

Assessment of some oxidative stress markers in humans exposed to pesticides in Okagwe and Ihe-Nta, Abia State, Nigeria

ABSTRACT

Aim: To assess the levels of some oxidative stress markers in humans exposed to pesticides in Okagwe and Ihe-Nta, Abia State, Nigeria.

Study design: A cross-sectional study.

Place and Duration of Study: Abia-ADP, Ohafia Zone and Department of Biochemistry, University of Port Harcourt, Nigeria, between August 2018 and September 2021.

Methodology: A total of 160 human subjects participated in this study. They consisted of 80 pesticides exposed agricultural workers in open field and 80 non-exposed volunteers, who served as control subjects. All the participants gave written informed consent, while the Ministry of Agriculture, Abia State, Nigeria gave Ethical Approval for this study. Venous blood samples were collected from all participants and dispensed into plain sample containers. The blood samples were allowed to clot and were centrifuged to obtain serum samples, which were used to determine oxidative stress markers: superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), glutathione (GSH), total antioxidant capacity (TAC), vitamin A, C and E and acetylcholinesterase (ACHE). Data generated were subjected to statistical analysis using IBM Statistical Package for Social Sciences (SPSS) Version 23, and results were considered statistically significant at $P < .05$.

Results: There were significant decrease ($P < .05$) in serum SOD 40.38 ± 2.81 (IU/L), CAT 63.52 ± 7.04 (IU/L), ACHE 1.43 ± 0.46 (n/ml), GSH, 62.19 ± 6.54 (n/ml), vitamin A 3.23 ± 0.83 (IU/L), vitamin C 0.74 ± 0.38 (IU/L), vitamin E 4.27 ± 0.91 (IU/L), GPx 211.78 ± 27.95 (IU/L) in pesticide exposed agricultural workers compared to the values for the control subjects.

Conclusion: This study showed that there was oxidative stress, evidenced by the significant increases in measured oxidative stress parameters in human subjects exposed to pesticides in Okagwe and Ihe-Nta communities.

Keywords: *Oxidative stress markers, pesticides, Okagwe, Ihe-Nta, Abia State, Nigeria*

1. INTRODUCTION

The use of pesticide is considered to be harmful to the environment and human existence. The extensive use of pesticides for crops, fruits and vegetables causes serious problems on non-target organisms leading to a number of pathological and disordered biochemical processes, viz; immune-deficiency associated with dysregulation, allergies, autoimmunity and dysfunction at neuromuscular synapses [1]. Pesticides are classified into Insecticides, Weedicides or Herbicides, Rodenticides and Fungicides [2]. Insecticides are types of pesticides that are used to specifically target and kill insects [3], and this includes organophosphates (BaytexEC50, Harcros Demro Malathion, Parathion, diazinon, Fenthion, dichlorvos, chlorpyrifos, ethion), carbamates (Baygon Fly Bait), Organochlorine(Aldrin) and Pyrethroids(Baygon mosquito coil).

Herbicides are substances that are toxic to plants, and are used to destroy unwanted vegetation. One commonly used herbicide in the world today is paraquat which is the trade

name of N, N – dimethyl 4-bipyridinium dichloride. It is highly toxic to animals and humans. Acute exposure results in high mortality rate between 60-80% [4]. Herbicides or Weedicides are classified in number of ways based on how they are used. Example of herbicides include Paraquat (Gramoxone), Propanil – (3, 4DPA), Glyphosate (Round up) and Chlorophenoxy. Human and animal exposure to pesticide may lead to Oxidative stress [5]. Oxidative stress is an imbalance between free radicals and antioxidant in the body. Free radicals are oxygen containing molecules with an uneven number of electrons. The uneven number allows them to easily react with other molecules, these reactions are called oxidation and it can be beneficial or harmful [6]. In other words free radical can be said to be a molecule that has one or more unpaired electrons, making it highly reactive with other molecules.

Pesticides have been shown to induce the production of reactive oxygen species (ROS) which ultimately lead to the oxidative stress [5][7]. Oxidative stress occurs when the production of reactive oxygen species overrides the free radicals quenching capacity/antioxidant capacity of the cell which leads to the damage of cellular biomolecules (Nucleic acids, lipids and proteins) involved in structural organization of the cell [8][9]. An extensive survey on available literature indicates that pesticide induced oxidative stress has been considered as a possible mechanism of toxicity [9][10]. Pesticides may increase the rate of lipid peroxidation (LPO) by altering the activity of both enzymatic antioxidant (Superoxide dismutase, catalase and glutathione peroxidase) and non-enzymatic antioxidant (total antioxidant capacity, Vitamin A, Vitamin C, Glutathione and Vitamin E) reserves of the cell, which causes oxidative stress [11].

The impact of pesticide induced oxidative stress ranges from tissue injury and aging to the onset of various known and unknown diseases [9]. The combined exposure of chlorpyrifos (CPF) and metal like cadmium (Cd) has been reported to decrease mitochondrial potential and induces reactive oxygen species [12]. The aim of this study was to assess the levels of some oxidative stress markers in humans exposed to pesticides in Okagwe and Ihe-Nta, Abia State, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

This research was carried out at Okagwe and Ihe-nta in Ohafia Local Government Area of Abia State, Nigeria. The work was done with the assistance of the Agricultural extension workers who provided technical advice to the local farmers. Ohafia is located at longitude 7.5247°E and latitude 5.4309°N. People in this area are predominantly farmers, and they are involved in both subsistence and commercial types of farming. Land tenure system is commonly practiced there. Ohafia is one of the local Government Area (LGA) in Abia State, Nigeria. It is an Igbo speaking region. It has its ancestral capital at Achi-chi Elu and has its LGA headquarters at Ebem Ohafia, Abia State, Nigeria. The current estimated population of Ohafia LGA is put at 234,700 inhabitants. People in this area are mostly Christians while some are traditionalists.

2.2 Sample Size Determination

The sample size was determined according to the method of Naing et al. [13].

2.3 Study Population

Human subjects were investigated in this study. This comprised of one hundred and sixty (160) subjects, eighty (80) of these subjects comprised of both Agricultural extension workers and adult farmers who are living in the same study area exposed to pesticides, and eighty (80) non-farmers who are living in different locality not exposed to pesticides as control subjects.

2.4 Selection Criteria for Subjects

2.4.1 Inclusion Criteria

The human subjects were between the age ranges of 25-50years. They must have been using pesticides in farming. They must have been residents in the same locality of the farm. Those not having any history of chronic disease like Diabetes Mellitus and AIDS.

2.4.2 Exclusion Criteria

Those subjects that had history of chronic disease like Diabetes Mellitus and AIDS and those on steroids.

2.5 Sample Collection

With the use of a syringe, 10ml of blood sample was collected from the anti-cubital vein of the subjects. The blood samples were dispensed into lithium heparinized and plain containers. The lithium heparinized containers containing samples were stored at 4°C.

2.6 Laboratory Procedures

Principle and method of determination of enzymatic and non-enzymatic antioxidants superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), glutathione (GSH), total antioxidant capacity (TAC), vitamin A, C and E and acetylcholinesterase (ACHE).

2.6.1 Determination of Acetylcholinesterase (ACHE) Activity

This was determined by the method of Ellman et al. [14]. This is an improved Ellman method in that the Thiocholine produced by the activity acetyl cholinesterase forms a yellow color with 5, 5'-dithio-bis (2-nitrobenzoic acid). The concentration of the color is measured at 412nm, which is proportional to the activity of the enzyme in the sample.

2.6.2 Estimation of Superoxide Dismutase (SOD) Activity

SOD activity was determined using Auto-Oxidation method Misra and Fridorich, [15]. At an alkaline pH of 10.2, SOD is able to inhibit the autoxidation of epinephrine, Superoxide (O₂-) radical which is produced by the Xanthine oxidase reaction caused the oxidation of epinephrine to adrenochrome which later give rise to (O²⁻) which increases with increase P^H and epinephrine concentration.

2.6.3 Estimation of Catalase Activity

Catalase activity was estimated spectrophotometrically. Principle is based on the reaction of hydrogenperoxide with catalase to produce water and oxygen.

2.6.4 Estimation of Glutathione (GSH)

Principle: (Ellman's reagent) 5,5 -dithio-bis (2-nitrobenzoic acid reacts with glutathione to form 5-thio -2-nitrobenzoic acid.

2.6.5 Estimation of Glutathione Peroxidase (E.C.1.11.1.9)

Method used for the estimation of glutathione peroxidase was based on that of Paglia and Valentine [16]. Principle is based on the activity of glutathione Peroxidase was determined kinetically by measuring the reduction of NADP

2.6.6 Estimation of Plasma Total Antioxidant Capacity (TAC)

In an acidic medium (PH=5.2) and a suitable oxidant (FeCl₃), the chromogen (N,N- dimethyl P- Phenylendiamine Sulphate) develops a stable and coloured radical cation that is photometrically detectable at 505nm at 37°C. Antioxidant compounds in the sample reduce the radical cation of the chromogen, quenching the color then producing a discoloration to

their concentration. The absorbance values obtained for the samples are compared with a standard curve obtained using Trolox.

2.6.7 Methods for Vitamin Analysis

Methods Adopted for analysis where modified from Rutkowski, and Grzegorezyk [17] and Biesalski et al. [18] methods.

2.6.7.1 Determination of Vitamin A by UV-Spectrophotometric method

Vitamin A (carotenoid) standard (5 mg) was dissolved in isopropanol and taken as stock solution. An aliquot of the stock solution was diluted and its absorbance measured at a typical wavelength of 325 nm against isopropanol.

2.6.7.2 Determination of Vitamin E by UV-Spectrophotometric method

Tocopherol (vitamin E) standard (5 mg) was dissolved in isopropanol and taken as stock solution. The absorbance measured against isopropanol at 292 nm. Standard curve were prepared for each vitamin by appropriate dilution of the measured stock solution [18].

2.6.7.3 Determination of Vitamin C by UV-Spectrophotometric method

Vitamin C standard was dissolved in methanol to prepare stock solution and diluted to construct a calibration curve. The absorbance was measured at corresponding wavelengths.

2.7 Statistical Analysis

Statistical analysis of data was done using IBM SPSS version 23 computer software. Descriptive statistics and standard deviations were used to summarize the characteristics for the study population, and values presented as (mean±standard deviation). Student's paired-sample t-test were used to compare the significant difference in the levels of exposure between farmers and control group. Results were presented in tables and $P < .05$ were considered statistically significant.

3. RESULTS AND DISCUSSION

Table 1: Mean±SD values of Serum Enzymatic and Non Enzymatic Antioxidants of the Study Population Exposed to Pesticides In Okagwe.

Parameters/ Groups	Humans Exposed Mean±SD	Humans Control Mean±SD	P-value	Remark
SOD (IU/L)	39.0±2.36	62.8±5.90	<0.0001	S
GPX (IU/L)	205.8±16.8	259.4±20.1	<0.0001	S
CAT (IU/L)	67.1±6.08	88.7±8.86	<0.0001	S
TAC (mm/l)	195.3±18.6	378.0±18.3	<0.0001	S
ACHE (n/ml)	1.3±0.44	3.2± 0.65	<0.0001	S
MDA(n/ml)	36.4±2.32	12.6±1.00	<0.0001	S
GSH (n/ml)	60.6±7.12	86.1±13.25	<0.0001	S
VIT A (iu/l)	3.2±0.87	3.5±0.94	0.083	NS
VIT C (iu/l)	0.8±0.42	1.4±0.47	<0.0001	S
VIT E (iu/l)	3.9± 0.84	8.3±1.89	<0.0001	S

Key: S-significant, NS –non-significant.

Table 2: Mean±SD values of serum enzymatic and non-enzymatic antioxidants of the study population exposed to pesticides in Ihe-Nta.

Parameters/ Groups	Humans Exposed Mean	Humans Control Mean	P-value	Remark
SOD (IU/L)	41.7±2.55	68.0±5.85	<0.0001	S

GPX (IU/L)	222.8±15.0	281.3±23.5	<0.0001	S
CAT (IU/L)	59.8±5.99	78.5±8.5	<0.0001	S
TAC (mm/l)	201.2±25.6	387.6±15.5	<0.0001	S
ACHE (n/ml)	1.47±0.48	3.33±0.57	<0.0001	S
MDA(n/ml)	46.7±2.36	16.1±1.08	<0.0001	S
GSH (n/ml)	63.6±5.52	90.1±9.75	<0.0001	S
VIT A (IU/L)	3.25±0.79	3.58±0.96	0.100	NS
VIT C (IU/L)	0.59±0.25	1.32±0.45	<0.0001	S
VIT E (IU/L)	4.60±0.86	9.31±1.83	<0.0001	S

Key: S-significant, NS –non-significant.

Table 3: Mean± SD values of serum enzymatic and non enzymatic antioxidants of the study population exposed to pesticides in Okagwe and Ihe-Nta.

Parameters/G roups	Humans Exposed Mean	Humans Control Mean	P-value	Remark
SOD (IU/L)	40.3±2.81	65.6±6.23	<0.0001	S
GPX (IU/L)	211.7±27.9	271.1±23.9	<0.0001	S
CAT (IU/L)	63.5±7.04	82.7±11.27	<0.0001	S
TAC (mm/l)	382.8± 17.5	198.2±23.7	<0.0001	S
ACHE (n/ml)	1.43±0.46	3.28±0.63	<0.0001	S
MDA(n/ml)	41.50±5.63	14.40±2.06	<0.0001	S
GSH (n/ml)	62.19±6.54	88.1±11.73	<0.0001	S
VIT A (IU/L)	3.23±0.83	3.54±0.96	0.017	S
VIT C (IU/L)	0.74±0.38	1.36±0.48	<0.0001	S
VIT E (IU/L)	4.27±0.91	8.86±1.91	<0.0001	S

Key: S-significant, NS –non-significant.

In this study, SOD, CAT and GPX showed significant decreases in the exposed farmers as compared to the control subjects as could be seen in tables 1 and 2. These significant decreases indicate that there is oxidative injury caused by individual or combined exposure to pesticides. The result of the present study also showed significant increase in malondialdehyde, which could be attributed to decreased levels of glutathione peroxidase, super oxide dismutase and catalase, because these are first line antioxidant whose function is to neutralize any molecule with the potential of developing into a free radical.

The above results support the finding of Gultekin et al. [19], which says that pesticide lead to increase in MDA and decrease in the activities of SOD, GSH and CAT. Exposure with chlorpyrifos, methyl parathion and malathion singly or in mixture has also been known to cause dose-dependent decrease in the activities of antioxidant enzymes namely CAT, SOD, and GPX, in rat tissues. In addition, acetylcholinesterase activity, an indicator of organophosphate poisoning decreased in rat tissue in dose dependent manner, also that chlorpyrifos caused accumulation of malondialdehyde. It is important to note that the increase in MDA level observed in the present study can also be as a result of increased lipid peroxidation. Furthermore, the present study also showed significant decrease in the activities of ACHE, which is line with the finding of (Manno et al. [20], where it was reported that there was decrease in CAT and ACHE compared to the control in all concentration tested with organophosphate insecticide, and that the decrease in the activities of the ACHE could be attributed to its property as inhibitor of the neuron muscular enzymes [21].

The above result also agree with Singh et al. [22], in their finding which says that, the low levels of ACHE revealed among the exposed farmers indicate that there was exposure to organophosphate and that lower ACHE levels in exposed farmers are significantly

associated with DNA damage and reactive oxygen species (ROS). The non-enzymatic antioxidant fall into the group of second line of defense antioxidants. This group of antioxidants is often referred to as scavenging antioxidants. Example include Vitamin E, C, glutathione etc. This study also observed a statistical significant decrease in vitamin E, which could be attributed to its function as an effective chain breaking antioxidant, within cellular membrane where it inhibits lipid membrane peroxidation. The above finding is in line with the finding of Ronika et al. [23], who says that vitamin E act as an effective antioxidant for chlorpyrifos toxicity.

The significant decrease in the value of vitamin A and C observed in Tables 1 and 2 could be attributed to their protective role against reactive oxygen species produces during cellular metabolism and after active oxidation cause by chlorpyrifos and glyphosate. This is in line with the finding of Nalini, [24], which says that earlier studies have shown that vitamin A, C and E can maintain the optimum levels of antioxidant enzymes and molecules which scavengers out reactive oxygen species, thus, they can fight pesticide mediated toxic effects. It has also been reported that vitamin E plays a crucial role in restoring the antioxidant enzymes such as SOD and catalase in populations exposed to pesticide, also, that vitamin C supplements protected against liver damage in vineyard crop sprayers, which resulted in decreased aspartate transaminase and alanine transaminase activities. In addition to its antioxidant activity, that vitamin C is known to perform other actions that enhance its protective effect in organophosphate induced toxicity. Therefore, it is suggested that farmers, pesticide applicators, workers in the pesticide industry and other pesticide users, who come in regular contact with pesticides, may benefit from pretreatment with vitamin C. The non-statistical significant decrease observed in vitamin A in the present study could be attributed to the short duration of exposure to the pesticides.

4. CONCLUSION

This study showed that there was oxidative stress, evidenced by the significant increases in oxidative stress parameters, in human subjects exposed to pesticides in Okagwe and Ihe-Nta communities.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. Fukuyama, T., Kosaka, T. Tajima, Y, Ueda, H., Hayashi, K. Shutoh, Y. and Harrada T. Prior exposure to organophosphorus and organochlorine pesticides increase the allergic potential of environmental chemical allergens in a local lymph node assay *Toxicology letters*, 2010; 199: 347-56.
2. Amil, K.J.C., Singhal, V. and Kuanantechato, L. T. Environmental science and Engineering, edition; volume 6; toxicology, chapter 7, publisher: stadium press LLC, USA Editor: 2017; pp.140-58.
3. Cranshaw, W. and shelter D. Gardan insects of North America, 2nd Edition, Princeton University Press Princeton, New Jce, 2017; 704 PP.
4. Vale, J.A., Meredith, T.J. and Buckley, B.M. Paraquat Poisoning: Clinical Features and Immediate General Management. *Human Toxicology*, 2010; 6: 41-7.
5. Shama, D., kaar G, and Khera K.S. Triazophos-Induced Oxidative Stress and Instomorphological Changes in Ovary of Female Winter Rats. *Pest Biochemistry Physiology*, 2015; 8: 1129-8.
6. Timothy, J. Legg. Medical Reviewed by extensive Healthline Clinician network, New York. 2017.
7. Abdollahi, M., Ranjbar, A., Shadina, S., Nikfar, S., and Rezaie, A. Pesticides and Oxidative Stress: A Review, *Medical Science, Monitor*. 2004; 10: 141-7.
8. Casida, J.E., Quistad, G.B. Organophosphate Toxicity safety Asepects at Non Acetylcholinesterase Secondary targets. *Chemical Research Toxicology*, 2004; 17:983-98.
9. Agrawal, A. and Sharma, B. Pesticides Induced Oxidative Stress in Mammalian System: Review Article *International Journal of Biological Medical Research*, 2010; 1: 90-104.
10. Agrawal, A. Aponte-Mellado, A., Premkumar, B., J., Sharma, A and Gupta S. The Effect of Oxidative Stress on Female production: A Review *Reproduction Biological Endocrinology*, 2012; 20: 19-49.
11. Jaswal, S.V. A D, Gore, S. P, Shah, H.R, and Kamath R. M. Stressful life events, hopelessness and suicidal intent in patients admitted with attempted suicide in a tertiary care general hospital. *Journal of postgraduate's medicine*, 2016; 62; 102-4.
12. Xu, M.Y., Wang P., Sun Y.J., and Wu Y.J. Joint Toxicity of Chlopyrifos and Cadmium of the Oxidative Stress and Mitochondrial Damage in Neuronal Cells. *Food Chemical Toxicology*, 2017; 103: 246-52.
13. Naing, L., Winn, T. and Rusli, B.N. Practical Issues in Calculating the Sample Size for Prevalence Studies. *Archives of Orofacial Sciences*, 2006; 1: 9-14.
14. Ellman, G.L., Courtney, K.D., Andres, V.J. and Featherstone, R.M. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, 1961; 7: 88-95.
15. Misra, H.P. and Fridovich, I. The role of superoxide dismutase anion in the auto-oxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biochemistry*, 1972; 247: 3170-5.
16. Paglia, D.E. and Valentine, W.N. Studies on the Quantitative and Qualitative Characterization of Erythrocyte Glutathione Peroxidase. *Journal of Laboratory and Clinical Medicine*, 1967; 70: 158-69.
17. Rutkowski, M., and Gizegorczyk, K. Modifications of spectrophotometric methods for antioxidative vitamins determination convenient in analytic practice, *Acta science Political Technology Aliment*, 2007; 6(3): 17-28.
18. Biesalski, H., Brodda, K., Hafner, G., and Bassler, K. Rapid determination of vitamin A and vitamin E in human sample by isocratic adsorption *International journal of vitamin nutrition Resource*, 1986; 56(4): 313-7

19. F. Gultekin, N. Delibas, S. Yasar, and I. Kilinc, In vivo changes in antioxidant systems and protective role of melatonin and a combination of vitamin C and vitamin E on oxidative damage in erythrocytes induced by chlorpyrifos-ethyl in rats, *Archives of Toxicology*, 2000; 75 (2): 88–96.
20. Manno, M., Viau, V., and Cocker, J. Biomonitoring for Occupational Health risk assessment (BOHRA) *Toxicology Letters*, 2010; 192 (1): 3-16.
21. Goodman, and Brenda. Pesticide Exposure in Womb Linked to Lower IQ” *Health and Pregnancy*. WebMg. Archived from the Original. 2011.
22. Singh, N.K., Jyoti, Haque, M., Rath, S.S. and Ghosh, S. A comparative study on cypermethrin resistance in *Rhipicephalus (Boophilus) microplus* and *Hyalomma anatolicum* from Punjab (India). *Ticks Tick Borne Dis*, 2014; 5: 90–4.
23. Ronika, S., Poonam, G. and Jain D.k. Department of chemistry, Bareilly College, Bareilly-243005, Uttar Pradesh, India, 2011; 18(1): 73-6.
24. Nalini S. School of studies in Biochemistry, Jiwaji University. Issue of *Ecotoxicology and Environmental Safety*, 2012; 40(6): 423-8.

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