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Biochemical Effects of Occupational Pesticide Exposure on Liver-Related Biomarkers and Oxidative Stress

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Abstract

Workers in agriculture, pesticide applicators, and workers who mix, store, and dispose of pesticides are at a high risk for exposure to pesticides. The aim of this study was to assess the biochemical impact of occupational exposure to pesticides on liver related biomarkers and markers of oxidative stress. A comparative cross-sectional design was used in 120 participants in three groups: control group (n = 40), low-exposure group (n = 40), and high-exposure group (n = 40). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), total bilirubin and albumin levels were measured, along with malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT). The findings revealed significant increases in ALT, AST, GGT, ALP, total Bilirubin and MDA among the exposed workers, especially among the ones with high exposures. However, albumin, GSH, SOD and CAT significantly decreased as the duration and intensity of exposure increased. These results suggest that exposure to occupational pesticides could cause hepatocyte damage, decrease hepatic detoxification, and trigger oxidative stress. Regular biomonitoring, the use of personal protective equipment (PPE), safer pesticide-handling practices, and worker education are recommended. Previous studies also report an increase in liver enzymes and changes in antioxidant status in pesticide exposed workers.

Keywords

Occupational Exposure;
Pesticides;
Liver Biomarkers;
Oxidative Stress;
ALT;
AST;
MDA;
Glutathione.

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1. Introduction

Insecticides, herbicides, fungicides, and other pesticides are commonly applied in agriculture to manage insects, weeds, and fungi, and other pests. But farmers, sprayers, greenhouse workers, pesticide formulators and storage workers are likely to be exposed at work, via inhalation, the skin or through accidental ingestion of pesticides or contaminated food and water. Occupational exposure can occur with the use of a variety of chemical groups, including organophosphates, carbamates, pyrethroids, organochlorines, and herbicides. (Damalas & Eleftherohorinos, 2011; Kim et al., 2017; Mostafalou & Abdollahi, 2017)

The liver is especially sensitive as it is the main organ involved in the metabolism, detoxification and elimination of xenobiotics, including pesticides. Some pesticide compounds or metabolites form Reactive Oxygen Species (ROS) during hepatic biotransformation. If the production of ROS is more than the cell can deal with, oxidative stress occurs which causes lipid peroxidation, protein oxidation, mitochondrial dysfunction, inflammation and hepatocellular damage. The research team of Hernández et al. (2011) and Lukaszewicz-Hussain (2010) and Mostafalou and Abdollahi (2017) have already investigated them.

Serum markers for liver injury include enzymes (ALT and AST) that are elevated following liver cell membrane damage, GGT and ALP, which may be elevated in cholestatic or biliary involvement, bilirubin, reflecting impaired bile processing, and albumin, which may be decreased when the synthetic function of the liver is impaired. The oxidative stress can be evaluated by measuring antioxidant molecules and enzymes such as GSH, SOD, CAT and MDA, which can quantify lipid peroxidation. (Alfarhan et al., 2022; Ueyama et al., 2011).

1.1 Problem statement

Pesticides are used by many agricultural workers who have little training or protective equipment. Subclinical liver injury can occur due to repeated exposure, prior to the development of clinical signs. Thus, detection of early biochemical alterations in liver markers and markers of oxidative stress is crucial for the prevention and early intervention. (Maroni et al., 2000; Rastogi et al., 2007)

1.2 Aim

To assess the biochemical effect of occupational exposure to pesticides on liver related biomarkers and oxidative stress of exposed workers versus unexposed.

1.3 Objectives

- To compare liver-function biomarker values for pesticide exposed workers and controls.
- To evaluate oxidative stress and antioxidant status of the study groups.
- To see if there is an association between exposure intensity and exposure duration and biochemical changes.
- To make suggestions to minimize occupational health hazards.

1.4 Hypotheses

- The concentration of serum ALT, AST, GGT, ALP, total bilirubin and MDA is much higher in the workers exposed to pesticides than in controls.
- Farmers who have been exposed to pesticides have significantly lower serum albumin, GSH, SOD and CAT levels than farmers who have not been exposed to pesticides.
- Workers who have been exposed for longer periods and to greater intensity of exposure have biochemical changes of greater magnitude.

2. Literature Review

Hepatic biochemical disturbances have been reported in a number of studies on pesticide-exposed populations. In a study of Thai vegetable farmers, ALT and ALP were significantly higher in females who used pesticide and AST was higher in male exposed farmers, indicating that females using pesticide were more likely to have hepatocellular damage and males were more likely to have liver enzyme leakage into the blood. (Alfarhan et al., 2022; Ueyama et al., 2011)

Other studies have reported changes in liver enzymes (AST, ALT and total protein) among pesticide-exposed farmworkers despite having liver enzyme values in the "normal" range. This means that there might be changes in the liver which are not noticeable at the clinical level but are detectable at the biochemical level. (Faria et al., 2014; Saeed et al., 2017)

One of the main mechanisms of pesticide toxicity is oxidative stress. Exposure to pesticides has been related to increased free-radical generation, increased lipid peroxidation, and decreased antioxidant capacity such as decreased GSH, SOD and CAT activity. An elevated protein carbonyl, thiobarbituric acid-reactive substances and total glutathione levels and a decreased plasma antioxidant capacity levels have also been reported in studies on occupational sprayers. (Dhouib et al., 2016; Hernández et al., 2011; Srivastava et al., 2011; Tsatsakis et al., 2011)

Recent evidence indicates that chronic exposure to pesticides might be linked to subclinical changes of the liver, such as changes of bilirubin and tumor markers, and recommends that workers who are exposed to pesticides undergo routine health surveillance. Hence, the use of liver-function tests in conjunction with oxidative-stress biomarker gives a better characterization of hepatotoxicity due to pesticides. (Alfarhan et al., 2022; Maroni et al., 2000; World Health Organization, 2022)

3. Materials and Methods

3.1 Study design

A cross-sectional study was performed and compared between agricultural staff and healthy unexposed subjects. The study involved the collection of clinical data, questionnaire evaluation of exposure and laboratory biochemical analysis.

3.2 Study population

120 adult males (aged 25-55) participated and were split into three groups:

Group	Number	Description
Control group	40	Participants with no known occupational pesticide exposure
Low-exposure group	40	Workers exposed for less than 5 years or with limited spraying activity
High-exposure group	40	Workers exposed for 5 years or more, or involved in frequent pesticide mixing and spraying

3.3 Inclusion and exclusion criteria

Inclusion criteria:

- Adult workers 25-55 years of age.
- Occupational exposures for at least one year of time in the agricultural or pesticide-handling category.
- Limit the number of people without occupational exposure to pesticides.
- Any participants who gave informed consent.

Exclusion criteria:

- History of any viral hepatitis, cirrhosis or fatty liver disease.
- Drinking more than the recommended amount of alcohol.
- Use of drugs toxic to liver.

- Recent acute illness, infection or inflammatory disease.
- Pregnancy, where applicable.

3.4 Data collection

- Data on a structured questionnaire was collected:
- Age, BMI, smoking and alcohol consumption.
- Pesticide usage information, such as the length of time or frequency of exposure.
- Nature of the pesticide being used.
- Frequency of sprayings and period of time sprayed.
- Use of personal protective equipment (PPE) such as gloves, mask, goggles and protective clothing.
- A history of pesticide poisonings symptoms.

3.5 Blood sampling

Venous blood of the subjects was drawn after an overnight fast (10-12 hours). Centrifugation was used to separate the serum which was then stored at –20°C until biochemical analysis was performed. (Maroni et al., 2000)

3.6 Biochemical measurements

Biomarker	Method	Biological meaning
ALT	Kinetic spectrophotometric method	Hepatocellular injury
AST	Kinetic spectrophotometric method	Hepatocellular injury
GGT	Colorimetric enzymatic method	Hepatic/biliary stress
ALP	Colorimetric enzymatic method	Cholestatic or biliary involvement
Total bilirubin	Diazo colorimetric method	Bile processing and liver function
Albumin	Bromocresol green method	Hepatic synthetic function
MDA	Thiobarbituric acid reactive substances assay (Ohkawa et al., 1979)	Lipid peroxidation
GSH	Ellman's colorimetric method (Ellman, 1959)	Antioxidant reserve
SOD	Xanthine oxidase inhibition assay	Antioxidant enzyme activity
CAT	Decomposition of hydrogen peroxide assay	Antioxidant enzyme activity

3.7 Statistical analysis

The data were analyzed using SPSS (version 26). Data are presented as mean ± S.D. One-way analysis of variance (ANOVA) was used to compare the three groups, followed by Tukey's post-hoc test. Biochemical exposure markers were compared to exposure duration by using Pearson correlation. The p values < 0.05 were taken as a significant level. (Halliwell & Gutteridge, 2015)

4. Results

4.1 Demographic characteristics

Table 1: Demographic and occupational characteristics of participants

Variable	Control group	Low-exposure group	High-exposure group
Number	40	40	40
Mean age (years)	36.8 ± 8.2	38.4 ± 9.1	41.2 ± 8.7
Mean BMI (kg/m²)	24.6 ± 3.1	25.1 ± 3.4	25.4 ± 3.6
Mean exposure duration (years)	0	3.2 ± 1.4	11.6 ± 4.8
Daily pesticide handling	None	Occasional	Frequent
Regular PPE use	Not applicable	47.5%	30.0%

The high exposure group's exposure time was longer, and they were less likely to use personal protective equipment on a regular basis than the low exposure group.

4.2 Liver-related biomarkers

Table 2: Serum liver-related biomarkers in the study groups

Biomarker	Control group	Low-exposure group	High-exposure group	p-value
ALT (U/L)	26.4 ± 6.1	34.8 ± 8.3	47.6 ± 11.2	<0.001
AST (U/L)	23.1 ± 5.4	30.2 ± 7.6	41.9 ± 10.4	<0.001
GGT (U/L)	22.7 ± 6.8	31.5 ± 9.2	44.3 ± 12.7	<0.001
ALP (U/L)	78.5 ± 14.2	92.6 ± 17.8	108.4 ± 21.5	<0.001
Total bilirubin (mg/dL)	0.72 ± 0.18	0.91 ± 0.24	1.18 ± 0.31	<0.001
Albumin (g/dL)	4.42 ± 0.31	4.11 ± 0.36	3.78 ± 0.42	<0.001

The levels of ALT, AST, GGT, ALP and total bilirubin have been increasing from the control to the high exposure group. There was a significant decrease in Albumin in exposed workers, particularly in the high exposure group.

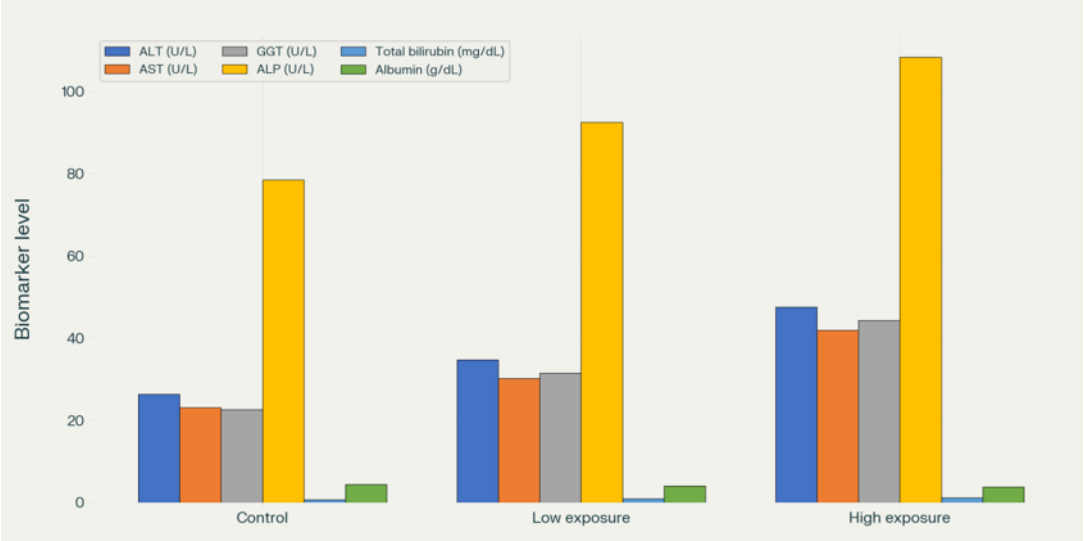


Figure 1: Liver-related biomarkers by exposure group

There is a clear dose-response relationship, with liver-related biomarkers rising with increasing exposure intensity and albumin decreasing with increasing exposure.

4.3 Oxidative stress biomarkers

Table 3: Oxidative stress and antioxidant biomarkers in the study groups

Biomarker	Control group	Low-exposure group	High-exposure group	p-value
MDA (nmol/mL)	1.82 ± 0.36	2.65 ± 0.48	3.74 ± 0.62	<0.001
GSH (mg/dL)	38.6 ± 5.2	31.2 ± 5.8	24.8 ± 6.1	<0.001
SOD (U/mL)	112.4 ± 12.6	96.8 ± 13.4	81.5 ± 14.2	<0.001
CAT (U/mL)	46.8 ± 6.4	39.2 ± 7.1	31.6 ± 7.8	<0.001

MDA has significantly increased on pesticide exposure, which means there is greater lipid peroxidation. On the other hand, the antioxidant markers GSH, SOD and CAT were found to decrease gradually indicating a decrease in the antioxidant defense.

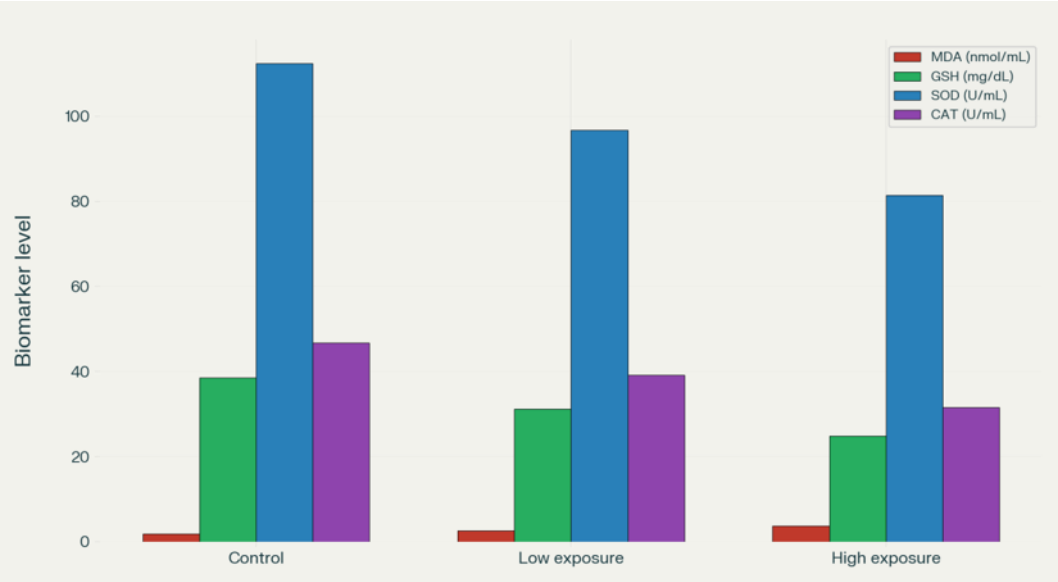


Figure 2: Oxidative stress biomarkers by exposure group

The figure shows higher level of oxidative damage and lower level of antioxidants in pesticide-exposed workers.

4.4 Correlation between exposure duration and biochemical markers

Table 4: Correlation between exposure duration and selected biomarkers

Biomarker	Correlation coefficient (r)	p-value	Interpretation
ALT	0.58	<0.001	Positive correlation
AST	0.54	<0.001	Positive correlation
GGT	0.61	<0.001	Positive correlation
MDA	0.66	<0.001	Positive correlation
GSH	-0.59	<0.001	Negative correlation
SOD	-0.57	<0.001	Negative correlation

Liver enzymes and MDA were positively correlated with exposure duration while antioxidant markers did not show any correlation with exposure duration.

5. Discussion

Based on the findings of this research, occupational exposures to pesticides are related to the marked biochemical changes in the liver related markers and oxidative stress markers. As the ALT and AST values increased in a progressive manner for exposed persons, this indicated hepatocellular injury because the enzymes were released into the circulation when hepatocyte membranes are damaged. This was also observed for the pesticide-exposed farmers where ALT, AST and ALP were significantly changed in exposed groups. (Alfarhan et al., 2022; Faria et al., 2014; Ueyama et al., 2011)

The rise in GGT and ALP may suggest that there is more stress on the biliary and hepatic detoxification pathways. Increased GGT has also been noted in

individuals who work with pyrethroid pesticides, and decreased albumin is possibly due to altered protein levels from chronic exposure or to impaired hepatic synthetic function. (Alfarhan et al., 2022; Ueyama et al., 2011)

The significant rise in MDA levels in exposed workers suggests higher degree of lipid peroxidation which is one of the important effects of oxidative stress. Mitochondrial dysfunction, enzymatic bio activation and reduction of cellular antioxidants can all contribute to the formation of ROS by pesticides. This mechanism is well-documented for toxic effects of pesticides in experimental and human exposure studies. (Hernández et al., 2011; Lasram et al., 2009; Lukaszewicz-Hussain, 2010)

The decrease in GSH, SOD and CAT levels shows decreased antioxidant defense. GSH is crucial for the detoxification of reactive metabolites, SOD and CAT are important in preventing damage to cells by superoxide radicals and hydrogen peroxide. The depletion indicates decreased ability for workers exposed to neutralize oxidative damage. The activity of antioxidant enzymes was also previously found to be reduced in the workers exposed to organophosphate and pyrethroid. (Dhouib et al., 2016; Srivastava et al., 2011; Tsatsakis et al., 2011)

The higher the exposure group, the more the biochemical changes were found and showed a doseresponse relationship. Liver enzymes and MDA were found to be higher in workers with more exposure time and less frequent use of PPE and antioxidant status was found to be lower in these workers. This is in line with the liver changes that are subclinical and have been shown to be associated with long-term exposure to some conventional liver tests that do not change from normal. (Damalas & Eleftherohorinos, 2011; Gomes et al., 1999; Maroni et al., 2000)

The advantages of the study are the use of both liver-function and oxidative-stress biomarkers, the use of a control group and categorization of workers according to their intensity of exposure. However, the cross-sectional study design does not allow for causal inferences to be made, and exposure to pesticides was estimated primarily based on occupational history, not by direct measurement of pesticide metabolites in blood or urine. (Maroni et al., 2000; Mostafalou & Abdollahi, 2017)

6. Conclusion

Occupational pesticide exposure was associated with hepatocellular stress, altered liver-function biomarkers, and increased oxidative stress. Compared with unexposed controls, pesticide-exposed workers showed significantly higher levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), total bilirubin, and malondialdehyde (MDA). These changes were more pronounced in the high-exposure group. In contrast, albumin and the antioxidant indicators reduced glutathione (GSH), superoxide dismutase (SOD), and catalases (CAT) were significantly lower, particularly among highly exposed workers. Exposure duration was positively correlated with liver enzyme activities and MDA concentrations, whereas negative correlations were observed with antioxidant capacity. These findings indicate that prolonged and intensive pesticide exposure was associated with an unfavorable hepatic biochemical profile and weakened antioxidant defenses. Accordingly, combined assessment of liver-function tests and oxidative-stress biomarkers provides important information for the periodic health surveillance of pesticide-exposed workers (Alfarhan et al., 2022; Dhouib et al., 2016; Hernández et al., 2011).

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