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GLYPHOSATE AND ALUMINUM PHOSPHIDE INDUCED DNA OXIDATION AND INFLAMMATION IN ALBINO RAT: POTENTIAL CHRONIC CONDITION FOR CANCER DEVELOPMENT

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ABSTRACT
Pesticides have been associated with increased risk of chronic disease such as cancer, yet their fundamental role that portends their association with such chronic disease is not yet well explored. This study evaluated the serum level of DNA oxidative and inflammatory markers in albino rats exposed to glyphosate and Aluminum phosphide. The chemicals were administered orally at intervals of one month up to third month, based on low and high dose assessment, to groups of male and female albino rats: Serum levels of 8-OHDG, IL-6 and IL-10 were determined using Enzyme Linked Immunosorbent Assay (ELISA). There was significant increase in mean level of 8-OHDG in male/female rats exposed to GLP on first, second and third exposures respectively (low dose P=0.052/0.052, P=0.037/0.026, P=0.742/0.011, respectively); (high dose. P=0.000/0.019, P=0.027/0.004, P=0.019/0.019 respectively). There was significant increase in mean level of IL-6 in male/female rats exposed to GLP on first, second and third exposures respectively (low dose P=0.011/0.008, P=0.007/0.002, P=0.000/0.000, respectively); (high dose. P=0.001/0.005, P=0.000/0.003, P=0.000/0.000 respectively). There was significant increase in mean level of IL-10 in male/female rats exposed to GLP on first, second and third exposures respectively (low dose P=0.802/0.254, P=0.008/0.003, P=0.000/0.004, respectively); (high dose. P=0.407/0.008, P=0.003/0.043, P=0.000/0.010 respectively). There was significant increase in mean level of 8-OHDG in male/female rats exposed to ALP on first, second and third exposures respectively (low dose P=0.025/0.026, P=0.026/0.009, P=0.025/0.021, respectively); (high dose: P=0.002/0.001, P=0.073 and 0.170 respectively). There was significant increase in mean level of IL-6 in male/female rats exposed to ALP on first, second and third exposures respectively (low dose P=0.016/0.007, P=0.026/0.005, P=0.000/0.000, respectively); (high dose: P=0.002/0.007, P=0.002/0.002 P=0.000/0.007 respectively). There was significant increase in mean level of IL-10 in male/female rats exposed to ALP on first, second and third exposures respectively (low dose P=0.154/0.182, P=0.001/0.007, P=0.000/0.000, respectively); (high dose: P=0.063/0.018, P=0.001/0.003 P=0.000/0.000 respectively). Glyphosate and aluminum phosphide mediated increase in the levels of DNA oxidative and inflammatory markers (8-OHDG, IL-6 and IL-10), thereby leading to deregulation of the immune system. It is also imperative that persistent exposure to these chemicals could sustain chronic inflammation and under this condition, such inflammatory cytokines as IL-6, could mediate chronic oxidative stress inducing persistent DNA damage. Exposure to GLYP and ALP could be a potent source of cellular oxidative stress and its persistence, mediating chronic inflammation, thus existing as underlying causal effects to many chronic diseases including Cancer.

KEYWORDS: Oxidative Stress, Inflammatory Markers, DNA Oxidation, Glyphosate, Aluminum Phosphide, Albino Rat.

INTRODUCTION
Pesticide-induced oxidative stress is caused by both reactive oxygen species (ROS) and reactive nitrogen species (RNS), which are associated with several diseases including cancer, inflammation, cardiovascular and neurodegenerative diseases. ROS and RNS can activate at least five independent signaling pathways including mitochondrial-induced apoptosis. Glyphosate

(GLYP) is a widely used pesticide; it is considered to be a safe herbicide for animals and humans because it targets 5- enolpyruvylshikimate-3-phosphate synthase. However, there has been increasing evidence that GLYP causes varying degrees of toxicity (Xiaojing et al., 2022).

Glyphosate, has the chemical name N-(phosphonomethyl)-glycine and has been industrially and agriculturally engaged with a record of about 48% usage globally (Zhong et al., 2018), considering its broad spectrum systemic effect. Glyphosate is an herbicide that acts on the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) (Helander et al., 2012). Glyphosate based herbicides (GBHs), goes with commercial formulation known as Roundup with glyphosate as its active ingredient. Currently, more than 1.4 billion pounds of glyphosate are applied to fields per year (Beckie et al., 2020; Larsen et al., 2021). The use of Glyphosate herbicides is widely recommended to improve crop yield and quality by reducing or inhibiting the growth of weeds as well as preserving agricultural produce by working as a desiccant for various grain crops including pulses (beans, peas, chickpea, lentils, etc) (Salazar-Flores et al., 2020). Among many herbicides, glyphosate-based herbicides (GBHs) are globally famous and widely used for the control of perennial weeds such as quack grass and thistle as well as by acting as a harvesting aid accelerating crop dry down (Richmond, 2018; UNEP, 2019). Studies have shown that glyphosate residues can be found in a variety of food products and exposure to this herbicide has been linked to several health concerns (Kalofiri et al., 2021). Some studies have suggested that glyphosate exposure may increase the risk of cancer, while others have linked it to developmental problems and endocrine disruption (Davoren et al., 2018; Shaw, 2021; Rani et al., 2021). GLYP has different toxic effects on different species, and these toxic effects have potentials of inducing DNA damages and subsequent accumulated damages and tumour development. The measure of DNA oxidative stress by these chemicals is yet to be properly estimated for adequate implication of these chemicals to suspected tumour development. DNA oxidation is induced by oxidative stress which is caused by excessive production of intracellular reactive oxygen species (ROS) or insufficient antioxidant defenses. GLYP-induced ROS generation plays critical roles in toxicity.

On the other hand, aluminium phosphide (ALP), a fumigant, has been enployed in the preservation of agricultural products and processed foods (Yousef et al., 2015). It has gained extensive use worldwide due to its low cost, efficacy, short half-life and low toxicity of its decomposition products. This report becomes controversial because it has also been reported to have highly toxic effect to both humans and animals on exposure (Shakeri and Mehrpour, 2013). Its widespread use poses the hazard of accidental and intentional poisoning to man.

ALP toxicity is potentially lethal and affects multiple systems with severe consequences. Its toxic effects are manifested in various forms including cardiotoxicity, neurotoxicity, electrolyteimbalance, hepatotoxicity, metb olic disturbances, haematological toxicity and renal toxicity, among others (Afolabi et al., 2018). The ALP exact mechanism (s) of action has to do with its toxic effects through the liberation of highly toxic gas, phosphine (PH_3), which is released in contact with water, moisture or gastric hydrochloric acid (Kariman et al., 2012). After the hydrolysis of ALP, the released phosphine diffuses to intracellular compartments; this induces cellular injuries mediated by oxidation. It has been reported that oxidative stress is the major contributor to ALP-induced cellular toxicity (Mehrpour et al., 2012). This report has not been clearly proven through the possible levels of biomarker detectable at the instance of ALP exposure. In this study, we measured DNA oxidation by the level of expression of 8-OH2DG biomarker. This is potential biomarker, evidence to DNA damage which accumulation could degenerate to tumorigenesis.

Pesticides are potential irritants and could disrupt physiological homeostatic balance, thus mediating cell injury and subsequent inflammatory responses. This could be made possible through skin irritation, respiratory impairment, or systemic effects Lopes-Ferreira et al., 2023. The crosstalk between pesticides and inflammation leads to more emphasis on adequate use of pesticides, their regulation and storage. Inflammation is a crucial component of innate immune response, often triggered by chemotactic actions of some soluble molecules or mediators, attracting neutrophils, monocytes, macrophages, to the site of irritation or infection. Pesticides may also work as an antigen, activating Toll-like receptors in epithelial cells' surface. This aggression incites the release of alarmins, and chemotactic and immune-activating proteins, such as IL-33, IL-25, TSLP, and HMGB1. Alarmins carry out neutrophil recruitment to the site, along with macrophages, and the consequent degranulation of these cells release proinflammatory cytokines, such as IL-6, IL-8, IL-1β, and TNF-α (Lopes-Ferreira et al., 2023).

MATERIALS AND METHODS

Chemicals: Glyphosate based herbicide (Glyphosate 1) and Aluminum phosphide (Celphos) were procured from Onitsha Main Market. Aluminium phosphide tablet (30g) was pulverized, and 4.5mg weighed out and added to their food for high dose group and 1.0mg for low dose group, while for Glyphosate 6.6ml/kg was added to their water for high group and 1.9ml/kg for the low dose group.

ANIMALS

Total number of fifty (50) male and female adult Albino rat (160±10g) were procured from the animal house of faculty of pharmaceutical sciences, Nnamdi Azikiwe University, Awka. The animals were randomly grouped

into 10 (n=5), and housed in plastic cages with free access to adequate diet, water ad libitum and normal light–dark cycle (12 h light/dark) and temperature (23±2°C), and left to acclimatize for 2 weeks before the study began (Arts *et al*, 2014). The animal handling and experimental procedures were approved by Ethical Committee on Animal Handling of Nnamdi Azikiwe University Awka Anambra State Nigeria (NAU/AREC/23/00095).

Experimental Approach

The groups were treated daily with the following: Group 1: male control (oral distilled water and food without pesticide); Group 2: female control (oral distilled water and food without pesticide); Group 3: male low dose Glyphosate (oral glyphosate 1000mg/kg/day); Group 4: female low dose Glyphosate (1000mg/kg/day); Group 5: male high dose Glyphosate (3500mg/kg/day); Group 6: female high dose Glyphosate (3500mg/kg/day); Group 7: male low dose ALP (1.1mg /kg /day); Group 8: female low dose ALP (1.1mg /kg /day); Group 9: male high dose ALP (5.0 mg /kg /day); Group 10: female high dose ALP (5.0 mg /kg /day).

The exposure doses of these pesticides were chosen based on the LD_{50} and no observed adverse effect level (NOAEL) of their use in previous studies (Moxon, 2002) and (Kimmel *et al*., 2013). The high dose of glyphosate was the half of the LD_{50} (½ of 7000mg/kg b.w = 3500mg/kg b.w (Turkmen and Dorgan, 2020), whereas the low dose (1000mg/kg bw) was the highest dose at which no adverse effect was recorded (Kimmel *et al*., 2013). The high dose of aluminium phosphide is the half of its LD_{50} (½ of 10mg/kg b.w =5mg/kg/b.w) (Dua and Gill, 2001), whereas the low dose (1.1mg/kg bw) is the highest dose at which no adverse effect was recorded (EFSA, 2008). Food and water were given *al bitum* and the remnants were discarded on daily bases. The animals were observed daily for general health and signs of toxicity. Individual body weights, together with feed consumption on a cage basis were determined daily. In this study, the National Institute of Health guidelines for laboratory animal care and use were followed.

Sample Preparation

At the start of the study, 1.5ml of the blood was collected via ocular puncture from the rat at the start of the study for pre-analysis and repeated every 4 weeks until the study ended, the study lasted for a period of 90days. The blood volume was put in a plain blood container and allowed to clot. The blood sample was centrifuged at 3500 rpm for 15 minutes; the serum was harvested and stored at −20°C until used for cytokine (IL-6 and IL-10) estimation.

Biochemical Analysis

Quantitative Estimation of 8-OH2DG IL-6 and IL-10 were done using Sandwich Method of Enzyme Linked Immunosorbent Assay as described by ABCAM USA.

RESULTS

Mean Levels of 8-OHDG, IL-6 and IL-10 at Pre-exposure Stage

Although significant difference was observed on mean levels of serum 8-OHDG values of both male and female albino rats, the values are within the normal range. However looking at the values between the variables, it was observed that the significant differences were a decrease in the mean value of female rats compared to the mean values of the male control group and a decrease in the mean value of male rats compared to the mean values of the female control group. The mean levels of Interleukin-6 (IL-6) of both male and female rats at this stage showed no significant difference within the variables (p=0.102). Although significant difference was observed on mean levels of serum Interleukin-10 (IL-10), values of both male and female albino rats, the values are within the normal range.

Table 1: Mean Levels of 8-OHDG, IL-6 and IL-10 at Pre-exposure Stage.

	8-OHDG (ng/ml)	**IL-6 (pg/ml)**	**IL-10 (pg/ml)**
GP 1	0.6.2±0.1	482.2±129.9	206.3±74.3
GP 2	0.9±0.2	763.0±117.6	325.0±108.4
GP 3	0.9±0.2	678.0±103.3	157.8±52.7
GP 4	0.7±0.2	729.0±87.3	232.2±59.2
GP 5	0.7±0.3	582.0±126.8	330.0±112.0
GP 6	0.6±0.2	774.0±121.6	370.2±60.0
GP 7	0.4±0.1	626.0±110.0	359.2±114.9
GP 8	0.4±0.1	739.0±118.5	219.8±74.0
GP 9	0.4±0.1	634.0±124.5	223.0±65.2
GP 10	0.3±0.1	684.0±97.0	360.6±62.9
F-value	**14.9**	2.0	**5.2**
P-value	**0.000**	0.102	**0.002**
	Post Hoc		
1 vs 7	0.167	0.678	0.919
1 vs 8	0.567	0.157	1.000
1 vs 10	**0.022**	0.286	0.112
2 vs 7	**0.018**	0.670	1.000

2 vs 8	**0.021**	1.000	0.727
2 vs 9	**0.015**	0.889	0.721
2 vs 10	**0.006**	0.960	0.999

Mean Levels of 8-OHDG, IL-6 and IL-10 at 1st Month Post exposure Stage

At one month post-exposure stage, the statistical analysis, showed significant increase in mean levels of 8-OHDG in both male and female rats within variables (p=0.000). Howell post hoc analysis showed that the significant increase was in mean level of 8-OHDG in male and female rats exposed to GLP (high dose. P=0.015 and 0.019 respectively) compared to the mean level of 8=OHDG in male control group without chemical exposure. The mean level of 8-OHDG was increased in male and female rats exposed to GLP (low dose P=0.052 and 0.052 respectively), but the increase was not statistically significant. On the other hand, statistically significant increase in mean level of 8-OHDG was seen in male and female rats exposed to ALP (low dose P=0.025 and 0.026 respectively), similarly, male and female rats exposed to ALP (high dose), also expressed significant increase in level of 8-OHDG (P=0.002 and 0.001 respectively). Still under first exposure, the mean levels of interleukin-6 (IL-6) in both male and female rats at this stage showed significant increase within variables (p=0.000). The post hoc analysis showed that the significant increase was in mean level of IL-6 in male and female rats exposed to GLP (low dose. P=0.011 and 0.008 respectively) as well as in same sexes exposed to GLP (high dose, P=0.001 and 0.005 respectively) compared to the mean level of the IL-6 in male control group without the chemical exposure. Similarly, significant increase was found in both male and female rats exposed to ALP (low dose P=0.016 and 0.007 respectively, as well as in those exposed to ALP (high dose P=0.002 and 0.007 respectively) compared to the mean level of IL-6 the male control group without the chemical exposure. On the other hand, the post hoc comparison with female control without chemical exposure, also showed that the significant increase within the groups was between the mean levels of interleukin-6 (IL-6) in male and female rats exposed to GLP (low dose. P=0.016 and 0.011 respectively) as well as in same sexes exposed to GLP (high dose, P=0.022 and 0.007 respectively). Similarly, significant increase was found in both male and female rats exposed to ALP (low dose P=0.022 and 0.009 respectively, as well as in those exposed to ALP (high dose P=0.003 and 0.010 respectively) compared to the mean level of IL-6 the female control group without the chemical exposure.

The mean levels of interleukin-10 (IL-10) in both male and female rats at this stage showed significant increase within variables (p=0.001). The post hoc analysis showed that the significant increase was in mean level of IL-10 in female rats exposed to GLP (high dose. P=0.008) as well as in female exposed to ALP (high dose, P=0.018) compared to the mean level of the IL-10 in male control group without the chemical exposure. Similarly, significant increase was found only in female rats exposed to GLP (high dose P=0.013), as well as in those exposed to ALP (high dose P=0.026) compared to the mean level of IL-10 in the female control group without the chemical exposure. Furthermore, Significant increase was found in female rats exposed to GLP (high dose P=0.020), as well as in those exposed to ALP (high dose P=0.022) compared with male rats exposed to (GLP low dose) See table 2.

Table 2: Mean Levels of 8-OHDG, IL-6 and IL-10 at 1st Month Post exposure Stage.

	8-OHDG (ng/ml)	**IL-6 (pg/ml)**	**IL-10 (pg/ml)**
GP 1	0.8±0.1	501.2±131.7	270.0±127.9
GP 2	1.7±0.5	786.0±114.4	333.0±109.6
GP 3	2.6±0.7	3546.0±821.6	378.2±75.7
GP 4	2.9±0.7	4720.0±1051.5	498.0±124.2
GP 5	2.7±0.5	3969.0±586.6	595.2±260.6
GP 6	3.3±0.8	4580.0±894.7	745.0±130.3
GP 7	2.4±0.5	3882.0±1001.7	702.4±248.1
GP 8	3.1±0.8	5583.0±1223.7	590.0±183.7
GP 9	3.0±0.4	3752.0±607.9	556.8±87.2
GP 10	2.7±0.3	4447.0±961.6	636.6±89.5
F-value	**39.9**	**56.7**	**6.1**
P-value	**0.000**	**0.000**	**0.001**
	PostHoc Analysis		
1 vs 3	0.052	**0.011**	0.802
1 vs 4	0.052	**0.008**	0.254
1 vs 5	**0.015**	**0.001**	0.407
1 vs 6	**0.019**	**0.005**	**0.008**
1 vs 7	**0.025**	**0.016**	0.154
1 vs 8	**0.026**	**0.007**	0.182

1 vs 9	**0.002**	**0.002**	0.063
1 vs 10	**0.001**	**0.007**	**0.018**
2 vs 3	0.543	**0.016**	0.997
2 vs 4	0.366	**0.011**	0.506
2 vs 5	0.297	**0.022**	0.595
2 vs 6	0.091	**0.007**	**0.013**
2 vs 7	0.600	**0.022**	0.248
2 vs 8	0.169	**0.009**	0.331
2 vs 9	0.055	**0.003**	0.112
2 vs 10	0.169	**0.010**	**0.026**

Mean Levels of 8-OHDG, IL-6 and IL-10 at 2nd Month Post Exposure Stage

At the second post-exposure stage, the statistical analysis, showed significant increase within variables (p=0.000) in the mean levels of 8-OHDG in both male and female rats. Games Howell post hoc analysis showed that the significant increase was in mean level of 8-OHDG in male and female rats exposed to GLP (low dose. P=0.037 and 0.026 respectively) compared to the mean level of 8=OHDG in male control group without chemical exposure. The mean level of 8-OHDG was also significantly increased in male and female rats exposed to GLP (high dose P=0.027 and 0.004 respectively). On the other hand, statistically significant increase in mean level of 8-OHDG was seen in male and female rats exposed to ALP (low dose P=0.026 and 0.009 respectively), similarly, female rats exposed to GLP (high dose), also expressed significant increase in level of 8-OHDG when compared with female control group.

Still under second exposure, the mean levels of interleukin-6 (IL-6) in both male and female rats at this stage showed significant increase within variables (p=0.000). The post hoc analysis showed that the significant increase was in mean level of IL-6 in male and female rats exposed to GLP (low dose. P=0.007 and 0.002 respectively) as well as in same sexes exposed to GLP (high dose, P=0.000 and 0.003 respectively) compared to the mean level of the IL-6 in male control group without the chemical exposure. Similarly, significant increase was found in both male and female rats exposed to ALP (low dose P=0.026 and 0.005 respectively), as well as in those exposed to ALP (high dose P=0.002 and 0.002 respectively) compared to the mean level of IL-6 the male control group without the chemical exposure. On the other hand, the post hoc comparison with female control without chemical exposure, also showed that the significant increase within the groups was between the mean levels of interleukin-6 (IL-6) in male and female rats exposed to GLP (low dose. P=0.008 and 0.003 respectively) as well as in same sexes exposed to GLP (high dose, P=0.000 and 0.004 respectively). Similarly, significant increase was found in both male and female rats exposed to ALP (low dose P=0.032 and 0.006 respectively, as well as in those exposed to ALP (high dose P=0.002 and 0.002 respectively) compared to the mean level of IL-6 the female control group without the chemical exposure.

The mean levels of interleukin-10 (IL-10) in both male and female rats at this stage showed significant increase within variables (p=0.000). The post hoc analysis showed that the significant increase was in mean level of IL-10 in male and female rats exposed to GLP (low dose. P=0.008 and 0.003 respectively) as well as in same sexes exposed to GLP (high dose, P=0.003 and 0.043 respectively) compared to the mean level of the IL-10 in male control group without the chemical exposure. Similarly, significant increase was found in both male and female rats exposed to ALP (low dose P=0.001 and 0.007 respectively), as well as in those exposed to ALP (high dose P=0.001 and 0.003 respectively) compared to the mean level of IL-10 in the male control group without the chemical exposure. On the other hand, the post hoc comparison with female control without chemical exposure, also showed that the significant increase within the groups was between the mean levels of interleukin-10 (IL-10) in male and female rats exposed to GLP (low dose. P=0.013 and 0.005 respectively). However only the male rat exposed to GLP (high dose) showed significant increase in IL-10 P=0.005. Significant increase was found in both male and female rats exposed to ALP (low dose P=0.002 and 0.011 respectively), as well as in those exposed to ALP (high dose P=0.001 and 0.004 respectively) compared to the mean level of IL-10 in the female control group without the chemical exposure. See table 3.

Table 3: Mean Levels of 8-OHDG, IL-6 and IL-10 at 2nd Month Post Exposure Stage.

	8-OHDG (ng/ml)	IL-6 (pg/ml)	IL-10 (pg/ml)
GP 1	0.75±0.08	509.0±129.8.3	273.6±127.4
GP 2	1.80±0.5	780.0±124.8	333.6±111.6
GP 3	3.1±0.9	5985.0±1289.9	720.0±118.3
GP 4	3.4±0.9	6922.0±1149.2	807.0±127.1
GP 5	2.8±0.7	5726.0±521.9	860.8±143.9
GP 6	3.4±0.6	7124.0±1287.9	1062.8±325.9

GP 7	2.6±0.6	5696.0±1746.7	962.2±152.8
GP 8	3.8±0.8	7534.0±1511.0	1003.2±211.6
GP 9	2.6±0.8	5344.0±810.9	856.8±81.6
GP 10	2.4±1.0	6723.2±1091.1	1057.0±193.9
F-value	**30.7**	**101.8**	**15.4**
P-value	**0.000**	**0.000**	**0.000**
		Post Hoc Analysis	
1 vs 3	**0.037**	**0.007**	**0.008**
1 vs 4	**0.026**	**0.002**	**0.003**
1 vs 5	**0.027**	**0.000**	**0.003**
1 vs 6	**0.004**	**0.003**	**0.043**
1 vs 7	**0.026**	**0.026**	**0.001**
1 vs 8	**0.009**	**0.005**	**0.007**
1 vs 9	0.073	**0.002**	**0.001**
1 vs 10	0.170	**0.002**	**0.003**
2 vs 3	0.298	**0.008**	**0.013**
2 vs 4	0.153	**0.003**	**0.005**
2 vs 5	0.375	**0.000**	**0.005**
2 vs 6	**0.027**	**0.004**	0.061
2 vs 7	0.497	**0.032**	**0.002**
2 vs 8	**0.032**	**0.006**	**0.011**
2 vs 9	0.740	**0.002**	**0.001**
2 vs 10	0.935	**0.002**	**0.004**

Mean Levels of 8-OHDG, IL-6 and IL-10 at 3rd Month Post Exposure Stage

At the third post-exposure stage, the statistical analysis, showed that the mean levels of 8-OHDG in both male and female rats were significant increased within variables (p=0.000). In post hoc analysis, the comparison with the mean 8-OHDG in male control group without any chemical exposure (1.9±0.6 3), showed that the significant increase was in mean level of 8-OHDG in female rats exposed to GLP (low dose: 3.4±0.7 P=0.011); mean level of 8-OHDG in male and female rats exposed to GLP (high dose 3.5±0.8, p=0.019 and 3.5±0.8, p= 0.019 respectively); mean level of 8-OHDG in male and female rats exposed to ALP (low dose: 4.0±1.0, p=0.025 and 3.7±0.9, p= 0.021 respectively); and mean level of 8-OHDG in male and female rats exposed to ALP (high dose: 3.7±0.8, p=0.017 and 3.6±0.8, p=0.017). See table 4.

Still under third exposure, the mean levels of interleukin-6 (IL-6) in both male and female rats at this stage showed significant increase within variables (p=0.000). In comparison with the mean level of the IL-6 in male control group without the chemical exposure (520.0±137.3), the post hoc analysis showed that the significant increase was in mean level of IL-6 in male and female rats exposed to GLP (low dose: 6192.0±483.5, p=0.000 and 7650.0±893.7, p=0.000 respectively) as well as in same sexes exposed to GLP (high dose: 6815.2±749.2, p=0.000 and 7194.0±808.7, p=0.000 respectively). Similarly, significant increase was found in both male and female rats exposed to ALP (low dose: 6662.0±691.2, p=0.000 and 7182.0±825.5, p=0.000 respectively), as well as in those exposed to ALP (high dose: 7088.0±829.1, p=0.002 and 7730.6±1675.1, p=0.002 respectively). On the other hand, the post hoc comparison with female control without chemical exposure, also showed that the significant increase within the groups was between the mean levels of interleukin-6 (IL-6) in male and female rats exposed to GLP (low dose: 6192.0±483.5, p=0.000 and 7650.0±893.7, p=0.000 respectively) as well as in same sexes exposed to GLP (high dose: 6815.2±749.2, p=0.000 and 7194.0±808.7, p=0.000 respectively). Similarly, significant increase was found in both male and female rats exposed to ALP (low dose 6662.0±691.2, p=0.000 and 7182.0±825.5, p=0.000, respectively), as well as in those exposed to ALP (high dose: 7088.0±829.1, p=0.002 and 7730.6±1675.1, p=0.002 respectively).

The mean levels of interleukin-10 (IL-10) in both male and female rats at this stage showed significant increase within variables (p=0.000). In comparison with the mean level of the IL-10 in male control group without the chemical exposure (269.0±130.6), the post hoc analysis showed that the significant increase was in mean level of IL-10 in male and female rats exposed to GLP (low dose: 1009.0±119.2, p=0.000 and 1158.6±232.9, p=0.004 respectively); as well as in male and female rats exposed to GLP (high dose: 1038.6±143.9, p=0.000 and 1343.4±321.7, 0.001 respectively); male and female rats exposed to ALP (low dose: 1146.0±146.5, p=0.000 and 1089.0±146.3, p=0.000 respectively); and in male and female rats exposed to ALP (high dose: 986.±78.7, p=0.000 and 1220.8±143.4, 0.000 respectively). On the other hand, the post hoc comparison with female control without chemical exposure, also showed that the significant increase within the groups was between the mean levels of interleukin-10 (IL-10) in male and female

rats exposed to GLP (low dose: 1009.0±119.2, p=0.000 and 1158.6±232.9, p=0.007 respectively); as well as in male and female rats exposed to GLP (high dose: 1038.6±143.9, p=0.000 and 1343.4±321.7, 0.015 respectively); male and female rats exposed to ALP (low dose: 1146.0±146.5, p=0.000 and 1089.0±146.3, p=0.000 respectively); and in male and female rats exposed to ALP (high dose: 986.±78.7, p=0.000 and 1220.8±143.4, 0.000 respectively). See table 4.

Table 4: Mean Levels of 8-OHDG, IL-6 and IL-10 at 3rd Month Post exposure Stage.

	8-OHDG (ng/ml)	IL-6 (pg/ml)	IL-10 (pg/ml)
GP 1	0.9±0.1	520.0±137.3	269.0±130.6
GP 2	1.9±0.6	780.2±112.9	332.6±108.7
GP 3	9.5±11.0	6192.0±483.5	1009.0±119.2
GP 4	3.4±0.7	7650.0±893.7	1158.6±232.9
GP 5	3.5±0.8	6815.2±749.2	1038.6±143.9
GP 6	3.5±0.8	7194.0±808.7	1343.4±321.7
GP 7	4.0±1.0	6662.0±691.2	1146.0±146.5
GP 8	3.7±0.9	7182.0±825.5	1089.0±146.3
GP 9	3.7±0.8	7088.0±829.1	986.±78.7
GP 10	3.6±0.8	7730.6±1675.1	1220.8±143.4
F- value	**30.8**	**202.2**	**28.1**
P-value	**0.000**	**0.000**	**0.000**
1 vs 3	0.742	**0.000**	**0.000**
1 vs 4	**0.011**	**0.000**	**0.004**
1 vs 5	**0.019**	**0.000**	**0.000**
1 vs 6	**0.019**	**0.000**	**0.001**
1 vs 7	**0.025**	**0.000**	**0.000**
1 vs 8	**0.021**	**0.000**	**0.000**
1 vs 9	**0.011**	**0.000**	**0.000**
1 vs 10	**0.017**	**0.007**	**0.000**
2 vs 3	0.828	**0.000**	**0.000**
2 vs 4	0.080	**0.001**	**0.007**
2 vs 5	0.112	**0.000**	**0.000**
2 vs 6	0.112	**0.001**	**0.015**
2 vs 7	0.089	**0.000**	**0.000**
2 vs 8	0.098	**0.001**	**0.000**
2 vs 9	0.053	**0.001**	**0.000**
2 vs 10	0.086	**0.008**	**0.000**

Table 5: Showing Correlation of the 8-OHDG, IL-6 and IL-10 Molecules at the First Exposure to GLP.

	GLP Low Exp. No. 10		GLP High Exp. No. 10	
Variables	r	p-value	r	p-value
OHDG vs IL-6	-0.095	0.793	-0.059	0.872
OHDG vs IL-10	-0.295	0.407	0.290	0.417
IL-6 vs IL-10	0.385	0.271	0.525	0.119

r= Correlation value.

Table 6: Correlation of 8-OHDG, IL-6 and IL-10 Molecules at the Second Exposure GLP.

	GLP Low Exp. No. 10	GLP High Exp. No. 10	
Variables	r	p-value	r	p-value
OHDG vs IL-6	0.075	0.837	-0.005	0.988
OHDG vs IL-10	0.291	0.414	0.149	0.682
IL-6 vs IL-10	0.173	0.632	**0.721**	**0.019**

r= Correlation value.

Table 7: Correlation of 8-OHDG, IL-6 and IL-10 Molecules at the Third GLP Exposure.

	GLP Low Exp. No. 10		GLP High Exp. No. 10	
Variables	r	p-value	r	p-value
OHDG vs IL-6	-0.338	0.339	-0.431	0.213
OHDG vs IL-10	-0.012	0.975	-0.127	0.726
IL-6 vs IL-10	0.112	0.474	0.276	0.440

r= Correlation value.

Table 8: Correlation of 8-OHDG, IL-6 and IL-10 Molecules at the First ALP Exposure.

	ALP Low Exp. No. 10		ALP High Exp. No. 10	
Variables	r	p-value	r	p-value
OHDG vs IL-6	**0.714**	**0.020**	-0.273	0.445
OHDG vs IL-10	-0.214	0.553	-0.031	0.933
IL-6 vs IL-10	-0.162	0.655	0.236	0.512

r= Correlation value.

Table 9: Correlation of 8-OHDG, IL-6 and IL-10 Molecules at the Second ALP Exposure.

	ALP Low Exp No. 10		ALP High Exp No. 10	
Variables	r	p-value	r	p-value
OHDG vs IL-6	0.425	0.221	-0.161	0.657
OHDG vs IL-10	**0.751**	**0.012**	-0.205	0.569
IL-6 vs IL-10	-0.158	0.664	0.421	0.226

r= Correlation value.

Table 10: Correlation of the CD4+, CD8+, 8-OHDG, IL-6 and IL-10 Molecules at the Third ALP Exposure.

	ALP Low Exp No. 10		ALP High Exp. No. 10	
Variables	r	p-value	r	p-value
OHDG vs IL-6	-0.088	0.810	0.373	0.289
OHDG vs IL-10	-0.128	0.725	-0.506	0.136
IL-6 vs IL-10	-0.242	0.501	-0.061	0.868

r= Correlation value.

DISCUSSION

In my bid to find the possible relative systemic chronic effects of Glyphosate and Aluminium phosphide in human using albino rat model, I exposed the animals to different doses of GLP and ALP at intervals of one month up to three months, with reference to control groups that are not exposed to such chemicals. The relevance of this work becomes useful considering that these chemicals may be seen as harmless at short term usage but there are tendencies of long term exposure toxicity, thus justifying the prolonged exposure of the animals to these chemicals to ascertain the effect on a long term bases. Glyphosate has long been regarded as harmless in animals (Peillex and Pelletier, 2020), yet, the International Agency for Research on Cancer (IARC) classified glyphosate as "probably carcinogenic" in humans. Also, increasing evidence shows that glyphosate and glyphosate-based herbicides exhibit cytotoxic and genotoxic effects, increase oxidative stress, and allegedly correlate with some cancers. Therefore, it is important to determine the relationship between the cause effect, risk factors and the pathological developments. Long term glyphosate and aluminum phosphide effects have been under reported, and there are lots of factors that encourage long term exposure in human, these may include persistent use of these chemical. Besides, Glyphosate is resistant to complete degradation due to the inert C-P linkage in the molecule (Van Bruggen *et al.*, 2021). It is broken down slowly in dead plant material, soil and water by various microorganisms (Carles *et al.*, 2019), indicating possible persistent when in contact with human, thus encouraging the need to under study the long term effect of the chemicals. Detection of the possible DNA oxidative effect of GLP and ALP was based on the expression of a very important DNA oxidative marker 8-hydroxy-2-deoxyguanosine (8-OH2DG). Increase in 8-OH2DG molecule was seen from the first month of exposure to both GLP and ALP. However, at low dose GLP, male and female rats showed no significant increase (p=0.052 and 0.052 respectively) in the level of 8-OH2DG, though the increment was relatively close to the significant level. On the other hand, ALP induced significant increase in 8-OH2DG level at low and high doses in both male and female. Suffice it to say that GLP at onset of exposure, may require higher doses (3500mg/kg/day) to stimulate

immune response. The increased level of 8-OH2DG is a pointer towards physiological stress and including DNA oxidation.

Earlier, research has shows that glyphosate and glyphosate-based herbicides exhibit cytotoxic and genotoxic effects, increase oxidative stress, and allegedly correlate with some cancers (Peillex and Pelletier, 2020). Basically, oxidative damage occurs when oxygen-derived free radicals attack the double bonds in unsaturated fatty acids found in membrane lipids, indicating lipid peroxidation (Sidthilaw et al., (2022). Moreover, when a cell is damaged by oxidative stress, it has a defense mechanism that produces antioxidants to destroy excess free radicals (Sidthilaw et al., (2022)). Glutathione (GHS) is an antioxidant compound with a sulfhydryl group (-SH) in its molecule which is found in almost every cell, playing a vital role in many cell processes, such as protecting cells from damage from oxidative stress (Gaucher *et al.*, 2018). The mechanism behind the glyphosate induce oxidation is out of the scope of this work, but research has shown that in vivo, oxidative stress caused by glyphosate is caused by a decrease in glutathione and an increase in the products of lipid peroxidation. The loss of glutathione comes from this antioxidant breaking down glyphosate through the activity of GHS-peroxidase (Sidthilaw *et al*., 2022). In line with the detected increase in serum levels 8-OH2DG in the animals, Jelić *et al*., (2018), reported that oxidative stress is possibly involved in the pathogenesis of cervical cancer, demonstrated by increased lipid peroxidation and an altered antioxidant defense system and higher levels of 8-OHdG. Increasing evidence shows that glyphosate and glyphosate-based herbicides exhibit cytotoxic and genotoxic effects, increase oxidative stress, and allegedly correlate with some cancers.

Reports on pathological effects of aluminum phosphides, show that inhaling Aluminum Phosphide can irritate the nose, throat and lungs causing coughing, wheezing and/or shortness of breath. Repeated exposure may damage the lungs, kidneys and liver. It liberates lethal phosphine gas when it comes in contact either with atmospheric moisture or with hydrochloric acid in the stomach, mediating toxicity such as cellular hypoxia due to the effect on mitochondria, inhibition of cytochrome C oxidase and formation of highly reactive hydroxyl radicals, Cellular injury due to lipid peroxidation is also suggested (Gurjar et al., 2011)). Similar to the effects of GLP, ALP poisoning, mediates decrease in the level of catalase and increase in the activity of superoxide dismutase (Chugh et al., 1997). The reduction of glutathione concentration in different tissues in AlP poisoning also explains the cellular injury as glutathione is a protecting factor against oxidation by catalysing the reduction of the oxygen peroxide in O_2 and H_2O. (Hsu *et al.*, 2002). Indicators of oxidative stress (reduced glutathione, malonyldialdehyed) reach peak levels within 48hrs of exposure of poison, approaching normalisation by day 5 (Chugh *et al.*, 1996). This explains the statistically significant rise in 8-OH2DG in rats exposed to low dose of ALP, thus indicating quick action of ALP compared to the group exposed to low dose GLP where 8-OH2DG was not significantly raised. However, as the exposure persisted, the serum level of 8-OH2DH continued to rise simultaneously.

Increase in serum level of IL-6 was also simultaneous with persistent exposure to GLP and ALP, indicating persistent inflammatory response. Interleukin-6 is a potent pro-inflammatory marker and is elevated in inflammatory diseases in humans. Interleukin-6 has been shown to be a key player in chronic inflammation. Expression of IL-6 is enhanced at the site of inflammation, and blockade of IL-6 and IL-6 signalling is effective at prevention and treatment in models of inflammatory diseases (including cancer, arthritis and colitis) (Lopes-Ferreira *et al.*, 2023). Earlier studies conducted on animal models or cells have shown that glyphosate induces inflammatory processes, increasing the expression and concentration of inflammatory cytokines, such as interleukin-6 (IL-6), IL-1β, and TNF-α, followed by an increase in the number of immune cells, like neutrophils and macrophages (Bai et al., 2022); Buchenauer *et al.*, 2022). The herbicide glyphosate presents surfactants in its formulations, which is responsible for its toxicity. Due to surfactants like polyethoxylated tallow amine (POEA), glyphosate-based herbicides can penetrate plants and act as herbicides. Nevertheless, the same can occur in animal cell membranes, allowing for bioaccumulation in plants and animals (de Brito Rodrigues *et al.*, 2019). Inflammatory effects were primarily observed in the liver and intestines of rats and mice during experiments, organs closely involved in absorption and metabolism (Panza *et al*., 2021; Rieg *et al*., 2022; Qi *et al.*, 2023) It is also noteworthy the study conducted by Buchenauer *et al*. (2022), which not only highlighted the significant inflammatory effects caused by glyphosate in mice, but also indicated that exposure of pregnant females affects the offspring's immune system, resulting in immunosuppression (reduced interferon gamma, IFN-γ, and expression) and alterations in the intestinal microbiome. In line with these reports, increased expression of IL-6 as detected in this study suggests that persistent exposure to GLP and ALP would sustain inflammatory response and possiblly mediate chronic inflammation. Chronic inflammation usually involves an imbalance of pro- and anti-inflammatory cytokines. Prolonged disruptions in this balance result in the development of diseases such as osteoarthritis and rheumatoid, autoimmune disorders, inflammatory bowel diseases, and cancer. Considering the outcome of this exposure experiment, GLP and ALP are both suggested to be capable of disrupting homeostatic and immune balance by overwhelming the physiological mechanism of the system, thus altering the normal inflammatory regulations. This is considered possible because the simultaneous rise in level of interleukin-10 is an indication of effort to suppress rising inflammatory and

cytotoxic pathways (elevated IL-6 and cytotoxic T cells). As reported by Carlini *et al.,* (2023), that interleukin-10 primarily acts as an anti-inflammatory cytokine, protecting the body from an uncontrolled immune response, mostly through the Jak1/Tyk2 and STAT3 signaling pathway. Additionally, IL-10 plays a fundamental role in maintaining host homeostasis at both local and global level, ensuring the fine equilibrium between pro- and anti-inflammatory immune response required to achieve an effective clearance of infecting pathogens and preventing at the same time, tissue damage occurrence (Ouyang and O'Garra, 2019). On the other hand, IL-10 can also have immunostimulating functions under certain conditions. Given the pivotal role of IL-10 in immune modulation, this cytokine could have relevant implications in pathologies characterized by hyperinflammatory state, such as cancer, or infectious diseases as in the case of COVID-19 and Post-COVID-19 syndrome (Carlini *et al.,* 2023). Such as envisaged through this study due to increased level of DNA oxidation and cytotoxicity and possible tissue damages, IL-10 can act as an endogenous danger signal, released by tissues undergoing damage in an attempt to protect the host organism from harmful hyperinflammation.

Furthermore, the pleiotropic effects of the molecules analyzed was put to test and as a result, IL-6 was found to increase as 8-OHDG increases in the presence of ALP. In comparison with evidence based study in C57BL/6J mice. Inhibition of IL-6 trans-signaling significantly reduces diabetes-induced oxidative damage at the systemic level (Robinson *et al.,* 2020). These findings provide further evidence for the possible role of IL-6 molecules in oxidative stress. The possible mechanism favouring the inflammation-oxidative stress development could be attributed to the neutrophil activity such as respiratory burst which may result in the production of high levels of ROS, leading to more intense cell aggression and death. While inflammation is typically a protective response, chronic, or excessive inflammation has been associated with a higher risk of inflammatory diseases, including autoimmune disorders like rheumatoid arthritis and inflammatory bowel disease.

This indicates that inhibition of IL-6 could mediate reduced systemic oxidative response or stress suggestive of immuno-suppressive therapy. Hence ALP is suggested here to be a potent oxidative stress inducer through pro-inflammatory cytokine activation. Moreover, the same positive correlation was found between IL-10 and 8-OHDG. The biomarker 8-OHdG or 8-oxodG has been a pivotal marker for measuring the effect of endogenous oxidative damage to DNA and as a factor of initiation and promotion of carcinogenesis (Qing *et al.,* 2019). On the other hand, Interleukin 10 (IL-10) is a cytokine with potent anti-inflammatory properties that plays a central role in limiting host immune response to pathogens, thereby preventing damage to the host and maintaining normal tissue homeostasis (Lyer and Cheng, 2012). Suffice it to say that increase in IL-10 in line with the 8-OHDG is imminent resulting from the imminent role of the immune system to check immunological excesses in the albino rat used. Similarly, the exposure *via* gavage was able to increase the mRNA levels of IL-1β, IL-6, TNF-α, MAPK3, NF-κB, and caspase-3 in the jejunum of rats exposed to the doses of 50 and 500 mg/kg bw/day (Tang et al., 2020). It is suggested that chronic GLP and ALP exposure is an inducer of oxidative stress.

CONCLUSION

From the study conducted, it is seen that glyphosate and aluminum phosphide cause increase in the levels of 8-OHDG, IL-6 and IL-10, thereby leading to deregulation of the immune system. These chemicals were also noted to cause increase in cells oxidation. It is also imperative that persistence of the exposure to these chemicals could sustain chronic inflammation and under this condition, such inflammatory cytokines as IL-6, TNF-alpha could mediate chronic oxidative stress inducing persistent DNA damage.

RECOMMENDATIONS

I will recommend the use of alternative measures in eliminating the pest. In a situation where there is no alternative, strict exposure controls should be implemented such as the use of personal protective equipment (PPE) to reduce exposure risk for farmers and anyone intending to use these chemicals. The users of these chemicals should be taught proper handling protocols to reduce exposures. Regulatory agencies should enforce strict policy on the usage, storage and handling of these chemicals, having evidence that these chemicals cause immune dysregulation and induction of oxidative stress.

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