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AL-HIKMAH JOURNAL OF HEALTH SCIENCES (AJOHS)

Official Publication of Faculty of Health Sciences

Al-Hikmah University Ilorin, Nigeria

www.alhikmahuniversity.edu.ng/centralJournal

Original Article

Selected Liver Function Indices in Male Farmers Exposed to Pesticides

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ARTICLE INFO

Article History
Received 01 November, 2023
Accepted 22 November, 2023
Available online 5 December, 2023

Keywords
Pesticide
Farmers
Liver Function Indices

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ABSTRACT

Introduction: Exposure to environmental toxins and ensuing liver health outcomes has attracted global attention. This study was aimed at assessing the liver function indices among farmers exposed to pesticides.

Methodology: This research was carried out on 130 apparently healthy male participants aged 18 years and above. The participants were randomly selected for this comparative cross-sectional study. The participants were further divided into two groups; the Pesticide-exposed group and the control group (non-exposed). Demographic characteristics of the participants were obtained by standard methods. Five milliliters of fasting blood samples were collected from each participant after an overnight fast (8 to 12 hours). A portion of the blood sample was dispensed into plain tubes for the estimation of serum Aspartate aminotransferase, alanine aminotransferase, albumin, and total protein. Statistical Package for Social Science (SPSS) 25.0 software was used for statistical analysis.

Results: The results from this study suggest that exposure to pesticides may have adverse effects on the liver as evidenced in the observed significant ($p \geq 0.05$) increase in serum AST, ALT, and decreased Albumin and Total protein values in the pesticide-exposed group when compared with the normal control.

Please cite this article as: Lawal A.Z., Ibrahim M., Mohammed Z.N., Ogunwale K.A., Garuba W.O., Popoola A.N., Adunmo G.O., Suleiman I.E., Busari A.O., Balogun M.O., Ibironke E.O., Adekunle C.A., Adedotun M and Nuhu A. (2023). Selected Liver Indices in Farmers Exposed to Pesticides. *Al-Hikmah Journal of Health Sciences, 3*(1), 18 - 23.

P-ISSN: 2811-1516
E-ISSN: 2811-1524

Introduction

Farming is the most predominant occupation among rural dwellers in Africa and it serves as a major source of economic drive and livelihood for many rural communities. Owing to the continuous loss of food crops and other commodities caused by the invasion of pests causing diseases, has led farmers to resort to the use of fertilizers and pesticides to get the best crop yield. The common categories of pesticides used by farmers are insecticides, rodenticides, avicides, herbicides and fungicides, most of which contain numerous natural and synthetic chemicals. The liver is the major organ in the body involved in the metabolism of chemical substances (including pesticides) that enter the body. These pesticides have been implicated to contain chemicals that may interfere with normal metabolic processes, biosynthetic, transport and endocrine functions (Radwan *et al.,* 2014). Farmers are exposed to different forms of pesticides that gain entrance into their body system through inhalation or skin contact during spraying of pesticides or ingestion of pesticide residues from contaminated farm produce (such as fruits, vegetables and grains). The general public may also be affected when these farm products are released into the market.

Despite the frequent and increased use of pesticides by the farmers, the effects of these chemicals on their health may remain undiagnosed early as long as they do not conduct any medical investigations. Consequently, these chemical residues may build up and have damaging effect to the entire body system over time once their toxicity level successfully overwhelms the liver functions (biotransformation, metabolism, detoxification and excretion). Thus, the study aimed at assessing the effects of pesticide exposure on hepatic (liver) functions of male farmers in Moro Local Government Area, Kwara State.

Methodology

Study area

This study was conducted in Malete and Asomu, Moro Local Government Area in Kwara State.

Kwara State is located in the north-central geopolitical zone of Nigeria and Ilorin is the capital city. Moro Local Government Area was created in the year 1976in Kwara state and has its headquarters in Bode Sa'adu. Towns and villages that make up Moro LGA include Lanwa, Ejidongari, Olooru, Ipaye, Malete, Aaro, Ige, and Odun-Ade. The current estimated population of Moro LGA is 213,448 inhabitants with the majority of the dwellers being members of the Igbomina sub-group of the Yoruba ethnic affiliation. The religions of Islam and Christianity are practiced in the area. Moro local government area is part of the Ilorin Emirate and also a part of the Kwara North senatorial district (Manpower, 2022).

Study design

This comparative cross-sectional study was conducted over a period of 3 months in the Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, Kwara State University, Malete. Ethical approval for this study was obtained from the Ministry of Health, Kwara State, Nigeria (ERC/MOH/2022/06//034).

Study participants

A total of 130 (65 controls and 65 subjects) apparently healthy male participants aged 18 years and above were randomly recruited for this study after informed consent.

Inclusion criteria

1. Apparently healthy male individuals.
2. Males that were within the age range of 18 - 60.
3. Males that were not on medication for liver disease.
4. Male individuals that gave consent.

Exclusion criteria

1. Male individuals that were immune-compromised.
2. Males that were not within the age range of 18 – 60.
3. Males that were on medication for liver disease
4. Male individuals who did not give consent.

Demographic indices

A semi-structured questionnaire was used to obtain data on age, gender, occupation, and medication status from the participants.

Sample collection

All the study participants were asked to fast overnight (between 8 to 12 hours). Five milliliters of venous blood were collected using a sterile needle and syringe from the medial cubital vein in the antecubital fossa. The blood was dispensed into plain tubes which were centrifuged at 3000rpm for five minutes to obtain serum for estimation of ALT, AST, Albumin and Total protein. After centrifugation, the serum was aspirated into clean vials. For assays not completed within 8 hours, the serum was stored at +2°C. For assays not completed within 48 hours, samples were frozen at –15°C. Frozen samples were thawed only once.

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Biochemical analysis

Alanine aminotransferase (ALT)

Serum ALT levels were assayed using commercial kits (Agappe Laboratories Limited, 2022). In the reaction, ALT catalyzes the reversible transamination of L-alanine and α-ketoglutarate to pyruvate and L-glutamate. The pyruvate is then reduced to lactate in the presence of lactate dehydrogenase (LDH) with the concurrent oxidation of NADH to NAD. The semi-auto analyzer monitors the rate of change in absorbance at 340 nm during 3minutes. The rate of change in absorbance per minute multiplied by the given factor (1745) is directly proportional to the ALT activity in the sample.

Aspartate aminotransferase (AST)

Serum AST levels was assayed using commercial (Agappe Laboratories ltd, 2022). In the reaction, the AST catalyzes the reversible transamination of L-aspartate and αketoglutarate to oxaloacetate and L-glutamate. The oxaloacetate is then reduced to malate in the presence of malate dehydrogenase with the concurrent oxidation of NADH to NAD. The semi-auto analyzer monitors the rate of change in absorbance at 340 nm for three minutes. The rate of change in absorbance per minute multiplied by the given factor (1745) is directly proportional to the AST activity in the sample.

Total protein

Serum levels of total protein were assayed using commercial biuret reagent test kits (El Shalom Laboratories Ltd, 2022). Biuret test is based on the reaction of the cupric ions Cu2+ with peptide bonds in an alkaline solution. These ions react with the nitrogen of the peptide bond to form a purple or violet-coloured complex. In an alkaline medium, cupric hydroxide is generated from copper sulfate of biuret reagent that helps in chelating the peptide bond with cupric ions to give violet or purple colour. The intensity of the colour formed is directly proportional to the amount of proteins present in the sample at a wavelength of 540nm.

Albumin

Serum levels of Albumin were assayed using commercial kits (EL Shalom Laboratories ltd, 2022). In the reaction, the albumin combines with Bromocresol Green (BCG) reagent to form a complex. The system monitors the change in absorbance at 630 nm. The change in absorbance is directly proportional to the concentration of albumin in the sample.

Data Analysis Method

All questionnaires were screened for completeness. Statistical Package for Social Science (SPSS) software version 25.0 was used for the analysis of data. Student test was used to find significant difference in mean and standard deviation. Analysis of variance was used for comparison of variables. Pearson's correlation coefficient was used to find the relationship between quantitative variables, p-value less than 0.05 ($p<0.05$) were considered significant.

Results

Table 1: Socio-Demographic Characteristics of Study Participants

Variables	Pesticide Exposed Farmers	Non-exposed group	X^2	P-value
	Frequency (%) N=65	Frequency (%) N=65		
Age (Years)			14.25	0.001*
18-35	28 (43.1)	49 (75.4)		
36-50	30 (46.2)	12 (18.5)		
>50	7 (10.8)	4 (6.1)		
Mean±SD	35.76 ± 9.03	27.33 ± 6.86		
Educational level			41.69	0.001*
None	35 (53.9)	8 (12.3)		
Primary	19 (29.2)	11 (16.9)		
Secondary	7 (10.8)	19 (29.2)		
Tertiary	4 (6.2)	27 (41.5)		
Duration of exposure (Years)				
<5	5 (7.7)	-		
5-10	26 (40.0)	-		
>10	34 (52.3)	-		

(*) means significance at $p<0.05$

P-ISSN: 2811-1516

E-ISSN: 2811-1524

Table 1 shows the comparison of the Socio-demographic characteristics of the study participants. The mean age of pesticide-exposed farmers was 35.76±9.03 years with majority of the farmers within the age of 36-50 years (46.2%). The control group has the mean age of 27.33±6.86 years with the majority within the age of 18-35 years (75.4%). Based on the level of education, the majority of pesticide exposed farmers have no formal education (53.9%). Among the non-exposed group, majority had tertiary level of education (41.5%). Duration of exposure to pesticides showed that 52.3% were exposed for more than 10 years, 40.0% were exposed for 5-10 years and 7.7% were exposed for less than 5 years.

Table 2: Comparison of AST, ALT, Total Protein, Albumin in Pesticide Exposed Farmers and Non-exposed Groups

Parameters	Pesticide exposed Farmers	Non-exposed groups	t-value	p-value
AST (U/L)	22.88±19.94	15.31±11.93	2.730	0.008*
ALT (U/L)	22.68±19.47	16.62±14.35	1.952	0.005*
Total Protein (g/L)	67.20±7.49	73.63±6.72	-5.178	0.001*
Albumin (g/L)	32.34±7.44	38.35±5.79	-5.286	0.001*

(*) means significance at $p<0.05$

AST- Aspartate transaminase, ALT- Alanine transaminase, U/L- Units per Liter, g/L- gram per Liter.

Table 2 shows a comparison of AST, ALT, Total Protein Albumin levels in pesticide-exposed farmers and control group. There were significant ($p<0.05$) increases in AST and ALT levels among pesticide-exposed farmers compared to non-exposed groups ($p<0.05$). However, a significant decrease in Total protein and Albumin was observed among pesticide-exposed farmers compared to non-exposed groups ($p<0.05$).

Table 3: Comparison of AST, ALT, Total Protein and Albumin among Pesticide exposed Farmers Based on Duration of exposure to Pesticides

Variables	<5YEARS Mean ± SD	5-10 YEARS Mean ± SD	>10YEARS Mean ± SD	F-value	P-value
AST (U/L)	28.80±18.03	19.04±20.65	24.94±19.68	0.882	0.419
ALT (U/L)	22.20±18.09	27.35±22.96	19.18±16.31	1.312	0.277
Total Protein (g/L)	68.40±7.83	66.58±7.34	67.50±7.75	0.176	0.839
Albumin (g/L)	32.60±8.08	32.23±6.91	32.38±7.95	0.006	0.994

AST- Aspartate transaminase, ALT- Alanine transaminase, U/L- Units per Liter, g/L- gram per Liter.

Table 3 shows the comparison of AST, ALT, Total Protein, and Albumin in pesticide-exposed Farmers based on the Duration of exposure to Pesticides. There were no significant differences in the levels of AST, ALT, Total protein, and Albumin when compared with duration of exposure to pesticides at ($p>0.05$).

Discussion

The liver, the body's primary metabolic organ, is thought to provide a strong protection against xenobiotics, which are environmental toxins, and metabolic toxins (Manfo *et al.,* 2014). The most common serum biomarkers for liver damage are aspartate transaminase (AST) and alanine transaminase (ALT). In this study, AST and ALT were significantly increased among pesticide-exposed individuals compared the control group. The observation agrees with El-Nahhal, (2016), who reported liver alterations in farmers from Gaza following occupational exposure to pesticides. Other investigations from Shahzad *et al.,* (2016), Araoud *et al.,* (2012) and Jamal *et al.,* (2016) have also demonstrated increased ALT and AST activity in individuals using pesticides. Elevated levels of ALT (A cytosolic enzyme that is primarily expressed by hepatocytes) in farmer serum samples indicate liver cell lysis and enzyme leakage, both of which point to the liver's cytotoxicity as a result of the agrochemicals. AST is also found in the liver and other organs such as the heart and skeletal muscle, while ALT has low concentrations in the skeletal muscle and kidney, and is chiefly produced in the hepatocytes (Kumar *et al.,* 2014). These changes indicated that frequent pesticide exposure resulted in the alteration of mainly the liver function in the farmers. In humans, higher AST and ALT were reported in tobacco farmworkers and farm workers in Gadap Karachi, Pakistan (Patil *et al.,* 2003; Azmi *et al.,* 2006).

P-ISSN: 2811-1516
E-ISSN: 2811-1524

Moreover, this present study showed that Total protein and Albumin were significantly decreased among pesticide-exposed individuals compared to the non-exposed group. Previous studies by (Borges *et al.*, 2007; Al-Sarar *et al.* 2009) have reported similar findings. A recent study, using mice as ~~a~~ model animal, revealed that deltamethrin (a synthetic pyrethroid pesticide) caused a decrease in total proteins (Desai, 2015). The decrease in serum proteins, especially albumin, might be due to an alteration in protein metabolism of free amino acids and their production in liver under the influence of pesticides (Attia, 2006). Another explanation of protein decline may be the result of protein loss either due to protein synthesis reduction/degradation or increase in the proteiolytic activity (Yeragi *et al.*, 2003). Total protein is made up of albumin which is synthesized by the liver together with globulin. Albumin has been suggested as a potential biomarker to bio monitor relatively low exposure levels to pesticides (Tarhoni *et al.*, 2008) (remove the underline). The level of albumin in the serum is dependent on multitude of factors including nutritional, liver function, urinary and gastrointestinal clearance (Aroonvilairat *et al.*, 2015, Hassanin *et al.*, 2018) (remove the underline). Lower albumin levels is an important pointer to a reduced synthetic function of the liver and has been associated with higher risk of ischemic heart disease (Arques, 2018, Ronit *et al.*, 2020).

In addition, this study showed that there was no significant correlation between AST, ALT, Total protein, Albumin and duration of exposure to pesticides. This could be considered as an early stage of hepatocyte degradation associated with the regeneration of new hepatocytes because the alteration in AST, ALT, Total protein and Albumin in pesticides exposed individuals still fall within the reference range which shows. The study also showed that majority of the pesticides exposed farmers had no formal education which has contributed to their indifference to the use of personal protective equipment which could have adverse effects to their health.

Conclusion

There was significant alteration in AST, ALT, Total protein and Albumin in pesticide-exposed farmers. However, the values for the parameters evaluated were within the reference range. This may be considered as gradual damage to the liver with or without overt signs and symptoms. This study could not find a significant relationship between duration of exposure to pesticides and AST, ALT, total protein and Albumin level. However, this may be due to the regenerative ability of the liver.

Conflict of Interest

The authors declare that there is no conflict of interest

Author's Contribution

Study conception and design: MI, LAZ and MZN. Data analysis and interpretation of results: BMO, AGO, SIE and BAO. Draft manuscript preparation: LAZ, MI, NA, CAA and AM. All authors reviewed the results and approved the final version of the manuscript. All authors agreed to be responsible for all aspects of the work to ensure the accuracy and integrity of the published manuscript.

Funding

This study did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit- sectors.

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