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EFFECT OF QUERCETIN ON LIVER OXIDATIVE STRESS PARAMETERS INDUCED BY BUTYLPARABEN IN MALE WISTAR RATS

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ABSTRACT

Parabens are hormonally active chemicals widely used as preservatives in foods, cosmetics, toiletries and pharmaceuticals. Quercetin is a flavonoid with antioxidant property found in many fruits, plants and vegetables. The study investigated the effect of quercetin on the liver oxidative stress parameters induced by butylparaben in wistar rats. Thirty albino rats divided into six (6) groups of five rats were administered with 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 all dissolved in corn while the control were fed rat diet and water ad libitum with corn oil three (3) times weekly for sixty (60) days. The rats were sacrificed and liver harvested, homogenized and used for malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and glutathione peroxidase (GPx) estimation using thiobarbituric acid method, Ellman's method, epinephrine method and Rotruck et al method respectively while biuret and bromocresol green methods were used to estimate the blood Total protein and albumin levels respectively. The result showed significant difference($p<0.005$) in concentrations of MDA (µmol MDA/mg protein) (2.62±0.44, 7.65±0.50 and 3.21±1.14), GSH (Units/mg protein) (12.65±1.52, 6.50±1.70, and 14.58±3.05), SOD (Units/mg protein) (90.67±8.30, 62.67±4.35 and 81.04±8.41),GPx (Units/mg protein) (1.16±2.81, 1.05±0.56 and 1.17±0.55), Total protein (g/L) (58.41±2.10, 55.08±3.60 and 58.41±2.10) and Albumin (g/L) (45.20±1.05, 40.69±4.82 and 42.14±1.51) for Control, (Bp) and (Bp+Q) respectively. The result has shown that quercetin caused a reversal of butylparaben-induced toxicity on liver oxidative stress parameters.

Contribution/Originality: The study investigated the effect of quercetin on the liver oxidative stress parameters induced by butylparaben in wistar rats.

1. INTRODUCTION

Parabens are hormonally active chemicals that is being used as preservatives in foods, cosmetics, toiletries, and pharmaceuticals base on their relatively low toxicity profile and because of a long history of safe use, they are frequently detected in human fluids and tissues [1]. Parabens are alkyl esters of *p*hydroxybenzoic acid and typically include methylparaben, ethylparaben, propylparaben, butylparaben, isobutylparaben, isopropylparaben and

benzylparaben [2]. The common effect of paraben include (a) Allergic Reactions [3](b) Estrogenic Activity [1] (c) Premature Aging [4] and (d) Potential Interference with Endocrine System Function [5].

Quercetin (3,3',4',5,7-pentahydroxyflavone) is a name derived from the Latin word "Quercetum" meaning Oak Forest which belongs to the flavonols that the human body cannot produce [6]. It is yellow color and is poorly soluble in hot water, quite soluble in alcohol and lipids and is insoluble in cold water. Quercetin is said to be one of the most widely used bioflavonoids for the treatment of metabolic and inflammatory disorders. It is one of the most abundant dietary flavonoids found in fruits (mainly citrus), green leafy vegetables as well as many seeds, buckwheat, nuts, flowers, barks, broccoli, olive oil, apples, onions, green tea, red grapes, red wine, dark cherries, and berries such as blueberries and cranberries [7].

Quercetin has been reported to exert many benefit on health such as protection against osteoporosis, lung cancer, and cardiovascular disease while high intake flavonoids has been shown to reduce risk of cardiovascular disease. Progressive disorder of the lung parenchyma and airways or also known as chronic obstructive pulmonary disease (COPD) which happens to be the third-leading cause of death in the USA. Therapies thus far for COPD, unfortunately, is said to be partially effective with possibilities of side effects. Increasing evidence indicates that quercetin supplementation may be beneficial in it treating this condition [8]. In the previous preclinical study, it has been demonstrated an increase in plasma quercetin levels significantly decreased lung inflammation and prevented disease progression Quercetin is a flavonoid with antioxidant properties and is the most commonly consumed in the human diet [9].

The aim of this study is to investigate the effect of Quercetin on liver oxidative stress parameters induced by butylparaben in wistar rat using Malondialdehyde (MDA), Glutathione (GSH), Glutathione peroxidase (GPx), Superoxide dismutase (SOD) activities as well as Total protein and Albumin concentrations as indicators.

2. MATERIALS AND METHOD

2.1. Study Animals

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.

2.2. Butlyparaben

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

2.3. Quercetin

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

2.4. Reagents

Malondialdehyde (MDA), Superoxide Dismutase (SOD), Glutathione (GSH), Glutathione Peroxidase (GPX) reagents were clinically prepared in laboratory. While Protein and Albumin reagent were purchased from Randox chemically prepared reagents.

2.5. Animal Experiment

Thirty albino rats were divided into six (6) groups of five rats labelled as 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 organs were harvested for the determination of oxidative status. While the blood samples were collected in a plain container for the estimation of protein and albumin levels.

2.6. Biochemical Analysis

2.6.1. Homogenization

The liver from the animals were rinsed with ice cold potassium chloride solution then blotted with filter paper and weighed. The Liver were then chopped into bits and homogenized in nine volumes of the homogenizing buffer (0.1M Phosphate buffer Saline, pH 7.4). The homogenizing buffer was prepared 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 and then made up to a 100 ml with distilled water. The resulting homogenate was centrifuged at 10,000 g for 10 minutes in a cold centrifuge (4^{o}C), to obtain the post mitochondrial fraction. The supernatant was collected and used for biochemical analyses.

Lipid peroxidation was assessed by estimating the Malondialdehyde (MDA) as thiobarbituric acid reactive substances (TBARS) in the experimental samples using the method of Ohkawa, et al. [10]. with minor modifications. This is based on the Principle that thiobarbituric acid (TBA) in acidic medium, interacts with MDA generated from peroxidation of fatty acids to yield a pink coloured solution with maximum absorbance at 532nm.

Four hundred microliter (0.4 ml or 400ul) of the sample was mixed with Five hundred microliter (0.5 ml or 500ul) of 20% Trichloroacetic acid (TCA) and 1.6 ml of Tris-KCl buffer. The mixture was centrifuged at 3000g. Five hundred microliter (0.5 ml) of 0.75% TBA was then added to the supernatant obtained. It was placed in a steaming water bath at 80^{o}C for 60 minutes. The pink coloured mixture obtained was cooled in ice while the absorbance was measured at wavelength of 532nm. Lipid peroxidation in units/mg protein was computed with a molar extinction coefficient of 1.56 x 10^5 $M^{-1}cm^{-1}$ [11].The concentration of the unknown was determined using the formular MDA (units / mg protein) = Absorbance x volume of mixture divided by E532nm x volume of sample x mg protein

Glutathione (GSH) Assay or Reduced glutathione (GSH) were determined by Moron, et al. [12] method. 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.Into a test tube containing 0.5 ml of precipitating solution, 0.5 ml of sample was added, vortex and centrifuged at 10,000 rpm for 15 minutes. Into separate test tube containing 4.5 ml of Ellman's reagent, 0.5 ml of the supernatant was added and the absorbance of the reaction mixture was read at 412 nm against a reagent blank. Serial dilutions of the GSH stock solution were prepared and absorbance of the yellow colour formed on addition of Ellman's reagent was read within 30 min at 412 nm against a blank solution containing 1.5 ml of Ellman's reagent and 0.5 ml phosphate buffer to prepare a Standard Graph of absorbance against concentration of reduced GSH. The result of the unknown was extrapolated from the Standard Graph prepared.

Superoxide dismutase (SOD) activity was determined by Misra and Fridovich [13] using the ability of superoxide dismutase to inhibit the autoxidation of epinephrine at Ph 10.2 thus making this reaction a basis for a simple assay for this dismutase. 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 outcomes led to the

proposal 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. An aliquot of Two hundred microliter (0.2 ml) of the diluted sample was added to 2.5 ml of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer. The reaction was started by the addition of 0.3 ml of freshly prepared 0.3 mM adrenaline to the mixture and quickly mixed by inversion. Into the reference cuvette was added 2.5 ml buffer, 0.3 ml of substrate (adrenaline) and 0.2 ml of water with increase in absorbance at 480 nm monitored every 30 seconds for 150 seconds.

Glutathione peroxidase **(GPx)** assay was determined according to Rotruck, et al. [14]. 0.5ml of phosphate buffer solution, 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 were added to a clean test tube for the glutathione peroxidase assay respectively. All the reaction mixture was incubated at 37°C for 3 minutes. 0.5ml of TCA was added to the tube and thereafter centrifuged at 3000rpm for 5 minutes. 2ml of K_2HPO4 and 1 ml of DTNB was added to 1 ml of each of the supernatants in their respective test tubes. The absorbance was read at 412 nm against a blank. Glutathione peroxidase activity was observed by plotting the standard curve and the concentration of the remaining GSH was extrapolated from the curve. GSH consumed = 245.34 – GSH remaining

Glutathione peroxidase activity was calculated by H_2O_2 consumed divided by protein(mg).

Total Protein concentration was carried out using Biuret method. This is based on the principle that Cupric ions, in an alkaline medium interact with protein peptide bonds resulting in the formation of a coloured complex. 5.0ml of Biuret reagent was dispensed into tubes labeled blank, standard, test, and control. 0.1ml of distilled water, standard, sample and control were pipetted into their respective tubes, mixed and incubated at 25°C for 30minutes. The absorbances of each tube were measured at wavelength of 546nm using the content of blank tube to zero the spectrophotometer. The concentration of total protein was calculated by dividing the absorbance of sample against absorbance of standard and then multiplied by concentration of standard [15].

Albumin estimation was carried out using Bromocresol green (BCG) method as reported by Doumas, et al. [16]. The measurement of serum albumin is based on its quantitative binding to the indicator 3,3'5,5' tetrabromo-m cresol sulphonephthalein (bromocresol green BCG). The albumin BCG – Complex absorbs maximally at 578nm, the absorbance being directly proportional to the concentration of albumin in the sample. 0.01ml of distilled water, standard, sample and control was pipetted into tubes labeled blank, standard, sample and control while 3ml of Bromocresol green reagent was added to each tube, mixed and incubated at 25 °C for 5minutes. The absorbances of each tube read at 578nm against the reagent blank. The concentration of albumin was extrapolated by dividing the absorbance of sample against absorbance of standard and then multiplied by concentration of standard.

2.7. Quality Control

External quality control sera produced by Randox Diagnostics London were purchased in Port Harcourt and assayed along with the analyses.

2.8. Statistical Analysis

The data obtained was subjected to statistical analysis using statistical package for social sciences (SPSS) version 20 for windows. The 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 $p< 0.05$.

3. RESULT

The result of the study shows that MDA (µmol MDA/mg protein) was 2.62±0.44, 7.65±0.50, 4.73±0.52, 2.31±0.52, 2.63±0.22 and 2.64±0.36 at of 0.00, 100Bp, 100Bp+25Q, 100Bp+50Q, 100Bp+100Q and 100Bp+200Q

respectively (p<0.05). The result of the study shows that GSH (Units/mg protein) was 12.65±1.52, 6.50±1.70, 12.75±2.08, 14.69±3.53, 13.08±0.72, 17.87±2.37 at concentrations of 0.00, 100Bp, 100Bp+25Q, 100Bp+50Q, 100Bp+100Q and 100Bp+200Q respectively (p<0.05). The result of the study shows that SOD (Units/mg protein) was 90.67±8.30, 62.67±4.35, 75.33±4.47, 84.16±9.18, 82.22±9.62, 84.16±9.95 at concentrations of 0.00, 100Bp, 100Bp+25Q, 100Bp+50Q, 100Bp+100Q and 100Bp+200Q respectively (p<0.05).

Also, GPx (Units/mg protein) was 115.76±2.81, 105.03±0.56, 116.80±0.76, 117.38±0.51, 117.09±0.26, 116.82±0.37 at concentrations of 0.00, 100Bp, 100Bp+25Q, 100Bp+50Q, 100Bp+100Q and 100Bp+200Q respectively (p<0.05). The result of the study shows that Total protein(g/L) was 58.41±2.10, 55.08±3.60, 57.49±5.09, 58.36±1.31, 58.21±3.39, 59.59±2.62 at concentrations of 0.00, 100Bp, 100Bp+25Q, 100Bp+50Q, 100Bp+100Q and 100Bp+200Q respectively (p<0.05). Albumin (g/L) was 45.20±1.05, 40.69±4.82, 42.31±1.95, 41.69±2.14, 42.06±1.15, 42.50±1.49 at concentration of 0.00, 100Bp, 100Bp+25Q, 100Bp+50Q, 100Bp+100Q and 100Bp+200Q respectively (p<0.05) as shown in Table 1.

Table-1. Effect Of Quercetin (Q) On Oxidative Stress Parameters And Proteins Of Male Wistar Rats Exposed To Butylparaben (Bp).

Concentration (mg/kg)	MDA (µmol MDA/mg protein)	GSH (Units/mg protein)	SOD (Units/mg protein)	GPx (Units/mg protein)	Total Protein (g/L)	ALB (g/L)
0.00	2.62±0.44	12.65±1.52	90.67±8.30	115.76±2.81	58.41±2.10	45.20±1.05
100BP	7.65±0.50	6.50±1.70	62.67±4.35	105.03±0.56	55.08±3.60	40.69±4.82
100BP+25Q	4.73±0.52	12.75±2.08	75.33±4.47	116.80±0.76	57.49±5.09	42.31±1.95
100BP+50Q	2.31±0.52	14.69±3.53	84.16±9.18	117.38±0.51	58.36±1.31	41.69±2.14
100BP+100Q	2.63±0.22	13.08±0.72	82.22±9.62	117.09±0.26	58.21±3.39	42.06±1.15
100BP+200Q	2.64±0.36	17.87±2.37	84.16±9.95	116.82±0.37	59.59±2.62	42.50±1.49
F	99.305	13.538	7.967	61.073	0.656	1.136
P	0.000	0.000	0.000	0.000	0.663	0.393

The Overall Effect of Quercetin on Oxidative Stress And Proteins Induced By Butylparaben in Wistar Rats showed that MDA (µmol MDA/mg protein) was 2.62±0.44, 7.65±0.50 and 3.21±1.14(p<0.005) while GSH (Units/mg protein) was 12.65±1.52, 6.50±1.70, and 14.58±3.05 (p<0.005) in control, Bp and Bp+Q groups respectively. SOD (Units/mg protein) was 90.67±8.30, 62.67±4.35 and 81.04±8.41 (p<0.005) while GPx (Units/mg protein) was 1.16±2.81, 1.05±0.56 and 1.17±0.55 (p<0.005) in control, Bp and Bp+Q groups respectively. Total protein (g/L) was 58.41±2.10, 55.08±3.60 and 58.41±2.10 (p<0.005) while, Albumin (g/L) was 45.20±1.05, 40.69±4.82 and 42.14±1.51 (p<0.005) for Control, (B) and (Bp+Q) groups respectively as shown in Table 2.

Table-2. Overall Effect of Quercetin On Oxidative Stress And Proteins Exposed to Butylparaben.

Concentration	MDA (µmol MDaA/mg protein)	GSH (Units/mg protein)	SOD (Units/mg protein)	GPx (Units/mg protein)	Total Protein (g/L)	ALB (g/L)
Control	2.62±0.44	12.65±1.52	90.67±8.30	1.16±2.81	58.41±2.10	45.20±1.05
Bp	7.65±0.50	6.50±1.70	62.67±4.35	1.05±0.56	55.08±3.60	40.69±4.82
Bp + Q	3.21±1.14	14.58±3.05	81.04±8.41	1.17±0.55	58.41±2.10	42.14±1.51
F	46.625	17.849	16.928	171.00	1.559	3.382
P	0.000	0.000	0.000	0.000	0.243	0.061

4. DISCUSSION

This study showed that the administration of butylparaben caused a marked increase in MDA a great marker of lipid peroxidation and a decrease in GSH, SOD and GPx concentrations in the experimental animals when compared to their respective controls. This is similar to previous study by Shah and Verma [17] who reported that butylparaben are capable of producing reactive oxygen species in the plasma membrane of cells and suppression of

anti-oxidative system leading to altered redox potential of cell thereby causing lipid peroxidation suggestive of considerable hepatocytic oxidative stress. Also Beazley, et al. [18] reported that numerous xenobiotics exert their toxicity by inducing oxidative stress where there is insufficient antioxidant activity causing excessive accumulation of free radicals, which damage cellular compounds such as proteins, carbohydrates, DNA and lipids

The result of the study also shows that administration of butylparaben caused a significance decrease in the protein and albumin concentrations in the experimental animals when compared to their respective controls. This is similar to previous study by Abdel-Misih and Bloomston [19] who reported that the liver is the prime organ involved in metabolism of xenobiotics and it is thereby prone to be attacked by the free radicals produced by them resulting in tissue injury and thus, affecting its hepatic roles.

Also, the result of the study further shows that the administration of various concentrations of quercetin to the butylparaben treated rats caused a significant decrease in MDA with increase in GSH, SOD and GPx concentrations previously reduced by butylparaben. This is suggestive that quercetin has an antioxidant property which scavenge the endogenous free radicals induced by butylparaben and reversing its toxicity. Quercetin increases the body's antioxidant capacity by regulating levels of GSH. This is because, once oxygen free radicals are generated in the body, superoxide dismutase (SOD) quickly captures O^{2-} and transforms it into H_2O_2. This enzyme further catalyzes the decomposition of H_2O_2 to H_2O. This reaction requires GSH as a hydrogen donor. Animal and cell studies found that quercetin induces GSH synthesis [20]. It was also reported that the application of quercetin therapy in hepatic ischemia increased GSH levels, an effect that enhanced the antioxidant capacity of rats [21]. When quercetin was applied at high doses, the dynamic balance of GSH (under the action of GSH peroxidase) was affected, H_2O_2 was converted to H_2O and GSH is oxidized to GSSG (oxidized glutathione).

The administration of various concentrations of quercetin to the butylparaben treated rats revealed marked increase in the proteins level. This is suggestive that quercetin antioxidant activity has reverses damages of the cellular compound (proteins) previously induced by butylparaben.

5. CONCLUSION

The study shows that administration of butylparaben caused liver oxidative damage by increasing the MDA concentration while lowering the GSH, SOD and GPx. The administration of quercetin reversed the changes by lowering the MDA and increasing the concentrations of SOD, GSH, and GPx to help the anti-oxidative defense of the body.

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