Research Article

Integrative Analysis of DNA Damage, Oxidative Stress, and Serum Mineral Composition in Pesticide-Exposed Agricultural Workers from South Punjab, Pakistan

Abdul Ghaffar[1*], Kashfa Akram[1], Habiba Jamil[1], Riaz Hussain[2], Ghulam Abbas[3], Fozia Afzal[4], Ahrar Khan[5,6], Rabia Tahir[7], Muhammad Ahmad Chishti[1] and Shahnaz Rashid[3]

[1]Department of Zoology, The Islamia University of Bahawalpur, 63100, Pakistan; [2]Department of Pathology, University College of Veterinary and Animal Sciences, The Islamia University of Bahawalpur, Bahawalpur-63100, Pakistan; [3]Centre of Excellence in Marine Biology University of Karachi, 75270, Pakistan; [4]Department of Zoology, The Islamia University of Bahawalpur, Rahim Yar Khan Campus, 64100, Pakistan; [5]Shandong Vocational Animal Science and Veterinary College, Weifang, 261061, China; [6]Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan; [7]College of Animal Science and Technology, Sichuan Agricultural University, Chengdu, China.

Abstract | This study investigates the adverse effects of pesticide exposure on agricultural workers in South Punjab, Pakistan. A sample of 100 exposed and 100 unexposed individuals with clinical abnormalities was selected from districts Bahawalpur, Lodhran, and Rahim Yar Khan. Clinical abnormalities, including throat infection, nasal allergy, skin allergy, eye irritation, uric acid levels, muscle and respiratory infections, hepatitis, restlessness, and chest tightness, were significantly more prevalent among pesticide-exposed individuals. Hematological parameters revealed significant decreases in hemoglobin, white blood cell count, erythrocytes, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), lymphocytes, and neutrophils, alongside significant increases in mixed cell count and monocytes. Serum profiles displayed decreased glucose levels and increased levels of urea, creatinine, bilirubin, alanine transaminase (ALT), alkaline phosphatase, very low-density lipoprotein (VLDL), cholesterol, and triglycerides in exposed workers. Conversely, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), creatine phosphokinase, and serum minerals were notably reduced in exposed workers compared to unexposed counterparts. Prolonged pesticide exposure induces oxidative stress, evidenced by elevated production of free radicals and alterations in antioxidant defense enzymes. Comet assay analysis detected DNA damage in pesticide-exposed workers, correlated with increased age and duration of exposure. In conclusion, pesticides elicit oxidative stress and lead to hematological, serological, and genetic abnormalities in exposed agricultural workers.

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***Correspondence** | Abdul Ghaffar, Department of Zoology, The Islamia University of Bahawalpur, 63100, Pakistan; **Email:** dr.abdul.ghaffar@iub.edu.pk
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Introduction

Extensive use of pesticides, herbicides, insecticides has increased the contamination level of our ecosystem leading to adverse impacts over living organisms. Two million tons of pesticide constituents are applied annually to destroy or stop pests, fungi, herbs, insects, rodents and ticks for improvement in the production of crops (Marrs and Ballantyne, 2004; De *et al*., 2014). Pesticides are complex mixtures containing active component and other constituent like additives, emulsifying agents and solvents. Furthermore, many agricultural implementations of insecticide having various formulations and combinations applied on the time of the growing season (Mostafalou and Abdollahi, 2013). Exposure to pesticides increase the risk of health ailments in human beings, different type of cancer, oxidative stress by greater construction of free radicals and damage to DNA, RNA, DNA repair proteins (Abdollahi *et al*., 2004; Mostafalou and Abdollahi, 2013; Kim *et al*., 2017) including detoxification and scavenger enzymes or change antioxidant defense mechanisms (Possamai *et al*., 2007; Hernandez *et al*., 2013). These chemicals can accumulate within the body of human by ingestion, inhalation and through skin contact and bioaccumulation at various trophic levels (Damalas and Koutroubas, 2016).

Genotoxic biomonitoring of population showed that chemical mutagens are warning system for genetic ailments. Occupational submission to pesticides concerned with increased risk of carcinomas like Alzheimer's disease, Hodgkin's lymphoma, non-Hodgkin's lymphoma, multiple lymphoma, colon and bladder cancer, gall bladder sarcoma, Leukemia, lung cancer, pancreatic cancer, Parkinson's ailment and reproductive abnormalities (Gauthier *et al*., 2001; Shukla *et al*., 2001; Baris *et al*., 2004; Beane-Freeman *et al*., 2005; Orsi *et al*., 2009; Andreotti *et al*., 2009; Koutros *et al*., 2009; Bonner *et al*., 2010; Bertrand *et al*., 2010).

Pakistan has wide climatic diversity, which offers great opportunities for growing a variety of vegetable crops in regional markets to meet demand across the country throughout the year. Vegetable production in the country is well-diversified in terms of a range of species grown as more than thirty-five types of vegetables are grown in Pakistan from low to high elevation areas in rain fed to irrigated land and low to high input systems. Different vegetables like tomato, chili, eggplant, potato, cucumber, gourds and okra are grown in large quantities by using different technologies in different zones in different season (Ahmad *et al*., 2005; Maalik *et al*., 2013; Tahir and Altaf, 2013). In Pakistan, control of pesticide residues in the food chain is not meticulous because there is no relative legislation for the management of pesticides and other harmful chemicals for monitoring their residues. Farm workers involved in spraying activities ignoring basic preventive measures when handling pesticides, which trigger numerous health concerns. In addition, Pakistan has no official training on safety measures for insecticide use due to lack of effective legislation and knowledge of potential risks (Latif *et al*., 2011; Yawar *et al*., 2012; Arafa *et al*., 2013; Khan and Damalas, 2015; Damalas and Koutroubas, 2016).

In recent years the demand of crop yield in Pakistan increased quickly due to native feeding. Different types of pesticides are used for the better growth and protection of crops throughout the country mainly insecticides. There are five types of acaricides, 39 types of herbicides, 108 types of insecticides, 30 types of fungicides and six types of rodenticides are registered in Pakistan (Zia *et al*., 2009; Anwar *et al*., 2011). Agricultural employers also exposed to insecticide when they are working in the field, which is not related to pesticide application (Bradman *et al*., 2009; Coronado *et al*., 2004; Quandt *et al*., 2004). Epidemiological studies suggested that larger submission to insecticides have harmful impacts such as endocrine, cancer at different sites, neurologic, nephrotoxic, respiratory and reproductive disorders an understandable linkage have to be accepted (Ntzani *et al*., 2013; McCauley *et al*., 2006). Epidemiological studies have suggested that there is association between the insecticides submitted workers and respiratory ailments like airway inflammation, coughing, wheezing, asthma, chronic obstructive pulmonary disease and lung cancer (Hoppin *et al*., 2007, 2009). Therefore, current study was planned to determine the genotoxicity, hematological-serological alterations and oxidative stress induced by pesticides in workers exposed to it (Widowati *et al*., 2022).

Materials and Methods

Study area and sampling

Survey conducted to collect blood samples of 100 exposed and 100 unexposed workers by using

disposable syringes from district Bahawalpur (Saddar and Ahmedpur), Lodhran and Rahim Yar Khan. For hematology, serum biochemistry and to measure DNA damage, 5ml blood taken, 2.5ml in CBC vials containing EDTA as anticoagulant and 2.5 ml in plain CBC vials. Sampling of the pesticide-exposed workers done in the morning when they were spraying in the cotton fields. The ages of the pesticide occupational workers were approximately 18 to 59 years, all the workers were male, and their exposure period was 3 to 35 years. After taking the blood of the farmers, the samples were carefully transported to the laboratory in iced cooler. Study conducted from July 2018 to October 2018 at the Aquaculture, Genetic Toxicity and Molecular Biology Laboratory in the Department of Zoology, The Islamia University of Bahawalpur Punjab, Pakistan. This survey was done in the month of July to October when pesticides use is on peak in cotton and sugarcane fields. Following parameters were focused during study: Age, marital status, children, smoking and economic status and clinical abnormalities were cough, throat infection, sneezing, nasal allergy, skin allergy, eye irritation, hepatitis, economic status, flu, kidney problem, diabetes, blood pressure, muscle problem, fever, uric acid, stomach problem, respiratory problem, restlessness, vomiting, dizziness, nervousness, heart problem, chest tightness and backbone tightness i.e., lumbago.

Hematological assay

Collected blood samples inspected using an automatic analyzer (Bk-5000vet; Biobase Biodustry Co. Ltd, Shandong, China) for haematological assay. Haematological parameters were calculated are listed as: white blood cells (WBC), Red blood cells, haemoglobin (Hb), hematocrit (HCT), platelet count (PLT), lymphocytes (LYM), neutrophils (NEUT), monocytes, eosinophils, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC). Biochemical Analysis of blood samples was carried out by using spectrophotometer with the use of commercially available kits of different parameters. First, collected blood samples were centrifuged by centrifugation machine (EBA-20, Hettich Zentrifugen Co. Ltd, Germany). at 1500 rpm for 15 min. Then serum got was separated from plasma through pipetting in Eppendorf tubes. Serum enzyme tests were carried out in from the laboratory. Parameters which were taken for biochemical analysis are Glucose, Urea, creatinine, Bilirubin, alanine aminotransferase (ALT), Alkaline Phosphatase (ALP), Aspartate aminotransferase (AST), lactate dehydrogenase (LDH), Lipid Profile (total cholesterol, triglycerides, HDL-C, LDC-C), serum minerals (sodium and potassium, calcium and phosphorous) Cardiac Enzymes (CPK and AST).

Comet assay

Blood samples were collected and processed for the assay according to the protocol outlined by Singh *et al*. (1988) with modifications. Thin smears of both 1% normal melting point agarose and 1% low melting point agarose were applied to frosted glass slides. Subsequently, these prepared slides, containing cells suspended in low melting agarose, were solidified and promptly immersed in freshly prepared lysing buffer solution. Following this, the slides were placed in a horizontal electrophoresis tank and subjected to electrophoresis in darkness for 25 minutes at 25 V. Following electrophoresis, the slides were neutralized and stained with ethidium bromide. Subsequent examination of all slides was conducted under a fluorescent microscope, with DNA damage assessed by measuring the tail length of 50 cells in each tissue sample (Ghaffar *et al*., 2021).

Statistical analysis

The data collected in this study underwent statistical analysis using MS Excel and SPSS 15 software. Results are presented as mean±SD for hematological and serum analyses, comparing exposed and unexposed workers through multiple comparisons. The grades of DNA damage in exposed and unexposed workers were compared using Duncan's multiple tests, accounting for variations in age.

Results and Discussion

Clinical abnormalities

Survey indicated that diseases were more common in pesticides exposed group as compared to unexposed workers. The percentages of clinical abnormalities of the pesticide exposed workers and unexposed workers are shown in Table 1. A notably high prevalence of various health issues was observed among pesticide-exposed workers compared to their unexposed counterparts. These included throat infection, cough, nasal allergy, skin allergy, eye irritation, hepatitis, kidney problems, fever, elevated uric acid levels, respiratory infection, restlessness, vomiting, nervousness, and

tightness in the backbone. Additionally, farmers frequently reported experiencing headaches, burning or watery eyes, and neurological symptoms such as forgetfulness, lack of concentration, and increased tiredness reported by Monger *et al.* (2023).

Table 1: *Percentage of clinical abnormalities in the pesticide exposed and unexposed workers.*

Clinical abnormalities	Unexposed % (N=100)	Exposed % (N=100)
Throat infection	17%	37%
Cough	18%	22%
Sneezing	81%	37%
Nasal Allergy	40%	76%
Skin Allergy	32%	58%
Eye irritation	17%	47%
Hepatitis	04%	05%
Kidney problem	02%	03%
Diabetes	5%	3%
Blood Pressure	10%	05%
Fever	03%	05%
Uric acid	21%	29%
Stomach problem	18%	11%
Respiratory infection	03%	07%
Restlessness and vomiting	16%	35%
Nervousness	15%	39%
Cardiac problem	05%	02%
Chest, backbone tightness	18%	48%

Similar patterns of pesticide poisoning conditions have been documented in prior studies by del-Prado (2007); Khan *et al.* (2010), and Mwabulambo *et al.* (2018). The reported clinical symptoms align closely with those documented among the exposed farmers in this study, corroborating the reality of the general health effects associated with pesticide exposure within farming communities. These clinical abnormalities are indicated by the pesticide-exposed workers may be due to the exposure of pesticide within their immune system (Henneberger *et al.*, 2013).

Demographic characteristic of pesticide exposed workers

Table 2 provides in-depth demographic profiles of pesticide-exposed workers, including age distribution, education, marital status, agricultural background, pesticide training history, monthly household income, economic standing, and no. of sibling. Additionally, Table 3 furnishes a comprehensive depiction of observed clinical abnormalities among both pesticide-exposed and unexposed workers, elucidating the behavioral and demographic factors influencing health outcomes within this cohort. In previous studies have revealed that pesticide exposure is greater in Pakistan agricultural workers (Srivirojana *et al.*, 2005). There are large numbers of pesticide exposed workers who were affected from the harm impacts of pesticides. Therefore, we decided to examine this problem and adopted possible safety measures to deal with pesticides exposure (Aroonvilairat *et al.*, 2015). The age of workers that use pesticides were from 18 to 59 years and exposure time of pesticides workers was 3 to 35 years (Chuisseu *et al.*, 2015) reported similar examinations. Additionally, the study results align closely with those of previous research, including studies by Staudacher *et al.* (2020); Afata *et al.* (2022), and Lelamo *et al.* (2023).

Table 2: *Demographic characteristic of pesticide Exposed workers in and around the district Bahawalpur.*

Age (years)	
15-25	22%
26-35	38%
36-45	21%
46-55	16%
≥ 56	3%
Education level	
None	59%
1-5th grade	27%
6-8th grade	14%
Marital status	
Married	89%
Unmarried	11%
Agricultural experience	
2-10	45%
≥15	55%
Received training about pesticides	
Yes	13%
No	87%
Monthly household income	
5000 ≤ less	23%
6000-8000	66%
9000-11000	11%
Economic status	
Poor	87%
Middle	13%
Number of siblings	
None	14%
1-5	64%
5-10	22%

Table 3: *Detailed behavioral and demographic characteristic of pesticide-exposed (n=100) and unexposed (n=100) workers for observed clinical abnormalities.*

Characteristics	Exposed	Unexposed	Exposed	Unexposed	Exposed	Unexposed	Exposed	Unexposed	Exposed	Unexposed	Exposed	Unexposed	Exposed	Unexposed n=100	Exposed	Unexposed	Exposed	Unexposed	Exposed	Unexposed	Exposed	Unexposed	Exposed	Unexposed	Exposed	Unexposed	Exposed	Unexposed	Exposed	Unexposed	Exposed	Unexposed	Exposed	Unexposed	Exposed	Unexposed
Clinical abnormalities	Throat infection (%)		Cough (%)		Sneezing (%)		Nasal Allergy (%)		Skin Allergy (%)		Eye Irritation (%)		Hepatitis (%)		kidney problem (%)		Diabetes (%)		Blood pressure (%)		Fever (%)		Uric acid (%)		Stomach problem (%)		Respiratory infection (%)		Restlessness and vomiting (%)		Nervousness		Heart Problem (%)		Chest, backbone tightness (%)	
Level	37	17	22	18	81	37	76	40	58	32	47	17	5	4	3	2	5	3	5	10	5	3	29	21	18	11	7	3	35	16	39	15	2	5	48	18
Age 15-25	24	10	20	04	17	05	18	10	22	27	19	18	40	19	0	05	4	5	0	0	40	05	24	03	06	06	43	2	17	7	21	10	0	0	25	5
26-35	27	13	23	07	37	28	26	20	31	20	39	29	40	14	0	5	21	14	0	5	0	12	14	7	16	13	14	9	17	16	15	18	0	0	13	44
36-45	23	20	20	24	23	32	33	29	23	16	06	36	0	38	50	27	38	25	20	30	0	21	35	34	28	23	14	20	26	23	26	21	50	20	31	25
46-55	19	27	27	29	17	22	17	35	17	25	21	11	20	19	50	28	15	3	20	30	40	35	17	39	28	25	14	37	31	27	28	27	50	40	25	18
≥56	07	30	10	36	06	13	06	06	07	12	15	06	0	10	0	35	22	2	60	35	20	27	10	17	22	33	15	32	09	27	10	24	0	40	6	8
Wear dress coat, closed shoes and hat	43	72	70	87	58	73	65	91	59	65	45	75	60	75	65	75	50	7	45	56	57	70	57	65	52	65	48	67	53	67	57	70	57	70	62	73
Use gloves only	11	50	35	70	34	64	32	77	28	60	30	50	35	45	46	57	45	5	40	53	45	65	49	57	50	59	46	59	40	55	45	65	47	65	30	39
Wear neat clothes daily	41	89	44	76	40	65	45	88	45	70	47	57	56	70	60	75	58	6	59	61	62	75	55	67	55	65	41	65	43	67	57	71	62	75	58	73
Only hand-wash with soap	57	87	62	98	67	83	62	93	75	81	60	75	68	80	53	67	65	7	51	60	60	65	53	65	52	75	60	78	61	76	61	65	60	65	59	65
No. times hand-wash with soap (1-2)	68	26	60	39	61	30	75	40	70	55	52	45	60	45	60	45	56	45	55	43	50	35	50	41	60	42	61	50	57	45	57	39	54	35	55	42

Hematological analysis

Results of blood parameters expressed in Table 4 as mean and Standard Deviation. Hemoglobin level, WBCs, RBCs, HCT, MCV, MCH, MCHC, lymphocytes, neutrophils, and eosinophils showed a notable decrease in pesticide-exposed workers compared to their unexposed workers. Moreover, these parameters exhibited significant decreases in exposed workers with clinical abnormalities. Conversely, mixed cell count (MXD) and monocytes in pesticide-exposed individuals significantly increased in comparison to the unexposed group, particularly among those with clinical abnormalities such as blood pressure issues, skin allergies, cardiac problems, and diabetes.

The observed slight decrease in neutrophils, eosinophils, and lymphocytes can be attributed to the presence of clinical abnormalities, which weaken the immune system. Conversely, the increase in mixed cell count and monocytes in pesticide-exposed workers may be linked to clinical abnormalities. This finding is consistent with the study by Garg *et al.* (2004). Furthermore, monocyte levels were significantly elevated in pesticide-exposed workers above 36 years of age, albeit within the reference range, when compared with unexposed workers. This increase in monocyte count could be attributed to the phagocytic activity of eosinophils and the activity of neutrophils. These results closely resemble those reported by Al-Sarar*et al.* (2009). It's worth noting that similar findings have been documented in previous studies, such as the one conducted by Aroonvilair *et al.* (2015).

Table 4: *Mean ± S.D values of hematological parameters in different diseases of pesticide exposed and unexposed individuals.*

Diseases		Hematological parameters										
		Hemoglobin	RBC	WBC	Hematocrit	MCV	MCH	MCHC	Lympho-cytes	Neutrophils	Monocytes	Eosinophils
Blood pressure	Exposed	13.25±.36	4.78±.161	7.43±.975	44.76±2.11	93.66±3.94	27.76±.747	29.85±1.23	39.51±2.98	43.53±3.00	10.33±3.00	5.33±1.49
	Unexposed	14.72±.28	5.67±.138	9.53±.625	47.67±2.77	95.57±1.52	28.67±2.29	30.89±2.67	41.67±2.32	55.33±2.85	10.09±1.00	5.43±1.15
Diabetes	Exposed	12.51±.45	4.81±.203	8.16±.808	45.33±2.17	94.18±3.06	25.93±.867	27.78±1.21	37.75±4.55	52.13±5.10	6.66±1.68	4.00±.447
	Unexposed	15.67±.63	5.83±.189	11.00±.198	49.33±2.69	96.44±1.55	28.33±1.44	32.33±2.82	40.90±3.93	56.63±4.01	8.65±2.55	5.70±.721
Cardiac problem	Exposed	12.46±.420	5.05±.120	8.88±.772	44.08±2.05	87.41±4.56	24.70±1.31	28.43±1.10	37.30±4.71	50.08±5.45	8.33±2.48	4.83±1.44
	Unexposed	17.67±.705	5.67±.185	9.93±.562	49.63±2.76	94.63±3.62	29.00±1.04	33.75±2.25	42.87±2.65	52.43±3.45	9.78±1.51	4.83±1.722
Throat infection	Exposed	12.28±.562	4.64±.138	7.21±.620	42.18±2.21	95.56±4.37	26.11±.929	27.26±1.31	38.41±4.82	51.01±5.84	7.00±2.60	4.33±1.56
	Unexposed	15.33±.998	5.17±.174	10.37±.298	50.78±1.82	96.99±3.42	28.89±1.67	31.59±2.48	40.67±3.45	60.67±4.837	8.81±2.68	5.23±1.47
Kidney problem	Exposed	11.70±.471	4.70±.242	7.70±.321	42.18±2.03	89.96±2.27	24.56±.827	27.80±1.15	34.50±5.39	56.11±6.30	6.33±1.76	3.66±.557
	Unexposed	16.40±.317	4.99±.221	10.67±.15	48.53±2.42	93.98±4.86	30.73±1.31	32.79±2.57	41.83±3.52	62.27±5.56	9.33±2.83	4.87±.777
Hepatitis	Exposed	12.03±.545	4.70±.141	7.30±.505	41.11±1.84	91.75±3.85	25.35±.827	27.46±.918	34.90±3.73	59.86±3.72	3.66±.557	2.33±.333
	Unexposed	17.63±.338	5.00±.159	9.80±.531	45.55±1.97	94.62±2.65	29.49±2.75	33.67±2.37	40.81±3.20	65.59±6.85	8.67±1.77	4.78±.533
Asthma	Exposed	12.03±.482	5.06±.175	8.38±.777	43.18±2.54	85.41±4.56	23.76±1.23	28.15±1.05	36.65±4.28	50.33±3.06	8.33±2.48	5.16±1.79
	Unexposed	16.66±.452	5.67±.270	9.63±.435	46.75±2.08	92.67±2.78	29.89±2.98	32.00±1.63	41.63±3.27	66.33±4.10	9.35±1.51	5.67±1.60
Uric acid	Exposed	11.83±.364	4.88±.208	7.80±.554	42.50±2.34	87.65±5.57	24.36±1.43	28.15±1.03	39.80±4.95	47.00±3.20	7.50±2.77	4.50±1.94
	Unexposed	15.71±.764	5.45±.208	9.00±.338	47.99±2.63	91.84±2.12	30.57±1.75	34.36±2.58	42.41±3.49	69.30±5.74	9.38±1.89	4.00±1.08
Nasal allergy	Exposed	12.56±.367	4.98±.184	6.78±.544	46.01±2.03	92.68±4.44	25.23±.969	27.55±1.26	41.31±3.12	44.90±3.14	9.83±3.21	6.83±2.34
	Unexposed	16.88±.447	6.00±.128	9.33±.431	49.62±1.00	93.76±2.30	28.73±1.08	34.59±1.16	43.67±2.89	60.57±4.72	9.33±2.87	6.33±2.02
Skin allergy	Exposed	12.63±.504	4.86±.134	6.63±.702	45.48±2.50	93.26±4.34	25.95±.595	27.93±1.14	40.68±5.19	45.10±3.72	9.33±2.67	5.66±1.68
	Unexposed	15.39±.562	5.50±.133	10.33±.169	47.69±2.25	95.69±1.00	29.90±2.40	33.68±1.41	42.33±2.52	63.70±6.26	9.07±2.14	5.67±1.55
Stomach problem	Exposed	11.98±.406	4.50±.137	6.75±.647	43.38±1.99	96.73±4.75	26.46±1.11	27.80±1.23	38.75±4.90	49.31±6.01	6.50±2.76	4.33±1.60
	Unexposed	17.47±.513	5.98±.289	10.00±.295	50.63±1.89	96.00±1.08	29.37±2.23	32.71±1.55	41.78±2.75	65.32±6.22	10.00±1.86	4.33±1.55

Serum analysis

Results of different serological parameters expressed in (Tables 5, 6) as mean and Standard Deviation. Glucose, Alkaline Phosphatase level, HDL-C, LDL-C, sodium level, calcium level, potassium level and phosphorus level in pesticide-exposed workers remarkably decreased as compared to the unexposed individuals. These parameters significantly decreased in exposed workers with clinical abnormalities kidney problem, uric acid, stomach problem and hepatitis as compared to unexposed individuals with same clinical abnormalities. While urea level, cholesterol, triglyceride, VLDL, CK-MB, lactate dehydrogenase, creatinine phosphokinase, creatinine, bilirubin, ALT in pesticide exposed workers significantly increased as compared to the unexposed individuals. These parameters significantly increased in exposed workers with clinical abnormalities blood pressure, skin allergy, cardiac problem and diabetes as compared to unexposed individuals with

Table 5: *Mean ± S.D values of serum parameters in different diseases of pesticide exposed and unexposed individuals.*

Diseases/Groups		Glu	Urea	Creat.	Bili.	ALT	ALP	Chol.
Blood pressure	Exp.	116.16±4.78	31.66 ±4.39	0.90±.05	0.52±.05	32.33±5.41	88.50±7.01	168.33±10.59
	Unexp.	131.16±4.56	15.89 ±3.25	0.60±.04	0.51±.05	31.71±4.48	110.56±5.06	176.3333±11.67
Diabetes	Exp.	111.1±5.85	24.50±2.81	1.00±.06	0.62±.016	36.66±6.81	91.83±7.51	199.66±14.17
	Unexp.	127.21±4.55	20.00±3.21	0.7±.05	0.53±.06	28.27±3.81	102.23±8.08	163.66±10.83
Cardiac problem	Exp.	118.5±5.09	25.16±2.13	0.96±.05	0.66±.09	39.67±10.80	80.83±4.46	187.00±13.43
	Unexp.	127.5±5.51	17.22±2.53	0. 67±.04	0.57±.04	31.678±7.84	107.45±5.42	171.0±12.51
Throat infection	Exp.	117.16±4.70	18.66±2.26	1.61±.68	0.60±.08	30.33±6.50	99.16±2.86	156.83±11.06
	Unexp.	135.16±4.90	21.76±3.45	0.81±.45	0.52±.09	28.09±5.53	105.18±5.61	165.83±13.08
kidney problem	Exp.	113.50± 2.5	22.33±3.20	0.91±.08	0.61±.02	39.83±7.52	98.33±6.35	195.16±16.45
	Unexp.	116.40± 3.15	13.36±4.25	0.84±.07	0.60±.06	31.58±6.77	104.67±3.78	153.17±14.48
Hepatitis	Exp.	113.83±4.35	21.16±4.11	1.65±.67	0.53±.04	39.66±7.98	87.16±8.03	181.66±10.62
	Unexp.	117.59±4.35	20.23±4.35	0.75±.55	0.51±.05	31.88±5.64	101.98±6.45	155.66±12.87
Asthama	Exp.	111.83±3.11	21.16±3.39	0.86±.09	0.56±.04	30.33±5.42	90.50±9.82	192.16±18.58
	Unexp.	122.53±3.11	15.34±2.89	0.79±.08	0.53±.08	31.76±6.32	99.35±8.33	167.16±16.23
Uric acid	Exp.	122.16±4.49	20.50 ±3.30	0.81±.07	0.55±.08	25.50±2.62	94.66±8.76	176.16±13.57
	Unexp.	116.19±3.39	18.65 ±4.00	0.64±.05	0.51±.05	29.61±3.82	97.72±7.48	179.16±13.59
Skin allergy	Exp.	111.83±3.11	27.00±5.93	0.96±.08	0.63±.09	32.33±8.15	92.16±9.35	178.33±12.29
	Unexp.	127.82±4.2	22.87±4.79	0.83±.06	0.58±.07	29.43±4.34	104.19±8.67	169.33±11.22
Nasal allergy	Exp.	118.16±4.37	21.16±2.58	0.90±.08	0.65±.08	23.83±2.19	89.16±8.53	166.66±22.78
	Unexp.	124.25±3.37	14.15±2.90	0.71±.04	0.60±.06	30.82±5.21	99.58±6.49	150.66±18.44
Stomach problem	Exp.	121.33±4.37	21.33±2.88	0.91±.09	0.58±.09	30.16±6.51	99.16±2.86	156.16±11.18
	Unexp.	135.37±4.57	21.33±2.88	0.76±.0.7	0.55±.07	33.15±4.55	99.43±4.18	151.16±13.56

same clinical abnormalities. Liver enzymes Aspartate aminotransferase AST and Alanine aminotransferase ALT were remarkably increased in pesticide exposed worker as compared to non-exposed workers but within reference range. This study is similar to previous study of (Chuisseu *et al.*, 2015) reported similar alterations in activity of liver enzymes. Pesticides disrupt the liver function because liver absorbed most of the pesticides and metabolized Kazmi *et al.* (2023). Our research is also similar with the previous study (Al-Sarar *et al.*, 2009). In serum profile, glucose level was remarkably decreased in exposed workers. Urea, Creatinine, Cholesterol, Bilirubin, (ALT), Very low-density lipoprotein (VLDL), Lactate Dehydrogenase (LDH) and Triglycerides were notably greater in exposed workers in comparison with unexposed individuals. Creatinine Phosphokinase (CPK) and Serum minerals were significantly decreased in pesticide-exposed workers as compared to unexposed workers (García-García *et al.*, 2016).

Genotoxicity

Genotoxic changes were represented in (Table 7 and Figures 1, 2, 3, 4, 5, 6, 7, 8, 9) showed the number and percentage of undamaged, slightly damaged, damaged and highly damaged cells according to age groups of

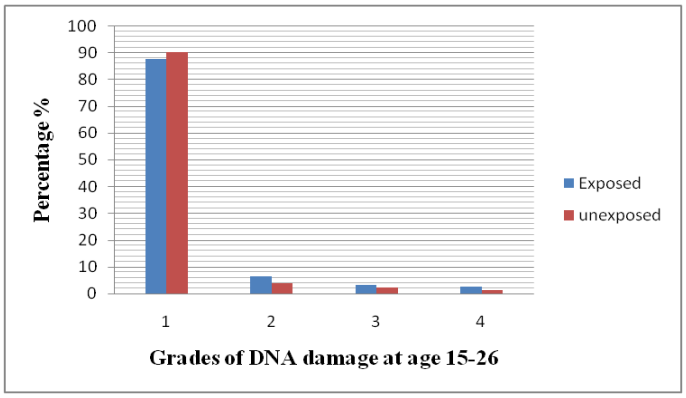

Figure 1: *Comparison of damaged DNA of exposed and unexposed workers according to age 15-26.*

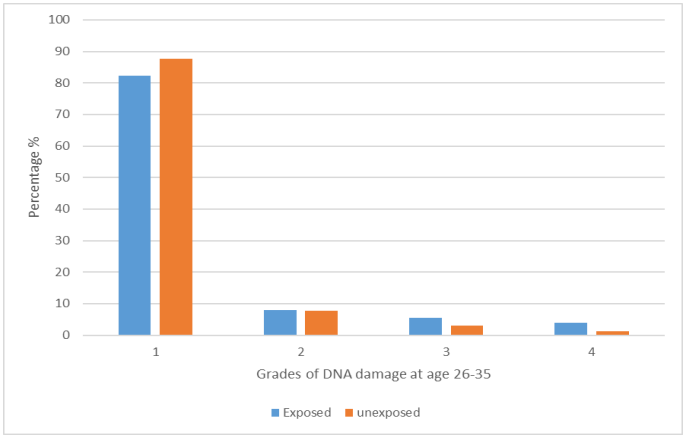

Figure 2: *Percentage of damaged DNA of exposed and unexposed workers according to age 26-35.*

Table 6: *Mean ± S.D values of serum parameters in different diseases of pesticide exposed and unexposed individuals.*

Diseases/ Groups		TRIG.	HDL-C	LDL-C	VLDL	CK-MB	CPK	LDH	Na	K	Ca	P
Blood pressure	Exp.	150.00± 19.21	30.83± 1.07	110.50± 16.84	40.33± 2.29	63.00± 17.40	112.83± 11.80	596.00± 242.2	139.00± 1.50	3.95± 0.123	9.08± 0.221	3.96± 0.170
	Unexp.	150.23± 11.33	36.90± 2.06	124.67± 10.90	27.22± 3.54	49.89± 3.65	170.22± 11.24	238.00± 9.89	145.81± 2.55	4.81± 0.156	8.99± 0.323	5.00± 0.190
Diabetes	Exp.	167.33± 21.45	34.66± 1.66	132.16± 6.73	38.66± 1.25	49.83± 13.76	139.16± 11.11	425.50± 58.86	136.83± 1.72	3.81± 0.218	8.73± 0.333	4.35± 0.291
	Unexp.	145.45± 15.47	35.98± 2.77	133.21± 7.45	29.78± 1.12	45.21± 5.36	140.28± 15.81	235.67± 5.47	138.53± 2.79	4.87± 0.203	8.98± 0.433	4.85± 0.411
Cardiac problem	Exp.	139.83± 18.82	34.00± 2.50	119.33± 12.96	36.83± 1.60	59.16± 13.31	128.50± 14.70	501.50± 13.15	139.50± 2.78	4.06± 0.117	9.05± 0.147	4.16± 0.170
	Unexp.	147.75± 13.67	37.66± 3.51	121.33± 10.76	25.90± 4.49	41.54± 2.79	135.99± 18.77	240.45± 10.52	151.40± 2.86	5.67± 0.587	10.00± 0.576	4.16± 0.162
Throat infection	Exp.	201.16± 15.99	31.00± 2.88	97.33± 15.89	38.00± 3.04	34.66± 2.67	109.83± 17.13	320.00± 33.90	136.83± 2.07	4.00± 0.223	8.68± 0.279	3.95± 0.368
	Unexp.	145.33± 14.88	35.45± 1.56	135.66± 13.33	26.81± 2.90	44.67± 7.37	128.53± 12.68	229.34± 13.20	154.88± 1.30	5.33± 0.321	9.83± 0.178	5.00± 0.256
kidney problem	Exp.	190.16± 10.00	34.00± 2.67	119.00± 13.29	36.83± 1.68	40.00± 2.63	134.16± 7.90	445.16± 53.19	137.66± 1.40	3.65± 0.251	8.68± 0.406	4.15± 0.209
	Unexp.	137.67± 12.00	38.44± 1.67	129.43± 9.35	29.56± 1.45	44.25± 2.22	147.66± 16.13	236.33± 11.11	148.76± 2.33	4.00± 0.333	9.33± 0.213	4.55± 0.336
Hepatitis	Exp.	183.66± 29.35	33.66± 3.06	108.83± 16.38	34.16± 5.36	39.66± 5.37	132.83± 19.86	403.33± 53.82	136.00± 2.14	4.08± 0.238	8.55± 0.278	4.18± 0.327
	Unexp.	149.87± 16.65	39.78± 3.33	130.76± 7.54	28.67± 2.87	49.00± 3.56	142.87± 18.64	267.39± 9.33	146.91± 3.76	4.81± 0.812	9.50± 0.499	4.18± 0.602
Asthama	Exp.	168.33± 24.80	31.50± 2.23	113.00± 15.68	39.83± 2.94	55.33± 13.94	129.16± 13.43	526.83± 12.53	137.66± 2.51	3.98± 0.151	9.11± 0.218	4.01± 0.207
	Unexp.	132.43± 18.70	36.56± 2.32	138.78± 8.62	26.55± 2.23	48.77± 5.67	135.42± 12.82	266.47± 15.05	139.17± 4.19	4.94± 0.145	9.67± 0.220	4.71± 0.323
Uric acid	Exp.	176.16± 17.25	31.16± 2.21	111.83± 15.18	40.83± 3.44	41.83± 7.011	125.00± 14.01	519.33± 12.39	139.16± 2.74	4.16± 0.122	9.23± 0.206	4.15± 0.209
	Unexp.	144.45± 13.22	35.67± 3.00	119.54± 9.77	23.82± 4.88	45.56± 4.78	127.51± 12.61	247.56± 4.88	138.19± 4.75	4.18± 0.293	9.63± 0.302	4.67± 0.236
Skin allergy	Exp.	197.83± 16.93	33.16± 2.35	104.66± 13.66	40.50± 2.43	55.00± 14.08	135.83± 21.06	694.00± 23.27	136.33± 2.34	3.91± 0.113	8.91± 0.284	3.90± 0.296
	Unexp.	147.54± 10.85	38.16± 1.74	132.84± 10.43	21.43± 2.73	43.43± 2.77	139.20± 19.05	234.55± 4.74	140.57± 3.58	4.91± 0.177	9.59± 0.251	5.00± 0.265
Nasal allergy	Exp.	181.83± 4.17	30.33± 1.85	95.16± 14.56	36.31± 0.47	37.16± 3.94	114.00± 14. 87	428.83± 77.70	137.33± 2.72	4.16± 0.166	9.03± 0.18	3.78± 0.356
	Unexp.	138.67± 11.19	39.22± 2.65	137.77± 11.57	25.44± 1.26	39.41± 5.43	126.69± 13. 73	235.47± 50	147.35± 2.45	5.56± 0.167	9.96± 0.24	5.00± 0.329
Stomach problem	Exp.	178.16± 10.28	32.33± 2.52	110.50± 15.65	37.66± 2.74	34.16± 2.60	115.66± 15.31	344.83± 32.88	135.33± 1.49	4.05± 0.229	8.63± 0.272	3.95± 0.368
	Unexp.	142.66± 12.00	40.33± 1.95	125.45± 12.66	18.32± 5.33	41.81± 4.51	170.65± 15.29	245.88± 19.89	145.49± 3.15	5.43± 0.213	9.76± 0.185	4.29± 0.256

exposed and unexposed workers. In microscopic field total (100×5×15) 7500 cells were observed. These tables indicated the comparison of DNA damage in pesticide exposed and unexposed workers according to their age groups. Rate of percentage of DNA damage highly increased in 35-46 age groups. The percentage of overall DNA damage was significantly increased with the increase in the age of pesticide exposed workers because of the increase of exposure time. The current study suggested that a longer exposure to

Table 7: *Grades of damaged DNA of exposed and unexposed workers according to their ages.*

Age groups (years)	Grades of DNA damages							
	Undamaged		Slightly damaged		Damaged		Highly Damaged	
	No.	%	No.	%	No.	%	No.	%
15-25								
Exposed	2776	87.5	207	6.5	105	3.3	82	2.5
Unexposed	2522	90.1	106	3.7	69	2.4	40	1.4
26-35								
Exposed	2487	82.4	240	7.9	168	5.5	121	4.0
Unexposed	2360	87.7	210	7.8	82	3.0	36	1.3
36-45								
Exposed	2270	76.0	301	10.0	256	8.5	159	5.3
Unexposed	2440	88.2	135	4.8	87	3.1	52	1.8
46-55								
Exposed	2654	79.5	287	8.7	258	7.7	138	4.1
Unexposed	2491	88.0	158	5.5	135	4.7	45	1.5
≥ 56								
Exposed	2372	78.0	268	8.8	255	8.3	143	4.7
Unexposed	2462	89.8	133	4.8	107	3.9	39	1.4

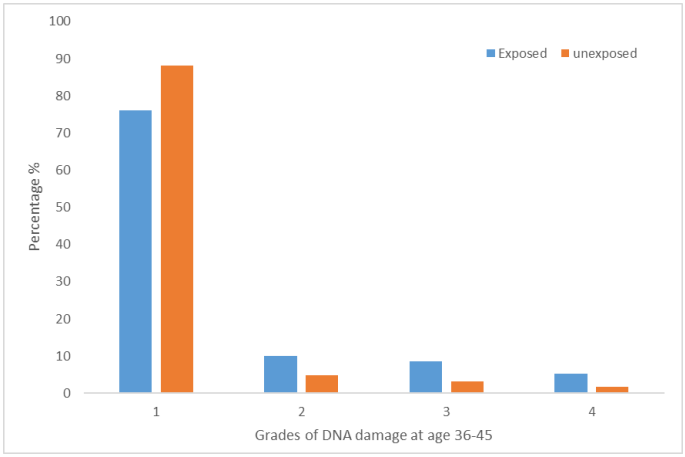

Figure 3: *Percentage of damaged DNA of exposed and unexposed workers according to age 36–45.*

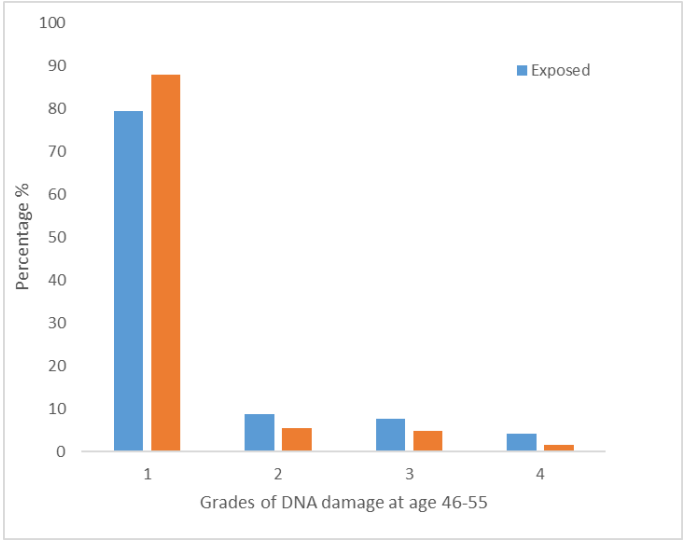

Figure 4: *Percentage of damaged DNA of exposed and unexposed workers according to age 46–55.*

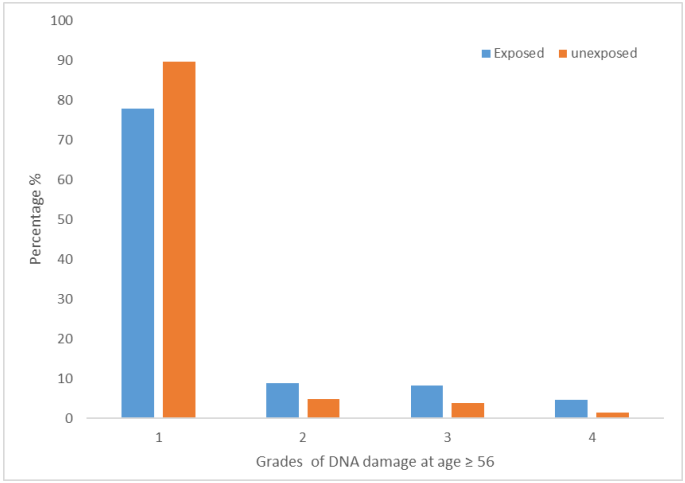

Figure 5: *Percentage of damaged DNA of exposed and unexposed workers according to age ≥56.*

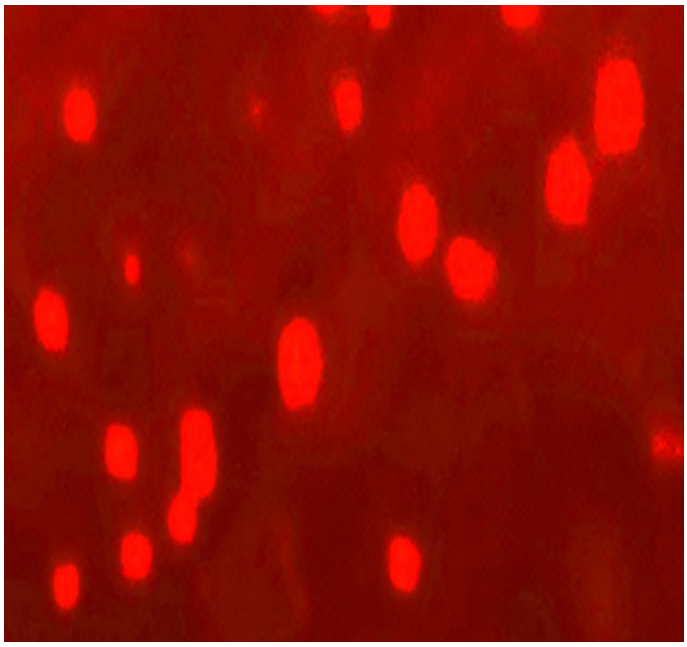

Figure 6: *Undamaged DNA of pesticide exposed workers.*

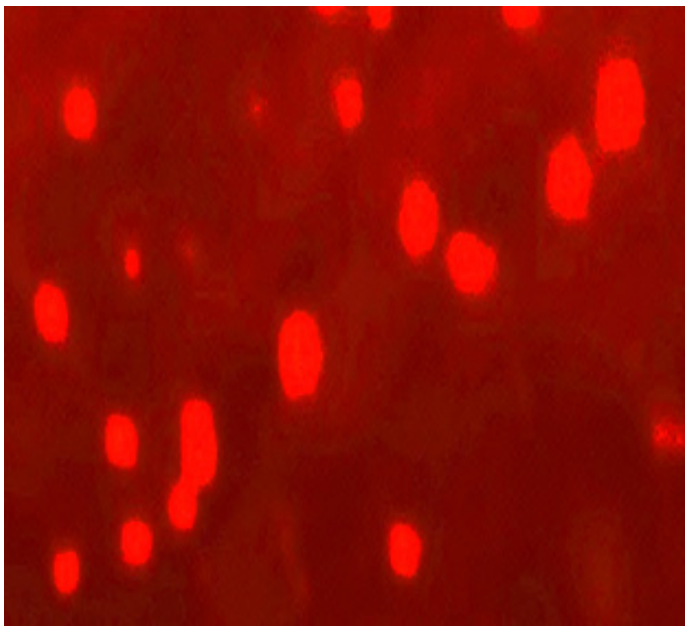

Figure 7: *Slightly damaged DNA of pesticide exposed workers.*

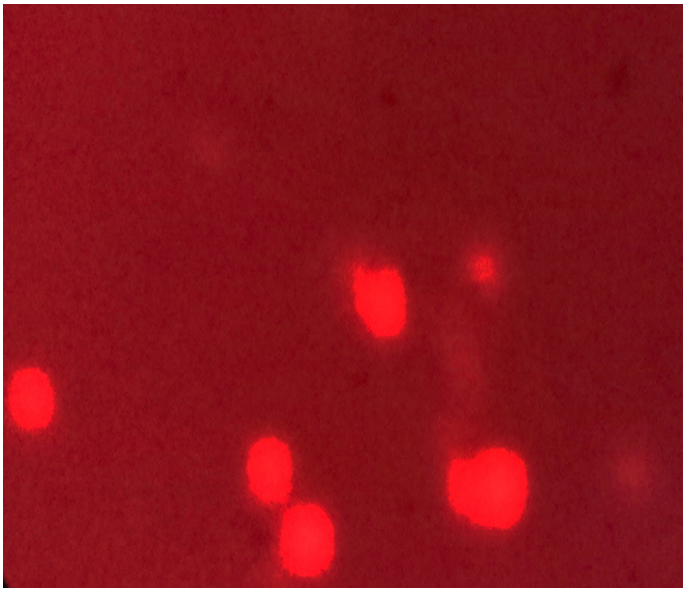

Figure 8: *Moderately damaged DNA of pesticide exposed workers.*

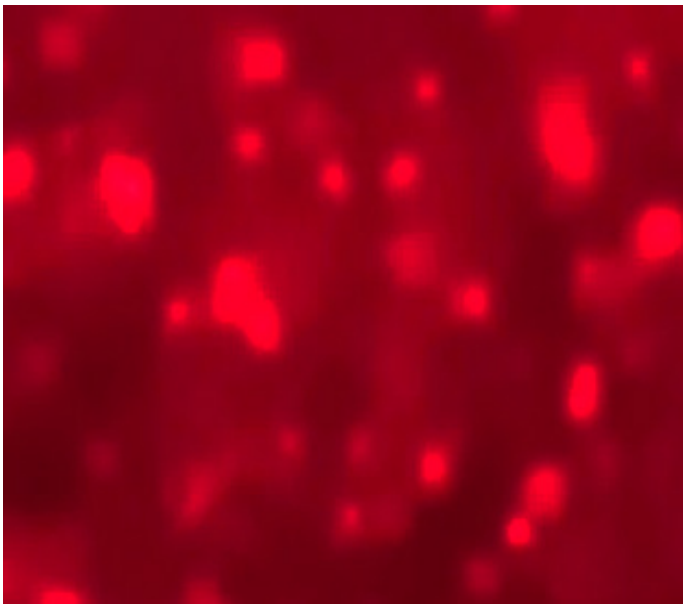

Figure 9: *Highly damaged DNA of pesticide exposed workers.*

insecticide has harmful impacts on health and cause oxidative stress (Aziz *et al.*, 2022). Various pesticides cause oxidative stress due to the creation of free radicals and alteration in antioxidant defense enzymes. This study is like the previous research (Lozano-Paniagua *et al.*, 2018). In this study, analysis of DNA damage was done by the procedure of comet assay. All the solutions were freshly prepared in the laboratory and fresh blood of pesticide exposed and unexposed individuals were taken to make slides (Al-Saeed *et al.*, 2023). The samples were promptly observed after preparation. Cells were categorized based on the degree of damage, ranging from undamaged to highly damaged cells. Significantly greater DNA alterations were observed in exposed individuals compared to those who were unexposed. This study shares similarities with previous research (Collins, 2004).

Conclusions and Recommendations

Pakistan has wide climatic diversity, which offers great opportunities for growing throughout the year. Variety of hazardous pesticide remain exposed in environment to beat pest control. Pesticide exposure may acute and chronic effects on exposed individual's mild toxicity to severe. Pesticides have been associated with neurological, endocrine, psychological, immunological, respiratory, hematological, dermatological, nephritic, hepatic issues. It has been concluded that pesticides cause clinical and hematological abnormalities, parameters like serum biochemical alterations, oxidative stress and genotoxic effects in pesticides exposed workers. In future, there is a need to form training institutes that teach the farmers about safety measures. The pesticides are toxic chemicals that induce harmful effects in exposed workers. Must underscore the need for intervention to improve workplace and safety. Worker's health surveillance participates to enhance interdisciplinary strategies providing quality of life at work. Engineering measures should be taken to get rid of these chemicals and aware train the occupational workers about the hazardous impact of these pesticides. There is also need to start a medical program to treat the pesticide exposed workers who indicate clinical abnormalities in their bodies. For treatment natural herbs and artificially made supplements also vitamins C and E are recommended.

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Novelty Statement

This research presents a pioneering comprehensive investigation into the effects of pesticides in a structured and systematic manner, providing valuable insights to the workers exposed to pesticide.

Author's Contribution

Abdul Ghaffar: Supervision, conceptualization, formal analysis.
Kashfa Akram: Executed sampling and data collection and laboratory work.
Riaz Hussain: Formal analysis, data curation.
Habiba Jamil: Writing review and editing.
Ghulam Abbas: Data curation.
Fozia Afzal, Ahrar Khan and Rabia Tahir: Helped in review and editing.
Muhammad Ahmad Chishti and Shahnaz Rashid: Helped in data curation and format setting of the MS.

Conflict of interest
The authors have declared no conflict of interest.

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