EVALUATION OF QUERCETIN ON TESTICULAR FUNCTION OF MALE WISTAR RATS TREATED WITH BUTYLPARABEN

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Received: 29th October, 2024 **Accepted:** 25th December, 2024 **Published**: 31st December, 2024

ABSTRACT

Background: Parabens are widely used as preservatives in many foods, cosmetics, toiletries and pharmaceuticals. Quercetin is a pigment found in many plants, fruits, and vegetables which helps fighting free radicals, reducing inflammation among others.

Aim: The aim of the study is to determine the effect of quercetin on testicular function of male Wistar rats treated with butylparaben.

Methodology: Thirty Wistar rats divided into 6 groups of five (5) rats were fed rat diet with water ad libitum with corn oil to serve as control while groups B, C, D, E and F received 100mg/kg of butylparaben (BP), 100mg/kg of BP and 25mg/kg of quercetin (Q), 100mg/kg of BP and 50mg/kg of Q, 100mg/kg of BP and 100mg/kg of Q, 100mg/kg of BP and 200mg/kg of Q respectively all dissolved in corn oil. The rats were administered the mixture for 60days, 3 times in a week. The rats were sedated using chloroform and blood collected for testosterone estimation using enzyme linked immunosorbent assay, the epididymis was also harvested and the semen extracted and used for semen analysis.

Results: The result of the experiment showed that mean motility (%) was 75.60±5.18, 66.60±7.60, 66.80±9.23, 74.50±6.40, 79.33±1.15 and 79.00±1.15 37 at concentrations (mg/kg body weight) 0.00, 100BP, 100BP+ 25Q, 100BP + 50Q, 100BP + 100Q and 100BP + 200Q respectively while mean abnormal sperm morphology (%) was 25.00±3.53, 28.00±5.70, 24.00±6.52, 24.50±5.26, 19.33±1.15 and 17.50±5.00 at respective concentrations. Mean viability (%) was 84.60±4.56, 63.00±6.71, 79.60±6.39, 78.25±7.68, 78.33±2.89, 84.50±5.26 while sperm count (10^6/ml) 560.00±96.17, 324.00±132.78, 346.00±148.59, 437.50±125.00, 316.67±57.74 and 507.50±65.00 and testosterone concentration (ng/ml) was 2.18±1.16, 0.44±0.42, 0.70±0.9, 1.04±0.75, 1.72±0.36, 1.37±0.37 at the following concentrations in mg/kg body weight 0.00, 100BP, 100BP+ 25Q, 100BP + 50Q, 100BP + 100Q, 100BP + 200Q respectively.

Conclusion: The result of the study has shown that quercetin caused reversal of reproductive parameters induced by butylparaben

Keywords: butylparaben, toxicity, Reproductive, Quercetin

INTRODUCTION

Parabens, alkyl esters of p-hydroxybenzoic acid, mainly including Butylparaben (BP), Methylparaben (MP), Ethylparaben (EP), and Propylparaben (PP), have been widely used as preservatives in foods, pharmaceuticals, cosmetics, and industrial products because of their broad antimicrobial spectra with relatively low toxicity, good stability, and non-volatility (Brausch and Rand, 2011). Parabens are also detected in human breast tissue (Harvey and Everett 2004), human milk (Schlumpf *et al.*, 2010), human urine and serum (Carrasco-Correa *et al.*, 2015) in recent years.

Citation: George-Opuda, I. M., Adegoke, O.A., Njoku, C. R-C., Briggs N. O., Elekima, I., Elechi-Amadi K.N., Udeme, I.E., and Egwuda, J.O. (2024): Evaluation Of Quercetin On Testicular Function Of Male Wistar Rats Treated With Butylparaben *BJMLS 9(2): 149 - 156*

Furthermore, parabens possess estrogenic activity (Routledge *et al.*, 1998, Darbre and Harvey, 2008, Lange *et al.*, 2014) and antiandrogenic activity (Satoh *et al*., 2005, Chen *et al*., 2007), which were classified as potential endocrine-disrupting chemicals (EDCs) by the Endocrine Society (Diamanti-Kandarakis *et al*., 2009). Several studies showed that parabens can promote the proliferation of MCF-7 human breast cancer cells (Charles and Darbre 2013, Khanna *et al.*, 2014), affect the male reproductive system in mice (Zhang *et al*., 2014), inhibit the mitochondrial respiratory capacities (Nakagawa and Molde´us 1998) and induce oxidative stress in the cell (Nishizawa *et al.*,2006, Popa *et al.*, 2011, Shah and Verma 2011). The effects of paraben include allergic reactions, premature aging, estrogenic activity and potential interference with endocrine system function. These compounds are known to exert a weak estrogenic activity, with butylparaben showing the most potent activity among methyl, ethyl and propyl esters (Oishi, 2001). Stronger evidence is found regarding effects on sperm count after developmental exposure to parabens (Kang *et al.*, 2002 and Yang *et al.*, 2015). Reduced epididymal sperm count and motility were seen in rat offspring of dams exposed subcutaneously to 100 and 200mg butylparaben/kg body weight/day during gestation and lactation (Kang *et al.*, 2002). Research suggests that butylparaben which is used in cosmetics and skin care product have more endocrine disrupting effects (Edwin and Routledge 1998).

Quercetin, is a plant flavonol from the flavonoids group of polyphenols, is a pigment found in many plants, fruits, and vegetables (Małgorzata 2008). It has a lot of health benefits and help to prevent a range of conditions. These benefits of quercetin include fighting free radicals, reducing inflammation, preventing neurological diseases, relieving allergic symptoms and preventing infections. Quercetin Content in Selected Foods include Apple with skin (4.42 mg/100g), Broccoli, Raw 3.21 mg/100g), Raw Onions (13.27 mg/100g), Spinach, raw (4.28 mg/100g), Black Tea Leaves, dry 04.66 mg/100g),Green Tea Leaves, dry (255.55 mg/100g), Red Wine (0.84 mg/100g), Cocoa powder, unsweetened (20.10 mg/100g) and Cranberries, raw (14.00 mg/100g)(Mohle 1985).

The aim of the study is to determine the effect of quercetin on reproductive parameters induced by butylparaben in male Wistar rats using Semen parameters such as total sperm count, sperm morphology, sperm viability and sperm motility and hormone Testosterone as indicators.

Materials and Methods

Thirty albino of inbreed rats weighing 112.5 $\pm$ 7.32g were purchased from Animal Science Department of Delta State University, Abraka. They were kept in the animal farm of Pharmacology Department, Madonna University, Elele Rivers State. The animals were acclimatized in the animal farm two (2) weeks before the study. The rat feeds were obtained from Elele market, Rivers State.

Butlyparaben

200g of commercially prepared Butylparaben (BP) were obtained from Lobachemie Chemicals.

Quercetin

200g of commercially prepared Quercetin (Q) purchased from Sigma Chemicals.

Reagents

Seminal fluid and other reagents were clinically prepared in laboratory.

Animal Experiment

Thirty albino rats divided into six (6) groups of five rats namely, group A, B, C, D, E, and F. The group A albino rats were fed with only rat diet and water *ad libitum* with corn oil to serve as a control group. Group B albino rats were administered with 100mg/kg butylparaben dissolved corn oil. Group C albino rats were administered with 100mg/kg butylparaben (Bp) and 25mg/kg quercetin (Q) dissolved in corn oil.

Group D albino rats were administered with 100mg/kg butlyparaben and 50mg/kg quercetin dissolved in corn oil. Group E albino rats were administered 100mg/kg butylparaben and 100mg/kg of quercetin dissolved in corn oil. While, group F albino rats were administered 100mg/kg of butylparaben and 200mg/kg of quercetin both dissolved in corn oil. The substances were administered three (3) times a week for sixty (60) days. At the end of the experimental procedures, the rats were induced sleep by sedating with chloroform, they were slaughtered and the liver organ and their epididymis were harvested for the determination of oxidative status while the blood samples were collected in a plain container for the estimation of Testosterone.

Semen Analysis

After the sacrifice of the rats, their epididymis was harvested and semen extracted and placed on a clean grease free slide. The semen was emulsified in eosin and examined under the microscope using 10X to focus and 40X to view. The semen was examined for motility, morphology, viscosity, viability, PH, total sperm count.

The Sperm Motility Determination was done according to Wyrobeck and Bruce (1978). Small drop of semen suspended in the semen incubation medium (trisaminomethane, citric acid and fructose) was placed on a slide. A cover-slip was placed on the drop and it was examined under the microscope for the estimation of spermatozoa motility. Observation was done at 100X magnification.

Sperm Viability (Live-Dead Count) was done according to Wyrobeck and Bruce (1978). The live-dead staining principle is based on the observation that eosin B penetrates and stain the dead sperm cells, whereas, the viable cells repel this stain. In order to benefit from the live-dead method, staining should be done without delay. The live-dead count supplements, rather than replaces the motility tests. Aliquots of sperm suspension in semen incubation medium were placed on a slide and stained with 1% eosin B and 5% nigrosin. The slide was examined at 100X magnification. At least 100 stained and unstained cells were counted and the percentage of each group was estimated.

The Sperm Morphology Examination was carried out according to Wyrobeck and Bruce (1978). The examination is performed to determine the presence and extent of occurrence of morphologically abnormal forms of spermatozoa. The same stained smear used for live-dead count was used for the detection of abnormal sperm cells. Cell morphology was studied under high power (400X).

The Epididymal Sperm Number (Sperm Count) in rat epididymis was estimated according to the method of Pant and Srivastava (2003). Epididymal sperm number was estimated by crushing excised cauda epididymis in suspension medium containing 140 mmol of NaCl, 0.3 mmol of KCl, 0.8 mmol of Na_2HPO_4, 0.2 mmol of KH_2PO_4 and 1.5 mmol of D-glucose (pH adjusted to 7.3). Sperms were collected and counted using hemocytometer under Phase Contrast Olympus Trinocular microscope at x 200 magnification. The values were expressed as an average of total sperm counts per ml of suspension (Cheesbrough, 2000).

The principle of Testosterone Estimation was based on mixing biotinylated antibody, enzyme-antigen conjugated and a serum containing the native antigen results to a competition reaction between the native antigen and the enzyme-antigen conjugate for a limited number of antibody binding sites. The interaction is illustrated by the following equation.

$$Ag^{Enz} + Ag + Ab_{btn} \rightleftharpoons AgAb_{Bnt} + Ag^{Enz}Ab_{Btn}$$

Where Ab_{btn} = biotinylated antibody (Constant quality), Ag=Native antigen (Variable quality), Ag^{Enz}=Enzyme-Antigen Conjugate (Constant variable), $Ag^{Enz}Ab_{btn}$= Enzyme-Antigen Conjugate- antibody complex, $AgAb_{btn}$= Antigen-antibody complex, Ka=Rate constant of association, k_a=Rate constant of dissociation

The microplate wells were formatted for each serum reference, control and patient specimen to be assayed in duplicate. Ten microlitre (10µl) of the appropriate serum reference, control and specimen were pipetted into the assigned well. Fifty microlitre (50µl) of the ready to use testosterone enzyme reagent was added to all the wells. The microplates were swirled gently for 30 seconds to mix. Fifty microlitre (50µl) of the testosterone biotin reagent was added to all the wells. The microplates were swirled gently for 30 seconds to mix. The microplates were covered and incubated for 60minutes at room temperature. The content of the microplate was discarded by aspiration. Three hundred and fifty microlitre (350µl) of the wash buffer was added, and decanted by tapping and blotting. This step was repeated two additional times for a total of three washes. One hundred (100µl) of the working substrate solution was added to all the wells and incubated at room temperature for fifteen minutes. Fifty microlitre (50µl) of the stop solution was added to all the wells and gently mixed for 20 seconds. The absorbance in each well were read at 450nm (using a reference wavelength of 630nm to minimize well imperfection) in a microplate reader (Tietz 1995).

Statistical Analysis

The data was analyzed using statistical package for social sciences (SPSS) version 20 for windows and results were expressed as mean ± standard error of mean (mean ± SEM) while analysis of variance (ANOVA) was used to determine the difference between treatments. Statistical significance as obtained at $p< 0.05$.

Result

The result of the study also showed that mean motility (%) at concentrations (mg/kg) of 0.00, 100BP, 100BP + 25Q, 100BP + 50Q, 100BP + 100Q and 100BP + 200Q was 75.60±5.18, 66.60±7.60, 66.80±9.23, 74.50±6.40, 79.33±1.15 and 79.00±1.15 ($P<0.05$) while mean Abnormal sperm morphology (%) was 25.00±3.53, 28.00±5.70, 24.00±6.52, 24.50±5.26, 19.33±1.15, 17.50±5.00 ($P> 0.05$) respectively. Mean viability (%) at concentrations (mg/kg) of 0.00, 100BP, 100BP + 25Q, 100BP + 50Q, 100BP + 100Q and 100BP + 200Q was 84.60±4.56, 63.00±6.71, 79.60±6.39, 78.25±7.68, 78.33±2.89 and 84.50±5.26 ($P<0.05$) while Sperm count (10^6/ml) was 560.00±96.17, 324.00±132.78, 346.00±148.59, 437.50±125.00, 316.67±57.74 and 507.50±65.00 ($P<0.05$) respectively. Testosterone concentration (ng/ml) at concentrations (mg/kg) of 0.00, 100BP, 100BP + 25Q, 100BP + 50Q, 100BP + 100Q and 100BP + 200Q was 2.18±1.16, 0.44±0.42, 0.70±0.9, 1.04±0.75, 1.72±0.36 and 1.37±0.37 ($P<0.05$) as shown in table 1 below.

Table 1: Effect of Quercetin (Q) on Sperm Quality Parameters and Testosterone Concentration in Male Wistar Rats Treated with Butylparaben (BP).

Concentration (Mg/Kg)	Motility (%)	Abnormal Sperm Morphology (%)	Viability (%)	Sperm Count(10^6/ml)	Testosterone (ng/ml)
0.00	75.60±5.18	25.00±3.53	84.60±4.56	560.00±96.17	2.18±1.16
Please 100BP	66.60±7.60[a]	28.00±5.70	63.00±6.71 [a]	324.00±132.78 [a]	0.44±0.42 [a]
100BP+25Q	66.80±9.23 [a]	24.00±6.52	79.60±6.39[b]	346.00±148.59 [a]	0.70±0.90[a,b]
100BP+50Q	74.50±6.40[b]	24.50±5.26	78.25±7.68	437.50±125.00[a,b]	1.04±0.75[a,b]
100BP+100Q	79.33±1.15[b]	19.33±1.15[a,b]	78.33±2.89[b]	316.67±57.74[a]	1.72±0.36[a,b]
100BP+200Q	79.00±1.15[b]	17.50±5.00a,[b]	84.50±5.26[b]	507.50±65.00[b]	1.37±0.37[a,b]
F	3.482	2.458	8.673	3.591	3.032
P	0.020	0.068	0.000	0.018	0.037

a= significant when compared with Control
b= significant when compared with Butylparaben

The overall effect of quercetin on sperm quality parameters and testosterone concentration induced by butylparaben in male wistar rats showed that Motility (%) for control, butylparaben and butylparaben plus quercetin groups was 75.60±5.17,66.60±7.60 and 74.12±7.81 ($p<0.05$) while Abnormal sperm morphology (%) was 25.00±3.53, 28.00±5.07 and 21.62±5.63 ($p>0.05$) respectively. Viability (%) for control, butylparaben and butylparaben plus quercetin groups was 84.60±4.56, 63.00±6.70 and 80.25±6.00 ($p<0.05$) while Sperm Count(10^6/ml) was 5.60±43.01, 3.24±59.38 and 4.03±126.90 ($p<0.05$) while testosterone (ng/ml) was 2.18±1.15, 0.44±0.42 and 1.14±0.74 ($p<0.05$) respectively as shown in table 2 below.

Table 2 Overall Comparison of Quercetin (Q) on Sperm Quality Parameters and Testosterone Concentration of Male Wistar Rats Treated with Butylparaben (BP).

	Motility (%)	Abnormal Sperm Morphology (%)	Viability (%)	Sperm Count(10^6/ml)	Testosterone (ng/ml)
Control	75.60±5.17	25.00±3.53	84.60±4.56	560.00±43.01	2.18±1.15
BP	66.60±7.60[a]	28.00±5.70	63.00±6.70[a]	324.00±59.38[a]	0.44±0.42 [a]
BP+ Q	74.12±7.81[b]	21.62±5.63[a,b]	80.25±6.00[b]	403.00±126.90[a,b]	1.14±0.74[a,b]
F	2.373	2.959	20.340	4.883	5.172
P	0.116	0.072	0.000	0.017	0.015

a= significant when compared with Control
b= significant when compared with Butylparaben

DISCUSSION

This research work also showed that butylparaben exposure causes decreased sperm motility, viability, sperm count and testosterone concentration when compared with their respective controls. It also caused an increase in abnormal sperm morphology when compared with the control. This is suggestive that butylparaben act like endocrine disruptors and exogenous chemicals that often interfere with the normal hypothalamo–pituitary–gonadal axis, mimicking hormones, blocking hormonal action or triggering inappropriate hormone activity hence the resultant reproductive dysfunction.

A review by Rotimi *et al* (2022) reported that reduction and concentration in the activities of testicular function biomarkers has been shown to correlate with impaired hypothalamic-pituitary-testicular axis and oxidative stress. This is similar to study by Oishi, (2001), Kang *et al*. (2002) and Dallinga *et al*. (2002). Oishi, (2001) reported that butylparaben caused dose dependent decrease in relative weight of epididymis, epididymal sperm reserve, sperm count, daily sperm production and serum testosterone in albino rats Kang *et al*. (2002) reported that butylparaben at 100mg/kg body weight induces testicular toxicity while reduced epididymal sperm count and motility were seen in rats exposed subcutaneously to 100 and 200mg Butylparaben/kg body weight/day during gestation and lactation. It also correlates with Dallinga *et al*. (2002) which stated that paraben may not only impair sperm motility, but also adversely affect sperm concentration, semen volume and sperm morphology.

On administration of various concentration of quercetin, there was a reduction in abnormal sperm morphology and increase in the sperm motility, viability, sperm count and testosterone concentration previously reduced by butylparaben. This is suggestive that quercetin has antioxidant property which enables it inhibit lipid peroxidation and reverse endocrine disrupting effect of butylparaben hence the increase in sperm motility, viability, sperm count and testosterone concentration. Rotimi *et al.*(2022) reported that Quercetin possessed pharmacological activities that help to combat cellular reproduction related assault such as altered sperm function, reproductive hormone dysfunction, dysregulated testicular apoptosis, oxidative stress and inflammation. Some reports emphasize that the dosage is essential for its expected biological activity (Tang *et al*. 2020). It has been reported that Quercetin supports apoptosis at high doses and shows antioxidant effects at low doses (Reyes-Farias and Carrasco-Pozo 2019). Therefore, in this study, Quercetin was used at a dose of 25- 200mg/kg to reveal its effects at lower and higher doses.

In line with this result, it is suggestive that quercetin interfered by reacting with the radicals formed by butylparaben resulting in increased in the sperm motility, viability, sperm count and testosterone concentration. Quercetin ameliorate testicular toxicity by inhibiting the generation of reactive oxygen species (ROS), with the help of two antioxidant pharmacophores present in the ring structure. The radical scavenging property of quercetin may alter signal transduction of oxidative stress induced apoptosis, prevent inflammation and increase sperm quality in relation to the hormonal concentration.

CONCLUSION

The study shows that administration of butylparaben caused reduction in sperm count, motility, viability and testosterone concentration while the administration of quercetin increased sperm count, motility, viability and testosterone concentration to help the anti-oxidative defense of the body.

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