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Research Article

Oral Supplementation of Coconut Oil Attenuates Propanil-induced Oxidative Stress in the Testes of Rats

¹Chiagoziem Anariochi Otuechere, ¹Oghenetega Jonathan Awaworoko, ¹Simisola Tinuala, ²Scott Fayemi and ¹Bamidele Adewale Salau

¹Department of Biochemistry, Redeemer's University, Ede, Osun State, Nigeria

²Department of Biological Sciences, Redeemer's University, Ede, Osun State, Nigeria

Abstract

Background and Objective: Pesticides have been implicated in oxidative stress, which is associated with many disorders, including testicular dysfunction. This study, therefore, investigated the ameliorative effect of coconut oil (CCO) on male Wistar rats exposed to propanil (PPN), a commonly used herbicide. **Materials and Methods:** The study comprised 4 groups of 5 rats each designated as: control, CCO (2.7 mL kg⁻¹), PPN (200 mg kg⁻¹) and PPN (200 mg kg⁻¹) + CCO (2.7 mL kg⁻¹). Rats were given their various treatments for 7 days and the ameliorative effect of CCO was assessed using antioxidant indices (superoxide dismutase, glutathione-S-transferase, glutathione peroxidase, reduced glutathione and malondialdehyde levels). **Results:** The study revealed that PPN exposure significantly ($p < 0.05$) disturbed antioxidant status, increased lipid peroxidation (LPO), alkaline phosphatase (ALP) and acid phosphatase (ACP) levels in testes of rats. Treatment with CCO, however, restored the depleted antioxidant status in rat testes, decreased LPO by 50.4% and reduced ALP activity by 19.6%. **Conclusion:** The results showed that testicular oxidative stress, a threat to male fertility induced by propanil exposure, could be attenuated by oral supplementation of coconut oil.

Key words: Coconut oil, herbicide, oxidative stress, testicular toxicity, fertility

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Corresponding Author: Bamidele Adewale Salau, Department of Biochemistry, Redeemer's University, Ede, Osun State, Nigeria Tel: +234-8077117362

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Herbicides are widely used for weed control and extirpation of specific disease vectors. However, prolonged use of herbicides has led to contamination of the environment and has increased human vulnerability to diseases. Several studies linking exposure to herbicides and diseases such as cancer, diabetes, neurodegenerative and reproductive disorders have been reported by Davoren and Schiestl¹, Otuechere *et al.*², Abolaji *et al.*³ and Garcia *et al.*⁴. Propanil is a highly selective herbicide used for the control of grasses and broad-leaf weeds⁵. The herbicide has been implicated as a pollutant of ground and surface water⁶.

Furthermore, the toxicity of propanil to non-target organisms has been reported by Otuechere *et al.*⁷ and Pereira *et al.*⁸. Several studies on the adverse effects of pesticides on reproductive well-being in animals have been reported. Thiocyclam, a broad-spectrum insecticide, at a dose of 15.98 mg kg⁻¹ for 65 days, increased lipid peroxidation and also caused DNA damage in the testes of exposed rats⁹. Additionally, Roundup herbicide, at the dose of 3.6 mg kg⁻¹ for 12 weeks, impaired spermatogenesis and endocrine balance in albino rats¹⁰. Oxidative stress has implicated as a key mechanism in pesticide-induced toxicities. However, the use of antioxidant molecules or medicinal plant extracts has been deployed to mitigate oxidative damage in susceptible tissues. Coconut oil, obtained from Coconut (*Cocos nucifera* L.), has a wide range of health benefits^{11,12}. Its antioxidant properties have been attributed to its rich poly-phenolic content. The coconut oil, also rich in medium-chain saturated fatty acids, especially myristic acid, Che Man and Abdul Manaf¹³ has variously been used as a protective agent against different chemical models of testicular toxicity. Coconut oil, administered at the dose of 10 mL kg⁻¹ for 56 days restored the derangements in testicular and seminal fluid parameters following the administration of highly active antiretroviral therapy to rats¹⁴. In the same vein, coconut oil extract has also been reported to ameliorate the testicular toxicity in Sprague-Dawley rats co-exposed to antiretroviral therapy and alcohol^{15,16}. Ekaye *et al.*¹⁷ further observed normal testicular architecture as a result of intervention with coconut oil following exposure of Norwegian rats to untreated refinery effluent over a treatment duration of 9 weeks.

Even though research has shown that coconut oil acted as free radical scavengers against environmental toxicants,

there is paucity of studies on the ameliorative effects of coconut oil on pesticide-induced testicular toxicity. The present study, therefore, investigated the ameliorative effect of coconut oil on alterations in biomarkers of oxidative stress in the testicular organ of albino rats exposed to propanil, an acylanilide herbicide.

MATERIALS AND METHODS

Study site: The present research was conducted at the Redeemer's University Campus at Mowe, Lagos, Nigeria, during January and March, 2013 for 50 days.

Chemicals and reagents: Technical grade propanil was obtained from Harvest Field Industries Limited, Lagos, Nigeria. Reduced glutathione (GSH), bovine serum albumin (BSA), 1-chloro-2, 4-dinitrobenzene (CDNB), trichloroacetic acid (TCA) and 5, 5-dithio-bis (2-nitrobenzoic) (DTNB) were obtained from Sigma-Aldrich Chemical Co. (St Louis, Missouri, USA). Other chemicals were of analytical grade.

Procurement of coconut oil: CCO, with the registered trade name Aquila®, was sourced from the Redemption Camp, Mowe, Ogun State, Nigeria. According to the manufacturers' leaflet, Aquila® was made via cold press without additives. CCO is liquid at room temperature and solid at temperatures lower temperatures.

Animal husbandry: Adult, 8 weeks old male rats were purchased from the University of Ibadan Animal Facility, Ibadan, Nigeria. Animals were transported and acclimatized at the Redeemer's University Animal House, Nigeria, for 2 weeks. The rats were housed in wire-meshed cages and provided with food and water *ad libitum*. They were kept at standard conditions of temperature and humidity and fed with commercial rat diet (Ladokun Feeds, Nigeria Ltd., Ibadan, Nigeria). All institutional and national guidelines for the care and use of laboratory animals were followed¹⁸.

Experimental design: The rats were divided into 4 groups of 5 rats in each group. Group 1 received normal saline at a dose of 2.7 mL kg⁻¹ b.wt., while group 2 received CCO at a dose of 2.7 mL kg⁻¹/b.wt., group 3 received PPN at a dose of 200 mg kg⁻¹/b.wt., while group 4 received both PPN

(200 mg kg⁻¹/b.wt.)+CCO (2.7 mL kg⁻¹/b.wt.). The propanil dose used in this study was based on previously published data². Animals were treated by gavage once daily for 7 days. Rats were sacrificed after an overnight fast by cervical dislocation. Testes were then carefully removed from the scrota of the rats, rinsed in ice-cold 1.15% potassium chloride and homogenized in 4 volumes of ice-cold 0.01 M potassium phosphate buffer (pH 7.4). The homogenates were centrifuged at 12 000 g for 15 min to obtain post mitochondrial supernatant fraction (PMF), which was kept at -20°C until analysis.

Biomarkers of testicular function: Alkaline phosphatase (ALP) and acid phosphatase (ACP) activities were determined in testicular PMF according to the instructions of the manufacturers (Randox diagnostic kits, UK).

Biomarkers of testicular oxidative stress: The protein content was determined according to the biuret method of Gornall *et al.*¹⁹ Lipid peroxidation (LPO) was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) according to the method of Varshney and Kale²⁰. Glutathione-S-transferase (GST) activity was determined by the method of Habig *et al.*²¹. The method of Beutler *et al.*²² was followed in estimating the level of reduced glutathione (GSH). The superoxide dismutase (SOD) activity was determined by the procedure of Misra and Fridovich²³ while glutathione peroxidase (GPx) activity was determined according to the method of Rotruck *et al.*²⁴.

Statistical analysis: All values were expressed as mean±standard error of mean (SEM). Intergroup differences between the groups were determined by one-way analysis of variance (ANOVA), while the post hoc test was performed using Tukey's test (GraphPad Prism 5). Values were regarded as significantly different at p<0.05.

RESULTS

Influence of CCO on testicular SOD activity: The SOD activity was significantly depleted following the administration of PPN when compared to the control group. However, co-treatment with PPN+CCO restored the SOD to near-normal levels (Fig. 1a).

Influence of CCO on testicular GPx activity: GPx activity was significantly depleted in the PPN group compared to the control. However, treatment of animals with PPN+CCO significantly restored GPx activity to near-normal levels in the PPN+CCO group compared to the PPN-treatment group (p<0.05, Fig. 1b).

Influence of CCO on testicular GST activity: GST activity was significantly depleted in the testes of rats exposed to PPN when compared to the control group. However, the treatment of animals with PPN+CCO restored the PPN-induced reduction of GST to levels comparable to the control group (Fig. 1c).

Influence of CCO on testicular GSH level: Treatment of rats with PPN elicited a significant decrease in GSH levels when compared with the control group. Co-treatment of rats with PPN+CCO preserved the GSH contents to near-normal levels (Fig. 1d).

Influence of CCO on testicular LPO level: Rats treated with PPN experienced a significant increase in lipid peroxidation level in the testes when compared with the control groups. When rats were administered PPN+CCO, a remarkable depletion of PPN-induced elevation in LPO was observed (Fig. 1e).

Influence of CCO on testicular ALP activity: Figure 1f shows the influence of CCO on ALP activity in the testes of rats exposed to PPN. There was a significant increase in the enzyme activity of ALP in the group of rats fed with PPN when compared to the control. Although the animals exposed to PPN+CCO experienced a significant reduction in ALP activity when compared to the PPN group, CCO was unable to reverse the PPN-induced elevation in testicular ALP activity.

Influence of CCO on testicular ACP activity: Figure 1g shows the influence of CCO on ACP activity in the testes of rats exposed to PPN. There was a significant increase in ACP activity in the group administered PPN compared to the control group. The activities of ACP were not significantly different in the normal and CCO administered groups. However, treatment of animals with PPN+CCO was unable to reverse the PPN-induced elevation in testicular ACP activity.

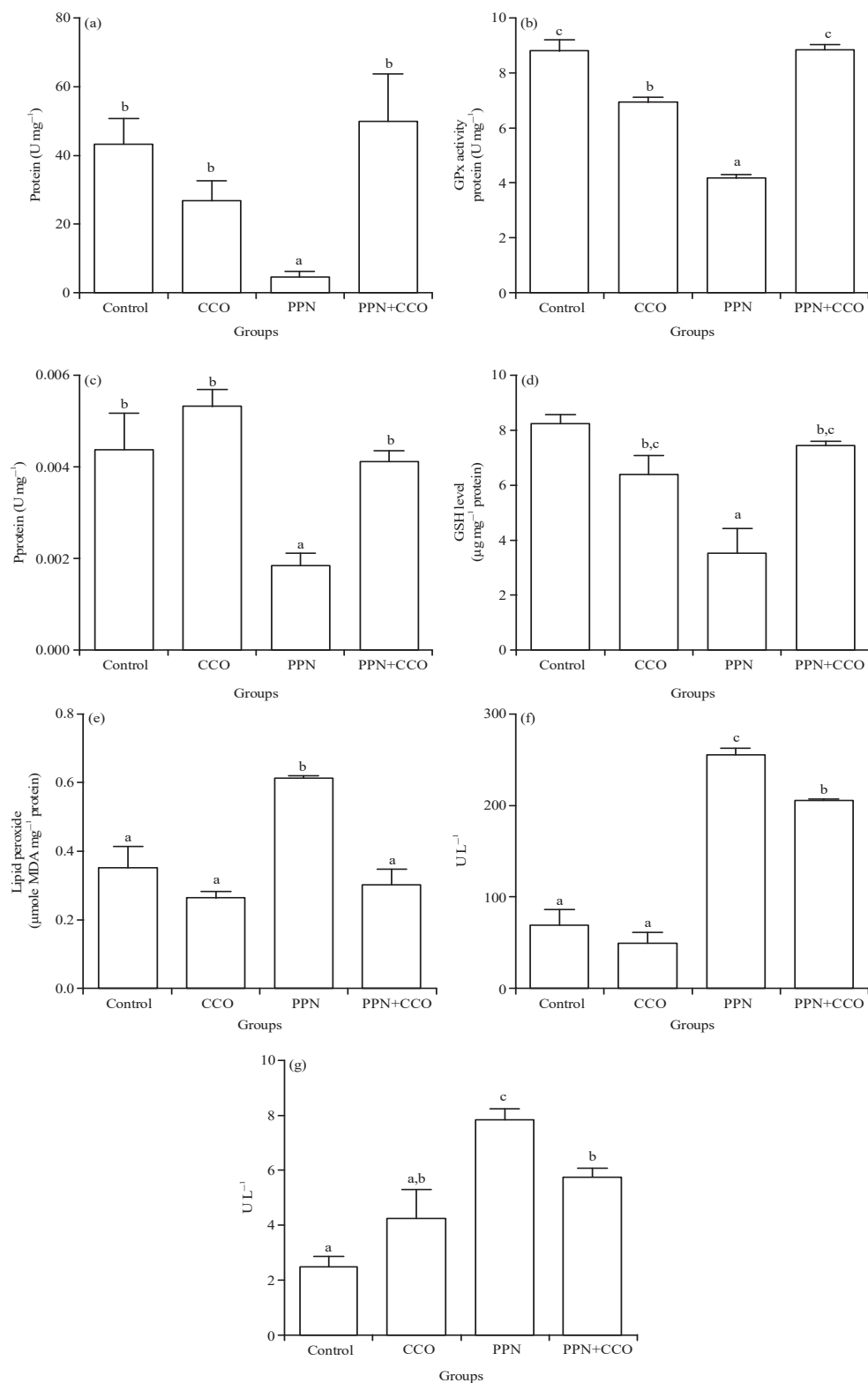


Fig. 1(a-g): Influence of CCO on (a) SOD, (b) Gpx, (c) GST, (d) GSH, (e) LPO, (f) ALP and (g) ACP activity in the testis of rats exposed to PPN

CCO: Coconut oil, PPN: Propanil, ACP: Acid phosphatase, bars are expressed as Mean $\pm$ SEM for 5 rats/group, mean values were compared using one way ANOVA, level of significance was assessed using Tukey's test at $p < 0.05$, bars with different superscripts are significantly different at $p < 0.05$

DISCUSSION

The present study evaluated the effect of a commonly used herbicide, propanil, on antioxidant: oxidant status in the testes of rats. Previously, chlorpyrifos and carbendazim-induced oxidative damage in the liver, kidney, spleen and testis have been reported by Abolaji *et al.*³ and Salihu *et al.*²⁵. An essential part of the male reproductive organ is the testis, the site of sperm and testosterone production. The testes are prone to oxidative damage and synergy between enzymatic and non-enzymatic antioxidants could suppress oxidative stress in the testes²⁶. In a previous study, the contributory role of oxidative stress in the reprotoxic effect of cyclophosphamide was reported by Abarikwu *et al.*²⁷. Significant diminutions in testicular SOD and GST activities after treatment with propanil were also observed in this study. By another study, the decrease in the activity of these antioxidant enzymes was also accompanied by a significant elevation in LPO within the testis²⁸.

The loss of activity of superoxide dismutase in the propanil- treated group, followed by a concomitant decrease in catalase activity, is an indication of oxidative stress in the testes of rats, a cellular event capable of impairing testicular homeostasis²⁹. The highly cytotoxic superoxide anion is detoxified by superoxide dismutase radical to form hydrogen peroxide and molecular oxygen, the hydrogen peroxide formed is catabolized into water and oxygen via the action of another antioxidant enzyme, catalase³⁰. Hence, the significant loss in activity of testicular antioxidant enzymes in rats exposed solely to propanil suggested that the herbicide caused oxidative damage in the tissue because of the generation of harmful free radicals. Propanil-induced testicular oxidative stress was, however, ameliorated by oral supplementation with coconut oil. These observations are in agreement with existing information regarding the protective and antioxidant properties of coconut oil in the testis, kidney and liver of rodents subjected to different toxicants^{31,32}. Similar to a previous study, lipid peroxidation levels in this present study were significantly increased in testes of the rats exposed to propanil-only group compared to the control groups³³. The elevated lipid peroxidation was, however, reduced in rats treated with coconut oil, thereby preventing or ameliorating testicular cell damage and improving testicular function³⁴. Furthermore, the observed diminution in GSH and GPx levels in PPN-treated rats was restored to normal by feeding rats with coconut oil^{35,36}.

Alkaline and acid phosphatases are hydrolase enzymes involved in the removal of phosphate groups or phosphomonoesters from organic molecules³⁷. Such

dephosphorylation could lead to a loss of metabolic/cellular function or activity of the dephosphorylated macromolecule. In the present study, ALP and ACP activities were significantly elevated in testes of rats exposed to propanil compared to the control. The increased phosphatase activities indicated intense dephosphorylation of testicular biomolecules, which could be an underlying cause of testicular dysfunction. Intervention with CCO, however, could not alleviate the elevations in the activities of these phosphatases.

CONCLUSION

Although the testis is not the primary target of propanil, sub-acute exposure to the herbicide elicited oxidative stress in the testicular organ. However, the findings of this research suggest the potential beneficial effect of coconut oil on oxidative stress in the testes of rats. Further studies on the influence of coconut oil on sperm parameters and endocrine function in the testes of rats exposed to propanil are warranted.

SIGNIFICANCE STATEMENT

This research highlighted the toxicity of a herbicide, propanil on the male reproductive system, especially the testes via the mechanisms of oxidative stress and derangement of testicular specific enzymes. This study also reported on the amelioration of this toxicity using the bioactive principles found in coconut oil. This study will not only serve as a precautionary note to occupationally exposed individuals but also provides baseline data for a therapeutic approach for the treatment of testicular dysfunction. This present study will also help related researchers to uncover the critical molecular endpoints of pesticide-induced reprotoxicity and new theories on chemopreventive strategies may also be arrived at.

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