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PROTECTIVE ROLE OF OMEGA H3 IN PROPOXUR-INDUCED TESTICULAR TOXICITY: INSIGHTS INTO OXIDATIVE STRESS AND INFLAMMATORY PATHWAY MODULATION

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ABSTRACT

Propoxur, a widely used carbamate pesticide, used in agriculture and household pest control has raised concerns with respect to its potential toxicity towards non-target organisms. Studies from previous research show that propoxur exposure can cause testicular toxicity, potentially affecting male reproductive function. This study investigates omega H3 antioxidant and anti-inflammatory response in mitigating propoxur exposure-induced testicular toxicity. A total of 24 male rats were exposed to propoxur via inhalation. The study evaluated its impact on oxidative stress markers, inflammatory mediators and testicular histology. Oxidative stress parameters measured were Superoxide Dismutase (SOD) and Malondialdehyde (MDA). Inflammatory markers were on Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-Kb) and P53 gene with Ki67 protein. The study found that omega H3 had a protective effect on oxidative stress markers, with lower MDA levels ($p<0.05$) and higher SOD levels ($p>0.05$) in treated groups compared to the propoxur-only group. Propoxur exposure led to substantial structural damage in the seminiferous tubules and alterations to the testicular basal membrane which were mitigated by omega H3. Propoxur exposure triggers oxidative stress and inflammatory responses that lead to dysregulation of P53, which was ameliorated by omega H3 intervention. In conclusion, this study suggests that Omega H3 could serve as a potential therapeutic agent; mitigating the adverse effects of propoxur exposure- induced testicular toxicity.

Keywords: Propoxur, testicular toxicity, omega H3, oxidative stress, anti-inflammatory
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INTRODUCTION

Propoxur (PPX), a carbamate pesticide is a known agricultural agent that causes reproductive toxicity partly through induction of cellular oxidative stress [1]. Pesticides have been identified as one of the factors that can result in reduced male fertility due to their role in male reproductive organs and germ cells toxicity [2]. Exposure to *PPX* insecticide has obvious deleterious effects on testicular micro-structure and reproductive hormones using rat model [3]. Studies have raised concerns about potential toxic effects of propoxur exposure, particularly on the male reproductive system. Previous research has shown that propoxur can induce oxidative stress, inflammation, and apoptosis (cell death) in testicular tissues, leading to impaired fertility [4].

The widespread use of carbamate insecticides, like propoxur, for agricultural and household uses has raised concerns resulting from their potential toxic effects, especially on the male reproductive system [5]. Pesticide exposure has been associated with testicular toxicity, characterized by oxidative stress, inflammation, and apoptosis in testicular tissues, ultimately leading to impaired fertility and hormonal imbalances [6, 7].

There is a growing interest in identifying compounds that can ameliorate or mitigate the effects of propoxur exposure on the testes. Omega-3 fatty acids, such as those found in fish oil supplements, have gained attention due to their antioxidant and anti-inflammatory properties [8]. In particular, a supplement, Omega H3, a synthetic derivative designed to enhance the bioavailability and potency of omega-3s, has shown promising results in preliminary studies. A study showed that omega-3 supplementation results in higher antioxidant activity in human seminal fluid also enhancing sperm count, motility, and morphology [9].

Several studies have demonstrated the potential of omega-3 fatty acids in attenuating oxidative stress and inflammation in various pathological conditions [4]. Specifically, in the context of testicular toxicity, Omega H3 supplementation has been reported to reduce lipid peroxidation and restore antioxidant enzyme activities in rats with induced testicular toxicity [10].

The proposed study aims to further investigate the potential protective effects of Omega H3 against propoxur-induced testicular toxicity. Specifically, it evaluates whether Omega H3 supplementation can reduce oxidative stress, mitigate inflammation, and inhibit apoptosis in testicular cells exposed to propoxur. Understanding the mechanisms by which Omega H3 may ameliorate these toxic effects could pave the way for developing interventions to safeguard male reproductive health in agricultural workers and populations at risk of propoxur exposure.

METHODOLOGY

Experimental Animals

Twenty-four (24) adult-male Wistar rats (*Rattus norvegicus*) weighing (160g - 180g) were procured and used for the experiment. They were housed in the animal house of the Faculty of Basic Medical Sciences, college of health sciences, university of Ilorin, at room temperature and maintained under 12 hours dark cycle. The rats were randomly divided into four (4) groups with each group comprising of six (6) rats. Then the rats were acclimatized in animal houses for two weeks, fed a rat diet and tap water. Ethical approval was obtained from the University of Ilorin Ethical Committee. The duration of the experiment was eight weeks. The protocol used for the study is summarized in Table 1.

Table 1: Shows the Grouping of experimental animals and the Dosage of drugs administered

GROUPS	DOSAGE	ROUTE OF ADMINISTRATION	DURATION
PROPOXUR (PPX) (Group 2)	3ppm/KgBW of Propoxur (PPX) for 30 Mins	Inhalation	56 DAYS
OMEGA H3 (Group 3)	300mg/KgBW of Omega H3	Orally (by gavage)	56 DAYS
PPX + OMEGA H3 (Group 4)	3ppm/KgBW of Propoxur (PPx) for 30mins 300mg/KgBW of Omega H3	Propoxur by Inhalation Omega H3 Administered Orally	56 DAYS
CONTROL (Group 1)	0.5ml/KgBW Distilled Water	Orally	56 DAYS

HISTOLOGICAL ANALYSIS

At the completion of treatment, the animals were sacrificed with an anesthetic agent using sodium pentobarbital (50 mg/kg, *i.p.*) as a chemical method of euthanasia. The blood sample was collected by cardiac puncture into heparinized tube. The samples were centrifuged at 704 g (5 min, room temperature). Plasma was stored (−80 °C) until the period of biochemical analysis. Routine histological processing was performed using the hematoxylin and eosin staining procedure. Isolated testicles had been properly fixed in Bouin's solution, and the testicles were then dehydrated with ascending grades of alcohol, cleared in xylene, and infiltrated with molten paraffin wax before being embedded in molten

paraffin wax to form a tissue block. The rotary microtome was used to section the paraffin block containing the tissue at a thickness of 4 µm. After floating in a water bath at 40 ° C, the sections were then transferred to a glass slide and stained with hematoxylin and eosin stains.

The slides were examined under a light microscope (magnification 200x).

Immunohistochemistry

The expression of P53 and Ki67 was determined using immunohistochemical staining. Tissue sections were deparaffinized in xylene and rehydrated through graded alcohols to water. Antigen retrieval was performed by heating sections in citrate buffer (pH 6.0) in a microwave oven for 15 minutes. Endogenous peroxidase activity was quenched by incubating sections in 3% hydrogen

Photomicrography

The slides were mounted on the microscope with the cover slip facing the objective lenses and secured on the stage using the stage clips. The coarse and fine adjustment knobs were used to focus and resolve the specific region of the tissue for viewing. An Amscope camera was inserted into one of the ocular lenses and connected to a laptop. The micrographs were captured on the laptop using the Amscope software application at magnifications of X200. The histological sections were examined to observe any changes in the structure of the testes.

Qualitative analysis

Histological slides prepared were observed under a Microscope at low and high magnifications and photographed using an amscope and then comparisons were made between the control and treated groups.

Statistical analysis

All quantitative analysis were analyzed using GraphPad (version 8) software and presented as mean and standard error of mean (mean±SEM) to show statistical significance and comparison between each groups using the analysis of variance (ANOVA). Comparison was done between all experimental groups and differences were determined using $p<0.05$, giving credence to the statistical significance of the comparisons.

RESULTS ANALYSIS

Body Weight

At the end of the 56-day treatment period, the mean value of animals administered Propoxur only showed a significant loss in weight compared to the control. Animals administered Omega H3 only showed significant body weight gain compared to control. However, animals administered Propoxur then Omega H3 showed

no significant change in body weight compared to all other groups.

The results obtained are summarised in Table 2.

Table 2: Table showing the initial and final weights, and weight differences of CONTROL, PPX, OMEGA H3 and PPX + OMEGA H3 groups.

GROUPS	INITIAL WEIGHT(g)	FINAL WEIGHT(g)	RELATIVE WEIGHT(g)
CONTROL (Group 1)	154.2 ± 3.277	256.2 ± 5.472	39.81 ± 1.264
PROPOXUR (Group 2)	150.6 ± 3.458	211.0 ± 2.702	38.62 ± 2.848
OMEGA H3 (Group 3)	156.6 ± 1.720	270.6 ± 4.490	42.13 ± 1.784
PROPOXUR + OMEGA H3 (Group 4)	152.2 ± 3.277	258.3 ± 5.472	41.08 ± 1.264

Data are presented as mean and standard error of mean (mean ± SEM). Analysis of variance (ANOVA) was used to analyze the data at $p \leq 0.05$.

OXIDATIVE STRESS PARAMETERS

SOD (Superoxide Dismutase):

Table 3 below indicates values used in a Tukey post hoc test, which revealed a significant decrease ($p < 0.05$) in the Propoxur only group compared to the control group, Omega H3 group. Also, there is no significant differences between the Propoxur + Omega H3 group, Omega H3 only group and the control group. Analysis of variance (ANOVA) with mean ± SEM was used.

Malonialdehyde (MDA):

Table 4 below indicates values used in a Tukey post hoc test, which revealed a significant decrease ($p < 0.05$) in MDA is observed in Control group compared to animals administered Propoxur, Propoxur + Omega H3. MDA level is reduced in animals administered Omega H3 compared to animals administered Propoxur.

INFLAMMATORY MARKER

Nuclear Factor-kappa B (NFkB)

Table 5 below indicates values used in a Tukey post hoc test, which revealed a significant decrease ($p < 0.05$) in NFkB is observed in Propoxur group compared to animals in the control group and the Omega H3 group. NFkB level is increased in animals administered Propoxur + Omega H3 compared to animals administered Propoxur only.

Table 3:

CONTROL (U/ml)	PROPOXUR (U/ml)	OMEGA H3 (U/ml)	PROPOXUR + OMEGA H3 (U/ml)
1.771428571	1.580901	1.223468	1.956726
1.675573	1.524398	1.8	1.8
1.750994	1.539006	1.9	1.7
1.802127	1.4	2	1.5

Table 4:

CONTROL (µM)	PROPOXUR (µM)	OMEGA H3 (µM)	PROPOXUR + OMEGA H3 (µM)
0.467789	0.68516129	0.459877	0.724013
0.501218	0.657852	0.6	0.6
0.489756	0.599871	0.65	0.65
0.3	0.690613	0.5	0.5

Table 5:

CONTROL (µM)	PROPOXUR (µM)	OMEGA H3 (µM)	PROPOXUR + OMEGA H3 (µM)
47.483	35.773	32.988	44.786
46.878	37.243	33.256	45.243
48.143	38.311	31.992	43.988
47.321	38.285	32.679	44.587

DISCUSSION

Oxidative stress parameters

The protective impact of Omega H3 is further supported by the oxidative stress indicators examined in this study, such as malondialdehyde (MDA) and superoxide dismutase (SOD). When comparing the Propoxur-only group to the control, the SOD levels were considerably lower, indicating considerable oxidative stress. On the other hand, rats given Omega H3 treatment had a

marked rise in SOD levels, indicating improved antioxidant defense systems. Similarly, the group that only received Propoxur had much higher MDA levels, an indication of oxidative damage and lipid peroxidation, whereas the group that received Omega H3 had significantly lower MDA levels. This is in line with the study of Mohammad *et al*. [12].

Effect of Omega H3 on Body Weight

Omega H3, a supplement known for its rich composition of essential fatty acids, vitamins, and minerals, has been observed to have a positive impact on the overall health and well-being of laboratory animals, including Wistar rats. In the context of this study, Omega H3 administration to the Wistar rats led to a stabilization or slight increase in body weight. This effect can be attributed to the nutritional and antioxidant properties of Omega H3, which support metabolic functions and improve the nutritional status of the animals.

The essential fatty acids in Omega H3 are crucial for maintaining cell membrane integrity and function, and the vitamins and minerals aid in various biochemical processes. This nutritional support helps in maintaining an optimal body weight, as the rats receiving Omega H3 did not exhibit the significant weight loss often associated with oxidative stress and toxicity. The overall health benefits provided by Omega H3 contribute to the maintenance of body weight, reflecting its role in promoting general well-being and mitigating stress-related weight changes. These findings were similar to those reported by Bays *et al.* [13].

Effect of Propoxur on Body Weight

The oxidative stress induced by Propoxur leads to the production of reactive oxygen species (ROS), which can damage cellular structures and disrupt metabolic processes. This metabolic disruption can impair nutrient absorption and utilization, leading to weight loss. Additionally, the toxic effects of Propoxur may cause gastrointestinal distress and reduced appetite, further contributing to the observed decrease in body weight. The weight loss observed in the Propoxur-exposed rats is a clear indicator of the systemic toxicity exerted by the pesticide.

Effect of Propoxur Plus Omega H3 on Body Weight

The combined treatment of Propoxur and Omega H3 provided a significant protective effect against the weight loss typically induced by Propoxur exposure. Rats treated with both Propoxur and Omega H3 showed a less pronounced reduction in body weight compared to those treated with Propoxur alone. This suggests that Omega H3

mitigates the adverse effects of Propoxur on body weight.

Omega H3's antioxidant properties play a crucial role in counteracting the oxidative stress induced by Propoxur. By reducing the levels of ROS and enhancing the body's antioxidant defense mechanisms, Omega H3 helps in maintaining cellular integrity and metabolic functions. This protective effect likely contributes to better nutrient absorption and utilization, thus preventing the severe weight loss observed in the Propoxur-only group.

Additionally, the nutritional benefits provided by Omega H3 support overall health and metabolic efficiency, helping to counterbalance the toxic effects of Propoxur. This results in a more stable body weight, indicating that Omega H3 can effectively ameliorate some of the systemic toxic effects of Propoxur.

Apoptosis and Cellular Integrity

The effects of Propoxur and Omega H3 on apoptotic markers and testicular cellular integrity were also investigated in this study. Biochemical indicators demonstrated that exposure to propoxur was linked to increased apoptosis and damage of cellular integrity. Treatment with omega H3 attenuated these effects by lowering apoptosis and maintaining cellular architecture. This finding aligns with the established anti-apoptotic characteristics of Omega H3, which contribute to preserving cell viability and functionality in hazardous environments [4, 14].

Comparative Analysis with Control Groups

A comparative study of the control, Propoxur-only, and Omega H3-treated groups demonstrated how effective Omega H3 is at preventing damage caused by Propoxur. Function of the control group were normal, but the Propoxur-only group displayed notable departures from the norm. The protective effects of Omega H3, however, were highlighted when the structural and functional integrity of the group treated with Omega H3 was nearly identical to that of the control group.

Oxidative Stress and Gene Regulation in Propoxur-Induced Testicular Toxicity

In the context of investigating omega-3 fatty acids' potential to mitigate propoxur exposure-induced testicular toxicity, the roles of P53 and Ki67 are crucial. Propoxur exposure triggers oxidative stress and inflammatory responses that lead to dysregulation of P53, a protein central to controlling apoptosis and managing cellular stress [11]. Elevated P53 levels reflect increased apoptosis in response to the cellular damage caused by propoxur. Concurrently, Ki67 expression, a marker of cell proliferation, often rises as a compensatory mechanism to repair the damaged testicular tissue, potentially

exacerbating the overall toxicity if the repair is ineffective [15]. Understanding these dynamics helps elucidate how omega-3 fatty acids could counteract propoxur-induced oxidative and inflammatory stress, potentially normalizing P53 and Ki67 expression and thus reducing testicular damage and apoptosis [16, 17].

Conclusion

In conclusion, this study provides evidence that Omega H3 significantly reduces Propoxur-induced testicular toxicity. The data indicates that Omega H3 boosts antioxidant defenses, decreases oxidative stress and inflammation, and inhibits apoptosis, preserving testicular structure and function. These findings show the potential of Omega H3 as a therapeutic agent for controlling testicular toxicity caused by exposure to toxicants such as Propoxur.

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