RESEARCH ARTICLE

Journal of Ecophysiology and Occupational Health, *Vol 24(4), DOI: 10.18311/jeoh/2024/43442, 415-425, December 2024*

ISSN (Online) : 0972-4397
ISSN (Online) : 0974-0805

Occupational Exposure to Mixed Pesticides in Northern West Bengal Tea Gardens: Impact on Acetylcholinesterase Inhibition, Oxidative Stress, and Antioxidant Markers Among Female Tea Pluckers

Ananya Bose[1,2], Sudipta Kundu[3], Oly Banerjee[1,4], Siddhartha Singh[1], Anindita Banerjee[1], Ranajit Mandal[5], Bithin Kumar Maji[1] and Sandip Mukherjee[1*]

[1]Department of Physiology, Serampore College, Hooghly - 712201, West Bengal, India; sm_kdc@yahoo.co.in
[2]Paramedical Section, Bengal Institute of Pharmaceutical Sciences, Kalyani, Nadia - 741235, West Bengal, India
[3]Department of Physiology, Kalka Dental College, Meerut - 250006, Uttar Pradesh, India
[4]Department of Medical Laboratory Technology, School of Allied Health Sciences, Swami Vivekananda University, Bara Kanthalia - 700121, West Bengal, India
[5]Department of Pathology, Bhatpara State General Hospital, Jagatdal - 743125, West Bengal, India

Abstract

Due to the potential health impacts of pesticides, biomonitoring of pesticide exposure is currently a subject of significant public interest. The purpose of the present research was to evaluate the levels of oxidative stress in female tea pluckers who are vulnerable to being exposed to a combination of pesticides in the northern part of West Bengal, India. Data were collected from 82 permanent female tea pluckers of two tea gardens in Jalpaiguri district and 68 women from the general population not directly exposed to the pesticides but living in the same geographical area. Acetylcholinesterase (AChE), Catalase (CAT), reduced glutathione (GSH), and Superoxide Dismutase (SOD) activity were measured, and levels of Lipid Peroxidation (LPO) and Nitric Oxide (NO) were determined in plasma. The student's t-test analyzed the disparity between the exposed and non-exposed groups. Correlation coefficient analysis was conducted to determine the relationship between AChE and biomarkers of oxidative stress. Compared to the control group plasma AChE activity was significantly decreased (17.42%) among female tea pluckers ($p \leq 0.001$). In comparison to the control group, female tea pluckers showed a significant increase in LPO and NO levels as markers of oxidative stress, as well as a significant decrease in GSH levels. Enzymatic antioxidants SOD and CAT were significantly reduced in the exposed group. Significant correlations were observed between AChE and biomarkers of oxidative stress. In conclusion, the alterations in different biochemical parameters indicate that female tea pluckers were occupationally exposed to mixed pesticides (containing organophosphates) in tea gardens. It is therefore imperative to highlight the usage of personal protective equipment by the pluckers and environmentally friendly pesticide alternatives in the tea gardens. Additional research is recommended to connect our results with the negative health impacts seen in chronic pesticide exposure, in which oxidative damage is believed to be a key factor.

Keywords: Acetylcholinesterase, Antioxidant Enzyme, Female Tea Plucker, Oxidative Stress, Occupational Exposure, Pesticide

**Author for correspondence*

Article Received on: 03.04.2024 Revised on: 13.06.2024 Accepted on: 21.06.2024

1. Introduction

Pesticides are a group of chemicals extensively used worldwide in intensive agriculture to enhance yields by eradicating harmful insects and managing disease-causing vectors[1]. There are substantial variations among pesticides in terms of their mechanisms of action, absorption, metabolism, excretion, and noxious effects on humans[2]. Pesticides used in agricultural and other work environments can enter the body through three main routes: oral, dermal, and inhalation. Insufficient knowledge, lack of education, indigence and other factors contribute to inappropriate and hazardous usage of pesticides, resulting in harmful health impacts[3]. Susceptibility to pesticides is predicted to rise in developing nations due to the prevalence of high temperatures, lack of proper nutrition and dehydration[2]. Exposure to pesticides is linked to numerous negative health outcomes, such as immunotoxicity, neurological damage, changes in reproductive and developmental characteristics, and cancer[4]. Numerous clinical symptoms, such as nausea, vomiting, runny nose, altered gait, sleep disturbances, paresthesia, anxiety, dizziness, headache, and abdominal pain, have been associated with chronic organophosphate toxicity[5]. According to the reports of the World Health Organization (WHO), approximately 3 million workers in developing nations suffer from serious pesticide poisoning every year, leading to the eventual deaths of around 18,000[6].

The main crop cultivated in the Terai and Dooars regions of West Bengal, India, is tea. The tea industry requires a huge labour force at every stage of production, from plantation to manufacturing, and a significant majority of the labour force is made up of women, mostly for the task of picking tea leaves[7]. Tea plantations frequently use a mixture of ethion, dicofol, heptachlor, chlorpyrifos, cypermethrin, beta-endosulfan, endosulfan sulfate, alpha-endosulfan, and deltamethrin as pesticides[6]. Female pluckers are highly susceptible to exposure to pesticides directly or indirectly at their workplace through inhalation, skin contact or ingestion of contaminated food[8]. Pesticides exert their toxic effects by overstimulating the cholinergic system as a result of enhanced acetylcholine buildup at neuro-neuronal and neuro-muscular junctions caused by acetylcholinesterase (AChE) inhibition[6,9,10]. A reduction in acetylcholinesterase activity in populations exposed to pesticides is strongly correlated with symptoms of pesticide poisoning[4,11]. Inhibition of AChE results in impairment of respiratory, myocardial, and neuromuscular transmission[12].

Pesticide metabolism leads to the production of highly reactive metabolites as well as Reactive Oxygen Species (ROS) and free radicals[1]. The ROS generated can interact with the major cellular macromolecules, leading to damage to carbohydrates, proteins, lipids, and nucleic acids. The disparity between the production of Oxygen-Free Radicals (OFRs) and the body's antioxidant defence system leads to oxidative stress, which has significant health consequences[13]. Antioxidant enzymes such as Glutathione Peroxidase (GPx), Glutathione Reductase (GR), Superoxide Dismutase (SOD), and Catalase (CAT) protect cells against intracellular oxidative stress[14,15]. Through compromising the antioxidant defences, oxidative stress is a major factor in developing metabolic, endocrinological, and neurological disorders[2,16]. Exposure to pesticides raises the levels of glutamate, which triggers the activation of N-Methyl-D-Aspartate (NMDA) receptors, leading to the generation of NO[17,18].

The current study was designed to measure the AChE activity, oxidative stress, and antioxidant biomarkers in the blood plasma of female tea pluckers who might be exposed to a combination of pesticides in their work environment.

2. Materials and Methods

2.1 Study Area and Selection of Subjects

This study was conducted in two tea gardens in Jalpaiguri, West Bengal, India from November 2021 to September 2023. Eighty-two (82) permanent female tea pluckers having a minimum work experience of two years were randomly selected for the study.

2.2 Inclusion and Exclusion Criteria

We included the female pluckers in our study if they fulfilled the following criteria-

1. Permanent worker
2. Minimum age ≥ 20 years, Upper age limit- 50 years
3. At least 2 years of work experience.

We excluded the female pluckers in our study if –

1. They were pregnant
2. They had an absence rate of ≥ 20%.

For the control group, data were collected from sixty-eight (68) women from the general population not directly exposed to pesticides but from the same geographical area. Written consent was taken from every participant and ethical clearance was taken from the Human Ethics Committee of Serampore College, West Bengal, India.

2.3 Clinical Assessment

Medical personnel examined both cases and controls for basic demographic data (age, height, weight), any health issues, tobacco and alcohol consumption patterns and occupational history with the aid of a well-structured questionnaire[19,20].

2.4 Blood Sampling and Processing

5ml venous blood was collected in the heparinized tube from 43 female tea pluckers and 31 women from the control group. To separate plasma the blood was centrifuged for 10 minutes at 4000 rpm at 4°C[12].

2.5 Measurement of Acetylcholinesterase Activity

AChE activity was assayed by the method of Ellman *et al.*[21] modified by Chambers JE and Chambers HW[22]. The substrate acetylthiocholine iodide was added to a phosphate buffer solution of DTNB (5,5'- dithio-bis- 2-nitrobenzoic acid) called Ellman's reagent. DTNB was used to quantify the number of thiol groups in the sample. The absorbance was measured at 410 nm. The whole recording was taken for about 5 minutes and AChE activity was expressed in µmoles/min/ml of plasma.

2.6 Estimation of Nitric Oxide Production

The amount of nitrite in plasma was estimated by using the principle of Griess reaction[23]. This nitrite comes from the decomposition of nitric oxides in oxygenated solutions. 100 µL of Griess reagent was incubated with 100 µL of plasma sample for 10 minutes at room temperature in the dark. ELISA Reader (thermo scientific) was used to measure the absorbance at 550 nm. Results were expressed as µM nitrite/mg of protein.

2.7 Assessment of Lipid Peroxidation

Thiobarbituric Acid (TBA) test was used to estimate malondialdehyde MDA as a marker of lipid peroxidation[24]. The results were expressed as nM of MDA/mg of protein.

2.8 Estimation of Superoxide Dismutase (SOD) Activity

Nitro Blue Tetrazolium (NBT) strategy (SOD inhibits the reduction of NBT) was used to measure Superoxide Dismutase (SOD) activity[25]. A 560 nm filter was used to measure the absorbance of blue formazan. The amount of SOD that produced a 50% decrease in the rate of degradation of NBT was equal to one unit of SOD activity/mg of protein, which was obtained by converting the relative absorbance.

2.9 Estimation of Catalase (CAT) Activity

Beer's method was used to estimate the catalase activity[26]. The difference in absorbance per unit time was used to calculate the CAT activity. Results were expressed in U/mg of protein.

2.10 Estimation of Reduced Glutathione (GSH)

The GSH level was quantified based on the reaction of DTNB (5,5' dithio-bis-2-nitrobenzoic acid) with the sulfhydryl group of reduced glutathione (GSH) which forms yellow-colored TNB (5-thio-2-nitrobenzoic acid) with maximum absorbance at 412 nm[27]. Results were expressed as mM/mg of protein.

2.11 Estimation of Plasma Protein

Lowry's method using bovine serum albumin as standard was used to estimate plasma protein[28].

2.12 Statistical analysis

The data were expressed as Mean ± SEM. To compare the means of the various groups, a two-tailed student's t-test was performed. Correlations between the selected variables were determined by Pearson's r correlation. Differences are considered significant if $p<0.05$ level. SPSS was used for statistical analysis.

3. Results

Table 1 represents the demographic profile and habits of exposed and non-exposed individuals. Age, weight, height, Blood Pressure (BP), Body Mass Index (BMI), heart rate, area of residence, educational and marital status and addiction habits were included. BMI was lower but the Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) were higher in the plucker population compared to the control group. The majority of the plucker population (60.98%) was illiterate and were more likely to be addicted (76.83% tobacco consumers and 8.54% drinkers).

AChE activity measurement is widely used to assess exposure to organophosphate and carbamate compounds found in pesticides, which are potent inhibitors of AChE. AChE activity in the study population has been represented in Figure 1. Analysis showed a substantial difference in AChE activity between the female tea pluckers and the control group (2.641±0.54 vs. 2.182±0.54 µmoles/min/ml of plasma, p<0.001). AChE activity was reduced by 17.42% among pluckers.

Table 1. Comparison of demographic features of female tea pluckers and control subjects

Variables	Plucker (n=82)Mean±SD	Control (n=68)Mean±SD
Age (Y)	35.95±7.36	27.24±8.60
Height (cm)	149.49±5.75	147.65±5.51
Weight (kg)	45.70±9.75	48.22±9.92
BMI (kg/m$^{2)}$	20.42±4.01	22.02±3.97
Blood pressure (mmHg)		
SBP	129.18±29.48	119.74±14.50
DBP	87.68±18.30	77.53±8.33
Heart rate (beats/min)	90.91±10.56	85.87±10.97
Ethnicity		
Urban	0	0
Rural	82(100%)	68 (100%)
Education		
Illiterate	50 (60.98%)	13 (19.12%)
School	32(39.02%)	46 (67.65%)
Graduate	0	7 (10.29%)
Postgraduate	0	2 (2.94%)
Work experience	16.12±8.84 (4-40Y)	0
Marital status		
Married	81 (98.78%)	51 (75%)
Unmarried	1 (1.22%)	17 (25%)
Widow	0	0
Tobacco consumption		
Yes	63(76.83%)	28(41.18%)
No	19(23.17%)	40(58.82%)
Alcohol consumption		
Yes	7(8.54%)	0
No	75(91.46%)	68(100%)

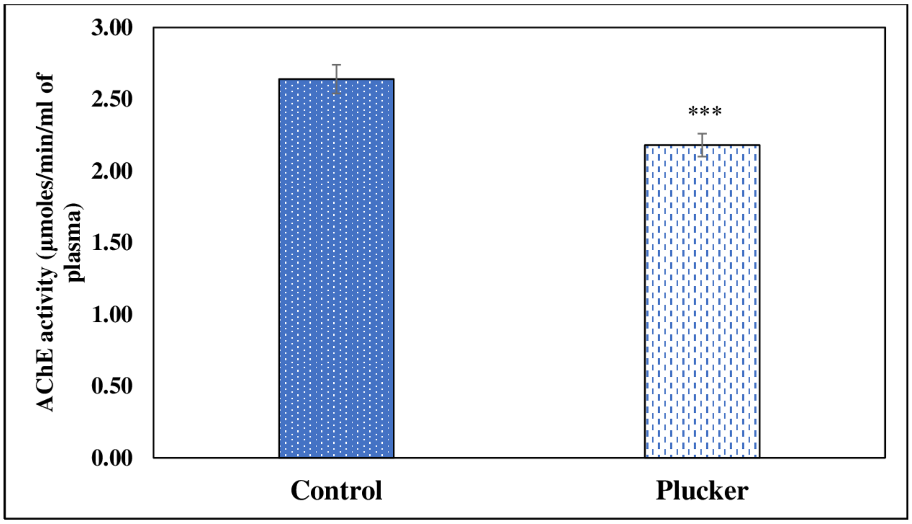

Figure 1. Acetylcholinesterase activity (µmoles/min/ml of plasma) in pesticide-exposed female tea pluckers (n=43) and control (n=31) subjects. Values are presented as Mean ± SEM. Based on the two-tailed student's t-test, the significance level is ***p < 0.001.

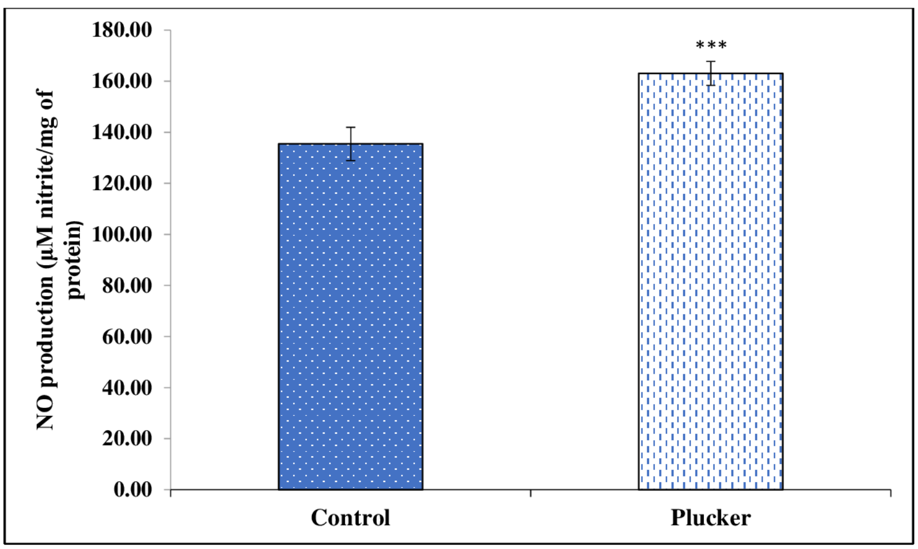

Figure 2. NO production (µM nitrite/mg of protein) in pesticide-exposed female tea pluckers (n=43) and control (n=31) subjects. Values are presented as Mean ± SEM. Based on the two-tailed student's t-test, the significance level is ***p < 0.001.

Considering oxidative stress, a condition marked by an imbalance between the body's antioxidant defences and the formation of ROS, it is especially important to measure the levels of NO and lipid peroxidation. Both NO and lipid peroxidation are key players in oxidative stress, and their measurement provides valuable insights into the extent of oxidative damage occurring in biological systems. The pesticide-exposed plucker group showed a considerably higher NO level (20.4%, $p<0.001$) in comparison to the unexposed control group (Figure 2). The reactivity of Thiobarbituric Acid Reactive Substances (TBARS) to MDA was used to measure the degree of lipid peroxidation. Reactions between ROS and polyunsaturated fatty acids result in the production of MDA, which is an important biomarker of oxidative stress (Figure 3). Compared to the control group, MDA activity was considerably higher (24.75%, $p<0.001$) in the plucker group exposed to pesticides.

Measuring the activity of SOD and catalase in oxidative stress is crucial for understanding the body's

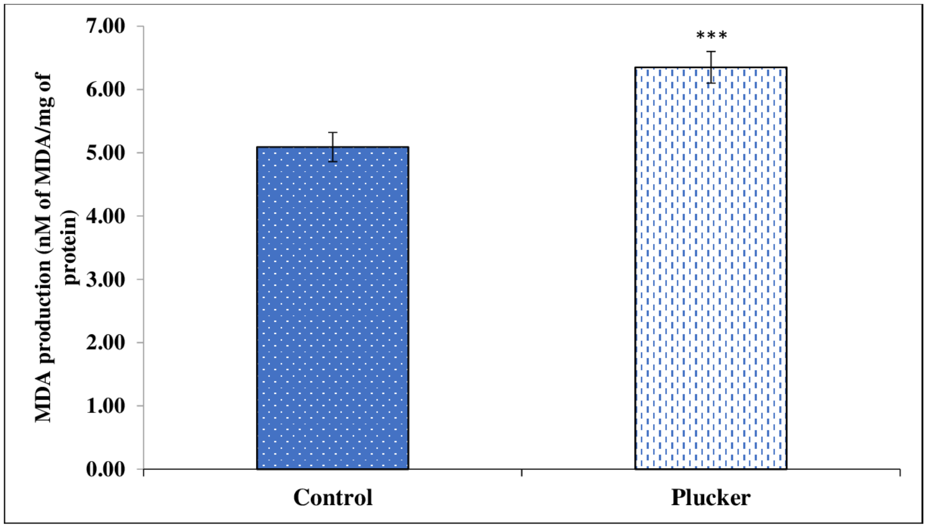

Figure 3. MDA production (nM of MDA/mg of protein) in pesticide-exposed female tea pluckers (n=43) and control (n=31) subjects. Values are presented as Mean ± SEM. Based on the two-tailed student's t-test, the significance level is ***$p < 0.001$.

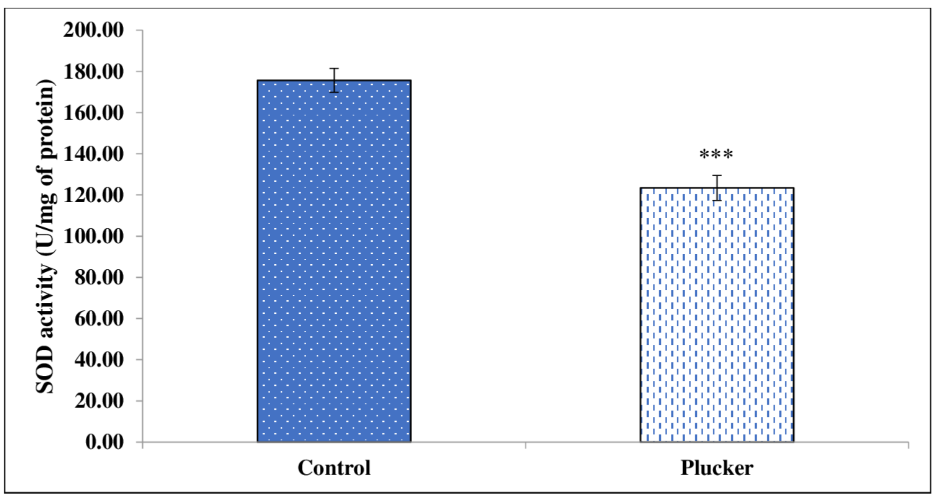

Figure 4. SOD activity (U/mg of protein) in pesticide-exposed female tea pluckers (n=43) and control (n=31) subjects. Values are presented as Mean ± SEM. Based on the two-tailed student's t-test, the significance level is ***$p < 0.001$.

antioxidant defence mechanisms and assessing the impact of oxidative stress on cellular health. Both SOD and catalase are key enzymes which protect cells against oxidative damage by scavenging reactive oxygen species. Superoxide radicals are converted by SOD into hydrogen peroxide and molecules of oxygen, while hydrogen peroxide is converted into water by catalase. Figure 4 depicts the SOD activity of the study population. Comparison between the unexposed control and exposed female pluckers showed a substantially reduced (29.74%, $p<0.001$) SOD activity among pluckers. Measurement of CAT activity in the study population showed (Figure 5) a significant decrease (27.12%, $p<0.001$) among pluckers.

Measuring GSH level is significant due to the pivotal role that GSH plays in cellular antioxidant defence mechanisms. GSH serves as one of the primary antioxidants in cells. The GSH level has been represented in Figure 6. The plucker group showed a substantial drop in GSH level (11.90%, $p<0.05$).

Table 2 illustrates Pearson's correlation coefficients between AChE and different variables of antioxidant enzymes and oxidative stress markers. According to our research AChE was substantially positively correlated with catalase, SOD and GSH (r=0.484, $p<0.01$; r=0.406, $p<0.01$; r=0.356, $p<0.05$) and substantially negatively

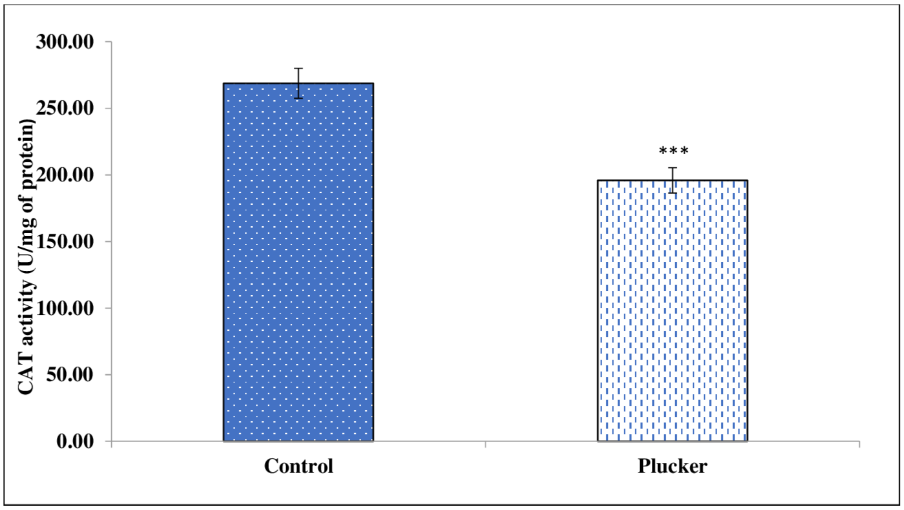

Figure 5. CAT activity (U/mg of protein) in pesticide-exposed female tea pluckers (n=43) and control (n=31) subjects. Values are presented as Mean ± SEM. Based on the two-tailed student's t-test, the significance level is ***p < 0.001.

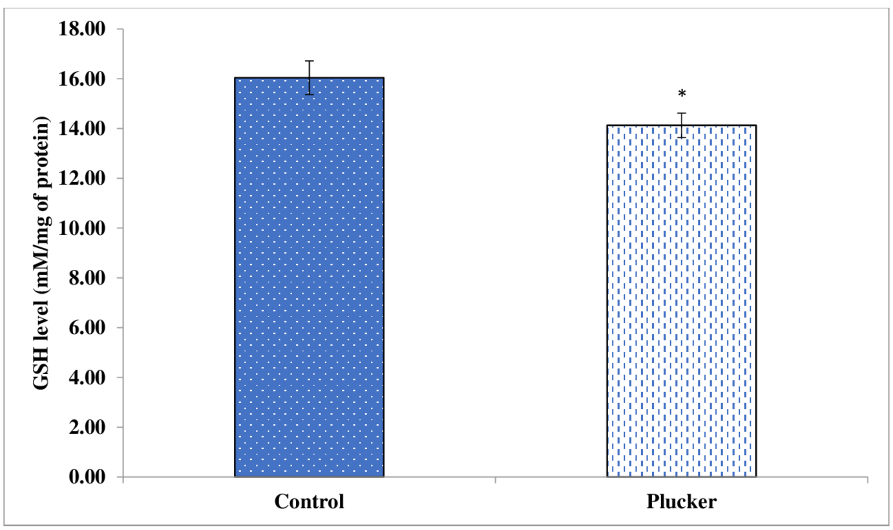

Figure 6. GSH level (mM/mg of protein) in pesticide-exposed female tea pluckers (n=43) and control (n=31) subjects. Values are presented as Mean ± SEM. Based on the two-tailed student's t-test, the significance level is *p < 0.05

Table 2. Pearson's correlation among different parameters of female tea pluckers

	AChE
CAT	0.484**
SOD	0.406**
GSH	0.356*
NO	-0.347*
LPO	-0.471**

Abbreviations: AChE, acetylcholinesterase; CAT, catalase; SOD, superoxide dismutase; GSH, reduced glutathione, NO, nitric oxide; LPO, lipid peroxidation. Significance level: $^{*}p<0.05$, $^{**}p<0.01$

linked to NO and LPO (r=-0.347, $p<0.05$; r=-0.471, $p<0.01$).

4. Discussion

Nearly 70% of the workforce in the tea industry is made up of women, who are traditionally the most skilled and potential pluckers. Female tea pluckers are more susceptible to work-related risks due to the prevalence of illiteracy and poor socioeconomic level. Lack of knowledge regarding the use of personal protective equipment like gloves, masks, and boots during plucking activity increases their risk of occupational exposure to pesticides.

Acetylcholine is the major neurotransmitter in the parasympathetic and preganglionic sympathetic nervous system and the neuromuscular junction. Acetylcholinesterase is an enzyme in the synaptic cleft that hydrolyzes acetylcholine and releases choline, which is used to reconstruct acetylcholine in the presynaptic neuron. A decrease in the level of acetylcholine causes continuous conduction of nerve impulses in the central nervous system, autonomic ganglia and neuromuscular junction. Organophosphates (phosphoric acid esters) and carbamates cause inhibition of acetylcholinesterase leading to increased accumulation of acetylcholine in synaptic cleft which can lead to muscle paralysis, convulsions, persistent glandular secretions, myosis, bronchoconstriction and death by asphyxiation. Various investigations have demonstrated that long-term pesticide exposure causes a range of negative health consequences, such as neurotoxicity, alterations in reproductive and developmental characteristics, immunological responses, and carcinogenesis[29-33]. The tea gardens in Terai and Dooars, West Bengal, use a diverse range of compounds as pesticides, including pyrethroids, organophosphates, and organochlorines[34,35]. AChE activity inhibition is frequently employed as a biomarker of exposure to carbamates and organophosphate pesticides in the workplace and environment[20]. The findings of our study suggest that the AChE activity in female tea pluckers was decreased by 17.42% when compared to that of controls due to the inhibition of AChE activity by occupational exposure to mixed pesticides which was supported by the earlier findings of Vidyasagar *et al.*[36] and also persistent with other previous studies[12,37,38]. Previous studies have demonstrated that inhibited AChE activity in workers exposed to pesticides is linked to cholinergic dysfunctions[2,39].

There was a dearth of research available to discuss how agriculture workers' exposure to pesticides could lead to neurodegenerative conditions like Alzheimer's disease[40,41]. The results of this study showed higher levels of NO and lipid peroxidation and decreased GSH, SOD and catalase activity among pesticide-exposed female tea pluckers, indicating that exposed agriculture workers experience increased oxidative stress because of pesticide toxicity[2,42-45]. Subchronic/chronic exposure to pesticides results in increased generation of ROS. Primarily their levels are balanced by first-line antioxidants such as GSH, SOD, and CAT and second-line antioxidants like vitamin E, vitamin C and vitamin A precursors. SOD converts superoxide anion into H_2O_2 which is further reduced by catalase and GSH to H_2O. When the amount of ROS outweighs the antioxidants lipid peroxidation of the cell membrane takes place. Besides this mitochondrial damage leads to increased cytosolic calcium causing phospholipid degradation and cytoskeletal damage which leads to increased production and accumulation of NO and MDA[13,16,43]. Compared to the control group, our results showed a 20.4% rise in NO activity and a 24.75% increase in MDA levels in the plucker population. Research on rodents (rats and mice) revealed that pyrethroids and organochlorines reduce antioxidant capacity, which leads to oxidative stress[46,47]. The reduction in GSH level by 11.90% in our investigation may be explained by the conjugation processes (consumption of GSH) inhibiting the cell's production of GSH. In our study, the SOD and catalase activity of pesticide-exposed pluckers were considerably lower than those of control participants by 29.74% and 27.12%, respectively which is in alignment with earlier research conducted by Prakasam *et al.* and López O *et al.*[48,49]. Previous studies revealed that chronic exposure to pesticides is associated with a positive correlation between AChE and SOD[49]. The coefficient correlation analysis demonstrates that AChE has a negative association with NO and LPO and a positive correlation with catalase, SOD, and GSH. A study by S.K. Rastogi[1] found a substantial negative correlation between AChE and MDA and a substantial positive correlation between AChE and GSH, which indicates increased lipid peroxidation among a pesticide-exposed population along with AChE inhibition, and the results support our findings in this study.

Increased oxidative stress levels ultimately lead to cell apoptosis via several pathways, including the NF-κB, Nrf2/Keap1/ARE, Ca^{2+}, TNF-α/TNFR1, STAT1, Nurr1, ASK1, and mitochondrial apoptosis pathways. The Ubiquitin-Proteasome System (UPS), which breaks down modified and misfolded proteins, may also be impacted by elevated levels of ROS and Reactive Nitrogen Species (RNS)[50]. Through the modification of reactive cysteines (Cys288, Cys273, and Cys151) and subsequent conformational change, ROS disturbs Keap1, causing IKKβ, Nrf2, and Bcl-2/Bcl-xL to be released from Keap1 and their ubiquitination and destruction to be suspended, leading to mitochondrial dysfunction, proteotoxicity and apoptosis[51-54].

5. Conclusion

The results of this study suggested that exposure to mixed pesticides (containing organophosphates and carbamates) was the cause of the decrease in enzyme activity and increase in oxidative stress indicators in female tea pluckers. It is therefore imperative to highlight the usage of personal protective equipment by the pluckers and environmentally friendly pesticide alternatives in the tea gardens. Antioxidants play an important protective role against chronic pesticide toxicity. Thus, supplementing with antioxidants may also be very beneficial in preventing the long-term harmful consequences of pesticide exposure. It is recommended that more research be done to connect our results to the harmful health impacts of long-term pesticide exposure, where oxidative damage is a pathophysiological factor.

6. References

1. Rastogi SK, Satyanarayan PV, Ravishankar D, Tripathi S. A study on oxidative stress and antioxidant status of agricultural workers exposed to organophosphorus insecticides during spraying. Indian J Occup Environ Med. 2009; 13(3):131-4. https://doi.org/10.4103/0019-5278.58916 PMID: 20442831; PMCID: PMC2862445.
2. Wafa T, Nadia K, Amel N, Ikbal C, Insaf T, Asma K, Hedi MA, Mohamed H. Oxidative stress, haematological and biochemical alterations in farmers exposed to pesticides. J Environ Sci Health B. 2013; 48(12):1058-69. https://doi.org /10.1080/03601234.2013.824285 PMID: 24007483.
3. Gilden RC, Huffling K, Sattler B. Pesticides and health risks. J Obstet Gynecol Neonatal Nurs. 2010; 39(1):103-10. https://doi.org/10.1111/j.1552-6909.2009.01092.x PMID: 20409108.
4. Simoniello MF, Kleinsorge EC, Scagnetti JA, Mastandrea C, Grigolato RA, Paonessa AM, Carballo MA. Biomarkers of cellular reaction to pesticide exposure in a rural population. Biomarkers. 2010; 15(1):52-60. https://doi.org/10.3109/13547500903276378 PMID: 19811113.
5. Dutta S, Bahadur M. Effect of pesticide exposure on the cholinesterase activity of the occupationally exposed tea garden workers of the northern part of West Bengal, India. Biomarkers. 2019; 24(4):317-24. https://doi.org/10.1080/1354750X.2018.1556342 PMID: 30512980.
6. 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; 2022:5563759. https://doi.org/10.1155/2022/5563759 PMID: 35096268; PMCID: PMC8791758.
7. Sivaram B. Productivity improvement and labour relations in the tea industry in South Asia. ILO Publications; 1996.
8. Kausar A, Giri S, Roy P, Giri A. Changes in buccal micronucleus cytome parameters associated with smokeless tobacco and pesticide exposure among female tea garden workers of Assam, India. Int J Hyg Environ Health. 2014; 217(2-3):169-75. https://doi.org/ 10.1016/j.ijheh.2013.04.007 PMID: 23706883.
9. Altuntas I, Delibas N, Demirci M, Kilinc I, Tamer N. The effects of methidathion on lipid peroxidation and some liver enzymes: role of vitamins E and C. Arch Toxicol. 2002; 76(8):470-3. https://doi.org/10.1007/s00204-002-0359-1 PMID: 12185416.
10. Cataño HC, Carranza E, Huamaní C, Hernández AF. Plasma cholinesterase levels and health symptoms in Peruvian farm workers exposed to organophosphate pesticides. Arch Environ Contam Toxicol. 2008; 55(1):153-9. https://doi.org/10.1007/s00244-007-9095-0 PMID: 18157496.
11. Kapka-Skrzypczak L, Sawicki K, Czajka M, Turski WA, Kruszewski M. Cholinesterase activity in blood and pesticide presence in sweat as biomarkers of children's environmental exposure to crop protection chemicals. Ann Agric Environ Med. 2015; 22(3):478-82. https://doi.org/10.5604/12321966.1167718 PMID: 26403119.
12. Dhananjayan V, Ravichandran B, Anitha N, Rajmohan HR. Assessment of acetylcholinesterase and butyrylcholinesterase activities in the blood plasma of agriculture workers. Indian J Occup Environ Med. 2012; 16(3):127-30. https://doi.org/10.4103/0019-5278.111755 PMID: 23776322; PMCID: PMC3683181.
13. 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.
14. Shadnia S, Azizi E, Hosseini R, Khoei S, Fouladdel S, Pajoumand A, Jalali N, Abdollahi M. Evaluation of oxidative stress and genotoxicity in organophosphorus insecticide formulators. Hum Exp Toxicol. 2005; 24(9):439-45. https://doi.org/10.1191/0960327105ht549oa PMID: 16235732.
15. Ogut S, Gultekin F, Kisioglu AN, Kucukoner E. Oxidative stress in the blood of farm workers following intensive pesticide exposure. Toxicol Ind Health. 2011; 27(9):820-5. https://doi.org/10.1177/0748233711399311 PMID: 21450927.
16. 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.

17. Pearson JN, Patel M. The role of oxidative stress in organophosphate and nerve agent toxicity. Ann N Y Acad Sci. 2016; 1378(1):17-24. https://doi.org/10.1111/nyas.13115 PMID: 27371936; PMCID: PMC5063722.
18. Vanova N, Pejchal J, Herman D, Dlabkova A, Jun D. Oxidative stress in organophosphate poisoning: role of standard antidotal therapy. J Appl Toxicol. 2018; 38(8):1058-70. https://doi.org/10.1002/jat.3605 PMID: 29516527.
19. Bentley ME, Griffiths PL. The burden of anaemia among women in India. Eur J Clin Nutr. 2003; 57(1):52-60. https://doi.org/10.1038/sj.ejcn.1601504 PMID: 12548297.
20. Kapeleka JA, Sauli E, Sadik O, Ndakidemi PA. Biomonitoring of Acetylcholinesterase (AChE) activity among smallholder horticultural farmers occupationally exposed to mixtures of pesticides in Tanzania. J Environ Public Health. 2019; 2019:3084501. https://doi.org/10.1155/2019/3084501 PMID: 31611921; PMCID: PMC6755286.
21. Ellman GL, Courtney KD, Andres V Jr, Featherstone RM. A new and rapid colourimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961; 7:88-95. https://doi.org/10.1016/0006-2952(61)90145-9 PMID: 13726518.
22. Chambers JE, Chambers HW. An investigation of acetylcholinesterase inhibition and ageing and choline acetyltransferase activity following high-level acute exposure to paraoxon. Pest Biochem Physiol. 1989; 33:125-31. https://doi.org/10.1016/0048-3575(89)90003-5
23. Raso GM, Meli R, Gualillo O, Pacilio M, Di Carlo R. Prolactin induction of nitric oxide synthase in rat C6 glioma cells. J Neurochem. 1999; 73(6):2272-7. https://doi.org/10.1046/j.1471-4159.1999.0732272.x PMID: 10582584.
24. Wills ED. Evaluation of lipid peroxidation in lipids and biological membranes. In: Snell K, Mullock B, editors. Biochemical toxicology: A practical approach. Oxford: IRL Press; 1987. p. 127-52.
25. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem. 1988; 34(3):497-500. https://doi.org/10.1093/clinchem/34.3.497 PMID: 3349599.
26. Beers RF Jr, Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem. 1952; 195(1):133-40. https://doi.org/10.1016/S0021-9258(19)50881-X PMID: 14938361.
27. Lakshmi Sundaram R, Vasanthi HR. Dalspinin isolated from *Spermacoce hispida* (Linn.) protects H9c2 cardiomyocytes from hypoxic injury by modulating oxidative stress and apoptosis. J Ethnopharmacol. 2019; 241:111962. https://doi.org/10.1016/j.jep.2019.111962 PMID: 31121210.
28. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951; 193(1):265-75. https://doi.org/10.1016/S0021-9258(19)52451-6 PMID: 14907713.
29. Cocco P, Satta G, Dubois S, Pili C, Pilleri M, Zucca M, *et al*. Lymphoma risk and occupational exposure to pesticides: results of the Epilymph study. Occup Environ Med. 2013; 70(2):91-8. https://doi.org/10.1136/oemed-2012-100845 PMID: 23117219.
30. Hagmar L, Strömberg U, Tinnerberg H, Mikoczy Z. The usefulness of cytogenetic biomarkers as intermediate endpoints in carcinogenesis. Int J Hyg Environ Health. 2001; 204(1):43-7. https://doi.org/10.1078/1438-4639-00071 PMID: 11725344.
31. Alavanja MC, Hoppin JA, Kamel F. Health effects of chronic pesticide exposure: cancer and neurotoxicity. Annu Rev Public Health. 2004; 25:155-97. https://doi.org/10.1146/annurev.publhealth.25.101802.123020 PMID: 15015917.
32. Hanke W, Jurewicz J. The risk of adverse reproductive and developmental disorders due to occupational pesticide exposure: an overview of current epidemiological evidence. Int J Occup Med Environ Health. 2004; 17(2):223-43.
33. McCauley LA, Anger WK, Keifer M, Langley R, Robson MG, Rohlman D. Studying health outcomes in farmworker populations exposed to pesticides. Environ Health Perspect. 2006; 114(6):953-60. https://doi.org/10.1289/ehp.8526 PMID: 16760000. PMCID: PMC1480483.
34. Bishnu A, Chakrabarti K, Chakraborty A, Saha T. Pesticide residue level in tea ecosystems of Hill and Dooars regions of West Bengal, India. Environ Monit Assess. 2009; 149(1-4):457-64. https://doi.org/10.1007/s10661-008-0222-9 PMID: 18286381.
35. Singh S, Bhutia D, Sarkar S, Rai BK, Pal J, Bhattacharjee S, *et al.* Analyses of pesticide residues in water, sediment and fish tissue from river Deomoni flowing through the tea gardens of Terai Region of West Bengal, India. Int J Fish Aquat Stud. 2015; 3:17-23.
36. Vidyasagar J, Karunakar N, Reddy MS, Rajnarayana K, Surender T, Krishna DR. Oxidative stress and antioxidant status in acute organophosphorous insecticide poisoning. Indian J Pharmacol. 2004; 37:76-9.
37. Singh S, Kumar V, Thakur S, Banerjee BD, Chandna S, Rautela RS, *et al.* DNA damage and cholinesterase activity in occupational workers exposed to pesticides. Environ Toxicol Pharmacol. 2011; 31(2):278-85. https://doi.org/10.1016/j.etap.2010.11.005 PMID: 21787695.
38. Crane AL, Abdel Rasoul G, Ismail AA, Hendy O, Bonner MR, Lasarev MR, *et al.* Longitudinal assessment of chlorpyrifos exposure and effect biomarkers in adolescent Egyptian agricultural workers. J Expo Sci Environ Epidemiol. 2013; 23(4):356-62. https://doi.org/10.1038/jes.2012.113 PMID: 23321857. PMCID: PMC3926695.

39. García-García CR, Parrón T, Requena M, Alarcón R, Tsatsakis AM, Hernández AF. Occupational pesticide exposure and adverse health effects at the clinical, haematological and biochemical levels. Life Sci. 2016; 145:274-83. https://doi.org/10.1016/j.lfs.2015.10.013 PMID: 26475762.
40. Jeong JY, Kim M, Park SH, Kim B, Oh SI, Kim E, *et al*. Residual level, histology, and blood biochemistry of tebuconazole: A repeated dose 28-day oral toxicity study in pigs. Food Sci Anim Resour. 2022; 42(4):712-22. https://doi.org/10.5851/kosfa.2022.e31 PMID: 35855269. PMCID: PMC9289805.
41. Kori RK, Singh MK, Jain AK, Yadav RS. Neurochemical and behavioural dysfunctions in pesticide exposed farm workers: A clinical outcome. Indian J Clin Biochem. 2018; 33(4):372-81. https://doi.org/10.1007/s12291-018-0791-5 PMID: 30319182. PMCID: PMC6170243.
42. Ahmadi N, Mandegary A, Jamshidzadeh A, Mohammadi-Sardoo M, Mohammadi-Sardo M, Salari E, *et al.* Haematological abnormality, oxidative stress, and genotoxicity induction in the greenhouse pesticide sprayers; investigating the role of NQO1 gene polymorphism. Toxics. 2018; 6(1):13. https://doi.org/10.3390/toxics6010013 PMID: 29414880. PMCID: PMC5874786.
43. Abdollahi M, Mostafalou S, Pournourmohammadi S, Shadnia S. Oxidative stress and cholinesterase inhibition in saliva and plasma of rats following subchronic exposure to malathion. Comp Biochem Physiol C Toxicol Pharmacol. 2004; 137(1):29-34. https://doi.org/10.1016/j.cca.2003.11.002 PMID: 14984701.
44. Muniz JF, McCauley L, Scherer J, Lasarev M, Koshy M, Kow YW, *et al.* Biomarkers of oxidative stress and DNA damage in agricultural workers: a pilot study. Toxicol Appl Pharmacol. 2008; 227(1):97-107. https://doi.org/10.1016/j.taap.2007.10.027 PMID: 18086483.
45. Oncu M, Gultekin F, Karaöz E, Altuntas I, Delibas N. Nephrotoxicity in rats induced by chlorpryfos-ethyl and ameliorating effects of antioxidants. Hum Exp Toxicol. 2002; 21(4):223-30. https://doi.org/10.1191/0960327102ht225oa PMID: 12099624.
46. Bagchi D, Bagchi M, Hassoun EA, Stohs SJ. *In vitro* and *in vivo* generation of reactive oxygen species, DNA damage and lactate dehydrogenase leakage by selected pesticides. Toxicology. 1995; 104(1-3):129-40. https://doi.org/10.1016/0300-483X(95)03156-A PMID: 8560491.
47. Maiti PK, Kar A, Gupta P, Chaurasia SS. Loss of membrane integrity and inhibition of type-I iodothyronine 5’-mono deiodinase activity by fenvalerate in the female mouse. Biochem Biophys Res Commun. 1995; 214(3):905-9. https://doi.org/10.1006/bbrc.1995.2372 PMID: 7575562.
48. Prakasam A, Sethupathy S, Lalitha S. Plasma and RBCs antioxidant status in occupational male pesticide sprayers. Clin Chim Acta. 2001; 310(2):107-12. https://doi.org/10.1016/S0009-8981(01)00487-9 PMID: 11498075.
49. López O, Hernández AF, Rodrigo L, Gil F, Pena G, Serrano JL, *et al*. Changes in antioxidant enzymes in humans with long-term exposure to pesticides. Toxicol Lett. 2007; 171(3):146-53. https://doi.org/10.1016/j.toxlet.2007.05.004 PMID: 17590542.
50. Lefaki M, Papaevgeniou N, Chondrogianni N. Redox regulation of proteasome function. Redox Biol. 2017; 13:452-8. https://doi.org/10.1016/j.redox.2017.07.005 PMID: 28715730; PMCID: PMC5512181.
51. Zhang J, Wang X, Vikash V, Ye Q, Wu D, Liu Y, *et al.* ROS and ROS-mediated cellular signalling. Oxid Med Cell Longev. 2016; 2016:4350965. https://doi.org/10.1155/2016/4350965 PMID: 26998193; PMCID: PMC4779832.
52. Tian H, Zhang B, Di J, Jiang G, Chen F, Li H, *et al.* Keap1: one stone kills three birds Nrf2, IKKβ and Bcl-2/Bcl-xL. Cancer Lett. 2012; 325(1):26-34. https://doi.org/10.1016/j.canlet.2012.06.007 PMID: 22743616.
53. Nordgren KK, Wallace KB. Keap1 redox-dependent regulation of doxorubicin-induced oxidative stress response in cardiac myoblasts. Toxicol Appl Pharmacol. 2014; 274(1):107-16. https://doi.org/10.1016/j.taap.2013.10.023 PMID: 24211725.
54. Kanzaki H, Shinohara F, Kajiya M, Kodama T. The Keap1/Nrf2 protein axis plays a role in osteoclast differentiation by regulating intracellular reactive oxygen species signalling. J Biol Chem. 2013; 288(32):23009-20. https://doi.org/10.1074/jbc.M113.478545 PMID: 23801334; PMCID: PMC3743476.