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PROTECTIVE EFFECT OF ONION EXTRACT AND QUERCETIN ON CAPECITABINE -INDUCED CYTOTOXICITY AND GENOTOXICITY IN GERM CELLS OF MICE

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ABSTRACT

Possible adverse effects of Capecitabine (CPB) treatment on reproductive functions and testicular DNA damage along with the potential protective effects of Quercetin and Onion extract on the germ cell toxicity were investigated. CPB was administered intraperitoneally (i.p.) at the dose of 5, 10 and 20mg/kg/bwt to male mice weighing 25g (n=5) daily for 21 days. Quercetin and Onion extract were given at the dose of 10mg/kg prior to CPB dosing each day. Body weight, testes and epididymis weights, testosterone and Luteinizing hormone levels (ELISA), sperm counts, motility, morphology and histology were evaluated. The DNA damage using halo assay and single-cell gel electrophoresis technique (COMET assay) was also measured. Reactive oxygen species (ROS) and Malondialdehyde (MDA) levels along with important antioxidant biomarker enzymes GSH, CAT and SOD were also evaluated. Raised ROS and MDA, the final product of fatty acid peroxidation levels with reduced testosterone and LH levels were measured. Depletion of antioxidant enzymes confirmed generation of oxidative stress following CPB treatment resulting to impaired functions of testicular tissue. DNA damage increased in a dose-dependent manner. However, quercetin and onion extract treatments significantly improved the testes weight, sperm count, motility and morphology as compared to only CPB- treated animals. Quercetin and onion extract co-treatment also significantly ($p<0.05$) restored the sperm DNA damage. Further, QUC and onion extract treatments showed protection against CPB-induced testicular toxicity as evident from testes histology. The present results confirmed damage of testicular tissue following CPB treatment. The studies also showed the ameliorative potential of quercetin from onion-extract against CPB -induced cytotoxicity and genotoxicity in male mice.

KEYWORDS: Capecitabine; Quercetin; Onion; Oxidative stress; Halo assay; single cell gel electrophoresis assay.

1.0 INTRODUCTION

Cancer continues to be a major concern as regards human health as its occurrence has been reported to be on a steady increase in recent years worldwide.[1] Capecitabine also known as Xeloda widely used in the treatment of gastric, breast and colorectal cancer is an oral prodrug of Fluorouracil (5- FU). The metabolism of Capecitabine involves its enzymatic conversion into its active compound 5-FU and this is conducted in three activation steps, the rate-limiting step is the final reaction catalyzed by thymidine phosphorylase.[2,3] It has been reported that there is marked increase in the activity of the enzyme thymidine phosphorylase in most tumors, including breast and colorectal cancers that exceed the levels in normal tissue from which the tumor arises.[2,3,4] Capecitabine is currently been used for four indications; monotherapy in the first line of treatment of advanced colorectal cancer, adjuvant treatment of patients with stage III (Duke's stage C) colon cancer, in combination with docetaxel in the treatment of locally advanced or metastatic breast cancer, and as monotherapy in advanced breast cancer after failure of a taxane- and anthracycline-containing chemotherapy or for patients for whom an anthracycline is contraindicated.[5,6] Capecitabine can be used as a monotherapy drug or in combination with other drugs.[2] Side effects that have been associated with Capecitabine include abdominal pain, vomiting, diarrhea, weakness, and rashes. Other severe side effects include blood clotting problems, allergic reactions, heart problems such as cardiomyopathy, and low blood cell counts.[7]

Quercetin (3,3',4',5,7-pentahydroxyflavone) in fruits and vegetables has attracted much attention for its beneficial health effect due to its potential antioxidant property.[8] Likewise Pharmacological properties of onion extract have been documented in scientific reports. Anti-inflammatory properties have been associated with onion extract.[9] The beneficial effects of quercetin which include its anti-oxidant and anti-inflammatory properties have been reported. Based on the literature available, there is insufficient information on the genotoxicity and cytotoxicity of Capecitabine in the germ cells and likewise on the alleviating effect of Quercetin and Onion extract on the genotoxicity and cytotoxicity of Capecitabine in germ cells of mice. Therefore the interest

of this study was to determine the alleviating effect of quercetin and onion extract on the genotoxicity and cytotoxicity of Capecitabine in germ cells of mice.

2.0 MATERIALS AND METHODS

2.1. Preparation of Onion Extract

Fresh onions (Allium cepa L., Nigeria) were purchased from a local market. The onions were washed with sterile distilled water and air-dried for 24 hours.

2.1.2. Extraction and Soxhlet polar fractionation

Method described by Guo-Qing Sh *et al.*,[10] was used for this process. Two hundred grams of onion skin powder was extracted with 6 L of 50% aqueous ethanol at 72°C for 2 hours in a water bath. The resulting extract was then cooled to room temperature (25±2 °C) and filtered through filter paper. This procedure was repeated two times and the combined extracts were concentrated under vacuum on a rotary evaporator near to cream, which was absorbed onto diatomaceous earth and air-dried. For soxhlet polar fractionation, the dried diatomaceous earth samples were sequentially extracted with ethyl acetate using a soxhlet extractor for 12 hours. The ethyl acetate extracts were collected for further processing.

2.1.3. pH gradient separation and Isolation

The ethyl acetate phase was extracted three times with 2% (w/v) $NaHCO_3$ solution, and the pH of the combined aqueous extracts was adjusted with concentrated hydrochloric acid to pH 2. The acidified aqueous was then extracted with ethyl acetate before being evaporated to dryness under vacuum to give the $NaHCO_3$ components of the ethyl acetate phase which was extracted with 5% (w/v) Na_2CO_3. The flavonoid, quercetin (QUC) in the $NaHCO_3$ component of the ethyl acetate phase was isolated by preparative TLC using toluene/ethyl, formate/formic acid as developing solvents.

2.2. Animals, Grouping and Treatments

Animals

All the animal experiment protocols were approved by the Institutional Animal Ethics Committee (IAEC) of College of Medicine, Ekiti state University, Ado-Ekiti, Nigeria. Experiments were performed on male Swiss mice (25g) procured from the central animal facility of the institute. The animals were kept at room temperature (25±2°C), with 50±10% humidity and a cycle of 12h light and 12h dark. Standard laboratory animal feed and water were provided ad libitum. Animals were acclimatized to the experimental conditions for a period of one week before the start of dosage.

2.2.1 Experimental procedures

Forty Swiss Albino mice weighing 25 kg were used for this study. The animals were randomly divided into eight groups I-VIII (n=5). 10 mg/kg Quercetin extract from onion was administered to the Capecitabine-treated animal, amount that is the equivalent in ratio to a 25 kg mouse via intraperitoneal route for seven days. Experimental mice were weighed before and at the end of the administration of the drug and extract. Below is the table showing the groups and treatments.

Table 1: Administration of Quercetin, Onion extract and Capecitabine (CPB).

Animal groups	Treatment (Dosage/Kg body /weight/day)
Group I	Vehicle-treated control
Group II	5mg/kg/day, CPB
Group III	10mg/kg/day, CPB
Group IV	20mg/kg/day, CPB
Group V	10mg/kg/day, QUC
Group VI	10mg/kg/day, Onion extract
Group VII	20mg/kg/day, CPB + 10mg/kg/day Onion extract
Group VIII	20mg/kg/day, CPB + 10mg/kg/day QUC

Mice were autopsied after seven days of treatment; testis and epididymis were excised and washed with phosphate-buffered saline (PBS) twice.

2.3. General observations, body and organ weights

Mice were observed daily for behavioral changes. Body weight was measured daily with the aid of a mono pan balance prior to administration of CPB. At autopsy, testes and epididymis were removed, blotted free of blood and adhering tissues and weighed. The organ weights were then converted per 100 g of body weight

2.4. Testicular cells preparation

Testicular cells were prepared following a protocol adapted from Malkov et al.[11] Briefly, testes were removed and decapsulated by making a small incision in the testis. The contents of the testes were collected through the incision into a 15 ml tube containing 5 ml ice-cold 1X PBS buffer (pH -7.4) and the contents were incubated for 40 min at 37° C with vigorous shaking. Then, the tubes were placed on ice and incubated to allow the seminiferous tubules to settle. The supernatants were discarded and the seminiferous tubules were washed twice in 10 ml of PBS twice.

2.5. Testicular Cells Viability Assay

Cell numbers were determined using a Neubauer hemocytometer and viable cells were analyzed by determining their ability to exclude the dye. The cytoplasm of a viable cell is clear, whereas a non-viable cell has a blue cytoplasm. We calculated cell viability (%) as follows: Cell viability (%) = (total number of viable cells (trypan blue-negative) cells)/ (total number of cells including trypan blue-positive and negative cells)* 100.

2.6. Measurement of Reactive Oxygen Species (ROS) Level

The ROS assay was also carried out. 50 µl of tissue homogenate and 1400µl sodium acetate buffer were transferred to a cuvette. After then, 1000ul of reagent

mixture (N,N-diethyl para phenylenediamine 6mg/ml with 4.37µM of ferrous sulfate dissolved in 0.1M sodium acetate buffer pH- 4.8) was added at 37^{o}C for 5min. The absorbance was measured at 505 nm using spectrophotometer (Molecular Devices.) ROS levels from the tissue were calculated from a calibration of H_2O_2 and expressed as U/mg of protein (1 unit=1.0mg H_2O_2/L).

2.7. Measurement of malondialdehyde level

One gram of testicular tissue was homogenized in 0.05 M phosphate buffer at pH=7.4, the solutions were centrifuged and supernatants were used for the evaluation of lipid peroxidation products. The degree of malondialdehyde (MDA) production is determined by spectrophotometric measurement based on the color produced by reaction of TBA with MDA. 300ml of trichloric acid 10% was added to 150 µl supernatant of centrifuged sample, and then centrifuged for 10 mins at 4^{o}C and 1000g. 300 µl of supernatant was transferred to a test tube and was incubated with 300 µl of thiobarbituric acid 0.67% at 100 ^{o}C for 25 min. 5 min after cooling the solution, the pink color due to the reaction of TBA-MDA appeared and was measured using a spectrophotometer at a wavelength of 535nm. Concentration of MDA was calculated using the coefficient of TBA-MDA complex absorption and was expressed as nmol/g.

2.8. Testicular Testosterone (T) and Luteinizing hormone (LH) concentrations

The testicular testosterone and luteinizing hormone levels in three mice from each group were measured. Testicular proteins were extracted with phosphate buffer (50 mM, pH -7.4) and centrifuged at 10,000 g for 20 min. The supernatant was used to estimate T and LH levels using ELISA, and were expressed in ng/ml.

2.9. Glutathione (GSH) level

Glutathione (GSH) activity was estimated by centrifuging an aliquot of 10% homogenates of the tissues in 100 mM Tris-HCL buffer (pH-7.4) containing 0.16 M KCL at 1000 × g for 5min. The supernatant was used to measure the rate of reduction of 5'5'-dithiobis-(2 nitrobenzoate) to 2-nitro-5 thiobenzoate. The absorbance was taken at 412 nm. Glutathione content was expressed in µM/mg protein.[12]

2.10. Superoxide dismutase (SOD) level

Superoxide dismutase (SOD) was measured using modified method of Kakkar *et al.*[13] Assay mixture containing sodium pyrophosphate buffer (pH-8.3, 0.052 M), nitroblue tetrazolium (300µM), NADH (780 µM) and appropriately diluted enzyme in total volume of 3 ml was incubated at 37^{o} C for 90 secs. The reaction was stopped by the addition of glacial acetic acid. The reaction mixture was mixed vigorously by adding n-butanol and was allowed to stand for 10 min before the collection of butanol layer. The intensity of chromogen in butanol was measured at 560 nm. The SOD activity was calculated in units/ml/min.

2.11. Catalase (CAT) level

Catalase was measured by the method described by Aebi.[14] Assay mixture consisting of 0.01 M phosphate buffer (pH-7.0), 0.2 M hydrogen peroxide (H_2O_2) and tissue homogenate was incubated at 37^{o} C for 1 min. The reaction was stopped by addition of potassium dichromate (5% w/v) and acetic acid. The remaining hydrogen peroxide was determined by measuring chromium acetate after heating the assay mixtures in a boiling water bath for 15 mins. The absorbance was read at 570 nm against control (without H_2O_2).The enzymatic activity was measured in µmol/min/mg protein.

2.12. Sperm count and Sperm Head morphology

Caudal epididymidis was removed from each mouse and cleaned off from the epididymal fat pad, and minced in a pre-warmed Petri dish containing 500 µl phosphate buffer saline solutions (PBS, pH -7.4) at 37 ^{o}C. For sperm count, an aliquot of this suspension was charged into the Neubauer's counting chamber and the spermatozoa were counted under light microscope. Total sperm count was calculated as the average of the spermatozoa count (N) in each chamber X multiplication factor (10^6) X dilution factor and was expressed in millions/ml.[15] The sperm morphology was also evaluated.[16] A smear of sperm was made on a clean slide, stained with haematoxylin and eosin and examined under a light microscope with an oil immersion lens. The sperm head morphology of spermatozoa was scored.[17,18,19]

2.13. Testis histology

Preparation and quantification of histological slides were done as standardized previously in our laboratory.[20,21] Testes samples were fixed in 10% formalin, dehydrated in ethanol and embedded in paraffin. Tissue sections (5m) were mounted on glass slide coated with albumin and dried at 30°C for 24 hrs. The sections were then deparafinized with xylene, rehydrated with alcohol and water. The rehydrated sections were stained with haematoxylin and eosin (H&E), mounted with DPX and examined under microscope. Histological quantification was performed by counting the normal number of seminiferous tubules in each slide. A graph was plotted between the normal numbers of seminiferous tubule at Y-axis vs. dose of Capecitabine at X-axis. Relative area was calculated by area of treatment group/area of control group.

2.14. Sperm comet assay

The sperm comet assay was performed essentially as described with some modified standard method.[18,20,22] Briefly, sperm sample (5 µl) containing 1–3×10^4 sperm ml^{-1} was suspended in 95 µl of 1% (w/v) low melting point agarose. From this suspension, 80 µl was applied to the surface of a microscope slide (pre-coated with 1% normal melting point agarose) to form a microgel and

allowed to set at 4 ^{o}C for 5 mins. Slides were dipped in cell lysis buffer (2.5M NaCl, 100mM EDTA, 10mM Tris HCl pH 10.0 containing 1% Triton X-100 and 40mM Dithiothreitol) for 24 hrs at room temperature and protected from light. Following the initial lysis, proteinase K was added to the lysis solution (0.5mg/ml) and additional lysis was performed at 37^{o}C for 24h. Following cell lysis, all slides were washed three times with deionized water at 10min intervals to remove salt and detergent from the micro gels. Slides were placed in a horizontal electrophoresis unit and were allowed to equilibrate for 20min with running buffer (500mM NaCl, 100mM Tris HCl and 1mM EDTA, pH 9) before electrophoresis (0.60V/cm, 250mA) for 30min. After electrophoresis, slides were neutralized and the DNA fluorochrome SYBR Green (1:10,000 dilutions) was applied for 1h. Slides were rinsed briefly with double-distilled water and cover slips were placed before image analysis. The main parameters of the comet DNA damage analysis include: tail length (TL), % DNA in comet tail (TDNA) and tail moment (TM). Samples were run in duplicate and 50 cells were randomly analyzed per slide for a total of 100 cells per sample and scored for comet tail parameters as defined by Olive.[39] Comet tail length is the maximum distance that the damaged DNA migrates from the center of the cell nucleus. The percentage of tail DNA is the total DNA that migrates from the nucleus into the comet tail. Tail moment is the product of the tail length and the percentage of tail DNA, which gives a more integrated measurement of overall DNA damage in the cell.

2.15. Data analysis

The data were analyzed using Statistical Package for Social Scientist (SPSS version 18). The results were expressed as mean± SEM. Significant differences among means of the groups were determined using one way analysis of variance (ANOVA) and where significant difference existed, it was followed by Duncan's multiple range tests. Mean was considered significant when $p \leq 0.05$.

3.0 RESULTS

3.1. Body weight and organ weight

Significant decrease in the body weight was observed after treatment with CPB as compared to the control groups. Pre-treatment with QUC and OE 10mg/kg resulted to slight recovery of body weight as compared to the group which received only CPB. The paired testes and epididymis weight in the groups which received protection with QUC and OE were found to be significant in comparison with the CPB -treated group. (Table1).

3.2. Cell Viability with QUC and Onion Extracts in CPB Treatment

The viability of testicular cells of mice treated with various doses of CPB (5, 10, and 20mg/kg) was determined using a Trypan blue-exclusion assay. Testicular cells of animals in CPB -treated groups exhibited significantly decreased viability compared to the testicular cells of mice in controls, QUC and Onion extract. CPB treatment decreased the number of viable cells in a dose-dependent manner as indicated by the number of trypan blue-positive cells ($P < 0.05$). There was a slight increase in viability of QUC and onion extract (10mg/kg) pre-treated cells, compared to the control cells ($P < 0.001$), suggesting that both QUC and onion extract at 10mg/kg protect against cytotoxicity, (Fig 1).

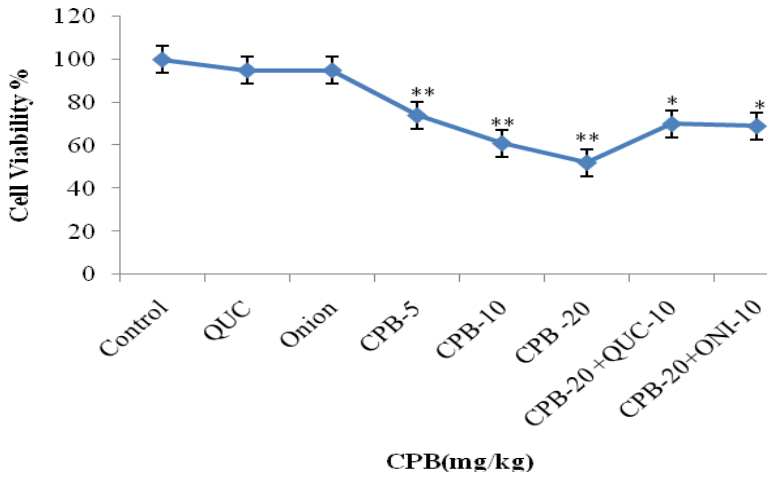


Fig. 1: Effect of CPB on the viability of testicular cells of mice as measured by the trypan blue exclusion method. A significant decrease in the number of trypan blue-negative cells was evident in the testicular cells of CPB-treated in dose dependent manner while slight increase of QUC and onion extract pre-treated cells compared to the cells treated with CPB only was measured. All the values are expressed as mean ± SEM, (n=5), ***P<0.001, **P<0.01 and*P<0.05.

3.3. Effect on Sperm count and sperm head morphology

Significant decrease in the sperm counts was observed in CPB -treated group as compared to the control groups (P<0.001). There was a significant recovery in the sperm counts in the groups which received QUC and OE treatment (10 mg/kg/day) prior to CPB treatment (P <0.05), (Figure 2a). Sperms with triangular and amorphous heads were classified as sperm head abnormalities. The sperm head abnormalities were found to increase significantly in the group which received CPB for 21days as compared to the control groups (P<0.001). A significant decrease in the % of abnormal sperms was observed in the group pre-treated with the 10mg/kg dose of QUC and OE in comparison with the CPB -treated group (P<0.05), (Figure 4b).

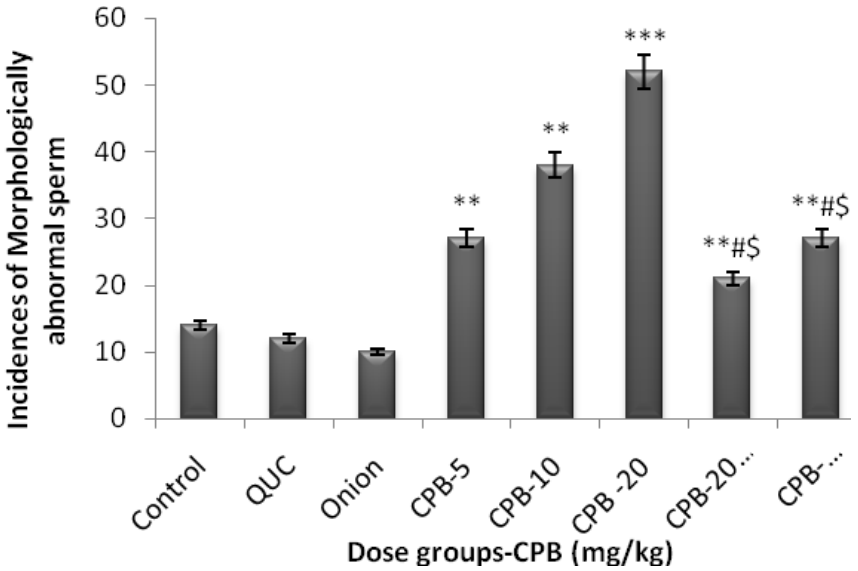
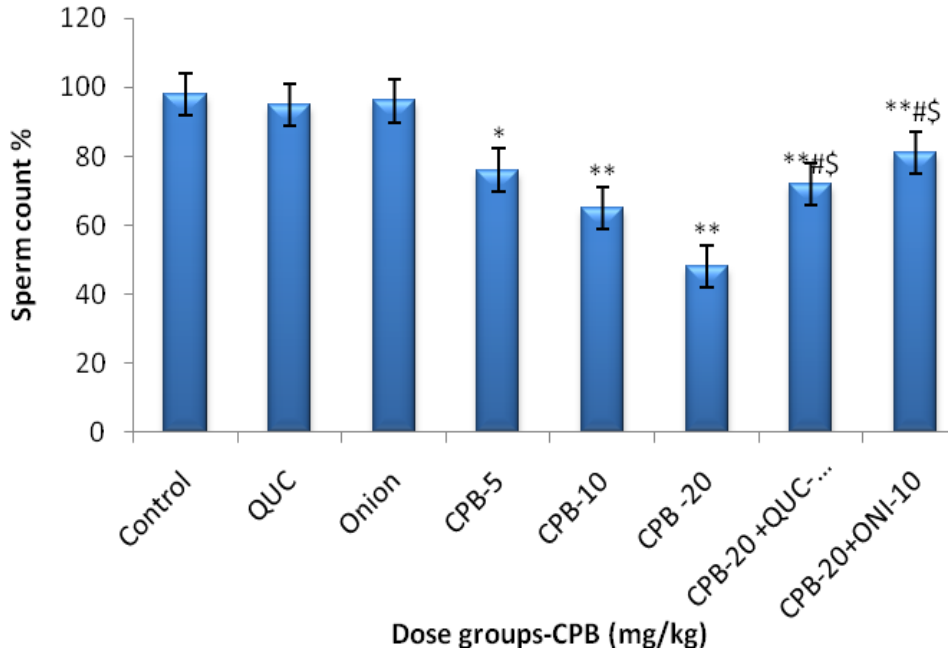


Fig. 2a. Protective effects of QUC and Onion extract against CPB induced abnormality in sperm head morphology. All the values are expressed as mean±SEM, (n=5), ***P<0.001, **P<0.01 and*P<0.05, # vs. control and $ vs. CPB-20.



(b)

Figure 2b. Protective effects of QUC and Onion extract against CPB- induced on sperm count. All the values are expressed as mean±SEM, (n=5), ***P<0.001, **P<0.01 and*P<0.05, # vs. control and $ vs. CPB-20

3.4. Effect of QUC and Onion extract on Reactive Oxygen species level

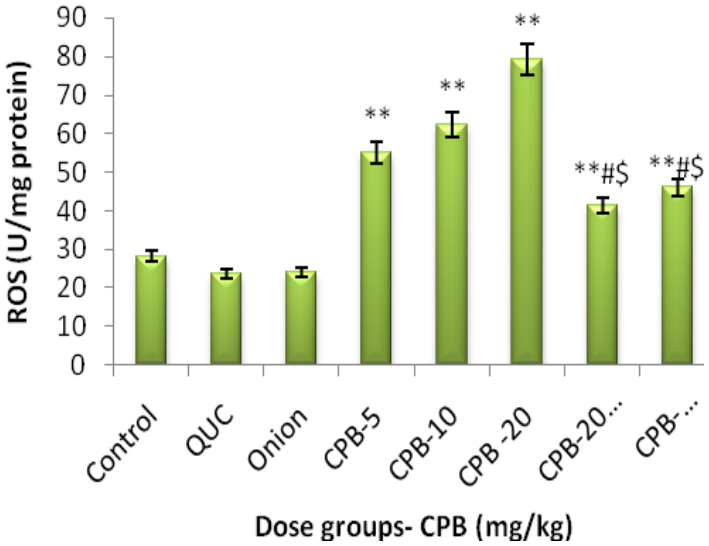


Fig 2: Bar chart showing the effect of CPB on testicular reactive oxygen species (ROS)generation level and protective effect of QUC and onion extract in mice. All the values are expressed as mean ± SEM, (n=5), ***P<0.001, **P<0.01 and*P<0.05, # vs. control and $ vs. CPB-20

3.5. Effect of QUC and Onion extract on MDA level of CPB-treated testicular tissue of mice

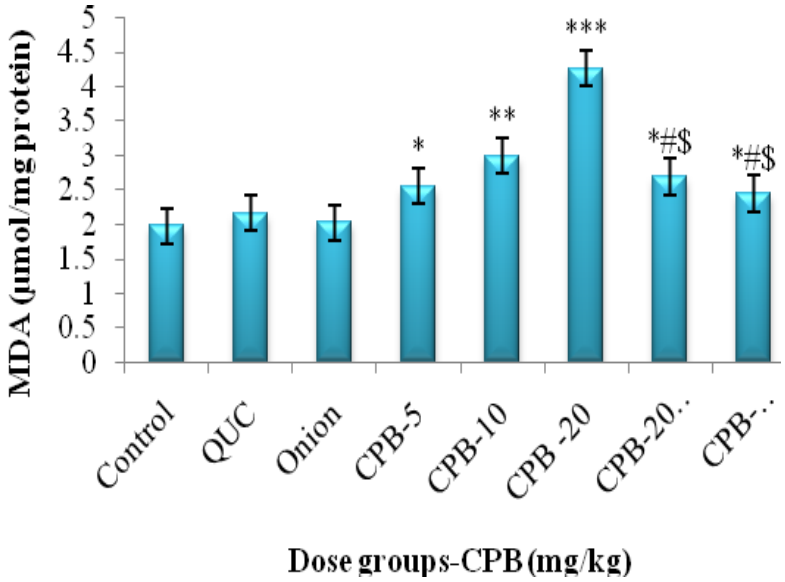


Figure 3: Bar chart showing the effect of CPB on testicular malondialdehyde (MDA)level and protective effect of QUC and onion extract in mice. All the values are expressed as mean ± SEM, (n=5), ***P<0.001, **P<0.01 and*P<0.05, # vs. control and $ vs. CPB-20.

3.6 Testis histology Observation

Vacuolization, disorganization and decreased spermatogonial and spermatid counts were seen in the histological observation of CPB-treated seminiferous tubules. The quantitative assessment of the seminiferous tubules was done based on the extent of damage induced by CPB (Fig. 5). Significant seminiferous tubule damage was observed at all the doses of CPB (5, 10, and 20mg/kg/day) as compared to the control group (P<0.01) after 21 days of treatment. However, decrease seminiferous tubule damage was observed and scored in groups pre- treated with QUC and OE in comparison with CPB-treated groups.

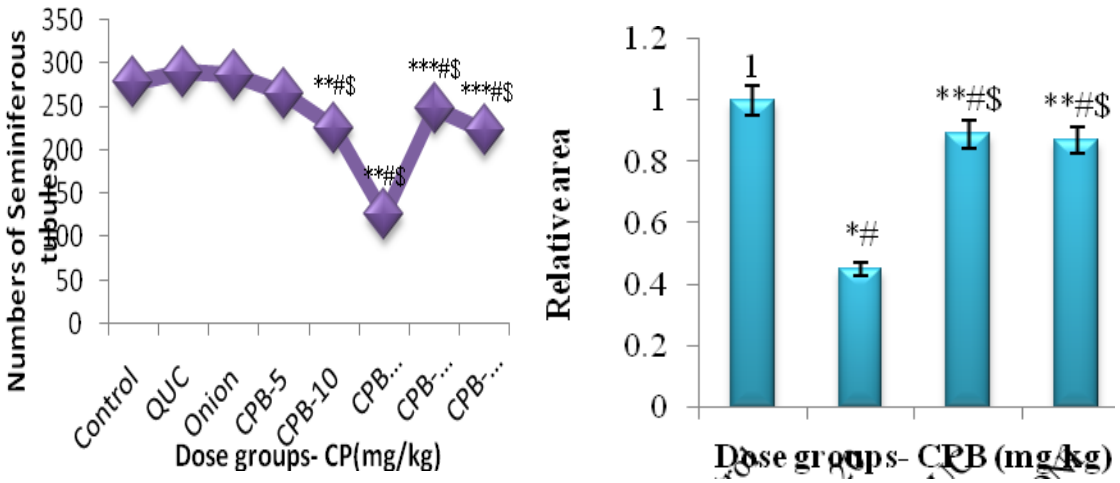


Fig. 4: Effects of QUC and Onion extract on CPB-induced germ cell toxicity. Comparison of normal seminiferous tubules was carried out among the control, CP- treated, CP+QUC and CP+ONI groups. Relative area was calculated by dividing the area of treatment group with the area of control group. All the values are expressed as mean ± SEM, (n=5), ***P<0.001, **P<0.01 and*P<0.05, # vs. control and $ vs. CPB-20.

3.7. Sperm comet and halo assay

Tail length, DNA in Tail, Tail movement showed a significant increase DNA damage in the sperm analyzed by using sperm comet assay at CPB 20mg/kg as compared with the control group (P<0.05) (Table 2). DNA damage in the testes was also revealed by the halo assay following CPB treatment (Fig. 10). After 21 days CPB exposure, the % of mild and extensively damaged testicular cells was found to be increased (P<0.01) as compared with the control group (Fig. 11). However, all in all the test, significant protective effect of QUC and Onion extract was seen.

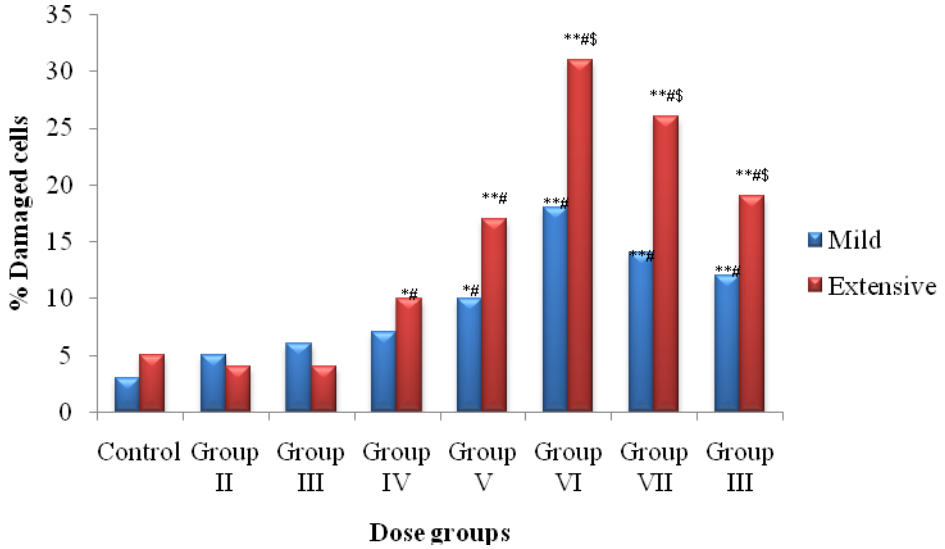


Fig: Ameliorative potential of Onion extract and QUC on % damaged cells following CPB- treatment determined by halo assay in testis. All the values are expressed as mean ± SEM, (n=5), ***P<0.001, **P<0.01 and*P<0.05, # vs. control and $ vs. CPB-20.

Table 1: Effects of Quercetin and Onion on Body, Testis, and Epididymis Weights in CPB-Treated Mice.

Body weight (g)	Vehicle-treated	5mg/kg/day CPB	10mg/kg/day CPB	20mg/kg/day CPB	10mg/kg/day QUC	10mg/kg/day OE	20mg/kg/day, CPB + 10mg/kg/day OE	20mg/kg/day, CPB + 10mg/kg/day QUC
Body weight (g)	26.07± 1.04	23.20 ± 0.07	21.16 ± 0.04*	17.85 ± 0.03*	25.77 ± 0.04	24.39 ± 0.10	23.56 ± 1.12**	24.62 ± 1.24***
Testis weight (mg)	129±2.01	122±1.06	114±1.31	111±0.12	106±1.01	132±0.12	128±0.21	131±0.31
Relative testis weight (mg/g body weight)	4.96±0.01	5.26±0.10	5.39±0.11	6.53±0.01	4.11±0.01	5.41±0.12	5.53±0.16	5.32±1.01
Epididymis weight (mg)	32.11± 1.72	31.26±0.61	28.24±0.45	25.13±1.38	33.21±0.22	34.14±0.16	36.29±0.72	34.17±0.54
Relative epididymis weight (mg/g body weight)	1.23±0.02	1.35±1.05	1.34±0.22	1.41±0.12	1.29±1.01	1.32±0.12	1.54± 0.03	1.39±0.09

Table 2: Effects of QUC and Onion Extract on reproductive Hormones and antioxidant enzymes in Testicular tissue of CPB-Treated Mice.

Body weight (g)	Vehicle-treated	5mg/kg/day CPB	10mg/kg/day CPB	20mg/kg/day CPB	10mg/kg/day QUC	10mg/kg/day OE	20mg/kg/day, CPB + 10mg/kg/day OE	20mg/kg/day, CPB + 10mg/kg/day QUC
Testosterone	3.92 ± 0.05	3.41 ± 0.01	3.02 ± 0.04	2.75 ± 0.01	3.77 ± 0.01	3.59 ± 0.01	3.17 ± 0.01	3.58 ± 0.05
LH	8.16 ± 0.11	6.42 ± 0.02	4.88 ± 0.01**	3.36 ± 0.15**	8.01±0.21	7.69±0.22	5.26±0.01	5.02±0.11
SOD (U/ml/min)	10.33 ± 0.15	8.16 ± 0.11**	6.72 ± 0.45***#$	3.24 ± 0.17**	11.79 ± 0.14	8.76 ± 0.01*	7.47 ± 0.10**#$	7.33 ± 0.02**#$
CAT (mmol/min)	46.11 ± 0.41	40.03 ± 0.18	36.09 ± 2.26*#	29.46 ± 0.34*#	55.84 ± 0.08**#	51.94 ± 0.12**#	32.88 ± 5.15***$#	34.56 ± 0.26***#$
GSH µM/mg protein	77.41 ± 1.40	62.11 ± 1.07***#	58.43±0.50***#	43.27±3.24***#	92.17 ± 1.52*	85.65 ± 1.10***	63.24 ± 2.52*	74.50 ± 4.22**$

Table 3: Effects of QUC and Onion extract on CPB induced sperm DNA damage as revealed by comet assay.

Dose groups	Tail length µm	%DNA in Tail	Olive tail movement	Tail movement
Control	15±0.01	11.36 ± 1.06	3±0.04	19±0.12
5mg/kg/day, CPB	27±0.12	20.3±0.02	2±0.11	31±0.08
10mg/kg/day, CPB	42±0.22	29.78± 0.02	2±0.07	47±0.16
20mg/kg/day, CPB	66±0.001	37.5±0.04	5±0.15	68±0.45
QUC (10mg/kg)	14±0.07	11.57±0.16	7±0.05	16±0.22
Onion Extract (10µl/ml)	16±0.11	12.7±0.01	13±0.12	20±0.01
20mg/kg/day, CPB + 10mg/kg/day Onion extract	32±0.04	20.79±0.16	7±0.13	36±0.18
20mg/kg/day, CPB + 10mg/kg/day QUC	39±0.02	23.53±0.17	6±0.14	44±0.04

DISCUSSION

The protective action of polyphenols has been reported for various pathological conditions.[23,24] Quercetin and onion extract belong to this class of polyphenols; their anti-oxidative and anti-inflammatory properties have likewise been reported. As cancer remains a prevalent disease all over the world, the development of anti-cancer drugs became expedient in order to serve health concerns. Toxicity of capecitabine in germ cells of mice is the main concern of this study as the toxicity of most anti-cancer drugs on fertility has been reported by various researchers; The ameliorative effect of jambolanon in the CPB-induced testicular damage has been reported likewise.[25] Present study investigated ameliorative potential of quercetin and onion extract on the cytotoxicity and genotoxicity induction following CPB administration.

Present findings showed that treatment with CPB resulted in decreased body weight, organ weight and cell viability and co-treatment with quercetin and onion extract caused a significant increase in the body weight, organ weight and cell viability of testicular cells following CPB administration.

Abnormalities in sperm morphology and decreased sperm count were observed in the testis of mice treated with CPB and this is in line with the report of testicular damage following treatment with CPB or fluouracil.[25,26,27] The viability of sperm is expedient in fertility and the viability of sperm is dependent on these parameters: Sperm morphology, sperm count and sperm motility, hence these parameters are important in the reproduction process. The observed decrease in the sperm count and abnormal sperm morphology could be said to be responsible for the infertility threat that has been associated with the anti-cancer drug Capecitabine, however pre-treatment with quercetin and onion extract caused a recovery of the normal morphology of the sperm and significant increase in sperm count of mice treated with CPB.

Oxidative stress was confirmed by increase level of reactive oxygen species and MDA activity in a dose dependent manner in the testicular tissue of mice treated with CPB. This could have been as a result of CPB causing damage to the anti-oxidant defense system making it defective in its response. Oxidative stress is a common pathology that has been implicated in male infertility. Induction of oxidative stress was as a result of increase in the free radical in testicular tissue resulting in deregulation of spermatogenesis.[21] The role of Oxidative stress has been suspected to be the major reason responsible for the decrease in sperm count, impaired sperm motility and abnormal spermatozoa leading to cytotoxicity.[23] however, it was observed that co-treatment with quercetin and onion extract decreased ROS and in MDA levels signifcantly. This could be attributed to the anti-oxidative and anti-inflammatory properties of quercetin and Onion extract.

In addition to this, significant seminiferous tubule damage was observed in a dose dependent manner in the caudal epididymidis of CPB treated mice indicating cytotoxicity of CPB in the testes of the mice. Heba and Abdelalim [25,28] reported testicular damage in the form of atrophied seminiferous tubules with focal disorganization of seminiferous tubules with marked depletion of the spermatogenic cell populations. Seminiferous tubules are the site of the germination, maturation and transportation of the sperm cells within the male testes and thus play a vital role in spermatogenesis hence the impaired sperm parameters observed in the testicular tissue of mice treated with CPB. Quercetin and onion extract caused a significant recovery of the seminiferous tubules in the testicular tissue of mice treated with CPB; this could be as a result of the abilities of quercetin and onion extract to reverse the damage of treatment with CPB on the seminiferous tubule. Significant increase in DNA damage was observed following Capecitabine. The sperm DNA integrity is important for the success of fertilization as well as normal development of the embryo, fetus and child and hence this could be said to be responsible for the degenreation of seminiferous tubules seen in this study. Oxidative stress causes increase in DNA breakdown. Besides that, evidence indicates that fragmentation of DNA is, commonly seen in infertile people's spermatozoa, is due to the ROS high concentration in the sperm.[29,30] In view of the present observations as well as the above cited reports, it is relevant to presume that quercetin and onion extract effectively ameliorate the cytotoxicity and genotoxicity induced by treatment with capecitabine, hence induction of oxidative stress which is known to be a major cause of infertility in men, and hence, the patient must first be administered quercetin or use onion extract to ameliorate CPB reproductive toxicity.

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