

## SJMLS - 9(2) - 002

**Effect of Quercetin on Reproductive Oxidative Stress Parameters Induced by Butylparaben in Male Wistar Rats**

Adebayo Olugbenga Adegoke <sup>1\*</sup>, Ibitoroko Maureen George-Opuda <sup>2</sup>, Kemzi Nosike Elechi-Amadi <sup>2</sup>, Iniabasi Emmanuel Udeme <sup>1</sup>

Department of Medical Laboratory Science, Madonna University, Elele, Nigeria <sup>1</sup>, Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria <sup>2</sup>

Author for Correspondence\*: bayoadeghq@yahoo.com. <https://dx.doi.org/10.4314/sokjmls.v9i2.2>

**Abstract**

Parabens such as butylparaben and methylparaben are commonly used as preservatives in many foods, cosmetics, toiletries and pharmaceuticals while Quercetin is a pigment found in many plants, fruits, and vegetables that helps fight free radicals. This study determined the effect of quercetin on reproductive oxidative stress parameters of male Wistar rats treated with butylparaben. Thirty Wistar rats divided into 6 groups of five (5) rats were administered 100mg/kg butylparaben (BP), 100mg/kg BP and 25mg/kg quercetin (Q), 100mg/kg BP and 50mg/kg Q, 100mg/kg BP and 100mg/kg Q, 100mg/kg BP and 200mg/kg Q respectively all dissolved in corn oil while control were fed rat diet with water ad libitum mixed with corn oil. The mixtures were administered thrice a week for 60 days. The rats were sedated, and the testes harvested, homogenized and used for malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and glutathione peroxidase (GPx) estimation using thiobarbituric acid, Ellman's, epinephrine and Rotruck *et al* methods respectively. The result of the study showed that MDA ( $\mu\text{mol MDA/mg protein}$ ) was  $3.29\pm 0.11$ ,  $5.06\pm 0.58$ ,  $3.38\pm 0.03$ ,  $3.44\pm 0.03$ ,  $3.43\pm 0.07$ ,  $3.35\pm 0.09$ ; GSH ( $\text{units/mg protein}$ ) was  $20.69\pm 0.31$ ,  $15.63\pm 2.39$ ,  $18.20\pm 0.69$ ,  $18.81\pm 0.77$ ,  $21.58\pm 1.44$ ,  $21.31\pm 0.66$ ; SOD ( $\text{units/mg protein}$ ) was  $84.26\pm 3.55$ ,  $54.63\pm 9.74$ ,  $78.52\pm 7.59$ ,  $81.48\pm 6.76$ ,  $85.19\pm 3.70$ ,  $83.33\pm 7.71$  and GPx ( $\text{units/mg protein}$ ) was  $83.16\pm 5.41$ ,  $49.79\pm 2.89$ ,  $68.03\pm 4.39$ ,  $102.04\pm 9.33$ ,  $103.21\pm 5.74$ ,  $105.9\pm 4.05$  at body

weight 0.00, 100BP, 100BP+ 25Q, 100BP + 50Q, 100BP + 100Q, 100BP + 200Q respectively. The result has shown that quercetin caused reversal of changes in oxidative stress parameter induced by butylparaben.

**Keywords:** butylparaben, toxicity, oxidative stress, Quercetin

**Introduction**

Parabens are alkyl esters of Para-hydroxybenzoic acid which 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 nonvolatility (Brausch *et al.*, 2011). These compounds and their salts are used primarily for their bactericidal and fungicidal properties. They are found in shampoos, commercial moisturizers, shaving gels, personal lubricants, topical/parenteral pharmaceuticals, suntan products, makeup and toothpaste (Golden *et al.*, 2005). Paraben are esters of para-hydroxybenzoic acid, from which the name is derived. Common parabens include; Methylparaben (E number E218), Ethylparaben (E214), Propylparaben (E216), Butylparaben and Heptylparaben (E209). Less common parabens include; Isobutylparaben, Isopropylparaben, Benzylparaben and their sodium salts. Estimated daily exposures to butylparaben based on conservative assumptions from exposure to various butylparaben-containing products include personal care products, adult (14,590 $\mu\text{g/day}$  through dermal route), Pharmaceuticals/OTC, adult

(1700 $\mu$ g/day Orally), Pharmaceuticals/OTC, child (Tylenol) (13,500  $\mu$ g/day orally) and Soil (dust) ingestion, pica (39.2  $\mu$ g/day orally). From Soil (dust) ingestion, hand-to-mouth a child (0.392  $\mu$ g/day orally), adult (0.196  $\mu$ g/day orally) and Soil adherence to skin (child) (0.431  $\mu$ g/day through the dermal route) while Indoor air a child (0.032  $\mu$ g/day through inhalation) and adult male (0.048 $\mu$ g/day through Inhalation) (Yazar *et al.*, 2011).

Quercetin, a plant flavanol from the flavonoid group of polyphenols, is found in many fruits and vegetables (Małgorzata, 2008). Leaves, grains, red onions and kale are common foods containing appreciable content of quercetin. It has a bitter flavor and is used as an ingredient in dietary supplements, beverages and foods. Humans cannot make quercetin in their body, but many fruits, vegetables and drinks contain it. Food and drinks that contain quercetin include; Grapes, Berries, Cherries, Apples, Citrus fruits, Onions, Buckwheat, Broccoli, Kale, Tomatoes, Red wine and Black tea (Małgorzata, 2008). Quercetin is considered to be a strong antioxidant due to its ability to scavenge free radicals and bind transition metal ions. These properties of quercetin allow it to inhibit lipid peroxidation (Hollman, 1997).

The aim of the study is to determine the effect of quercetin on reproductive oxidative stress parameters induced by butylparaben in male Wistar rats using malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and glutathione peroxidase (GPx) as indicators.

## Materials and methods

### Quercetin

Two hundred grams (200g) of commercially prepared Quercetin (Q) was purchased from Sigma-Aldrich, USA.

### Butylparaben

Two hundred grams (200g) of commercially prepared Butylparaben (BP) were obtained from Lobachemie Chemicals, Mumbai, India.

### Study Animals

Thirty (30) albino of inbred rats weighing 112.5

+ 7.32g were purchased from Animal Science Department of Delta State University, Abraka and kept in the animal farm of Pharmacology Department, Madonna University, Elele Rivers State. The rats were acclimatized in the animal farm two (2) weeks before the study. The rat feed was obtained from Elele market, Rivers State.

### Reagents

Commercially produced Protein and Albumin reagents were purchased from Randox Limited, UK while Malondialdehyde (MDA), Superoxide Dismutase (SOD), Glutathione (GSH), Glutathione Peroxidase (GPX) reagents were clinically prepared in our laboratory.

### Animal Experiment

Thirty albino rats divided into six (6) groups A, B, C, D, E, and F contained five rats each. The albino rats in group A were fed rat diet and water *ad libitum* with corn oil to serve as a control group while albino rats in Group B were administered with 100mg/kg butylparaben dissolved in corn oil. Group C albino rats were administered with 100mg/kg butylparaben (Bp) and 25mg/kg quercetin (Q) dissolved in corn oil while albino rats Group D were administered with 100mg/kg butylparaben and 50mg/kg quercetin dissolved in corn oil. Albino rats in groups E and F were administered 100mg/kg butylparaben and 100mg/kg of quercetin dissolved in corn oil and 100mg/kg of butylparaben and 200mg/kg of quercetin dissolved in corn oil respectively. The substances were administered three (3) times a week for sixty (60) days. At the end of the experimental procedures, the rats were induced to sleep by sedating with chloroform, they were slaughtered, blood samples were collected in plain containers for the estimation of protein and albumin levels while the testes were harvested for the determination of oxidative status.

### Homogenization

Ice cold potassium chloride solution was used to rinse the testes, blotted with filter paper and weighed. They were then chopped into bits and homogenized in nine volumes of the homogenizing buffer (0.1M Phosphate buffer Saline, pH 7.4) which was produced by dissolving 0.496 g of di-potassium hydrogen orthophosphate,

$K_2HPO_4$  and 0.973 g of potassium di-hydrogen orthophosphate,  $KH_2PO_4$  in 90ml of distilled water. The pH was adjusted to 7.4. The resulting homogenate was centrifuged at 10,000 g for 10 minutes in a cold centrifuge (4°C), to obtain the post mitochondrial fraction. The supernatant was collected and used for biochemical analyses.

#### **Malondialdehyde (MDA) estimation**

Malondialdehyde (MDA) a marker of lipid peroxidation was assessed as thiobarbituric acid reactive substances (TBARS) as described by Ohkawa *et al.* (1979) with modifications. The principle of the reaction showed that thiobarbituric acid (TBA), an acidic medium interacts with MDA generated from peroxidation of fatty acids to produce a pink-coloured solution with maximum absorbance at 532nm. In a test tube, 0.4 ml of the sample was mixed with 0.5 ml of 20% TCA and 1.6 ml of Tris-KCl buffer and centrifuged at 3000g. To the supernatant obtained, 0.5 ml of 0.75% TBA was added and placed in a steaming water bath at 80°C for 60 minutes. The pink- coloured mixture was cooled in ice and the absorbance measured at 532nm. Lipid peroxidation in units/mg protein was computed with a molar extinction coefficient of  $1.56 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$  (Adam and Seregi, 1982) while concentration of the unknown was determined using the formular  $\text{MDA (units / mg protein)} = \text{Absorbance} \times \text{volume of mixture} \div \text{E532nm} \times \text{volume of sample} \times \text{mg protein}$ .

#### **Glutathione (GSH) Assay**

Glutathione (GSH) Assay or Reduced glutathione (GSH) in the samples were estimated using Ellman's reagent as outlined by Moron *et al.* (1979). This is based on the principle that the reduced glutathione exists largely as non-protein sulfhydryl groups in samples and it interacts with Ellman's reagent (5', 5'-dithiobis-(2-nitrobenzoic acid) (DTNB) to generate a stable yellow coloured chromogenic solution with molar absorption at 412nm. In a clean test tube, 0.5 ml of sample was added to 0.5 ml of precipitating solution, vortex and centrifuged at 10,000 rpm for 15 minutes. Thereafter, 0.5 ml of the supernatant was added to 4.5 ml of Ellman's reagent in another test tube. The absorbance of the reaction mixture was read at 412 nm against a reagent blank. Also, a standard graph was produced from serial dilution of GSH stock

solution and Ellman's reagent against a blank of 1.5 ml of Ellman's reagent and 0.5 ml phosphate buffer. The result of the unknown was extrapolated from the Standard Graph prepared.

#### **Superoxide dismutase (SOD) estimation**

The ability of superoxide dismutase to inhibit the autoxidation of epinephrine at pH 10.2 makes this reaction a basis for a simple assay for this dismutase according to Misra and Fridovich (1972). Superoxide ( $O_2^-$ ) radical generated by the xanthine oxidase reaction caused the oxidation of epinephrine to adrenochrome and the yield of adrenochrome produced per  $O_2^-$  introduced increased with increasing pH and also increased with increasing concentration of epinephrine. These results suggest that autoxidation of epinephrine proceeds by at least two distinct pathways, only one of which is a free radical chain reaction involving superoxide ( $O_2^-$ ) radical and hence inhibitable by superoxide dismutase. Into a micro cuvette, 0.2 ml of the diluted sample was added to 2.5 ml of 0.05 M carbonate buffer of pH 10.2 to equilibrate in the spectrophotometer and the reaction started by the addition of 0.3 ml of freshly prepared 0.3 mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5 ml buffer, 0.3 ml of substrate (adrenaline) and 0.2 ml of water. The increase in absorbance at 480 nm was monitored every 30 seconds for 150 seconds.

#### **Glutathione peroxidase (GPx) assay**

Glutathione peroxidase (GPx) estimation was carried out according to Rotruck *et al.* (1973). Into a clean test tube, 0.5ml of phosphate buffer solution was added followed by addition of 0.1ml of  $NaN_3$ , 0.2ml of reduced glutathione reagent, 0.1ml of hydrogen peroxide, 0.5ml of sample solution and 0.6ml of distilled water. The reaction mixture was incubated at 37°C for 3 minutes while addition of 0.5ml 10% trichloroacetic acid (TCA) was done to stop the reaction. The content of the test tube was centrifuged at 3000rpm for 5 minutes. The Glutathione peroxidase **of the supernatant was estimated by adding** 2ml of  $K_2HPO_4$  and 1 ml of DTNB reagent to 1 ml of the supernatant in a test tube to produce a color which was read at 412 nm against reagents blank and results expressed as  $\mu\text{g}$  of GSH consumed/mg protein.

#### **Quality Control**

External quality control sera produced by Randox Diagnostics UK assayed along with the analyses for accuracy and precision.

**Statistical Analysis**

The data was analyzed using statistical package for social sciences (SPSS) version 21. Tools such as analysis of variance (ANOVA) were used to determine the difference between treatments. The results were expressed as mean ± standard error of mean.

**Results**

**Effect of various concentrations of Quercetin (Q) on Oxidative Stress in Male Wistar Rats Treated with Butylparaben (Bp)**

The result of the study showed that MDA (µmol

MDA/mg protein) at concentrations (mg/kg) of 0.00, 100BP, 100BP+ 25Q, 100BP + 50Q, 100BP + 100Q and 100BP + 200Q was 3.29±0.11, 5.06±0.58, 3.38±0.03, 3.44±0.03, 3.43±0.07 and 3.35±0.09 (p< 0.05) while GSH (units/mg protein) was 20.69±0.31, 15.63±2.39, 18.20±0.69, 18.81±0.77, 21.58±1.44 and 21.31±0.66 (P< 0.05) respectively. SOD (units/ mg protein) at concentrations (mg/kg) of 0.00, 100BP, 100BP+ 25Q, 100BP + 50Q, 100BP + 100Q and 100BP + 200Q was 84.26±3.55, 54.63±9.74, 78.52±7.59, 81.48±6.76, 85.19±3.70 and 83.33±7.71 (p< 0.05) while GPx (units/mg protein) was 83.16±5.41, 49.79±2.89, 68.03±4.39, 102.04±9.33, 103.21±5.74 and 105.9±4.05 (p< 0.05) respectively as shown in table 1 below.

**Table 1: Effect of Quercetin (Q) on Oxidative Stress in Male Wistar Rats Treated with Butylparaben (Bp)**

Concentration (mg/kg)	MDA (µmol MDA/ mg protein)	GSH (Units/mg protein)	SOD (Units/mg protein)	GPx (Units/mg protein)
0.00	3.29±0.11	20.69±0.31	84.26±3.55	83.16±5.41
100BP	5.06±0.58	15.63±2.39	54.63±9.74	49.79±2.89
100BP+25Q	3.38±0.03	18.20±0.69	78.52±7.59	68.03±4.39
100BP+50Q	3.44±0.03	18.81±0.77	81.48±6.76	102.04±9.33
100BP+100Q	3.43±0.07	21.58±1.44	85.19±3.70	103.21±5.74
100BP+200Q	3.35±0.09	21.31±0.66	83.33±7.71	105.9±4.05
F	31.386	13.558	10.676	65.692
P	0.000	0.000	0.000	0.000

a= significant when compared with Control

b= significant when compared with Butylparaben

**Effect of various concentrations of Quercetin (Q) on Oxidative Stress in Male Wistar Rats Treated with Butylparaben (Bp)**

The overall effect of quercetin on oxidative stress parameters induced by butylparaben in male Wistar rats for control, butylparaben and butylparaben plus quercetin showed that MDA (µmol MDA/mg protein) was 3.29±0.11, 5.05±0.58 and 3.39±0.06 (p<0.05) while GSH (Units/mg protein) was 20.67±0.31, 15.63±2.39 and 19.76±1.72 (p<0.05). SOD (Units/mg protein) for control, butylparaben and butylparaben plus quercetin was 84.26±3.54, 54.63±9.74 and 81.71±6.67 (p<0.05) while GPx (Units/mg protein) was 83.16±5.40, 49.77±2.89 and 92.68±18.07 respectively as shown in table 2 below.

**Table 2: Overall Effect of Quercetin (Q) on Oxidative Stress Parameters in Male Wistar Rats Treated with Butylparaben (BP).**

Group	MDA (µmol MDA/mg protein)	GSH (Units/mg protein)	SOD (Units/mg protein)	GPx (Units/mg protein)
Control	3.29±0.11	20.67±0.31	84.26±3.54	83.16±5.40
BP	5.05±0.58	15.62±2.39	54.63±9.74	49.77±2.89
BP+Q	3.39±0.06	19.76±1.72	81.71±6.67	92.68±18.07
F	89.745	11.013	27.037	12.340
P	0.000	0.001	0.000	0.000

a= significant when compared with Control

b= significant when compared with Butylparaben

## Discussion

The result of the study showed that there was increase in MDA the marker of lipid peroxidation with decrease in GSH, SOD, and GPx in male Wistar rats treated with butylparaben when compared with their respective controls. This is because butylparaben was capable of triggering reactive oxygen species (ROS) generation which caused lipid peroxidation hence the resultant increase in MDA. The body's response to butylparaben induced reactive oxygen species resulted in a decrease in GSH, SOD and GPx. This is because these enzymes worked to neutralize the effect of these reactive oxygen species. This is similar to studies by Aitken *et al.* (2006) and Koppers *et al.* (2010) which reported that parabens are capable of triggering reactive oxygen species (ROS) generation and creating a state of oxidative stress in human spermatozoa.

On administration of various concentration of quercetin, there was a reduction in MDA, with increase in the GSH, SOD, and GPx, 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 decrease in MDA and increase in the GSH, SOD, and GPx. In line with this result, it is suggestive that quercetin interfered by reacting with the radicals formed by butylparaben resulting in increased GSH, SOD and GPx concentration.

## Conclusions

The result of the study showed that butylparaben caused a reduction in the oxidative stress parameter with increased MDA suggestive of oxidative stress. However, treatment with quercetin caused a significant increase in GSH, SOD and GPx with reduced MDA suggestive of reversal of oxidative stress.

## Declaration of competing interest

The authors report no conflict of interest in this study.

## References

Adam Vizi, V. & Seregi, M. (1982). "Receptor dependent stimulatory effect of noradrenaline on  $N^+$  /  $K^+$  AT pase in rats brain homogenate role of lipid peroxidation" *Biochemistry and*

- Pharmacology*; **31**:2231-2236.
- Aitken, R. J., Gibb, Z., Baker, M. A., Drevet, J. & Gharagozoo, P. (2006). "Causes and consequences of oxidative stress in spermatozoa" *Reproductive Fertility Development*; **28**:1-10.
- Brausch, J. M. & Rand, G.M. (2011). "A review of personal care products in the aquatic environment: environmental concentrations and toxicity" *Chemosphere*; **82**:1518–1532.
- Golden, R., Gandy, J. & Vollmer, G.A. (2005). "A review of the endocrine activity of parabens and implications for potential risks to human health" *Critical Revised Toxicology*; **35**:435–458.
- Hollman, P. (1997). "Bioavailability of the dietary antioxidant flavonol quercetin in man" *Cancer Letters*; **114**:139-140.
- Koppers, A. J., Garg, M. L. & Aitken, K.J. (2010). "Stimulation of mitochondrial reactive oxygen species production by unesterified, unsaturated fatty acids in defective human spermatozoa" *Free Radical Biological Medicine*; **48**:112-119.
- Małgorzata, M. (2008). "Quercetin and its derivatives: chemical structure and bioactivity" *Journal of Food Nutritional Science*; **58(4)**:407-413.
- Misra, H. P. & Fridovich, I. (1972). "The role of superoxide anion in the autoxidation of epinephrine and a simple assay for the superoxide dismutase" *Journal of Biological Chemistry*; **247(10)**:3170-3175.
- Moron, M. S., Depierre, J. W. & Mannervik, B.U. (1979). "Biochemical and Biophysical Analysis. (BBA)" *General Subjects*; **52(94)**:632-635.
- Ohkawa, H., Ohishi, N. & Yagi, K. (1979). "Analytical Biochemistry"; **95(2)**:351-358.
- Rotruck, J. T., Pope, A. L., Ganther, H. E., Swanson, A. B., Hafeman, D. G. & Hoekstra, W.G. (1973). "Selenium: Biochemical role as a component of glutathione peroxidase"; **179**: 588-590.
- Yazar, K., Johnson, S., Lind, M. L., Boman, A. & Liden, C. (2011). "Preservatives and fragrances in selected consumer-available cosmetics and detergent" *Contact Dermatitis*; **64**:265-272.

**Citation:** Adebayo Olugbenga Adegoke, Ibitoroko Maureen George-Opuda, Kemzi Nosike Elechi-Amadi, Iniabasi Emmanuel Udeme Effect of Quercetin on Reproductive Oxidative Stress Parameters Induced by Butylparaben in Male Wistar Rats. *Sokoto Journal of Medical Laboratory Science*; **9(2)**: 17–21. <https://dx.doi.org/10.4314/sokjmls.v9i2.2>

**Copyright:** This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.