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Antioxidant Vitamins Attenuate Oxidative Stress Induced by Pesticides in Rats

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Abstract

Pesticides are extensively used in agricultural and domestic settings, raising concerns about their potential to induce oxidative stress and associated health risks. The present study investigates the impacts of acute and chronic exposure to pesticides, alone and in mixtures, and measures their harmful effect by assessing changes in Thiobarbituric Acid-Reactive Substances (TBARS) levels in various rat tissues. The study examines how two Organophosphate (OP) pesticides (monocrotophos and chlorpyrifos) and two Organochlorine (OC) pesticides (lindane and endosulfan), given alone or together, affected TBARS levels in rat tissues. Rats received 1.8mg Monochrotophos (MCP), 15.5mg Chlorpyrifos (CPF), 10mg Endosulfan (ENS), and 8.8mg Lindane (LND)/kg body weight (equal to 1/10 Lethal Dose (LD50)), mixed in 0.4ml peanut oil. Additionally, the protective role of antioxidant vitamins (vitamin A: 2.0 µg, vitamin E: 20.0 µg, and vitamin C: 120 µg/kg body weight) was assessed through pre-treatment prior to pesticide exposure. Results from both acute and chronic administration showed a significant, dose-dependent rise in TBARS levels. It was observed that the joint administration of pesticides caused more oxidative damage than the single administration of the pesticides. Pretreatment of the animals with antioxidants showed a reduction in TBARS levels, indicating their protection against oxidative damage. In conclusion, the results clearly demonstrate that the effect of pesticides on oxidative stress is dose-dependent, especially during exposure in combination, whereas vitamin supplements can be used as a possible approach to counteract these adverse effects.

Major Findings: Combined pesticide exposure significantly increased TBARS levels across all examined tissues in a dose- and duration-dependent manner, with greater oxidative damage than individual pesticide exposure. Pretreatment with vitamins A, C, and E substantially reduced TBARS levels, although values remained above controls, indicating partial protection against pesticide-induced oxidative stress.

Keywords: Lipid Peroxidation, Malondialdehyde, Organophosphate, Organochlorine, Reactive Oxygen Species, Thiobarbituric Acid Reactive Substances.

1. Introduction

Pesticides are man-made substances aimed at managing pests, yet they have evolved into common pollutants that endanger both human and environmental health. Employing various pesticide categories at the same time heightens the likelihood of cross-contamination and intricate exposures to combinations. Pesticides are harmful as they induce oxidative stress primarily by generating Reactive Nitrogen Species (RNS) and Reactive Oxygen

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Species (ROS). These molecules harm crucial components of cells, such as polyunsaturated fatty acids in the membrane of cells, vital structural and functional proteins, and genetic information contained in nucleic acids (DNA and RNA). The oxidative cascade starts with the generation of ROS, which target membrane lipids, causing peroxidation that eventually generates products of Lipid Peroxidation (LPO) like TBARS, acting as indicators of oxidative damage. This cascade leads to fractured DNA strands, modified DNA bases, protein oxidation, and elevated LPO. Elevated free radicals increase LPO. Diminished antioxidant defences are key factors in the toxicity associated with OC, OP, and pyrethroid pesticides[1,2]. Assessing LPO products, such as TBARS, and antioxidant levels aids in quantifying oxidative stress and assessing pesticide exposure.

Pesticides cause oxidative stress, RNS, and ROS generation in various cells and animal models. Oxidative stress is among the most significant mechanisms in pesticide toxicity[3]. The histopathological findings revealed significant degeneration in the pesticide groups. The levels of serum acetylcholinesterase activity, liver glutathione, total antioxidant status, acetylcholinesterase, catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, Na+/K+ ATPase, aryl hydrocarbon receptor, and paraoxonase were significantly lower, while serum tumor necrosis factor-α, liver malondialdehyde, homocysteine, nitric oxide, advanced oxidation protein products, total oxidant status, ROS, and oxidative stress index levels as well as aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, glutathione-S-transferase, arginase, and xanthine oxidase[4] and LPO was significantly increased in Wistar rats exposed to various categories of pesticides at an equal dose (0.1 LD50), with chlorpyrifos resulting in higher lipid oxidation and injury compared to other pesticides. Pesticide administration increased the concentration of nitric oxide in the plasma by more than twice. Administration of CPF resulted in significant increases in Nitric Oxide (NO) concentration in liver tissues[5]. In another study, it is clearly revealed that Thiamethoxam (TMX) is highly toxic in a dose-dependent manner, with oxidative stress and DNA damage playing a prominent role in the process[6].

The toxicological studies on pesticides address individual compounds[7], yet limited work explores the health effects of pesticide mixtures, despite evidence of their increased and synergistic toxicities[8–15]. Interactions in mixtures can be additive, synergistic, or antagonistic, underscoring the need for such research[9,16,17]. Most existing studies on pesticide toxicity primarily focus on individual compounds under controlled conditions, whereas real-world exposure typically involves complex mixtures that may exhibit enhanced or altered toxic effects. Additionally, limited research has systematically examined both acute and chronic exposure to combined OP and OC pesticides across multiple tissues using reliable oxidative stress biomarkers such as TBARS. Furthermore, although antioxidant vitamins are reported to mitigate oxidative damage, their protective role under mixed pesticide exposure remains insufficiently explored. Therefore, there is a clear need to investigate the combined, time-dependent toxicity of pesticide mixtures and the potential protective effects of antioxidant vitamins *in vivo*.

This study uniquely investigates the combined effects of OP and OC pesticide mixtures under both acute and chronic exposure, addressing a key gap in mixture toxicology. It also evaluates, for the first time in this context, the protective role of combined antioxidant vitamins (A, C, and E) as a preventive strategy against oxidative damage. It hypothesises that pesticide mixtures induce enhanced oxidative stress compared to individual exposures, and that pre-treatment with antioxidant vitamins significantly mitigates LPO and tissue damage.

2. Materials and Methods

2.1 Substances and Test Animals

Thiobarbituric acid, glacial acetic acid, sodium chloride, sodium dodecyl sulfate, butanol, pyridine, 1,1,3,3-tetramethoxypropane, and various other chemicals were procured from Sigma-Aldrich in St. Louis, MO. CPF, MCP, ENS, and LND pesticides were sourced from the Scientific Fertiliser Company in Tiruchirapalli, Tamil Nadu, India. Male albino Wistar rats (120 ± 20 g) were sourced from the animal facilities of Defence Research and Development Establishment (DRDE) in Gwalior, India. The animals were kept in accordance with the authorised regulations of the 'Committee for the Purpose of Control and Supervision of Experiments on Animals' (CPCSEA, India). Permission had been obtained prior to the experiment for animal use (Session - 2010/11) following the protocols of the Animal Ethics Committee (IAEC) at Jiwaji University, Gwalior, MP (Registration no. 1854/GO/Re/S/16/CPCSEA). Proper care was taken during the experiment to minimise animal usage and pain. Two groups were utilised for both acute and chronic studies.

2.2 Research Methodology

Rats were allocated into experimental groups (n = 6/group) to assess the level of LPO caused by both acute and chronic exposure to a pesticide combination. In the acute study, animals were sacrificed at 24, 48 and 72 hours following treatment. In the long-term exposure study, rats were sacrificed 24 hours following the last dose of the pesticide mixture.

2.3 Pesticides Exposure

Rats were exposed to acute and chronic pesticides. For the acute study, rats were exposed to each pesticide alone or to a pesticide mixture, given orally suspended in peanut oil for 1, 2, and 3 days, and for 2 months each day for the chronic study.

2.4 Immediate Pesticide Exposure

Rats were randomly assigned to 6 groups, with each group containing three animals. The initial group was administered 1.8 mg/kg body weight of MCP (1/10 LD50) via oral route in 0.4ml of peanut oil for a duration of 3 days. The second group was given 15.5mg CPF/kg (1/10 LD50) in the same manner. The third group was administered 8.8 mg of LND/kg (1/10 LD50), while the fourth group was given 10 mg of ENS/kg (1/10 LD50), with both substances mixed in 0.4ml of peanut oil over a duration of three days. The fifth group was given an equal volume of peanut oil and acted as the control. The experiment was conducted twice.

Animals were randomly distributed into 6 groups, each having 9 rats. Animals in the first and second groups received 0.05mg and 0.1mg of pesticide mixture/kg body wt orally, respectively (corresponding to a total of 1/20 and 1/10 LD50 of the pesticide mixture, with each pesticide's presence equal to 1/40 and 1/80 LD50, respectively, in the mixture, resulting in total amounts of 1/20 and 1/10 LD50). The third and fourth groups were administered an equal quantity of the pesticide mixture along with a blend of antioxidant vitamins A (2.0µg), E (20.0µg), and C (120µg/kg body wt). A and E were given vitamins mixed in peanut oil, whereas C was given vitamins mixed in water. The mixtures were then combined before application. These dosages were set according to standard human dietary recommendations and modified for rat metabolism to maintain physiological credibility without surpassing usual rat nutritional needs. Citing established rat Recommended Dietary Allowance (RDA) or earlier research on rat antioxidants could strengthen this reasoning and alleviate worries regarding excessive nutritional intake. The fifth and sixth groups were given the same volume (0.4ml) of peanut oil and an equal quantity of vitamin mixture, respectively, and acted as controls. Three rats were sacrificed through cervical dislocation from each group following one, two, and three days of exposure to the pesticide mixture. Liver, kidney, spleen, and brain tissue samples were rinsed with 0.9% Sodium Chloride (NaCl) to eliminate leftover blood and then prepared for the TBARS assay. The experimental procedure was conducted twice to guarantee consistency.

2.5 Prolonged Exposure to a Blend of Pesticides

Animals were randomly placed into 6 groups, with 6 rats per group. The first and second groups were given daily oral doses of a pesticide mixture dissolved in peanut oil at levels corresponding to LD_{50} and 2 × LD_{50}, respectively, for two months, whereas the third and fourth groups were exposed to the same pesticide mixture combined with a blend of antioxidant vitamins every day for the same duration. The fifth and sixth groups were given an equal volume (0.4ml) of peanut oil along with the same quantity of antioxidant vitamin mixture daily for the same duration, serving as the control group. The antioxidant amount was established according to previous research that showed the effectiveness of vitamins A, C, and E in lowering oxidative stress in similar contexts[18,19]. Rats in the first and third groups were given a total pesticide dose equal to the LD_{50}, whereas the other treatment group received a cumulative dose of 2 × LD_{50}. The control group was given only peanut oil for the same two-month period. All animals were euthanised one day after the last treatment, and tissue samples were gathered for the TBARS assay.

2.6 Measurement of Thiobarbituric Acid Reactive Substances

The assessment of TBARS was conducted using the procedure outlined by Ohkawa *et al.*[20].

2.7 Analysis of Statistical Data

Data are presented as mean ± S.E. Statistical evaluations were conducted utilising SigmaStat V-2.0 software. The one-way ANOVA was applied with Bonferroni's test.

3. Results and Discussion

Elevated production of ROS is linked with numerous diseases and the harmful effects of several compounds[21]. LPO commonly occurs due to free radical assaults on biological structures[22]. In this research, individual pesticide exposures notably increased TBARS levels in all rat tissues. This suggests heightened oxidative stress and LPO. MCP led to the highest increase among the pesticides evaluated. The brain tissue exhibited the greatest vulnerability to oxidative harm (Table 1).

Exposure to the pesticide combination also markedly elevated TBARS levels in all rat tissues in a dose-dependent fashion, with the highest elevation noted in the brain. For example, TBARS levels in the brain increased by roughly 2.3 times compared to the liver, emphasising the brain's greater susceptibility to oxidative harm. Extended exposure to pesticide combinations led to a time-related rise in TBARS levels in all tissues, signifying cumulative oxidative harm (Table 2).

Sixty days of pesticide exposure resulted in significant, dose-dependent TBARS increases in all rat tissues, with the highest values observed at $2LD_{50}$ doses. The brain consistently showed greater oxidative damage than other organs (Table 3).

The pre-treatment with antioxidant vitamins significantly diminished LPO caused by pesticides, decreasing TBARS levels across all tissues. In most tissues, TBARS levels dropped by roughly 48% when vitamins were administered alongside pesticides compared to when pesticides were given alone. The protective effect remained consistent across various doses and exposure times, with the brain exhibiting the most significant decrease. Pre-treatment with vitamins markedly decreased TBARS

Table 1. Changes in concentration of TBARS caused by single pesticides in various rat tissues

Organs	Control	MCP Pesticide	CPF Pesticide	ENS Pesticide	LND Pesticide
Liver	261.33±10.48	797.50±15.11***	453.77±52.83 *	719.57±14.04 ***	585.97±9.97 ***
Kidney	229.00±2.52	745.93±9.29 ***	397.46±50.48 *	665.74±9.42 ***	485.70±9.21 ***
Brain	169.33±2.67	619.43±11.61 ***	408.26±3.35 ***	594.98±14.49***	396.47±6.81 ***
Spleen	435.00±12.00	1010.18±22.59 ***	924.39±23.52 ***	987.93±16.29 ***	897.08±7.82 ***

Data are presented as mean ± S.E. derived from 6 sets of observations. MDA concentrations are represented as nmol g^{-1} of tissue. Animals were administered 1.8mg MCP, 15.5mg CPF, 10mg ENS, and 8.8mg LND/kg body wt (equivalent to 1/10 LD50), mixed in 0.4ml peanut oil, over three days. Rats were euthanised 24 hours following the final treatment. Significance was set at P<0.05 relative to the control. *denote P<0.05, **denote P<0.01, ***denote P<0.001 # P>0.05.

Table 2. Changes in concentration of TBARS caused by one, two, and three days of exposure to a pesticide mixture in various rat tissues

Organs	Exposure (One day)			Exposure (Two days)			Exposure (Three days)		
	In the Control	LD50 (1/20) Dose	LD50 (1/10) Dose	In the Control	LD50 (1/20) Dose	LD50 (1/10) Dose	In the Control	LD50 (1/20) Dose	LD50 (1/10) Dose
Liver	84.20 ± 9.33	366.95 ± 15.84 ***	435.78 ±24.00 ***	182.25 ± 12.93	444.15 ± 8.07 ***	477.42 ±32.75 **	207.25 ±11.23	575.06 ± 23.67 ***	N.D.
Kidney	228.73 ± 8.34	404.66 ± 16.91 ***	502.71 ± 25.39 ***	246.89 ± 16.87	484.22 ± 15.98***	592.08 ±4.24 ***	198.11 ±12.34	507.47 ± 10.35 ***	N.D.
Brain	104.48 ± 16.75	237.29 ± 9.55 **	244.77 ± 12.23 **	105.12 ± 9.97	270.75 ± 8.98 ***	302.10 ±15.00 ***	108.25 ± 5.79	276.00 ± 4.24 ***	N.D.
Spleen	481.64 ± 14.62	723.12 ± 37.69 **	788.07 ± 35.95 **	433.25 ±11.52	740.49 ± 26.47***	870.50 ±12.58 ***	402.75 ±38.60	773.75 ± 6.14 ***	N.D.

Data are presented as mean ± S.E. based on 6 observation sets. MDA concentrations are measured in nmole g^{-1} of tissue. Animals administered a pesticide mixture orally at dosages of 0.05 and 0.1 mg/kg body wt (1/20 and 1/10 LD50, with each pesticide at 1/40 and 1/80 LD50 within the mixture), using peanut oil, over a duration of three days. Controls received 0.4ml of peanut oil. Significance is established by P<0.05 relative to the control. *denotes P<0.05, **denote P<0.01, ***denote P<0.001 # P>0.05.

Table 3. Change in concentration of TBARS caused by long-term exposure to a pesticide combination in various rat tissues

Organ	Control	LD50 Dose	2 LD50 Dose
Liver	371.73 ± 21.04	974.14 ± 16.18***	1188.78±32.68***
Kidney	385.61 ± 24.85	1091.01±26.76***	1319.83±38.68***
Brain	246.50 ± 6.94	691.37 ± 17.90***	828.74 ± 13.39***
Spleen	832.14 ± 28.79	1723.28 ± 124.04*	2070.00 ± 171.47*

Data are presented as mean ± S.E. based on 6 sets of observations. MDA concentrations are measured in nmole g^{-1} of tissue. Animals were administered a pesticide blend (LD50 or 2LD50) in peanut oil orally for a duration of 60 days. Animals were euthanised 24 hours following the final treatment. Significance was determined at P<0.05 relative to the control. *denotes P<0.05, **denote P<0.001, ***denote P<0.0001 # P>0.05.

Table 4. Changes in concentration of TBARS caused by one, two, and three days of exposure to a pesticide blend in various rat tissues previously administered antioxidant vitamins

Organ	Exposure (One day)			Exposure (Two days)			Exposure (Three days)		
	In the Control	LD50 (1/20) Dose	LD50 (1/10) Dose	In the Control	LD50 (1/20) Dose	LD50 (1/10) Dose	In the Control	LD50 (1/20) Dose	LD50 (1/10) Dose
Liver	164.79 ± 4.15	251.38 ± 9.67**	301.03 ± 5.44***	130.66 ± 4.22	237.44 ± 4.71***	274.65 ± 4.27***	173.92 ± 4.34	286.48 ± 3.25***	327.52 ± 5.20***
Kidney	193.70 ± 4.68	247.32 ± 19.76*	325.05 ± 9.29***	153.76 ± 6.27	226.96 ± 4.03***	284.14 ± 3.36***	134.00 ± 5.54	219.63 ± 6.11***	270.15 ± 2.17***
Brain	97.61 ± 1.42	141.21 ± 10.39*	176.97 ± 4.55***	95.33 ± 1.22	168.47 ± 2.97***	213.76 ± 4.13***	102.77 ± 2.11	145.74 ± 6.37**	163.15 ± 3.16***
Spleen	301.44 ± 5.11	383.05 ± 16.47**	435.50 ± 15.12**	369.13 ± 7.83	458.10 ± 5.84***	510.34 ± 7.09***	375.87 ± 2.88	497.14 ± 5.86***	559.74 ± 16.20***

Data are presented as mean ± S.E. from 6 distinct observation groups; MDA levels are given in nmole g^{-1} of tissue. Animals were administered 0.05mg and 0.1mg of a pesticide mixture per kg body weight orally, corresponding to 1/20 and 1/10 LD50 of the pesticide mixture, respectively, with each pesticide present at 1/40 and 1/80 LD50, respectively, in the mixture. This was suspended in peanut oil alongside a combination of A vitamin (2.0µg), E vitamin (20.0µg), and C vitamin (120µg/kg body wt) for one day, and the rats were euthanised after 24 hours.

Table 5. Change in concentration of TBARS caused by prolonged exposure to a pesticide blend in various rat tissues pre-treated with antioxidant vitamins

Organ/Treatment	Control	LD50 Dose	2 LD50 Dose
Liver	260.83 ± 15.55	406.86 ± 17.00*	602.78 ± 24.20***
Kidney	298.01 ± 17.76	625.22 ± 13.77***	797.18 ± 20.42***
Brain	199.00 ± 6.32	302.11 ± 9.20***	381.03 ± 14.39***
Spleen	403.44 ± 17.50	695.42 ± 23.01***	896.89 ± 25.81***

Data are presented as mean ± S.E. from 6 distinct sets of data; MDA levels are reported in nmole g^{-1} of tissue. Animals, previously fed an antioxidant, received a pesticide mixture (dosage equivalent to LD50 and 2LD50) suspended in peanut oil for sixty days orally and were euthanised 24 hours post-final treatment. Significance is determined at P<0.05 relative to the control.

increases from 1/10 LD_{50} pesticide exposure over three days, although levels still exceeded control values in all tissues (Table 4).

Chronic supplementation with A, E, and C vitamins significantly reduced TBARS levels in all rat tissues exposed to LD_{50} and $2LD_{50}$ pesticide mixtures. Even in control rats, vitamin treatment slightly reduced TBARS levels, confirming its protective antioxidant effect (Table 5).

The impact of pesticide exposure on oxidative stress is caused by the uncontrolled production of ROS, which exceeds the capacity of the body's antioxidants to neutralise them. These reactive oxygen species

include superoxide anions, hydroxyl ions, and hydrogen peroxide, which cause oxidation of polyunsaturated fatty acids found in the cell membrane, leading to LPO [3]. The reactions involved in lipid peroxidation occur through an initiation stage, propagation stage, and termination stage, culminating in secondary products like Malondialdehyde (MDA), determined as TBARS. LPO results in membrane dysfunction, increased fluidity, and functional abnormalities. Vitamins A, C, and E help reduce oxidative damage by quenching free radicals and preventing the progression of LPO [5].

The current investigation showed elevated TBARS levels, which indicate LPO, in liver tissue, kidney tissue, brain tissue, and spleen tissue of rats treated with pesticides. Numerous studies in the literature indicate that LPO rises after exposure to different chemicals and radiation, in addition to a variety of diseases. Yavuz *et al*.,[23] discovered that exposure to methidathion resulted in cardiotoxicity, with significantly increased MDA levels observed in these patients, which could be improved by vitamin E and C treatment. The notable rise in TBARS levels due to the pesticide mixture also suggested a pronounced peroxidation effect, aligning with earlier studies[24]. In our research, every pesticide-treated tissue exhibited heightened LPO, indicated by increased TBARS levels, implying that ROS-induced oxidative damage is the primary toxic effect of the four pesticides examined.

LPO is a highly damaging process that changes the structure and function of the cell membrane, resulting in oxidative modification of essential targets. Altuntas *et al*.,[25] evaluated the *in vitro* and *in vivo* effects of methidathion on serum cholinesterase activity, liver injury, and LPO. They noted that MDA levels were found to be elevated in the methidathion group as compared to the control group. Exposure to Malathion increased serum MDA levels and the activity of various antioxidant enzymes. Kale *et al*.,[26] examined the impact of pyrethroids, specifically cypermethrin and fenvalerate, on MDA levels in rats, finding that these pesticides raised MDA levels and boosted the activities of antioxidative enzymes. Oral exposure to Dichlorodiphenyltrichloroethane (DDT) and LND led to a dose-dependent rise in serum TBARS levels.

Oxidative stress can damage membrane lipids, alter their fluidity and increasing membrane permeability, thereby disrupting cell function. It can also inactivate enzymes or membrane-bound receptors. LPO may further harm cells by producing oxidised products. A few of these may be chemically reactive species that can modify key macromolecules[27,28]. LPO in the brain is especially dangerous as compared to other organs because the brain is very prone to oxidative damage due to higher metabolic rate, low antioxidant defences, presence of easily oxidised Polyunsaturated Fatty Acids (PUFA), and high levels of transition metals[27,29–31]. Several other reports also support the findings that there is an elevation in the TBARS levels and production of ROS on different OP pesticide exposures[18,19,32–37].

4. Conclusion

This study shows that both single and mixed administration of OP and OC pesticides cause a significant increase in TBARS levels in the liver, kidneys, brain, and spleen of rats, thus causing oxidative damage. The impact is more marked in the case of mixed exposure, increases with increasing concentration and duration, and the brain is highly sensitive to the effect. Vitamin A, C, and E pretreatment successfully decreased the TBARS levels in all studied organs in both cases (acute and chronic). However, the vitamin treatment failed to normalise TBARS levels back to normal, indicating that it only mitigated the effect.

A key limitation of this study is its reliance on a single oxidative stress biomarker (TBARS), without assessment of additional antioxidant enzymes or molecular endpoints, which may limit comprehensive interpretation of oxidative mechanisms. Furthermore, the findings are based on an animal model and controlled exposure conditions, which may not fully represent complex real-world exposure scenarios. Despite these limitations, the study provides clear experimental evidence of enhanced toxicity from pesticide mixtures and the mitigating effect of antioxidant vitamins.

5. References

1. Abdollahi M, Ranjbar A, Shadnia S, Nikfar S, Rezaie A. Pesticides and oxidative stress: A review. Med Sci Monit. 2004; 10(6):141-147. PMID: 15173684.
2. El-Demerdash FM. Lipid peroxidation, oxidative stress and acetylcholinesterase in rat brain exposed to organophosphate and pyrethroid insecticides. Food Chem Toxicol. 2011; 49(6):1346-1352. https://doi.org/10.1016/j.fct.2011.03.018 PMid:21419823.
3. Sule RO, Condon L, Gomes AV. A common feature of pesticides: oxidative stress-the role of oxidative stress in pesticide-induced toxicity. Oxid Med Cell Longev. 2022;

2022(1):5563759. https://doi.org/10.1155/2022/5563759 PMid:35096268 PMCid:PMC8791758.

4. Coremen M, Turkyilmaz IB, Us H, Us AS, Celik S, Ozel AE, Bulan OK, Yanardag R. Lupeol inhibits pesticide-induced hepatotoxicity via reducing oxidative stress and inflammatory markers in rats. Food Chem Toxicol. 2022; 164:113068. PMID: 35483487 https://doi.org/10.1016/j.fct.2022.113068 PMid:35483487.
5. Yadav AS. Malondialdehyde and nitric oxide levels reveal comparative toxicity of three insecticides in Wistar rats. Toxicol Mech Methods. 2026; 36(3):376-388. https://doi.org/10.1080/15376516.2025.2601988 PMid:41379097.
6. Issa SY, Rahman SA, Gaber YM, Soliman NA. Toxicological impact of Thiamethoxam on adult male rats: Histopathological, biochemical, and oxidative DNA damage assessment. Toxicol Rep. 2025; 14:101983. https://doi.org/10.1016/j.toxrep.2025.101983 PMid:40206789 PMCid: PMC11979398.
7. Carpenter DO, Arcaro K, Spink DC. Understanding the human health effects of chemical mixtures. Environ Health Perspect. 2002; 110(1):25. PMCID: PMC1241145 https://doi.org/10.1289/ehp.02110s125 PMid:11834461 PMCid:PMC1241145.
8. Bailey HC, Miller JL, Miller MJ, Wiborg LC, Deanovic L, Shed T. Joint acute toxicity of diazinon and chlorpyrifos to *Ceriodaphnia dubia*. Environ Toxicol Chem. 1997; 16(11):2304-2308. https://doi.org/10.1002/etc.5620161115
9. Belden JB, Lydy MJ. Impact of atrazine on organophosphate insecticide toxicity. Environ Toxicol Chem. 2009; 19(9):2266-2274. https://doi.org/10.1002/etc.5620190917
10. Chauhan LKS, Chandra S, Saxena PN, Gupta SK. *In vivo* cytogenetic effects of a commercially formulated mixture of cypermethrin and quinalphos in mice. Mutat Res - Genet Toxicol Environ Mutagen. 2005; 587(1-2):120-125. https://doi.org/10.1016/j.mrgentox.2005.08.004 PMid:16185912.
11. DeLorenzo ME, Serrano L. Individual and mixture toxicity of three pesticides: Atrazine, chlorpyrifos, and chlorothalonil to the marine phytoplankton species *Dunaliella tertiolecta*. J Environ Sci Health B. 2003; 38(5):529-538. https://doi.org/10.1081/PFC-120023511 PMid:12929712.
12. Denton DL, Wheelock CE, Murray SA, Deanovic LA, Hammock BD, Hinton DE. Joint acute toxicity of Esfenvalerate and Diazinon to larval fathead minnows (*Pimephales promelas*). Environ Toxicol Chem. 2003; (2):336-341. PMID: 12558165 https://doi.org/10.1002/etc.5620220214 PMid:12558165.
13. Lushchak VI, Matviishyn TM, Husak VV, Storey JM, Storey KB. Pesticide toxicity: A mechanistic approach. Excli J. 2018; 17:1101. PMCID: PMC6295629.
14. Roloff BD, Belluck DA, Meisner LF. Cytogenetic studies of herbicide interactions *in vitro* and *in vivo* using Atrazine and Linuron. Arch Environ Contam Toxicol. 1992; (3):267-271. PMID: 1616310 https://doi.org/10.1007/BF00212084 PMid:1616310.
15. Singh A, Mahajan M, Kothari R, Singh NK, Singh RP. Mechanistic action of pesticides on pests and their consequent effect on fishes and human health with remediation strategies. Aqua Water Infrastruct Ecosyst Soc. 2023; 72(3):363-380. https://doi.org/10.2166/aqua.2023.233
16. Abaineh A, Ejigu D, Atlabachew M, Dejen E, Tilahun G. Risks of pesticides on aquatic ecosystems and pesticide management effectiveness in Ethiopia. Int J Environ Sci Technol. 2024; 21(13):8833-8848. https://doi.org/10.1007/s13762-024-05631-7
17. Navarro I, Royano S, Alonso C, de la Torre A, Martínez MÁ. Environmental exposure and risk assessment of pesticide mixtures in aquatic organisms from the Tagus River Basin. Ecotoxicol Environ Saf. 2025; 305:119221. https://doi.org/10.1016/j.ecoenv.2025.119221 PMid:41101120.
18. Kalender S, Kalender Y, Durak D, Ogutcu A, Uzunhisarcikli M, Cevrimli BS, Yildirim M. Methyl parathion-induced nephrotoxicity in male rats and protective role of vitamins C and E. Pestic Biochem Physiol. 2007; 88(2):213-218. https://doi.org/10.1016/j.pestbp.2006.11.007
19. Verma RS, Mehta A, Srivastava N. *In vivo* chlorpyrifos-induced oxidative stress: attenuation by antioxidant vitamins. Pestic Biochem Physiol. 2007; 88(2):191-196. https://doi.org/10.1016/j.pestbp.2006.11.002
20. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979; 95(2):351-358. https://doi.org/10.1016/0003-2697(79)90738-3 PMid:36810 PMCid:PMC8374294.
21. Halliwell B, Zentella A, Gomez EO, Kershenobich D. Antioxidants and human disease: A general introduction. Nutr Rev. 1997; 55(1):S44-S55. https://doi.org/10.1111/j.1753-4887.1997.tb06100.x PMid:9155225.
22. Stohs S J. Synthetic pro-oxidants: drugs, pesticides and other environmental pollutants. In Ahmad S. (eds) Oxidative stress and antioxidant defenses in biology. 1995. Boston, MA: Springer US. https://doi.org/10.1007/978-1-4615-9689-9_4
23. Yavuz T, Delibas N, Yildirim B, Altuntas I, Candır O, Cora A, Karaman N, Ibrisim E, Kutsal A. Vascular wall damage in rats induced by methidathion and ameliorating effect of vitamins E and C. Arch Toxicol. 2004;78(11):655-659. https://doi.org/10.1007/s00204-004-0593-9 PMid:15368089.
24. Banerjee BD, Seth V, Bhattacharya A, Pasha ST, Chakraborty AK. Biochemical effects of some pesticides on lipid peroxidation and free-radical scavengers. Toxicol Lett.1999; 107(1-3):33-47. https://doi.org/10.1016/S0378-4274(99)00029-6 PMid:10414779
25. Altuntas I, Delibas N, Sutcu R. The effects of organophosphate insecticide methidathion on lipid peroxidation and

antioxidant enzymes in rat erythrocytes: Role of vitamins E and C. Hum Exp Toxicol. 2002; 21(12):681-685. https://doi.org/10.1191/0960327102ht304oa PMid:12540039.
26. Kale M, Rathore N, John S, Bhatnagar D. Lipid peroxidative damage on pyrethroid exposure and alterations in antioxidant status in rat erythrocytes: A possible involvement of reactive oxygen species. Toxicol Lett. 1999; 105(3):197-205. https://doi.org/10.1016/S0378-4274(98)00399-3 PMid:10355540.
27. Astiz M, de Alaniz MJ, Marra CA. Antioxidant defense system in rats simultaneously intoxicated with agrochemicals. Environ Toxicol Pharmacol. 2009; 28(3):465-473. PMID: 21784044 https://doi.org/10.1016/j.etap.2009.07.009 PMid:21784044.
28. Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of oxidative damage in human disease. Clin Chem. 2006; 52(4):601-623. https://doi.org/10.1373/clinchem.2005.061408 PMid:16484333.
29. Abuja PM, Albertini R. Methods for monitoring oxidative stress, lipid peroxidation and oxidation resistance of lipoproteins. Clin Chim Acta. 2001; 306(1-2):1-7. PMID: 11282089 https://doi.org/10.1016/S0009-8981(01)00393-X PMid:11282089.
30. Desport JC, Couratier P. Stress oxydant et maladies neurodégénératives. Nutr Clin Metab. 2002; 16(4):253-259. https://doi.org/10.1016/S0985-0562(02)00172-3
31. Dringen R. Metabolism and functions of glutathione in brain. Prog Neurobiol. 2000; 62(6):649-671. https://doi.org/10.1016/S0301-0082(99)00060-X PMid:10880854.
32. Celik I, Suzek H. Effects of subacute exposure of dichlorvos at sublethal dosages on erythrocyte and tissue antioxidant defense systems and lipid peroxidation in rats. Ecotoxicol Environ Saf. 2009; 72(3):905-908. https://doi.org/10.1016/j.ecoenv.2008.04.007 PMid:18539328.
33. Mahjoubi-Samet A, Fetoui H, Zeghal N. Nephrotoxicity induced by dimethoate in adult rats and their suckling pups. Pestic Biochem Physiol. 2008; 91(2):96-103. https://doi.org/10.1016/j.pestbp.2008.01.009
34. Ranjbar A, Solhi H, Mashayekhi FJ, Susanabdi A, Rezaie A, Abdollahi M. Oxidative stress in acute human poisoning with organophosphorus insecticides: A case-control study. Environ Toxicol Pharmacol. 2005; 20(1):88-91. https://doi.org/10.1016/j.etap.2004.10.007 PMid:21783573.
35. Sharma Y, Bashir S, Irshad M, Gupta SD, Dogra TD. Effects of acute dimethoate administration on antioxidant status of liver and brain of experimental rats. Toxicology. 2005; 206(1):49-57. https://doi.org/10.1016/j.tox.2004.06.062 PMid:15590108.
36. Sulak O, Altuntas I, Karahan N, Yildirim B, Akturk O, Yilmaz HR, Delibas N. Nephrotoxicity in rats induced by organophosphate insecticide methidathion and ameliorating effects of vitamins E and C. Pestic Biochem Physiol. 2005; 83(1):21-28. https://doi.org/10.1016/j.pestbp.2005.03.008
37. Verma RS, Mehta A, Srivastava N. Comparative studies on chlorpyrifos and methyl parathion-induced oxidative stress in different parts of rat brain: Attenuation by antioxidant vitamins. Pestic Biochem Physiol. 2009; 95(3):152-158. https://doi.org/10.1016/j.pestbp.2009.08.004

Author Contribution

SKY: Original draft, Writing - review and editing, Formal analysis, Data curation, Investigation, Methodology. **NK:** Writing - review and editing. **BY:** Writing – review and editing. **SKM:** Writing - review and editing.

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