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Ameliorating potentials of antioxidants on the lead-induced immunotoxicity in male Wistar rats

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Abstract

Lead exposure is a significant environmental and public health concern, known for its detrimental effects on various physiological systems, including the immune system. The study was undertaken to establish the ameliorating action of antioxidants on lead-induced toxicity on immunoglobulins using male Wistar rats as experimental models. One hundred and sixty-two male Wistar rats with weights between 180 and 200 g were obtained from the Experimental Animal Farm of the University of Port Harcourt, Nigeria. The Wistar rats were housed in wooden animal cages in a well-ventilated experimental room. Handling of the animals was in accordance with relevant institutional and ethical guidelines as approved for scientific study. The control group (group 1) was orally given 0.5 ml of distilled water, while the treatment groups (groups 2 to 9) were orally given different substances as follows: 10 mg/kg body weight (BW) of lead only, 200 mg/kg BW of vitamin C only, 1000 iu/kg BW of vitamin E only, 10 mg/kg BW of lead + 200 mg/kg BW of vitamin C, 10 mg/kg BW of lead + 1000 iu/kg BW of vitamin E, 10 mg/kg BW of lead + 40 mg/BW levamisole, 10 mg/kg BW of lead + 200 mg/kg BW of vitamin C + 40 mg/BW levamisole, and 10 mg/kg BW of lead + 1000 iu/kg BW of vitamin E + 40 mg/BW levamisole, respectively, once a day. The experiment was conducted in three phases (phases 1 to 3), which lasted for 7 days (acute phase), 30 days (sub-acute phase), and 60 days (chronic phase). At the end of the experimentation for each phase, five rats were sacrificed, and blood samples were collected from each rat and examined for immunological parameters. The effects of treatment with lead and antioxidants were compared with the control group. There was a significant decrease in the concentrations of the immunoglobulins in the lead group with respect to the control in all the phases. There was also a significant increase in the concentrations of the immunoglobulins in groups 3 and 4 with respect to the control in phases 1 and 2 and a significant increase in the concentrations of the immunoglobulins in groups 5 to 9 with respect to the lead group in all three phases. The antioxidants have, therefore, demonstrated the ability to ameliorate the lead-induced toxicity on the immunoglobulins.

Keywords: Lead, Immunoglobulins, Antioxidants, Ameliorate and Toxicity.

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INTRODUCTION

Lead toxicity remains a significant public health concern due to its widespread environmental and occupational exposure. Lead is a potent toxicant that exerts deleterious effects on multiple organ systems, including the immune system, where it disrupts the production and function of immunoglobulins, essential components of humoral immunity. [1,2]Immunoglobulins, such as IgG, IgA, and IgM, play critical roles in pathogen defense, and their dysfunction can lead to increased susceptibility to infections and immune-related disorders.[3] Chronic lead exposure has been shown to impair immunoglobulin synthesis and alter immune responses, posing a significant risk to human health.

Antioxidants have emerged as a promising therapeutic approach to mitigate the adverse effects of lead-induced oxidative stress. Lead toxicity induces the generation of reactive oxygen species (ROS), which disrupt cellular homeostasis and contribute to immune system dysfunction.[4]Antioxidants, such as vitamin C, vitamin E, and selenium, counteract ROS, restoring cellular function and reducing oxidative damage. [1] Studies have highlighted the potential of antioxidants to protect immunoglobulins from lead-induced toxicity, preserving immune competence and reducing systemic inflammation. [5,6]

This study investigates the ameliorating effects of antioxidants on lead-induced immunoglobulin toxicity, with a focus on their role in modulating oxidative stress and enhancing immune function. Understanding these interactions can provide critical insights into developing interventions for lead toxicity and improving immune health.

MATERIALS AND METHOD

One hundred and sixty-two (162) male Wistar rats with weight between 180 and 200 g were obtained from the Experimental Animal Farm, University of Port Harcourt, Nigeria. The Wistar rats were housed in animal wooden cages in a well-ventilated experimental room. The rats were allowed to acclimatize for a period of two weeks before the commencement of treatments. The rats had free access to standard rat chow and clean water ad libitum. Handling of animals was in accordance with relevant institutional and ethical guidelines as approved for scientific study. After acclimatization, the rats were divided into nine (9) groups. The control group (group 1) was orally given 0.5ml of distilled water only, group 2 was given 10mg/Kg Body weight of Lead only, group 3 was given 200mg/Kg Body Weight of Vitamin C only, group 4 was given 1000iu/Kg Body Weight of Vitamin E only, group 5 was given 10mg/Kg Body Weight of Lead + 200mg/Kg Body Weight of Vitamin C, group 6 was given 10mg/Kg Body Weight of Lead + 1000iu/Kg body Weight of Vitamin E, group 7 was given 10mg/Kg Body Weight of Lead + 40mg/Body Weight Levamisole, group 8 was given 10mg/Kg Body Weight of Lead + 200mg/Kg Body Weight of Vitamin C + 40mg/Body Weight Levamisole while group 9 was given 10mg/Kg Body Weight of Lead + 1000iu/Kg Body Weight of Vitamin E + 40mg/Body Weight Levamisole. These administrations were done once a day. The experiment was conducted in three phases (phases 1 to 3). Phase 1 (acute phase) lasted for 7 days, phase 2 (sub-acute phase) lasted for 30 days while phase 3 (chronic phase) lasted for 60 days. At the expiration of the experimentation for each phase, five (5) rats were sacrificed from each group and blood samples were collected from each rat

in an EDTA bottle. The blood samples were collected, using the method of cardiac puncture, after each rat has been anaesthetized in a desiccator, using diethyl ether and examined for the immunological parameters (IGA, IGG and IGM). The effects of treatment with lead and antioxidants were compared with the control group.

STATISTICAL ANALYSES

The results were subjected to statistical analysis using statistical package for social sciences (SPSS) version 20.0. Data are presented as mean ± SEM. Difference of means were considered significant at P value less than 0.05.

RESULTS

The effects of the administration of antioxidants (Vitamin C, Vitamin E and Levamisole) on lead-induced toxicity on Immunoglobulins (IGA, IGG and IGM) after 7 days are presented in table 1 below. There was significant decrease in the concentrations of IGA, IGG and IGM in the lead group (group 2), when compared with the control group (group 1). Again, there was a significant increase in the concentration of IGM in groups 3 (Vitamin C group) and 4 (Vitamin E group), when compared with the control group. There was also a significant increase in IGA, IGG and IGM in groups 5 to 9, when compared with the lead treated group (Table 1).

Table 1: Ameliorating effects of antioxidants on lead-induced toxicity on Immunoglobulins after 7 days

Immunoglo-bulins	Grp 1	Grp 2	Grp 3	Grp 4	Grp 5	Grp 6	Grp 7	Grp 8	Grp 9
IGA	48.60 ±3.30	18.40[a] ± 1.47	52.20 ±3.02	55.40 ±2.40	34.00[b] ±3.00	30.60[b] ±5.83	34.00[b] ±4.51	56.80[b]± 2.25	56.40 [b] ± 2.62
IGG	81.40 ±6.46	21.40[a]±3.60	85.40 ±7.45	84.00± 5.15	37.60 [b]±4.00	44.40 [b]±2.38	45.00 [b]±2.86	66.20 [b]±2.56	51.20 [b]±3.14
IGM	81.20 ±3.00	29.80[a]±3.71	89.40[a]±2.27	90.00[a]±2.76	47.60[b] ±0.75	50.20[b]±2.54	49.60 [b]±4.07	84.00 [b]±2.17	63.60[b] ±2.40

[a] and [b] denote significant differences when compared with the control and lead groups respectively, at p<0.05.

The effects of the administration of antioxidants (Vitamin C, Vitamin E and Levamisole) on lead-induced toxicity on Immunoglobulins (IGA, IGG and IGM) after 30 days are presented in table 2 below. There was significant decrease in the concentrations of IGA, IGG and IGM in the lead group (group 2), when compared with the control group (group 1). Again, there was a significant increase in the concentration of IGA (in group 3), IGG (in groups 3 and 4) and IGM (in groups 3 and 4) when compared with the control group.

There was also a significant increase in the concentration of IGA (in groups 5 to 9), IGG (in groups 5, 7, 8 and 9) and IGM (in groups 5 to 9), when compared with the lead treated group

Table 2: Ameliorating effects of antioxidants on lead-induced toxicity on Immunoglobulins after 30 days

Immunoglo-Bulins	Grp 1	Grp 2	Grp 3	Grp 4	Grp 5	Grp 6	Grp 7	Grp 8	Grp 9
IGA	54.20 ±2.91	16.00 [a] ±3.00	34.60 [a] ±5.98	48.80 ±0.73	30.80 [b] ±1.28	32.20[b]±1.32	31.20[b]±4.82	40.64 [b] ±3.18	38.00 [b] ±4.84
IGG	89.40 ±6.45	26.02 [a] ±1.83	38.24 [a] ±2.99	47.31[a] ±5.36	53.88[b]±2.92	34.91 ±2.90	46.40[b]±1.27	57.68 [b] ±2.35	59.88 [b] ±2.88
IGM	85.80 ±2.06	10.10 [a] ±1.13	60.98 [a] ±5.83	65.76[a] ±2.87	65.60[b]±3.43	73.60[b]±4.34	60.40[b]±1.47	57.32 [b] ±3.79	64.40 [b] ±2.53

[a] and [b] denote significant differences when compared with the control and lead groups respectively, at p<0.05.

The effects of the administration of antioxidants (Vitamin C, Vitamin E and Levamisole) on lead-induced toxicity on Immunoglobulins (IGA, IGG and IGM) after 60 days are presented in table 3 below. There was significant decrease in the concentrations of IGA, IGG and IGM in the lead group (group 2), when compared with the control group (group 1). Again, there was a a significant increase in IGA, IGG and IGM in groups 5 to 9, when compared with the lead treated group

Table 3: Ameliorating effects of antioxidants on lead-induced toxicity on Immunoglobulins after 60 days

Immunoglo-Bulins	Grp 1	Grp 2	Grp 3	Grp 4	Grp 5	Grp 6	Grp 7	Grp 8	Grp 9
IGA	62.60 ±3.27	14.40[a]±1.57	69.20 ±3.25	69.20 ±2.65	52.60[b]±4.91	43.60 [b]±3.11	56.40 [b] ±7.17	64.80 [b] ±2.25	60.80 [b] ±6.27
IGG	96.60 ±7.08	26.80[a]±3.84	112.96 ±15.40	102.36 ±7.35	54.32 [b]±5.41 [b]	57.06 [b]±5.21	59.92 [b] ±4.39	74.92 [b] ±2.76	70.72 [b] ±5.74
IGM	94.00 ±1.70	9.68[a]±2.29	99.38 ±2.02	99.64 ±1.53	67.76 [b]±6.78	69.76 [b] ±2.90	61.16 [b] ±3.83	83.60 [b] ±1.82	74.92 [b] ±4.05

[a] and [b] denote significant differences when compared with the control and lead groups respectively, at p<0.05.

DISCUSSION

The findings of this study demonstrate the ameliorative effects of antioxidants on lead-induced immunoglobulin (IgA, IgG, and IgM) disorder, with notable variations in response observed across different time intervals (7days, 30days, and 60 days). These results underscore the therapeutic potential of antioxidants such as Vitamin C, Vitamin E, and Levamisole in mitigating the immunosuppressive effects of lead exposure toxicity.

The observed decrease in immunoglobulin levels (IgA, IgG, and IgM) in the lead-treated group (group 2) compared to the control group highlights the detrimental impact of lead toxicity on the immune system. Lead is known to disrupt immune homeostasis by inducing oxidative stress, which damages immune cells and impairs immunoglobulin production. [4] These findings align with prior research indicating that lead exposure negatively affects both cellular and humoral immunity, thereby compromising host defense mechanisms. [3]

Immunoglobulins (antibodies) are critical components of the immune system, mediating the body's defense against pathogens. Among the five major immunoglobulin classes, IgA, IgG, and IgM play distinct roles in immunity, with specialized physiological functions and distribution.

IgA is the predominant antibody found in mucosal surfaces, such as the respiratory and gastrointestinal tracts. It exists in two forms: monomeric IgA in the serum and dimeric IgA in mucosal secretions. The secretory IgA (sIgA) plays a critical role in mucosal immunity by preventing the attachment of pathogens to epithelial cells, thereby inhibiting colonization and infection.[7] It is transported across epithelial cells via the polymeric immunoglobulin receptor (pIgR), acquiring a secretory component that protects it from proteolytic degradation. [8] It is especially crucial in neonatal immunity, as it is abundant in colostrum and breast milk, providing passive immunity to infants.[9]Deficiencies in IgA production can result in increased susceptibility to respiratory and gastrointestinal infections, highlighting its protective function. [10]

IgG is the most abundant antibody in the serum and extracellular fluid, accounting for approximately 75% of total serum immunoglobulins. It is vital for long-term immunity and memory responses. IgG is subdivided into four subclasses (IgG1, IgG2, IgG3, and IgG4), each with distinct functions and affinities for antigens. [11] It is central to humoral immunity, providing neutralization of toxins and pathogens, opsonization (facilitating phagocytosis by macrophages), and activation of the classical complement pathway (Nimmerjahn & Ravetch, 2008). It is the only antibody class that crosses the placenta, providing passive immunity to the fetus. [12] Additionally, its long half-life, mediated by the neonatal Fc receptor (FcRn), ensures prolonged immune protection. [13]

IgM is the largest antibody in terms of molecular size and the first antibody produced during an immune response. It exists as a pentamer in circulation, which allows it to form multiple antigen-binding sites, enhancing its efficacy in agglutination and complement activation. [14]It plays a critical role in the primary immune response by neutralizing pathogens and activating the complement system through its high avidity for antigens. [15]It is also expressed as a monomer on the surface of immature B cells, where it serves as a B-cell receptor (BCR), essential for antigen recognition and B-cell activation. [16]

While all three immunoglobulins contribute to immune defense, they differ in their primary roles and distribution. IgA specializes in mucosal immunity, IgG is critical for systemic immunity and memory responses, and IgM is essential for initiating the primary immune response. Their coordinated action ensures both immediate and long-term protection against infections.

Abnormal levels of IgA, IgG, or IgM are associated with various diseases. Elevated IgA levels may indicate chronic inflammation or autoimmune conditions, while deficiencies

are linked to recurrent mucosal infections. [17] High IgG levels may occur in chronic infections or autoimmune diseases, while low IgG levels are associated with immunodeficiency disorders. [18]Elevated IgM levels are seen in Waldenström's macroglobulinemia, whereas deficiencies are linked to increased susceptibility to pyogenic infections.

The significant increases in immunoglobulin levels observed in antioxidant-treated groups (groups 3 to 9) relative to the lead-treated group suggest that antioxidants effectively counteract the oxidative damage caused by lead. Antioxidants such as Vitamin C and Vitamin E are potent scavengers of reactive oxygen species (ROS), which are heavily implicated in lead-induced oxidative stress.[1] This corroborates with findings by others, who reported that antioxidant supplementation can restore immune function in cases of heavy metal toxicity.[5]

The progressive improvement in immunoglobulin concentrations over 30 and 60 days of antioxidant treatment further supports the hypothesis that antioxidants can reverse lead-induced immune dysfunction. The long-term effects observed are particularly significant as they suggest that sustained antioxidant therapy may not only mitigate acute damage but also restore immune competence over time.

The results of this study are consistent with previous findings in the literature. For instance, research by others has demonstrated that Vitamin C and Vitamin E effectively ameliorate lead-induced reductions in immunoglobulin levels by reducing oxidative stress and preserving lymphocyte function. [6]Similarly, Levamisole, an immune modulator, has been shown to enhance antibody production in immunocompromised states, further supporting its beneficial role observed in this study. [2]

Significantly, the findings highlight a differential effect of individual antioxidants. For instance, Vitamin C and Vitamin E significantly improved immunoglobulin levels in the short term (7 days), while the groups receiving combined antioxidant therapy showed sustained improvement over 30 and 60 days. This suggests a possible synergistic effect when multiple antioxidants are administered, a phenomenon also reported in studies exploring combination therapies for lead toxicity. [1]

These findings have important implications for the development of therapeutic strategies against lead toxicity. The demonstrated efficacy of antioxidants in restoring immunoglobulin levels suggests their potential role as part of a comprehensive treatment regimen for individuals exposed to lead. Further research is however needed to explore optimal dosages, combinations, and long-term safety of antioxidant therapy in diverse populations.

This study therefore highlights the critical role of antioxidants in mitigating the immunosuppressive effects of lead toxicity. The observed improvements in immunoglobulin levels provide strong evidence for the therapeutic potential of antioxidants, paving the way for their application in clinical and public health settings.

CONCLUSION

These findings of this study underscore the efficacy of antioxidants in ameliorating lead-induced toxicity on immunoglobulins, with consistent improvements observed across all time points. The results suggest that antioxidants such as Vitamin C, Vitamin E, and

Levamisole may serve as therapeutic agents to mitigate lead-related immune dysfunction, with potential implications for managing environmental and occupational lead exposure. Further studies are however recommended to explore the underlying mechanisms and optimize their therapeutic protocols.

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COMPETING INTEREST / CONFLICT OF INTEREST

The authors have declared that no competing interests exist.

AUTHOR'S CONTRIBUTION:

Author D. V, Dapper, conceived the study, designed the protocol and coordinated the experiment, author B. J, Olatunde, undertook the task of the animal feeding, laboratory procedures and manuscript writing while the statistical analysis and data interpretation were performed by author S. O, Ojeka. All the three authors read through and approved the final manuscript.

ETHICAL APROVAL

All authors hereby declare that "Principles of laboratory animal care" were followed. All experiments have been examined and approved by the appropriate ethics committee.

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