothrin resulted in gradual decline in SOD, CAT, GPx and GR of Liver and Kidney. The decrease in SOD, CAT, GPx and GR is in dose and time dependent manner. The results of SOD, CAT, GPx and GR of the control group and experimental group of albino mice under Lambda cyhalothrin are presented in Table 1-4 and Figure 1-4. The results of Malonoldialdehyde content in the control and experimental albino mice treated with Lambda cyhalothrin under the study were given in theTable 5 and Figure5. The experimental animal mice induced by Lambda cyhalothrin showed the increase of malonoldialdehyde content in Liver andKidney.

The present results compared to control animal's treatment with Lambda cyhalothrin are in consistent with following authors, such as, the decreased activities of antioxidant enzymes were observed in rat kidney (Fetoui *et. al.,* 2010); and rat liver (Fetoui *et. al.,* 2009). They showed the induced effect of LCT on failure of antioxidant defense system to overcome the influx of ROS. The decreased levels in SOD activity in Lamdba cyhalothrin exposed mice may be due to the increased production of superoxide radical anions. Catalase scavenges H2O2 that has been generated by free radicals or by SOD in removal of superoxide anions. Chemical induces oxidative stress that leads to generation of free radicals and brought change in antioxidant and lipid peroxidation (Ender and Onder, 2006). Antioxidant enzymes act as free radical scavengers and slow down not only radical oxidation but also the accompanying damaging effects on the body (Mossa *et al.,* 2012; Marzouk et al.,, 2011; Dwivedi et al., 1998). The present study shows that increased concentration of lipid peroxidation in high dose exposure to Lambda cyhalothrin. The

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end product of peroxidation of polyunsaturated fatty acids is Malanoldehyde and is taken as a marker of lipid peroxidation (Buyukokuroglu*et al.,* 2018).

Hence it was concluded that, the regular dosing of synthetic pyrethroid Lamda cyhalothrin in low doses of may lead to gradual depletion in the levels of SOD, CAT, GR and GPx. The increased production of the free radicals and decreased levels of endogenous antioxidant enzymes indicates the Oxidative stress induced potentiality of Lambda cyhalothrin.

Table 1: Alterations in levels of SOD activity in Lambda cyhalothrin treated albino mice tissuses (Superoxide anion reduced/mg of protein/minute).

Tissue name	Control	10 days	20 days	30 days
Liver				
Mean	2.195	3.332	2.843	2.498
SD	±0.081	±0.137	±0.24	±0.233
PC		(51.39)	(29.51)	(13.87)
Kidney				
Mean	0.953	1.352	1.22	1.115
SD	±0.017	±0.170	±0.0752	±0.035
PC		(41.88)	(28.05)	(16.98)

±SD-Standard Deviation; PC - Percent Change over control;

One Way Anova

Source of Variation	DF	Liver	Kidney
		Mean Squares	Mean Squares
Between Groups	3	1.818*	0.188*
Within Groups	20	0.127	0.002
Total	23		

All the values are Significant at $P<0.05$

Table 2: Alterations in levels of catalase activity in Lambda cyhalothrin treated albino mice tissuses (µmoles of H_2O_2 decomposed /mg protein/min)

Tissue name	Control	10 days	20 days	30 days
Liver				
Mean	0.4885	0.704	0.5935	0.5677
SD	±0.00691	±0.00854	±0.00319	±0.00244
PC		(45.75)	(21.49)	(16.11)
Kidney				
Mean	0.1864	0.2999	0.2677	0.235
SD	±0.00438	±0.00522	±0.00388	±0.00898
PC		(60.83)	(43.56)	(26.11)

±SD-Standard Deviation; PC - Percent Change over control.

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One Way Anova

Source of Variation	DF	Liver Mean Squares	Kidney Mean Squares
Between Groups	3	0.8789*	0.0176*
Within Groups	20	0.00542	0.0043
Total	23		

All the values are Significant at $P<0.05$

Table 3: Alterations in levels of GPx activity in Lambda cyhalothrin treated albino mice tissuses. (µmoles of NADPH oxidized/mg protein/hour).

Tissue name	Control	10 days	20 days	30 days
Liver **Mean** **SD** **PC**	0.7791 ±0.0127	0.3842 ±0.0197 (50.68)	0.532 ±0.0188 (31.7)	0.6856 ±0.0163 (12.00)
Kidney **Mean** **SD** **PC**	0.6529 ±0.0168	0.3404 ±0.01931 (47.86)	0.4357 ±0.01835 (33.26)	0.5768 ±0.01405 (11.66)

±SD-Standard Deviation; PC - Percent Change over control.

One Way Anova

Source of Variation	DF	Liver Mean Squares	Kidney Mean Squares
Between Groups	3	0.2414*	0.042*
Within Groups	20	0.0115	0.00038
Total	23		

All the values are Significant at $P<0.05$

Table 4: Alterations in levels of GR activity in Lambda cyhalothrin treated albino mice tissuses (µmoles of NADPH oxidized/mg protein/hour)

Tissue name	Control	10 days	20 days	30 days
Liver **Mean** **SD** **PC**	0.7311 ±0.013	0.3559 ±0.015 (51.32)	0.5092 ±0.013 (30.34)	0.6456 ±0.022 (11.69)
Kidney **Mean** **SD** **PC**	0.6020 ±0.02197	0.309 ±0.01731 (48.67)	0.4074 ±0.01349 (32.33)	0.5236 ±0.02104 (13.05)

±SD-Standard Deviation; PC - Percent Change over control.

One Way Anova

Source of Variation	**DF**	**Liver Mean Squares**	**Kidney Mean Squares**
Between Groups	3	0.00506*	0.002*
Within Groups	20	0.000075	0.00009
Total	23		

All the values are Significant at P<0.05

Table 5: Alterations in malonoldialdehydecontent in Lambda cyhalothrin treated albino mice tissuses (µ moles of malonoldialdehyde formed /gm.wet wt. of tissue)

Tissue name	**Control**	**10 days**	**20 days**	**30 days**
Liver				
Mean	16.572	22.432	25.476	29.684
SD	±0.2343	±0.3868	±0.3066	±0.4430
PC		(35.360)	(48.844)	(69.811)
Kidney				
Mean	12.224	15.668	17.889	20.892
SD	±0.4455	±0.4230	±0.3894	±0.4282
PC		(28.174)	(39.253)	(60.061)

±SD-Standard Deviation; PC - Percent Change over control.

One Way Anova

Source of Variation	DF	Liver Mean Squares	Kidney Mean Squares
Between Groups	3	128.472	43.421
Within Groups	20	0.627	0.435
Total	23		

All the values are Significant at P<0.05

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Fig 1: Alterations in levels of SOD activity in Lambda cyhalothrin treated albino mice tissuses (Superoxide anion reduced/mg of protein/minute).

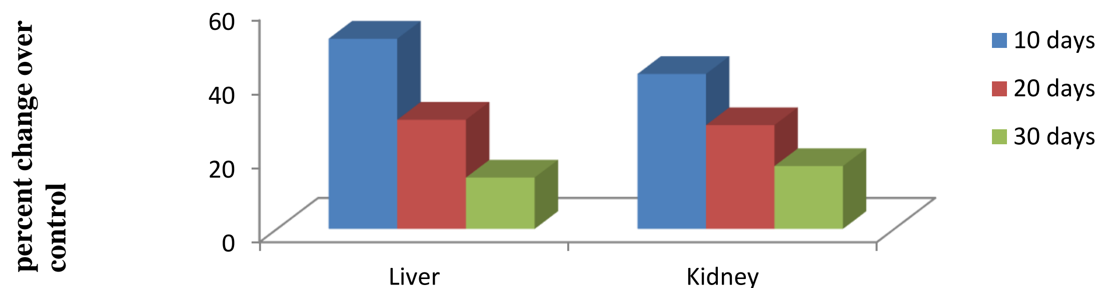


Fig 2: Alterations in levels of catalase activityin Lambda cyhalothrin treated albino mice tissuses (µmoles of H_2O_2 decomposed /mg protein/min)

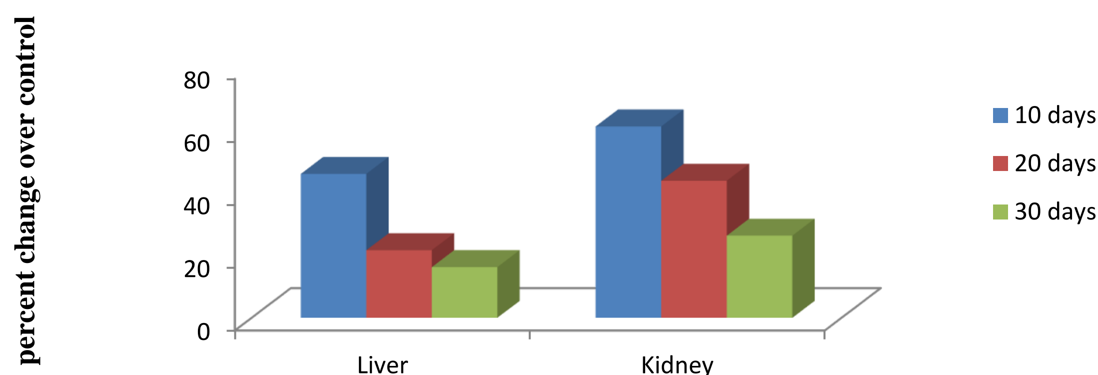
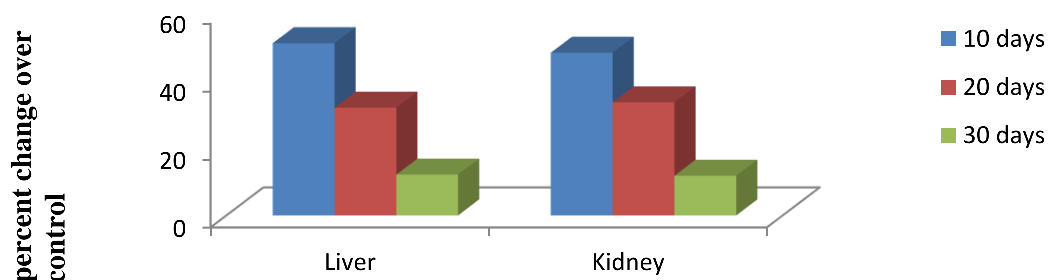
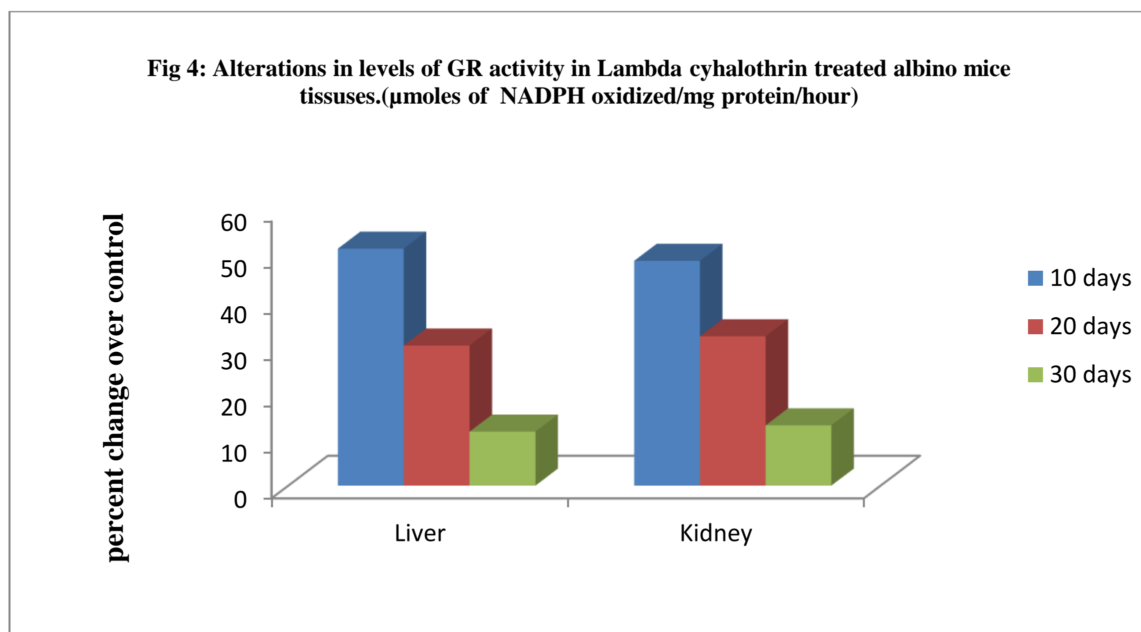
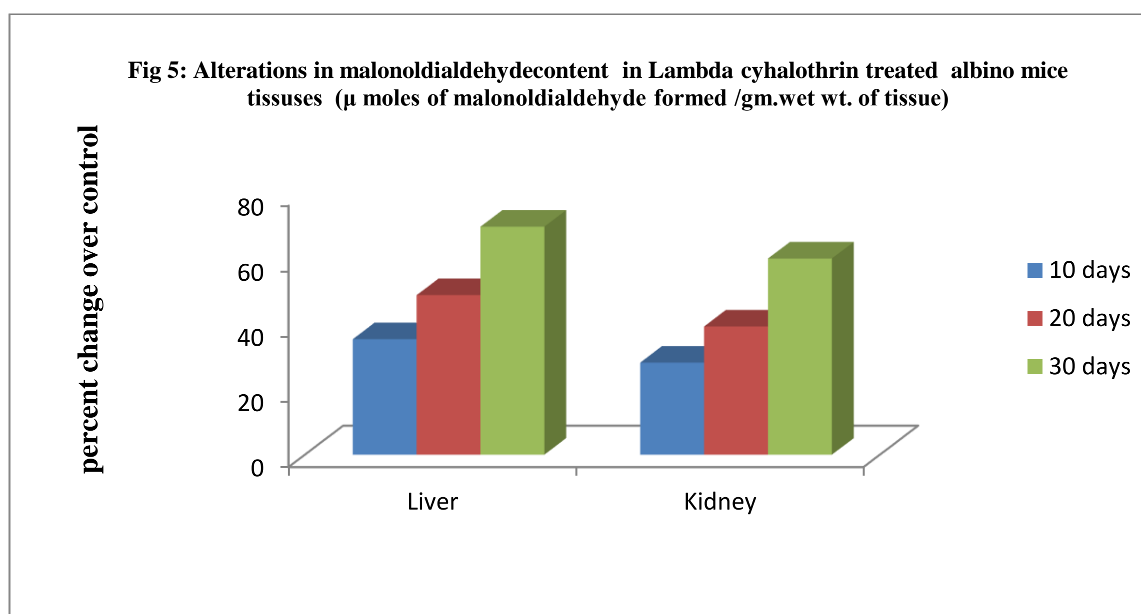


Fig 3: Alterations in levels of GPx activity in Lambda cyhalothrin treated albino mice tissuses. (µmoles of NADPH oxidized/mg protein/hour).







DISSCUSSION

In the present study, the activities of antioxidant enzymes (GPx, GST and GR) and GSH were decreased significantly in LCT-treated mice. In consistent with these results, the decreased activities of antioxidant enzymes were observed in rat liver (Fetoui et al., 2009), in rat kidney (Fetoui et al., 2010) and in fish liver (*Oreochromis niloticus*) (Piner and Üner, 2012). They indicated the failure of antioxidant defense system to overcome the influx of ROS induced by LCT exposure. LCT toxicity might be due to the release of cyanohydrins, which are unstable under

physiological conditions and further decompose to cyanides and aldehydes which in turn could act as a source of free radicals (World Health Organization, 1990). Depletion of tissue GSH is one of the major factors that permit lipid peroxidation and subsequent tissue damage (Huang et al., 2003). GSH is known to function as a substrate for GPx and GST. Decrease in the activity of SOD and CAT could be due to inactivation of the enzymes by cross-linking or due to exhaustion of the enzymes by increased peroxidation. Pesticides and numerous environmental stressors may induce oxidative stress, leading to generation of free radicals and alterations in antioxidants, or oxygen free radicals and alteration in antioxidants, or oxygen free radical, the scavenging enzyme system, and lipid peroxidation (Akhgari et al., 2002). Generation of reactive oxygen species and the resulting damage may be mediated directly by a toxic compound and its metabolites, or by alterations of metabolic links, which indirectly increase the process of free radical generation (Liczmanski 1988a; Southern and Powis, 1988).

Antioxidant enzymes are used by the organisms as natural endogenous protection against the generation of reactive oxygen species (Halliwell, 1984). Superoxide dismutases are metalloenzyme scavengers, which destroy superoxide radicals by converting them into hydrogen peroxide and oxygen by dismutation. However, the reaction product hydrogen peroxide must be removed, not only because it is mildly toxic and inactivates SOD enzymes, but also because it can form other more reactive and damaging free radical species, including the hydroxyl radical. SOD works in conjunction with two enzymes, glutathione peroxidase and catalase, that degrade hydrogen peroxide to water and oxygen .

Glutathione reductase converts oxidised glutathione back to glutathione (Halliwell, 1984). The normal pro-oxidant/antioxidant balance of the cell can be shifted in favour of pro-oxidants when the production of oxygen species is increased as following exposure to chemicals or drugs), or when levels of antioxidants are diminished. This state is called oxidative stress and can result in serious cell damage if the stress is massive or prolonged. The decreased activity of antioxidant enzymes observed in this study can therefore be explained in several ways. Pesticides may be metabolized to an oxygen free radical that re-oxidizes to make superoxide; the pesticide may itself be a free radical; or the pesticide may deplete antioxidant defences. The overall effect of pesticide is the production of more free radicals. The activity of superoxide dismutase, glutathione peroxidase and glutathione reductase were decreased, due to consumption of these enzymes to neutralize free radicals generated by pesticides.

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