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Rutin Positively Modulates Butachlor-Induced Testicular Oxidative Stress in Murine Models

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ABSTRACT

Background: Butachlor is a post-emergent herbicide used in the control of weeds. Exposure to herbicides has been found to lead to oxidative stress and impairment of male reproductive organ. Rutin, a flavonoid has been reported to have antioxidative potential.

Aim and Objective: This study was designed to investigate the ameliorative properties of Rutin on Butachlor-induced testicular oxidative stress in rats.

Material and Methods: Twenty-four male wistar rats weighing 250g–280g were divided into four groups of six animals each. Group A [control] received distilled water, Group B [Butachlor] received 100 mg/kg/BW Butachlor, Group C [Butachlor + Rutin] received 100 mg/kg/BW Butachlor and 100 mg/kg/BW Rutin. Group D [Rutin] received 100 mg/kg B.W. Rutin.

Results: Testicular Ascorbic acid and reduced glutathione [GSH] levels were significantly reduced in the Butachlor-treated group by 40.4% and 35.9% respectively; Superoxide Dismutase, catalase, and Glutathione-s-Transferase activities were down-regulated by 42.3%, 38.9%, and 42.8% respectively in Butachlor-treated group. Testicular Acid phosphatase and Alkaline Phosphatase activities significantly decrease while malondialdehyde and nitric-oxide levels were significantly increased relative to control. However, co-treatment of Butachlor and Rutin ameliorated the Butachlor-induced changes in the aforementioned parameters.

Conclusion: Rutin, due to its antioxidant properties conferred a protective effect against testicular oxidative stress induced by Butachlor exposure

Keywords: Antioxidants; Free Radicals; Flavonoid; Herbicide; Exposure; Toxicity; Anticarcinogenic; Cytoprotective; Antiplatelet; Antithrombotic; Vaso Protective; Anti-Inflammatory; Cardioprotective

Abbreviations: VLCFAs: Very-Long-Chain Fatty Acid; CDNB:1-Chloro-2,4-Dinitrobenzene; DTNB: 5', 5'-Dintrobenzoic Acid; PNPP: Para-Nitrophenyl Phosphate; TBA: Thiobarbituric Acid; TBAR: Thiobarbituric Acid Reactive; GST: Glutathione-S-Transferase; ALP: Alkaline Phosphatase; ACP: Acid Phosphatase Activity

Introduction

During recent years, intensive use of herbicides has raised increasing concern mainly due to their massive pollution of the environment, as these herbicides are directly or indirectly toxic to a wide range of organisms [1]. There is increasing evidence that reproductive abnormalities are increasing frequently in both humans and animals which is probably related to exposure to toxic contaminations in the environment [2]. Exposure to these toxic environmental chemicals increases the risk for sperm abnormalities, decrease fertility, a deficit of male children, and growth retardation. It is reported that pesticides may induce pathological changes in the testes and alter the reproductive function by altering sperm count, sperm shape, and sexual behavior or increase infertility in animals and human beings [2]. Butachlor is a member of the chloroacetanilide class of chemicals and it is an herbicidal active ingredient. This herbicide is used as a pre-emergence control for undesirable grasses and broadleaf weeds [3]. Extensive use of this herbicide over the years has led to deleterious effects on the soil flora and fauna, its mechanism of action is through the inhibition of non-sphingolipid very-long-

chain fatty acids [VLCFAs] biosynthesis, resulting in a lack of lipids, proteins, and lignin for the plant [4]. Exposure to butachlor includes eye contact, nose [nasal], and skin [dermal] [5]. Investigations into the metabolism and pharmacokinetics of butachlor have revealed species differences in the way that this molecule is bio transformed and eliminated from the body [6,7].

Therefore, its environmental impact has been extensively investigated based on toxicity assessment [8]. Butachlor is known to exert a genotoxic effect on amphibians and reportedly induce apoptosis in mammalian cells [9]. Furthermore, the toxicity of butachlor is due to not only the parent compound but also its degradation product such as dialkyl quinonimine [10]. Butachlor decreases the percentage of viable motile sperm and sperm velocity and also causes tumor formation in rodents [10]. Rutin also called Ruto side, quercetin-3-o-rutinoside, and sphorin from citrus fruit is the glycoside combining the flavonol quercetin and the disaccharide rutinose [α-L-rhamnopyranosyl-[1-6]-β-D-glucopyranose][11]. Rutin has been reported to exhibit various pharmacological properties including antioxidant, anticarcinogenic, cytoprotective, antiplatelet, antithrombotic, Vaso protective, anti-inflammatory, and cardioprotective effects [11-13]. The strong antioxidative capacity of Rutin has been proven by numerous studies, particularly for excellent scavenging activity [14]. However, only a few attempts have been made to observe the effects of butachlor on the male reproductive system. Hence, the present investigation was to evaluate the toxic effect of butachlor on testes and the ameliorative effect of rutin on butachlor-induced oxidative stress in the testes.

Materials and Methods

Chemicals and Reagents

Butachlor [Striker®] is a product of Sinochem Ningbo Ltd, 21 Jiangxia Street, Ningbo 315000, China. Rutin is a product of AK Scientific U.S.A, Glutathione [GSH], 1-chloro-2, 4-dinitrobenzene [CDNB], 5', 5'-dintrobenzoic acid [DTNB], Para-nitrophenyl phosphate [PNPP], Thiobarbituric acid [TBA], and Epinephrine were purchased from Sigma Chemical Company [London, UK]. All other chemicals and reagents were of analytical grade and were obtained from British Drug House Poole, London

Experimental Animals

Twenty-four male rats weighing between 250-280 g were used in this study. The animals were purchased from the animal breeding unit, veterinary medicine, University of Ibadan, Oyo State and were acclimatized to laboratory conditions for two weeks at the animal breeding unit, Department of Chemical Sciences, Ajayi Crowther University, Oyo, Nigeria, preceding the start of the study. The rats were contained in wire-meshed cages and provided with food and water ad libitum. Handling of the experimental animals is consistent with international principles on the care and use of experimental animals [15]. The animals were divided into four experimental groups of six animals each. The groupings of the rats are as follows:

- Group I (Control) received distilled water
- Group II (BUTA) received Butachlor (100 mg/kg body weight)
- Group III (RUT) received Rutin (100 mg/kg body weight)
- Group IV (BUTA + RUT) received Butachlor (100mg/kg body weight) + Rutin (100 mg/kg body weight)

The doses were delivered by oral gavage and the administration occurred once a day over a period of 14 days. The animals were sacrificed 24 hours after the last administration.

Preparation of Tissue Homogenate

The testes were removed, rinsed in ice-cold 1.15% KCl, blotted, weighed, and homogenized in 4 volumes of ice-cold 0.1M phosphate buffer [pH 7.4]. The homogenates were centrifuged at 12000 g for 10 minutes using Eppendorf [UK] refrigerated centrifuge. The supernatant, termed post mitochondria fraction was obtained and stored frozen for subsequent analysis.

Assay of Testicular Enzymes

The method of measurement of testicular acid phosphatase (ACP) activity was based on that of (Wright, et al. [16]) in which the hydrolysis of Para-nitrophenyl phosphate is determined by spectrophotometric measurement. Alkaline phosphatase activity was determined according to the method described by (Wright, et al. [17]).

Assay of Biomarkers of Oxidative Stress

The testicular ascorbic acid concentration was determined according to the method of Jagota and Dani [18]. The activity of catalase was determined according to the procedure described by Claiborne [19]. Superoxide dismutase activity was determined by the method of Sun and Zigman [20]. The level of reduced glutathione [GSH] in the samples was determined by the method described by Jollow et al [21]. Glutathione-S-transferase [GST] activity was determined by the method according to Habig et al. [22]. Lipid peroxidation was assayed by measuring the thiobarbituric acid reactive [TBAR] products present in the test sample using the procedure of Vashney and Kale,[23]. The level of testicular Nitric oxide (NO) was determined by the method of (Green et al. [24])

Statistical Analysis

The data were expressed as Mean ± SD. The data were analyzed using one-way ANOVA followed by GraphPad Prism® [v 6.01] for comparison between control and treated rats in all groups. P values less than 0.05 [$p<0.05$] were considered statistically significant.

Results

Rutin Attenuates Butachlor-Induced Alterations in Testicular SOD, CAT, and GST Activities in Rats

Attenuation of Rutin on Butachlor-induced changes in Testicular

SOD, CAT, and GST activities in rats was shown in (Table 1). Testicular SOD, CAT, and GST activities were significantly decreased by 42.3%, 38.9%, and 42.8% respectively in the Butachlor group compared with the control [P<0.05]. However, administration of Rutin significantly protects against the decrease in SOD, CAT, and GST activities relative to Butachlor-treated rats.

Table 1: Attenuation of Rutin on Butachlor-Induced Changes in Testicular Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione-S-Transferase (GST) Activities in Rat.

Treatment	SOD (units)	CAT (µmol H_2O_2 consumed/min)	GST (nmoL/min/mg protein)
CONTROL	1.42 ± 0.01	28 ± 0.71	77.75 ± 0.5
BUTA	0.82 ± 0.01*(42.3%)	17.1 ± 0.16*(38.9%)	44.5 ± 1.29*(42.8%)
RUT	1.40 ± 0.01	26 ± 0.82	76 ± 1.00
BUTA + RUT	1.29 ± 0.01*, a	23.2 ± 0.15*, a	69.25 ± 0.96*, a

Note: The results are expressed as Mean ± SD for six rats in each group.

Values in parenthesis represent a percentage (%) decrease.

* Significantly different from the control (P<0.05).

a - significantly different from the Butachlor group.

BUTA= Butachlor (100 mg/kg b.w);

RUT= Rutin (100 mg/kg B.W)

Rutin Attenuates Butachlor-Induced Alterations in Testicular Ascorbic Acid and GSH Levels

Attenuation of Rutin on Butachlor-induced changes in Testicular Ascorbic Acid and GSH are presented in Table 2. Ascorbic acid and GSH levels were significantly decreased in the butachlor-treated group by 40.4% and 35.9% respectively when compared with the control [P<0.05]. The co-administration of Butachlor and Rutin significantly protected the decrease in ascorbic acid and GSH when compared with the Butachlor group.

Table 2: Attenuation of Rutin on Butachlor-Induced Changes in Testicular Ascorbic Acid and Reduced Glutathione (GSH) in Rats.

Treatment	Ascorbic Acid (µg/mL)	GSH (µg/g Tissue)
Control	1.88 ± 0.02	7.30 ± 0.1
BUTA	1.12 ± 0.01*(40.4%)	4.68 ± 0.08*(35.9%)
RUT	1.59 ± 0.01	7.12 ± 0.08
BUTA+RUT	1.76 ± 0.02*, a	6.36 ± 0.11*, a

Note: The results are expressed as Mean ± SD for six rats in each group.

Values in parenthesis represent a percentage (%) decrease.

* Significantly different from the control (<0.05).

a- significantly different from the butachlor group.

BUTA= Butachlor (100 mg/kg b.w);

RUT= Rutin (100 mg/kg b. W)

Rutin Attenuates Butachlor-Induced Alterations in Malondialdehyde and Nitric Oxide Level

Figure 1 shows the protective effect of rutin on butachlor-induced alterations in malondialdehyde [MDA] and nitric oxide [NO] levels in the testes of rats. There was a significant increase in the levels of MDA and NO in the testes of butachlor-treated rats when compared with the control. However, the co-administration of Rutin and Butachlor significantly attenuated the alteration relative to the Butachlor-treated group.

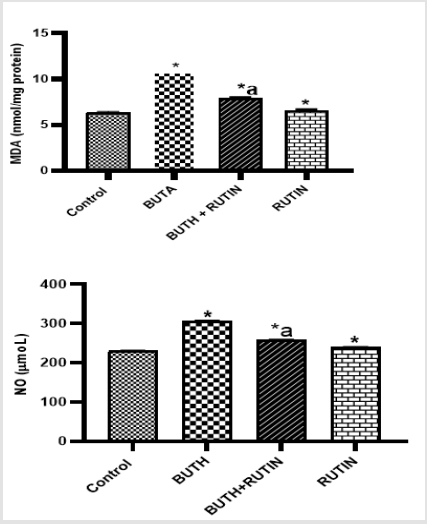

Figure 1: Protective effect of Rutin on butachlor-induced alterations in levels of (a) malondialdehyde (MDA) and (b) nitric oxide (NO) in the testes of rats.

Note: RUTIN= Rutin,

BUTA= Butachlor.

Each bar represents the mean ± SD (n=6).

* - significantly different compared with control (P < 0.05).

a- significantly different compared with butachlor (P<0.05).

Rutin Attenuates Butachlor-Induced Alterations in Alkaline Phosphatase (ALP) and Acid Phosphatase Activity (ACP)

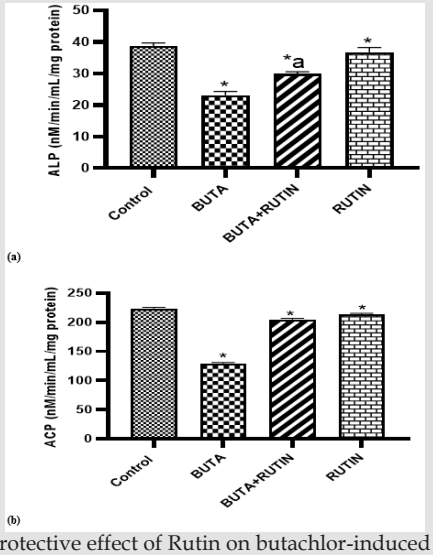

Figure 2: Protective effect of Rutin on butachlor-induced alterations in activities of (a) alkaline phosphatase (ALP) and (b) acid phosphatase (ACP) in the testes of rats.

Note: RUTIN= Rutin,

BUTA= Butachlor.

Each bar represents the mean ± SD (n=6).

* - significantly different compared with control (P < 0.05).

a- significantly different compared with butachlor (P<0.05).

The protective effect of rutin on butachlor-induced changes in ALP and ACP activities in the testes of rats was shown in Figure 2. There were a significant decrease in ALP and ACP activities in the Butachlor-treated group relative to the control (P<0.05). Combined treatment of butachlor and rutin was seen to significantly protect the decrease in testicular ALP and ACP activities when compared with the butachlor-treated group.

Discussion

Several conditions have been known to affect testicular functionality; these conditions include exposure to certain drugs and environmental toxicants. Although the testes possess defense systems, the resultant impact occasioned by the drug can lead to poor sperm quality, thereby resulting in male infertility [25]. Butachlor is a post-emergent herbicide used in the control of weeds. However, due to its improper use and persistence in the environment, animals and humans may be exposed to the herbicide. Exposure to herbicides has been found to lead to oxidative stress and impairment of the functions of the male reproductive organ [26,27]. Rutin is a flavonoid found in a wide variety of plants including citrus fruit and certain vegetables. It has the ability to scavenge free radicals, thereby preventing oxidative damage in the body [11]. The present study evaluated the protective effect of rutin on butachlor-induced oxidative stress in the testes. In this study, a significant reduction in testicular activities of Superoxide Dismutase [SOD], Catalase [CAT], and Glutathione-S-Transferase [GST] was observed as a result of Butachlor administration. The antioxidant enzymes SOD and CAT represent the primary intracellular antioxidant defense mechanism against oxidative stress. SOD catalyzes the reaction involving a rapid dismutation of superoxide radicals to hydrogen peroxide and di-oxygen, while CAT converts the hydrogen peroxide formed in this process and other cellular processes into water and molecular oxygen [28,29]. The reduction in the activities of SOD and CAT by butachlor may predispose the testes to oxidative damage.

Glutathione-S-Transferase [GST], on the other hand, is an enzyme involved in the detoxification of ingested xenobiotics. It also catalyzes the reduction of peroxide-containing compounds in the cell and this peroxidase activity exhibited by GST is however dependent on the n availability of GSH [30]. Thus, the balance of the enzyme system may be essential to get rid of superoxide anion and peroxide generated in the testis. Reports have shown that Rutin protects cells from H_2O_2-induced damage by inhibiting ROS generation [31]. Co-administration of Rutin offers protection against oxidative stress in the testes of the animal by increasing the activities of antioxidant enzymes in the experimental animals. Ascorbic acid is a vital antioxidant of the aqueous phase of the cell and rapidly scavenges free radicals. It also plays an important role in the regeneration of a membrane-bound antioxidant, vitamin E [32]. The ascorbic acid in the testes is maintained in a reduced state by GGSH-dependent dehydroascorbate reductase in the testes. The level of GSH is a measure of the cellular redox status [33]. Hence, alteration in GSH concentration may affect the overall redox status of the testis. The decrease in the antioxidant status of the rat implies an increased

susceptibility of the testicular tissues to radical species generated by the Butachlor. Membrane lipids succumb easily to deleterious actions of reactive oxygen species. Uncontrolled oxidative stress may result in membrane lipid peroxidation and, ultimately, testicular damage and loss of testicular functions [33].

In the present study, the increased levels of TBARS [Thiobarbituric acid reactive substances] which measures malondialdehyde [MDA] in the testis of rats treated with Butachlor reflected lipid peroxidation as a consequence of oxidative stress caused by the herbicide. Co-treatment with Rutin protected the testis cells through attenuation of lipid peroxidation and decrease the population of free radical derivatives as evident from the decreased level of testis TBARS. Furthermore, nitric oxide [NO] derived from inducible nitric oxide synthase [iNOS] has been associated with germ cell necrosis and the destruction of testis [34]. This was evident from the data generated from this study, indicating an increased level of NO in butachlor-treated animals which was ameliorated upon co-administration of butachlor and rutin. Therefore, Rutin offered protection against oxidative stress by scavenging free radicals. Activities of testicular marker enzymes such as acid phosphatase [ACP] and alkaline phosphatase [ALP] are considered functional indicators of spermatogenesis. In the spermatogenic cells, the specific activity of ACP and ALP increases as the germ cells differentiate from spermatocytes and spermatids [33]. In this study, a significant reduction in the activity of ACP and ALP as observed is characteristic of testicular atrophy associated with damage to germ cells by many xenobiotics [33]. However, co-treatment of Butachlor along with Rutin effectively attenuated the activities of ACP and ALP.

Conclusion

From this study, it may be suggested that Butachlor impairs the testicular antioxidant system and causes degenerative changes in the germ cells. However, Rutin, a potent antioxidant positively modulates the effect of the herbicide on the antioxidant status by attenuating the oxidative damage and effectively protects against butachlor-induced testicular oxidative stress, and this may be due to its intrinsic antioxidant properties.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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Authors Contributions

OET conceived the work; OET, KSA and OAT carried out the literature search and all experimental work, performed the statistical analysis and data interpretation, and wrote the draft of the manuscript. OET and KSA contributed to the design, analysis, and interpretation of data and critical review of the manuscript. OET, KSA and OAT supervised the work and contributed intellectual input in the discussion and overall presentation of the manuscript. All authors read and approved the final manuscript.

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