ISSN 1996-3351

Asian Journal of Biological Sciences

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OPEN ACCESS Asian Journal of Biological Sciences

ISSN 1996-3351
DOI: 10.3923/ajbs.2019.210.216

Research Article
Impact of an Acylanilide Herbicide Propanil on Biochemical Indices in Kidney of Diabetic Rats

[1]Chiagoziem Anariochi Otuechere, [2]Femi Ayoade and [1]Oladeji John Arogundade

[1]Department of Chemical Sciences, Redeemer's University, Ede, Osun State, Nigeria
[2]Department of Biological Sciences, Redeemer's University, Ede, Osun State, Nigeria

Abstract

Background and Objective: Propanil (PRO) is an acylanilide herbicide used to control barnyard grass and broad leaf weeds. This study evaluated the impact of PRO in streptozotocin (STZ)-induced diabetic rats. **Materials and Methods:** Diabetes was induced in rats by a single injection of STZ (60 mg kg^{-1} intraperitoneal, i.p.). The PRO was administered at the dose of 200 mg kg^{-1} (i.p. for 7 days), to both normal and diabetic rats. **Results:** The plasma levels of triglyceride, aspartate aminotransferase, creatinine and renal malondialdehyde level (an index of lipid peroxidation) were significantly increased in diabetic rats. In addition, the levels of high density lipoprotein-cholesterol, superoxide dismutase were depressed in the STZ-treated rats compared to the control group. The co-exposure of PRO increased absolute organ weight, catalase, glutathione peroxidase and malondialdehyde levels. **Conclusion:** Taken together, exposure to PRO exacerbated oxidative stress in renal tissue of diabetic rats.

Key words: Propanil, diabetes, acylanilide herbicide, antioxidant status, streptozotocin, lipoprotein-cholesterol

Received: December 06, 2018 Accepted: January 27, 2019 Published: March 15, 2019

Citation: Chiagoziem Anariochi Otuechere, Femi Ayoade and Oladeji John Arogundade, 2019. Impact of an acylanilide herbicide propanil on biochemical indices in kidney of diabetic rats. Asian J. Biol. Sci., 12: 210-216.

Corresponding Author: C.A. Otuechere, Department of Chemical Sciences, Redeemer's University, Ede, Osun State, Nigeria Tel: +234-8023887060

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

The herbicide, propanil (3, 4 dicholoropropionalide) is one of the widely used agricultural pesticides in the world in the control of weeds in rice and paddy farms. The incidence or prevalence of organ injury arising from the use of chemicals has continued to soar[1].

Exposure to propanil has been known to produce a variety of biochemical dysfunctions in experimental animals including hepatotoxicity in rats, derangement of intermediary metabolism in fish and nephro-toxicities in cell lines[2,3].

Diabetes mellitus (DM) is a chronic, metabolic disorder characterized by alterations to macromolecular metabolism resulting from absolute or relative insulin deficiency with dysfunction in organ systems. Aside the geometric increase in prevalence of DM in recent years, it is worrisome that populations previously unaffected or minimally affected by DM now report soaring prevalence figures[4]. It has been theorized that the dramatic increase in DM incidences could involve an additional factor, aside lifestyle and genetics. Interestingly, environmental pollutants have been implicated as a potential risk factor in the etiology of DM. Specifically, human studies on certain persistent organic pollutants, bisphenol A and phthalates lend credence to the possible association between exposure to environmental pollutants and the risk of diabetes[5,6].

During the last decades the use of pesticides has increased steadily in sub-saharan Africa in an effort to increase food production and control vector-borne diseases; however their use may have negative human health implications[7,8]. In a recent case-control study, occupational exposure to endosulfan organochlorine), mevinphos (organophosphate), carbaryl/sevin (carbamate) and benlate (fungicide) was positively associated with the prevalence of diabetes in Thai farmers recruited for the study[9]. Unfortunately, there are insufficient studies on the possible mechanisms of acylanilide herbicide in rodent model of STZ-induced diabetes. The objective of this study was to investigate the effect of exposure of propanil on renal biochemical parameters of diabetic rats.

MATERIALS AND METHODS

Animals: Adult male albino rats (140-200 g) obtained from the Covenant Farm Animal House located at Ibadan, Oyo state, Nigeria, were used in this study. The animals were kept in well-ventilated cages at room temperature and under controlled conditions of ambient temperature (25°C) at the Redeemer's University Animal House facility, Mowe, Ogun state, Nigeria, 12 h natural light and 12 h darkness, with free access to tap water and normal laboratory chow. Study was conducted in accordance with the Redeemer's University Research Ethics Committee for care and use of the laboratory animals in biomedical research. The approved study was referenced RUN08-09-1895.

Chemicals/reagents: Streptozotocin was procured from Sigma Chemical Co. (St. Louis, MO, USA). Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Total Cholesterol, High density lipoprotein cholesterol (HDLC) and Triglyceride were obtained from Randox commercial kits, UK. Technical grade herbicide, propanil (PRO) was purchased from Harvest Field Industries Limited, Lagos state, Nigeria. All other chemicals used were of analytical grade.

Experimental design: Twenty rats were divided into 4 groups of 5 rats each (n = 5) and treated according to the experimental design below:

Group A : Rats were given normal saline only and served as control

Group B : Rats were treated with 60 mg kg^{-1} b.wt., of Streptozotocin (STZ)

Group C : Rats were treated with 200 mg kg^{-1} b.wt., of Propanil (PRO)

Group D : Rats were treated with 60 mg kg^{-1} b.wt., of STZ in combination with 200 mg kg^{-1} b.wt., of PRO (STZ+PRO)

The dose of PRO (200 mg kg^{-1}) used was equivalent to quadruple dose of an earlier study[2]. A single dose of STZ (60 mg kg^{-1}) dissolved in 100 mM citrate buffer was administered to animals in groups B and D. About 5% glucose. Water was also given to rats to forestall the hypoglycemic effect of STZ. After 48 h of STZ induction, animals that showed higher blood glucose levels were considered as diabetic. After 7 days of STZ and PRO treatment, rats of all the groups were sacrificed. Blood was collected by cardiac puncture into EDTA tubes and plasma separated. Kidneys were excised, homogenized in phosphate buffer (0.1 M, pH 7.4), centrifuged at 4,500 g and the supernatants used for the various biochemical measurements.

Biochemical measurements

Plasma clinical parameters: Glucose level was estimated using ACCU-CHECK active glucometer (Roche) and the results were expressed as mg dL^{-1}, while ALP, AST, Triglyceride, High Density Lipoprotein-Cholesterol and Creatinine were measured according to the protocol indicated in manufacturers' manual.

Superoxide dismutase (SOD): The activity of SOD was determined using the method of Misra and Fridovich[10] based on the inhibition of autoxidation of epinephrine (pH 10.2) at 30°C.

Catalase (CAT): Catalase activity in tissue was assayed at room temperature following the procedure of Luck[11]. In this procedure, decomposition of hydrogen peroxide by catalase was measured at 240 nm for 3 min. The molar extinction coefficient of H_2O_2 used was 71 M^{-1} cm^{-1}.

Glutathione peroxidase (GPx): The activity of GPx was evaluated by the method of Rotruck *et al.*[12].

Malondialdehyde (MDA): The MDA level was measured as thiobarbituric acid reactive substances in renal tissues according to the method described previously[13]. Lipid peroxidation in mg of MDA formed/mg protein was computed with a molar extinction coefficient of 1.56×10^{-5} M^{-1} cm^{-1}.

Nitric oxide: Kidney nitrite (NO_2) and nitrate (NO_3) were estimated as index of nitric oxide (NO) production. Quantitation was based on the Griess reaction according to the method of Bryan and Grisham[14].

Estimation of proteins: The protein concentrations in the homogenate samples were determined by means of biuret method as described by Gornall *et al.*[15] and Otuechere *et al.*[16].

Statistical analysis: All values have been expressed as mean±standard error of mean (SEM) of 5 observations. Data were analyzed using one way analysis of variance (ANOVA) followed by Neuman Keul's post-test for analysis of biochemical data using Graph Pad Prism version 6. Values were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

Effects of propanil on absolute organ weight in STZ-diabetic rats: The effect of PRO and STZ co-exposure on absolute organ weight of the kidney is presented in Table 1. The absolute organ weight of PRO alone and in combination with STZ significantly increased when compared to the control. There was also significant increase in the STZ+PRO group when compared with STZ alone. Although the administration of STZ alone had no treatment related change on absolute organ weight, co-exposure of STZ+PRO caused an increase, indicating an early tissue hypertrophy and toxic potential[16].

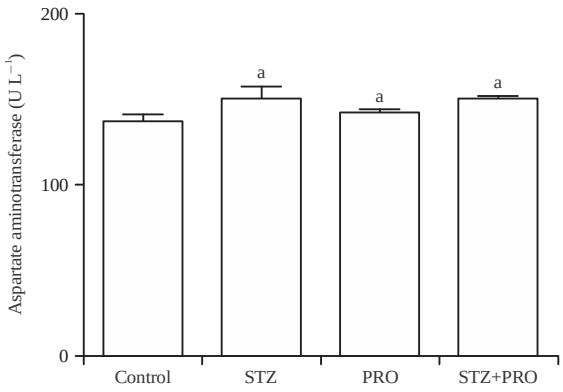

Fig. 1: Effect of propanil on aspartate aminotransferase activity in STZ diabetic rats
Values are Mean±SEM (n = 5), [a]Values differed significantly from control ($p<0.01$)

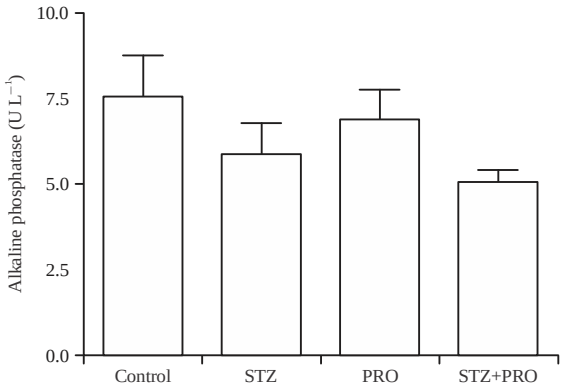

Fig. 2: Effect of propanil on alkaline phosphatase activity in STZ diabetic rats
Values are Mean±SEM (n = 5)

Table 1: Effect of PRO on the absolute kidney weights of STZ-diabetic rats

Treatments	Kidney (g)
Control	0.92±0.08
STZ	1.22±0.05
PRO	1.39±0.09[a]
STZ+PRO	1.68±0.19[a,b]

Values are Mean±SEM (n = 5), [a]Values differed significantly from control ($p<0.01$), [b]Values differed significantly from STZ alone ($p<0.01$)

Effects of propanil on plasma clinical parameters in STZ-diabetic rats: The effect of STZ and PRO co-treatment on aspartate aminotransferase (AST) activity is shown in Fig. 1. The activity of AST was significantly elevated in STZ, PRO and in STZ+PRO treated rats when compared with control.

The AST is a mitochondrial enzyme found in the heart, liver, skeletal muscle and kidney and is normally present in plasma. The elevated plasma AST was apparently due to

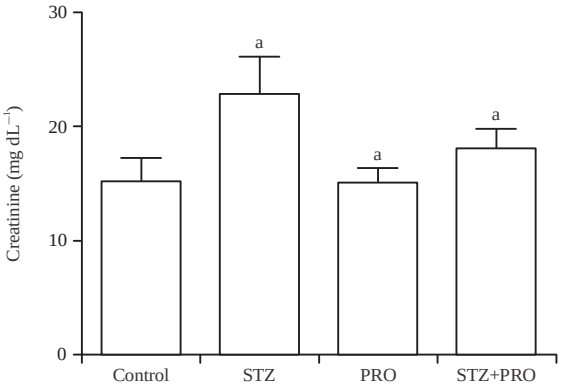

Fig. 3: Effect of propanil on creatinine levels in STZ diabetic rats

Values are Mean±SEM (n = 5), [a]Values differed significantly from control (p<0.01)

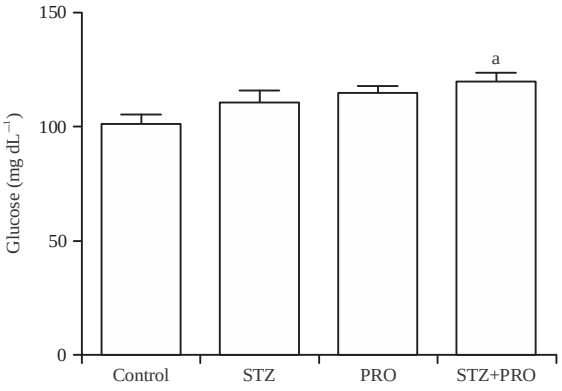

Fig. 4: Effect of propanil on glucose levels in STZ diabetic rats

Values are Mean±SEM (n = 5), [a]Values differed significantly from control (p<0.01)

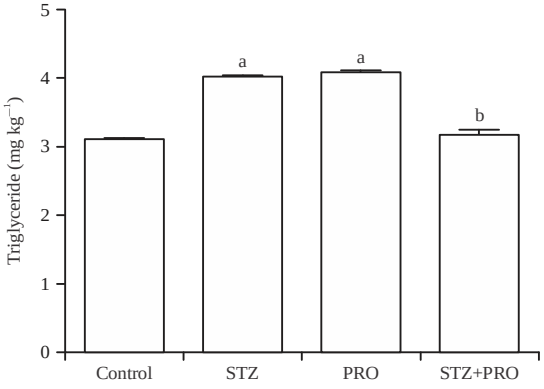

Fig. 5: Effect of propanil on Triglyceride levels in STZ diabetic rats

Values are Mean±SEM (n = 5), [a]Values differed significantly from control (p<0.05), [b]Values differed significantly from STZ alone (p<0.001)

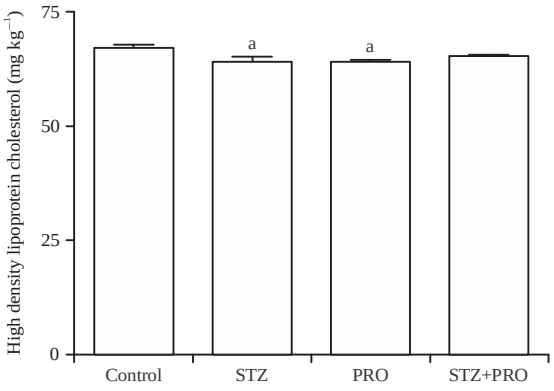

Fig. 6: Effect of propanil on high density lipoprotein levels in STZ diabetic rats

Values are Mean±SEM (n = 5), [a]Values differed significantly from control (p<0.05)

mitochondrial damage by reactive oxygen species induced by propanil. This observation concurred with an earlier report on the effect of short term exposure of atrazine in normal and diabetic rats[17]. Surprisingly, STZ and PRO co-treatment had no significant effect on alkaline phosphatase activity (Fig. 2).

In addition, the levels of creatinine and glucose in rats administered STZ+PRO were markedly increased, when compared with the control groups (Fig. 3, 4). Creatinine, an important indicator of renal health was significantly elevated in rats administered STZ+PRO. This could be as a result of increased muscular breakdown or impaired clearance of creatinine from the glomerulus[18]. This trend of elevation was similarly observed for glucose levels in rats administered STZ+PRO (Fig. 4).

Effects of propanil on lipid profile parameters in STZ-diabetic rats: Diabetes, experimentally induced in this study, significantly (p<0.001) increased triglyceride levels in the STZ and PRO alone group as depicted in Fig. 5.

The most characteristic lipid abnormality is hypertriglyceridemia due to over-production and/or under-utilization of triglyceride[19].

Contrariwise, significant depletions were observed in the HDL-C levels in the STZ and PRO alone treatment groups (Fig. 6).

Effects of propanil on antioxidant enzyme activities in STZ-diabetic rats: The SOD, a first line of defense against reactive oxygen species was non-significantly depleted across all treatment groups as shown in Fig. 7.

Catalase activity was significantly elevated in the STZ+PRO group compared to the control group (Fig. 8). On the other, the glutathione peroxidase activity was significantly

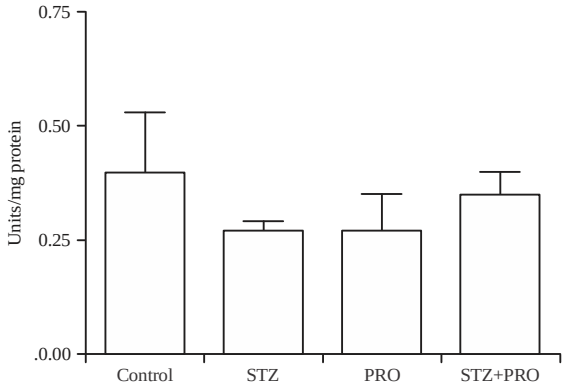

Fig. 7: Effect of propanil on renal superoxide dismutase activity in STZ diabetic rats
Values are Mean±SEM (n = 5)

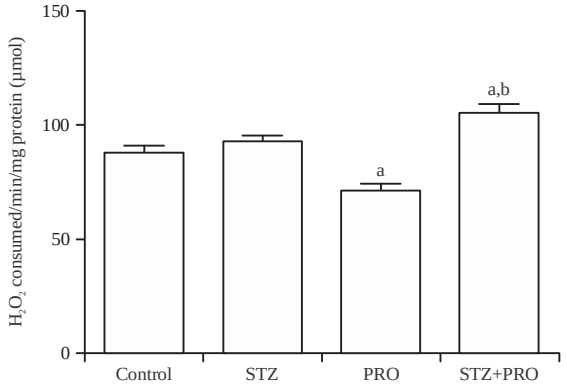

Fig. 8: Effect of propanil on renal catalase activity in STZ diabetic rats
Values are Mean±SEM (n = 5), [a]Values differed significantly from control (p<0.0.001), [b]Values differed significantly from STZ alone (p<0.001)

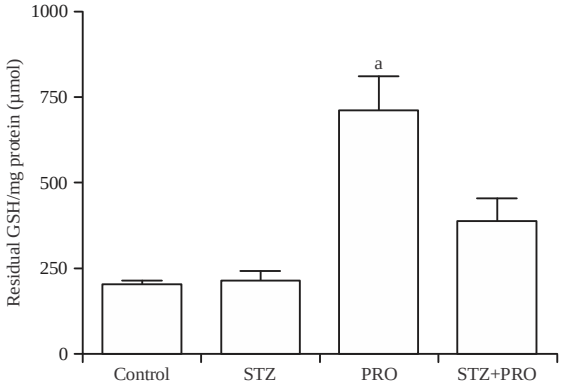

Fig. 9: Effect of propanil on renal glutathione peroxidase activity in STZ diabetic rats
Values are Mean±SEM (n = 5), [a]Values differed significantly from control (p<0.0.001)

elevated in the PRO alone group, while there was no significant increase in the STZ+PRO group in comparison with the control group (Fig. 9). This increase in catalase activity reflected a compensatory mechanism against increased oxidative stress[17].

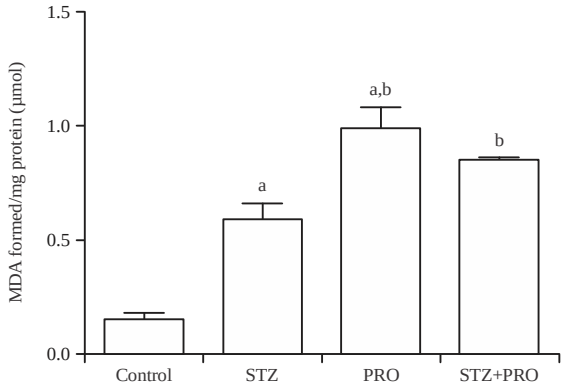

Fig. 10: Effect of propanil on renal lipid peroxidation levels in STZ diabetic rats
Values are Mean±SEM (n = 5), [a]Values differed significantly from control (p<0.0.001), [b]Values differed significantly from STZ alone (p<0.01)

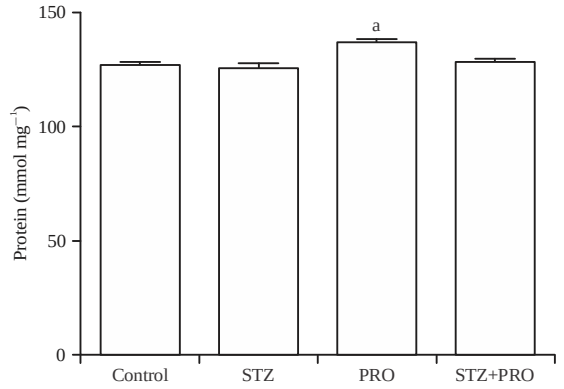

Fig. 11: Effect of propanil on renal nitric oxide levels in STZ diabetic rats
Values are Mean±SEM (n = 5), [a]Values differed significantly from control (p<0.0.001)

Effects of propanil on malondialdehyde and nitric oxide levels in STZ-diabetic rats: The level of MDA in the kidney of experimental diabetic rats is shown in Fig. 10. There was a significant elevation of MDA in all treatment groups compared to control rats. Furthermore, there was a significant increase in MDA levels in the STZ+PRO group when compared to the STZ alone group, an indication of a potentiated oxidative damage to the renal tissue in diabetic rats exposed to the herbicide.

Incidentally, there was only a significant increase in nitric oxide level in the PRO alone group when compared with the control group (Fig. 11). However, treatment of rats with STZ+PRO did not elicit any change in nitric oxide levels.

Mechanistic studies have suggested that pesticides could derange carbohydrate and fat metabolism via oxidative stress mediated mechanisms[20]. These results agreed with a previous report that short term exposure of atrazine at a dose of 300 μg kg^{-1} induced oxidative damage in the kidney of both normal and diabetic rats[17].

CONCLUSION

The present data showed that propanil intoxication enhanced lipid peroxidation, elicited hypertriglyceridemia and also disrupted the antioxidant system in an experimental model of diabetes. On the basis of this study, it is plausible that repeated administration of propanil augmented the pre-existing renal oxidative stress and dysfunction in diabetic rats.

SIGNIFICANCE STATEMENT

This study discovered the adverse effects of propanil exposure in the kidney of streptozotocin-induced diabetic rats. This study also provided baseline data that will help other researchers fill in critical gaps on the molecular mechanisms involved in the association between exposure to herbicides and the risk of diabetes that many researchers were unable to explore. Thus, a new theory on potentiation of diabetic complications due to exposure to herbicides may be arrived at.

ACKNOWLEDGMENT

Authors are grateful to the Department of Chemical Sciences, Redeemer's University for use of its facilities.

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