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(RESEARCH ARTICLE)

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Antioxidative properties of baicalin on methylparaben-induced testicular oxidative stress and oxidative DNA damage in a rat experimental model

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Abstract

Parabens, a group of related chemicals, are commonly used as preservatives in cosmetic and pharmaceutical products. Among them, methylparaben is the most frequently used. However, increasing concerns have been raised on the health risks of methylparaben regarding its widespread applications and potential endocrine-disrupting activities and reproductive impairments. Presently, there is no conclusive evidence either way. Therefore, this study aimed to determine the ability of baicalin to protect rat testes against methylparaben-induced toxicity based on some biochemical and histological parameters. Fourty-five adult male Wistar rats were divided into nine groups of five animals each. The animals were administered methylparaben alone at 1000 mg/kg/bw; three groups received different doses of baicalin and another three concomitantly received baicalin at the three doses and methylparaben at the fixed dose. The histology of the testes was examined together with oxidative stress markers such as catalase, reduced glutathione, and superoxide dismutase as well as 8-Hydroxydeoxyguanosine, a marker for oxidative DNA damage. Results show that the methylparaben-treated group exhibited a significant increase in oxidative stress with raised levels of malondialdehyde and 8-Hydroxydeoxyguanosine and reduced levels of catalase, reduced glutathione, and superoxide dismutase compared to the control and baicalin alone groups. Methylparaben also induced considerable degenerative changes in the histology of the testes. Co-administration of baicalin mitigated these adverse effects resulting in significant improvement in all oxidative stress markers and protection of the testes from histological damage. We conclude that exposure to methylparaben caused testicular toxicity which can be mitigated by concomitant administration of baicalin.

Keywords: Methylparaben; Baicalin; Oxidative stress; Toxicity

1. Introduction

Parabens are one of the most well-known preservatives in use and current literature classifies parabens as a group of endocrine-disrupting chemicals, EDCs [1]. There are increasing concerns about the health risks of parabens regarding their broad applications and potential endocrine-disrupting activities. Parabens might disturb the body's endocrine system by activating endocrine receptors and disrupting the synthesis and secretion of steroid hormones, suggesting their potential harm to the environment and human health [2].

Chemically, parabens are alkyl or aryl homologs of the p-hydroxybenzoic acid (p-HBA). They dissolve easily in alcohol, ether, and acetone. Physically, parabens are colourless crystal or crystalline powders at room temperature [3]. It has been reported that methyl-, ethyl-, propyl-, butyl-, and benzyl-paraben (MeP, EtP, PrP, BuP, and BzP, respectively) are the most commonly used parabens [1, 4].

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Methylparaben (CAS No. 99-76-3) is a methyl ester of p-hydroxybenzoic acid. Methylparaben is a stable, non-volatile compound that has been used as an antimicrobial preservative in foods, drugs, and beauty care products for over 50 years. Methylparaben is easily absorbed through the skin and from the gastrointestinal tract. Following absorption, it is hydrolyzed to p-hydroxybenzoic acid and conjugated, and the conjugates are rapidly excreted in the urine [5]. Methylparaben is widely used as an antimicrobial preservative in beauty care products, food products, and pharmaceutical formulations. It may be utilized either alone, in combination with other parabens, or with other antimicrobial agents. In cosmetics, methylparaben is the most frequently used antimicrobial preservative [6]. Different studies have reached contrasting conclusions about the effects of methylparaben on living organisms. Soni et al. [5] report that methylparaben is not carcinogenic or mutagenic; it is not teratogenic or embryotoxic and is negative in the uterotrophic assay. However, studies suggest that methylparaben may accumulate in the body, resulting in the formation of radicals [7, 8], endocrine disruption and reproductive toxicity [9].

Baicalin (7-glucuronic acid,5,6-dihydroxy-flavone) is one of the most commonly used bioactive flavonoids in Chinese medicine. It is a bioactive flavonoid isolated from Scutellariae Radix (the dried roots of *Scutellaria baicalensis* Georgi) and offers multiple potential pharmacological activities including anti-fibrotic, anti-pruritic, anti-bacterial, anti-oxidant, anti-inflammatory and anti-cancer effects [10, 11]. Baicalin has been shown to ameliorate oxidative stress [12] and reduce the levels of reactive oxygen species and malondialdehyde [13]. In diabetic nephropathy, baicalin alleviated oxidative stress, and its underlying mechanism was associated with the activation of the Nrf2-mediated antioxidant signalling pathway [14]. While the pharmacological activities of baicalin have been reported, whether it has protective effects on methylparaben-induced testiculotoxicity remains elusive. Further research is needed to remove any ambiguities about the potential health risks of exposure to methylparaben. Therefore, this study was carried out to assess the antioxidative effects of baicalin on methylparaben-induced testicular oxidative stress markers and DNA.

2. Materials and methods

2.1. Study Area

This study was carried out at the Anatomical Sciences Laboratory of the Department of Anatomy, College of Medicine, University of Nigeria Enugu Campus.

2.2. Methylparaben

Methylparaben (CAS 99-76-3; EC 202-785-7; Sharon Laboratories Limited, Israel), was purchased from Ejis Chemicals, 3960, Ikorodu Road, Kosofe, Lagos.

2.3. Baicalin

Baicalin (Botany Biosciences, USA) was obtained from Ossy Stores, Lagos Nigeria.

2.4. Animals

Fourty-five (45) adult male Wistar rats were used for this study. They were purchased from the Animal House of the Department of Anatomy, University of Nigeria Enugu Campus. The rats were randomly divided into nine (9) groups of five (5) rats each, housed in clean cages at room temperature (25–32°C) in a 12-hour light/darkness rhythm in the Animal House of the Department of Anatomy, University of Nigeria Enugu campus, under standard environmental conditions. The rats had free access to laboratory chow and drinking water and were allowed to acclimatize to their new laboratory environment and food for 2 weeks before the study.

2.5. Animal Treatment

- Group 1: (Normal control); 1ml/kg bw of distilled water for 28 days
- Group 2: (Vehicle Control); 1ml/kg bw of peanut oil for 28 days
- Group 3: 1000 mg/kg/bw of Methylparaben only (dissolved in peanut oil) for 28 Days
- Group 4: (Low Dose Baicalin) Baicalin only 50mg/kg/bw for 28 Days
- Group 5: (Medium Dose Baicalin) Baicalin only100mg/kg/bw for 28 Days
- Group 6: (High Dose Baicalin) Baicalin only 200mg/kg/bw for 28 Days
- Group 7: Protective (Low Dose) Baicalin 50mg/kg/bw + Methylparaben 1000mg/kg/bw for 28 Days
- Group 8: Protective (Medium Dose) Baicalin 100mg/kg/bw + Methylparaben 1000mg/kg/bw for 28 Days
- Group 9: Protective (High Dose) Baicalin 200mg/kg/bw + Methylparaben 1000mg/kg/bw for 28 Days

All treatments were administered by gastric gavage. Treatment was given once a day at 10.00 am. At the end of the experiment, the animals were sacrificed under ketamine anaesthetics. The testis and epididymis were dissected out of each rat, cleared of fat, and weighed separately. One testis was fixed in formol saline for histological studies and the other was macerated in distilled water and used for biochemical assays.

2.6. Biochemical Assays

2.6.1. Assay of catalase (CAT) activity

The principle of the assay is that the enzymatic reaction of catalase (CAT) in decomposing H_2O_2 can be quickly stopped by ammonium molybdate. The residual H_2O_2 reacts with ammonium molybdate to generate a yellowish complex. CAT activity can be calculated by the production of the yellowish complex at 405 nm against blank. Briefly, tissue samples were homogenized in 10 vol. of 50 mmol/l phosphate buffer, pH 7.4. (e.g. 0.5g tissue in 5ml phosphate buffer). The homogenates were centrifuged for 20 min at 3000 rpm. The supernatant was collected and used in the assay. Into a cuvette 200ul of the sample was added. Then 1ml of the substrate solution was added into the cuvette mix and allowed to stand for 1 minute. Then 1ml of molybdate solution was added into the cuvette and mixed for 2 minutes. The absorbance was read at a wavelength of 405nm [16].

2.6.2. Assay of superoxide dismutase (SOD) activity

The principle of the assay is that the enzyme superoxide dismutase can inhibit the autoxidation of pyrogallol. The autoxidation of pyrogallol in the presence of EDTA at pH 8.2 is 50%. The principle of this method is based on the competition between the pyrogallol autoxidation by $O_2\bullet^-$ and the dismutation of this radical by SOD. Briefly, the tissue samples were homogenized in 10 vol. of 50 mmol/l phosphate buffer, pH 7.4. (e.g. 0.5g tissue in 5ml phosphate buffer). The homogenates were centrifuged for 20 min at 3000 rpm. The supernatant was collected and used in the assay. Into a clean cuvette 50ul of the sample was added. Then 1ml of SOD assay buffer was added to the cuvette. After that 1ml of SOD chromogen solution was added to the cuvette and mixed. The absorbance was read immediately at 420nm, then read again after 1 minute [17].

2.6.3. Assay of reduced glutathione (GSH) activity

These spectrophotometric procedures are based on the method of Ellman [18], who reported that 5,5'-dithiobis- (2,-nitrobenzoic acid) is reduced by SH groups to form 1 mole of 2-nitro-5-mercaptobenzoic acid per mole of SH. The nitromercaptobenzoic acid anion has an intense yellow colour which when measured at a wavelength of 412nm can be used to measure SH groups. Briefly, 0.5g of the tissue was rinsed with PBS. It was then homogenized in 2ml protein precipitation reagent and centrifuged at 3000rpm for 10 minutes. The clear supernatant was used in the assay. Into clean cuvettes were placed 100ul of standard and samples. To the glass tube 800ul of GSH dilution buffer was added followed by 100ul GSH Chromogen which was mixed well. Absorbance was measured at 412nm within 5 minutes.

2.6.4. Estimation of lipid peroxidation (Malondialdehyde, MDA)

The principle of this assay is based on the reaction of a chromogenic reagent, 2-thiobarbituric acid, with MDA at 25°C. One molecule of MDA reacts with 2 molecules of 2-thiobarbituric acid via a Knoevenagel-type condensation to yield a chromophore with an absorbance maximum of 532 nm. Briefly, 0.5g of the tissue was homogenised in a 2.5 ml protein precipitation reagent. This was mixed and centrifuged at 3,000rpm for 5 minutes. The supernatant was used for the assay. Into glass test tubes was added 200µl of sample and 200µl of indicator solution and mixed well. The mixture was allowed to react for 45 minutes at room temperature. Then 300µl was transferred to a microplate and the absorbance of the resulting solution was measured at 532 nm. The pink colour was stable for several hours at room temperature [19].

2.6.5. Assay of 8-Hydroxydeoxyguanosine (8-OHdG)

To each well was added 50µL standard or sample. Immediately, 50µL biotinylated detection Ab was then added to each well and incubated for 45 min at 370C. The solution was then aspirated and washed 3 times. To each well 100µL HRP Conjugate to was added and incubated for 30 min at 370C then aspirated and washed 5 times. To this was added 90µL substrate reagent and incubated for 15 min at 370C. Then 50µL stop solution was added, read at 450nm immediately and results calculated.

2.7. Histology

Tissue sections from testes and epididymis were prepared for histologic examination by the method of Sheehan and Wrapshak [20]. Briefly, the specimens were cleared of fixative (Bouin's fluid), and dehydrated in graded alcohol. They

were then blocked out in paraffin and cut into 0.5 μ sections. The sections were deparaffinized, rehydrated and stained with haematoxylin and eosin. They were examined in a Ceti microscope fitted with an XLI camera and software at total magnifications ranging from between 100 and 1000.

2.8. Statistical Analysis

All quantitative data was analyzed using GenStat software for Windows (Release 17.1). One-way analysis of variance (ANOVA) was used to compare the mean differences. P-value less than 0.05 ($p<0.05$) was considered to be statistically significant. Duncan's New Multiple Range Test (DNMRT) was conducted for any of the tests that was found to be significantly different across the tables.

3. Results

3.1. Antioxidants

Depicted in table 1 is the analysis of variance (ANOVA) of the mean levels of antioxidant markers: catalase (CAT), superoxide dismutase (SOD), glutathione (GSH) and malondialdehyde (MDA). The table shows that there was a statistically significant difference when the mean differences of CAT, GSH, SOD and MDA were compared between the various groups of the experiment.

Table 1 The Mean Levels of Antioxidative Markers CAT, SOD, GSH and MDA Among Various Groups Of The Experiment

Group	Catalase (U/mg protein)	SOD (U/ml)	GSH (mM)	MDA (μM)
Group 1	3.81 a	1.23 a	1.03 c	0.66 b
Group 2	3.57 a	1.36 a	1.01 c	0.63 b
Group 3	2.09 b	0.92 b	0.55 d	1.22 a
Group 4	4.24 a	1.26 a	1.22 abc	0.63 b
Group 5	4.02 a	1.23 a	1.26 abc	0.59 b
Group 6	3.73 a	1.36 a	1.25 abc	0.64 b
Group 7	3.87 a	1.40 a	1.13 bc	0.56 b
Group 8	3.69 a	1.44 a	1.29 ab	0.62 b
Group 9	3.43 a	1.42 a	1.40 a	0.62 b
P-value	<0.001	<0.001	<0.001	<0.001
Cv (%)	12.40	8.90	11.60	8.60

Means with the same letter in a column are not significantly different from each other (DNMRT, $P > 0.05$); SOD = Superoxide Dismutase; GSH = Glutathione; MDA = Malondialdehyde

- Group 1 = (Normal control) Distilled Water at 1 ml/kg bw
- Group 2 = (Vehicle Control) Peanut oil at 1 ml/kg bw
- Group 3 = Methylparaben only (Dissolved in Peanut Oil) at 1000 mg/kg/bw
- Group 4 = (Low Dose Baicalin) Baicalin only at 50 mg/kg/bw
- Group 5 = (Medium Dose Baicalin) Baicalin only at 100 mg/kg/bw
- Group 6 = (High Dose Baicalin) Baicalin only at 200 mg/kg/bw
- Group 7 = Protective (Low Dose) Baicalin + Methylparaben at 50 mg/kg/bw + 1000mg/kg/bw
- Group 8 = Protective (Medium Dose) Baicalin + Methylparaben at 100 mg/kg/bw + 1000mg/kg/bw
- Group 9 = Protective (High Dose) Baicalin + Methylparaben at 200 mg/kg/bw + 1000mg/kg/bw

The mean level of CAT in the testes of adult male Wistar rats in group 3 was significantly lower ($p < 0.001$) than the mean level of CAT in the testes of adult male Wistar rats of all the other experimental groups.

The mean SOD levels in the testes of adult male Wistar rats in group 3 was significantly lower ($p < 0.001$) than the mean levels of SOD in the testes of adult male Wistar rats all the other experimental groups.

The mean value of GSH in the testes of adult male Wistar rats in group 3 was significantly lower ($p < 0.001$) than the mean level of GSH in the testes of adult male Wistar rats in all the other experimental groups. The mean values of GSH in the testes of adult male Wistar rats in groups 4, 5 and 6 were not significantly different from each other.

The mean value of MDA in the testes of adult male Wistar rats in group 3 was significantly higher ($p < 0.001$) than the mean values of MDA in the testes of adult male Wistar rats in all the other experimental groups.

3.2. 8-Hydroxydeoxyguanosine (8-OHdG)

Shown in Table 2 is the ANOVA comparison of the differences in the mean values of 8-Hydroxydeoxyguanosine (8-OHdG) in the testes of adult male Wistar rats among the different groups of the experiment.

The mean level of 8-OHdG in the testes of adult male Wistar rats in group 3 was significantly higher ($p<0.001$) than the mean level of 8-OHdG in the testes of adult male Wistar rat in groups 1 and 2. The mean level of 8-OHdG of adult male Wistar rats in groups 4, 5, 6, 7, 8 and 9 were significantly lower than that of group 3. The mean values of 8-OHdG in groups 2, 7 and 8 were similar; groups 4 and 5 also had similar values, not significantly different from each other.

Table 2 The mean level of 8-OHdG (sperm DNA fragmentation marker) in the testes of Adult Male Wistar rats among the various groups of the study

Group	8-OHdG (µg/ml)
Group 1	5.59 b
Group 2	5.28 bcd
Group 3	7.25 a
Group 4	5.14 cd
Group 5	5.14 cd
Group 6	5.06 d
Group 7	5.31 bcd
Group 8	5.28 bcd
Group 9	5.50 bc
P-value	<0.001
Cv (%)	3.70

Bars (means) with the same alphabet are not significantly different from each other (DNMRT, $P > 0.05$)

- Group 1 = (Normal control) Distilled Water at 1 ml/kg bw
- Group 2 = (Vehicle Control) Peanut oil at 1 ml/kg bw
- Group 3 = Methylparaben only (Dissolved in Peanut Oil) at 1000 mg/kg/bw
- Group 4 = (Low Dose Baicalin) Baicalin only at 50 mg/kg/bw
- Group 5 = (Medium Dose Baicalin) Baicalin only at 100 mg/kg/bw
- Group 6 = (High Dose Baicalin) Baicalin only at 200 mg/kg/bw
- Group 7 = Protective (Low Dose) Baicalin + Methylparaben at 50 mg/kg/bw + 1000mg/kg/bw
- Group 8 = Protective (Medium Dose) Baicalin + Methylparaben at 100 mg/kg/bw + 1000mg/kg/bw
- Group 9 = Protective (High Dose) Baicalin + Methylparaben at 200 mg/kg/bw + 1000mg/kg/bw

4. Discussion

Parabens are a group of substances that are widely used as antibacterial preservatives in packaged foods, pharmaceuticals, and cosmetic products. This has led to their residual presence in various environment matrices and inevitable human exposure [21]. Humans are usually exposed to parabens through skin absorption, ingestion, and inhalation of contaminated air [22]. In recent years, studies conducted in animal models showed the male reproductive toxicity caused by some parabens [23]. Much has already been written about parabens' toxicological effect, yet it remains a subject that is not consensual [24, 23]. Studies on gene expression profiles show that parabens such as

methylparaben cause toxicity by inducing oxidative stress, DNA double-strand breaks, and apoptosis as well as by altering fatty acid metabolism [25].

The administration of methylparaben in Group 3 increased lipid peroxidation and disturbed the testicular antioxidant state significantly, which was evidenced by the increase in the mean testicular Malondialdehyde (MDA) levels and the reduction in mean testicular levels of Superoxide Dismutase (SOD), Glutathione (GSH) and Catalase (CAT) compared to the control groups. Methylparaben can trigger the formation of free radicals in the human body [7]. Ateş et al. [26] found that methylparaben exposure increased lipid peroxidation. In their study on paraben's effects on rats' testes and genotoxicity, Martins et al. [27] found that parabens can affect the rat testis enzymatic antioxidant system, decreasing the cellular antioxidant capacity. Their finding is in agreement with those of this study. The groups administered baicalin only showed preservation of their antioxidant levels comparable to those of the control. In the groups co-administered with baicalin and methylparaben, baicalin mitigated the pro-oxidant activities of methylparaben, keeping the antioxidant levels significantly higher than those of the group administered with methylparaben only and comparable to those of the control groups.

It is known that there is a balance between oxidative stress and the body's antioxidant defence mechanisms. When the body's antioxidant capacities are exceeded by the production of reactive oxygen species (ROS), a metabolic imbalance known as oxidative stress occurs. These excess ROSs, formed in excess amounts during metabolism cause oxidative damage. Their actions include oxidation and disruption of biomolecules such as lipids, proteins, and nucleic acids, which are the main building blocks of cell membranes. Beyond disrupting essential molecules, ROS can also lead to the death of cells. Reactive oxygen species have also been linked to the pathogenesis of many different human disorders that disrupt the operation of the genetic mechanism. Nonetheless, antioxidants may mitigate such oxidative damage, thus preventing and alleviating illnesses caused by oxidative stress. Furthermore, when taken with food, exogenous antioxidants that cannot be produced by the body, can strengthen the body's antioxidant defences. These exogenous antioxidants, which can be sourced from fruits and vegetables are powerful and have extensive actions. In this context, antioxidants obtained from these sources have the ability to remove ROS easily [28]. Baicalin has been shown to possess potent antioxidant properties [10, 29]. In this study, baicalin, serving as an antioxidant, protected the administered animals from the pro-oxidant effects of methylparaben.

Methylparaben administration resulted in a statistically significant increase in the mean level of 8-Hydroxydeoxyguanosine (8-OHdG) in the testes of adult male Wistar rats in group 3 compared to the control groups (Table 2). 8-Hydroxydeoxyguanosine (8-OHdG) is a widely used biomarker of oxidative DNA damage [30]. Exposure to methylparaben has been shown to cause oxidative damage to DNA. Previous research has shown that exposure to methylparaben in various organisms can cause oxidative stress. The formation of 8-OHdG occurs due to metabolic interactions in the body that make methylparaben bioactivated to form by-products in the form of hydroxy radicals that attach to DNA Guanine. [31, 7]. Ishikawa et al. [32] observed that increased 8-OHdG expression in the testis was associated with deficient spermatogenesis. When rat testes were exposed to radiofrequency radiation emitted from cellular phones, single-strand DNA breaks and oxidative changes marked by increased 8-hydroxydeoxyguanosine (8-OHdG) were recorded [33]. Since co-administration of baicalin and methylparaben, resulted in attenuation of the testicular levels of 8-OHdG, it is thought that baicalin protected the testes from oxidative DNA damage by serving as an antioxidant, inhibiting the pro-oxidative actions of methylparaben.

The histological sections of the control groups (Fig 1 and Fig 2) exhibited normal testicular structure, with oval or rounded seminiferous tubules that were lined with a thick stratified germinal epithelium resting on a basement membrane. The spermatogenic cells were arranged in several layers between the basal lamina and lumen of the tubules that appeared as small round cells. Besides, spermatids were arranged in two to three rows close to the lumen of the tubules. Furthermore, the tubular lumens were filled with elongated mature spermatozoa. Histological sections showed that the administration of methylparaben caused marked depletion of spermatogenic cells (Fig 3) in the seminiferous tubules accompanied by vacuolization in the lumen of the tubes and sloughing of cells as well as loss of cell-to-cell adhesion. There was also a marked depletion in the volume of free mature spermatozoa that could be seen in the central areas of the tubular lumen. This depletion in sperm cell numbers may suggest an interruption in spermatogenesis. In the interstitial tissue, Leydig cells were also depleted in number. The groups that received baicalin showed preservation of their testicular architecture (Fig 4, Fig 5 and Fig 6). In the groups that were administered both baicalin and methylparaben, there was also preservation of their testicular architecture with intact seminiferous tubule boundaries, preserved germinal cells and free mature spermatozoa in the lumen (Fig 7, Fig 8 and Fig 9). These sections were comparable to those of the control groups. This shows that the baicalin was able to mitigate against methylparaben-induced injury in the testes. In a study on the effect of methylparaben on the testes of zebrafish, Hassanzadeh [34] observed that methylparaben caused histological alteration of the testis which consisted of general testicular atrophy, multi-nucleated gonocytes (MNGs), impaired germ cell, spermatogonial proliferation, Leydig cell hyperplasia,

interstitial fibrosis and apoptosis of Sertoli cells. He concluded that sub-chronic exposures of methylparaben could disrupt the histology of the testis and produce estrogenic and antispermatogenic activity in male zebrafish. Shen et al. [23] also associated methylparaben with declining sperm concentration in the testes. These negative associations between methylparaben and testicular tissue may be due to oxidative stress caused by methylparaben explained earlier.

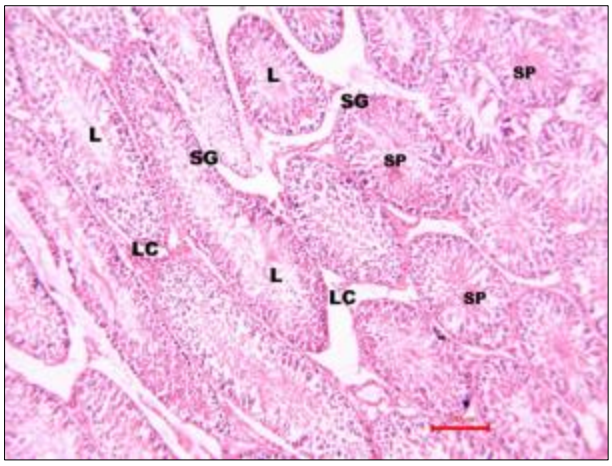

Figure 1 A photomicrograph of a section of the test is presented in this group (Group 1) showed normal seminiferous tubules with moderately active spermatogenesis. Spermatogonia (SG) resting upon the basement membrane, a cluster of spermatozoa (SP) in the lumen (L) and Interstitial tissue containing Leydig cells (LC) were observed. (H and E, × 100)

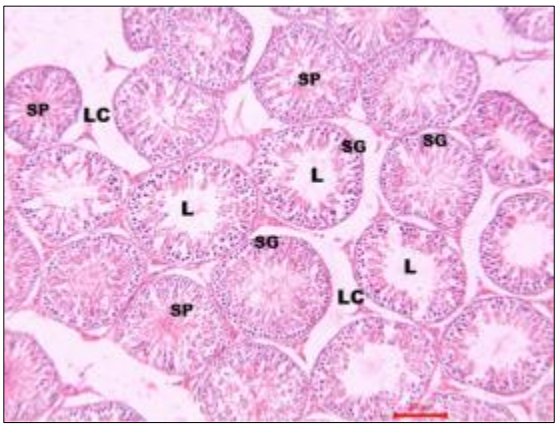

Figure 2 A photomicrograph of a section of the test is presented in this group (Group 2) showed normal seminiferous tubules (arrow) with moderately active spermatogenesis. Spermatogonia (SG) resting upon the basement membrane, a cluster of spermatozoa (SP) in the lumen (L) and Interstitial tissue containing Leydig cells (LC) were observed. (H and E, × 100)

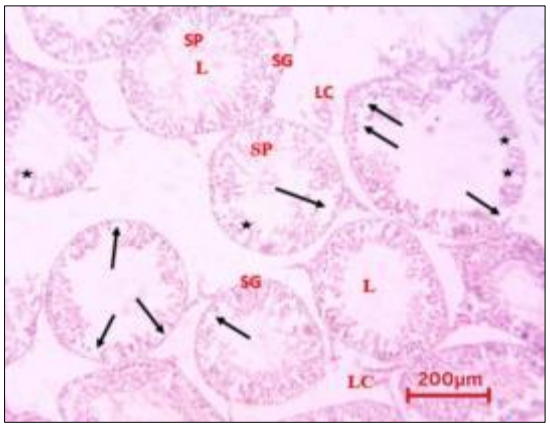

Figure 3 A photomicrograph of a section of the test is presented in this group (Group 3) showed marked depletion of spermatogenic cells (black arrows). Noticed vacuolations (black stars). Spermatogonia (SG), cluster of spermatozoa (SP) in the lumen (L) and Leydig cells (LC) which were also depleted in number in the interstitial tissue were observed. (H and E, × 100)

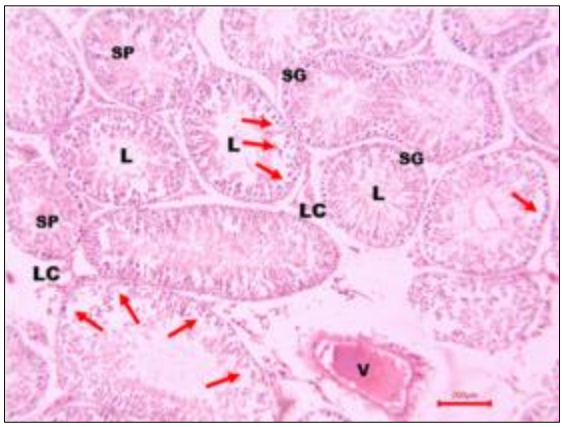

Figure 4 A photomicrograph of a section of the test is presented in this group (Group 4) showed mild depletion of spermatogenic cells (red arrows). Spermatogonia (SG), a cluster of spermatozoa (SP) in the lumen (L), Leydig cells (LC) in the interstitial tissue and an enlarged blood vessel (V) were observed. (H and E, × 100)

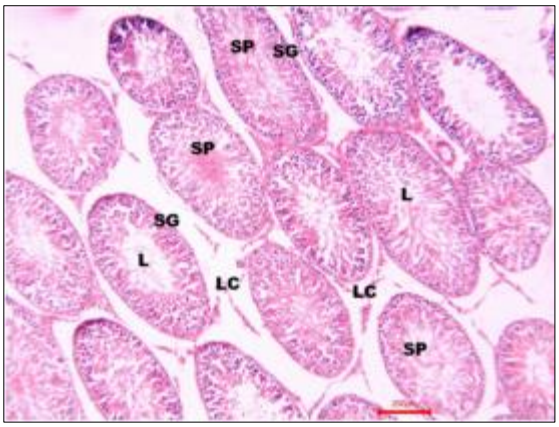

Figure 5 A photomicrograph of a section of the test is presented in this group (Group 5) showed normal seminiferous tubules (arrow) with moderately active spermatogenesis. Spermatogonia (SG) resting upon the basement membrane, a cluster of spermatozoa (SP) in the lumen (L), interstitial tissue containing Leydig cells (LC) and vein (V) were observed. (H & E, x100)

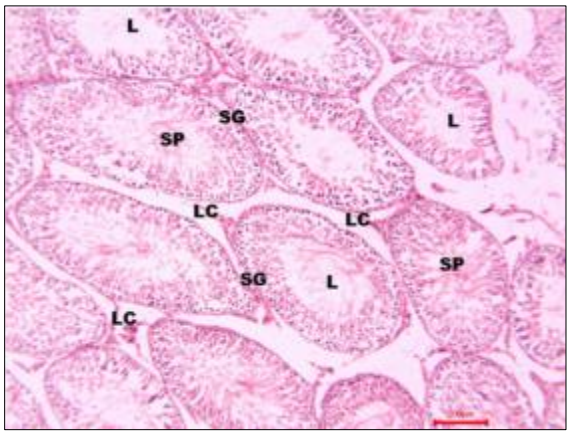

Figure 6 A photomicrograph of a section of the test is presented in this group (Group 6) showed relatively normal seminiferous tubules with moderately active spermatogenesis. Spermatogonia (SG) resting upon the basement membrane, a cluster of spermatozoa (SP) in the lumen (L), and interstitial tissue containing Leydig cells (LC) were observed. (H & E, x100)

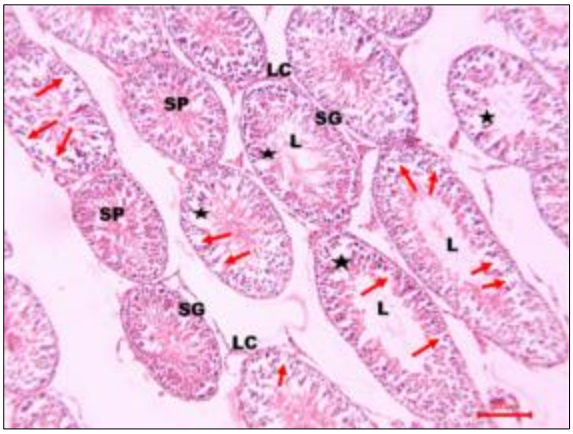

Figure 7 A photomicrograph of a section of the test is presented in this group (Group 7) showed mild depletion of spermatogenic cells (red arrows). Noticed few vacuolations. Spermatogonia (SG), cluster of spermatozoa (SP) in the lumen (L), Leydig cells (LC) in the interstitial tissue and enlarged blood vessel (V) were observed. (H and E, × 100)

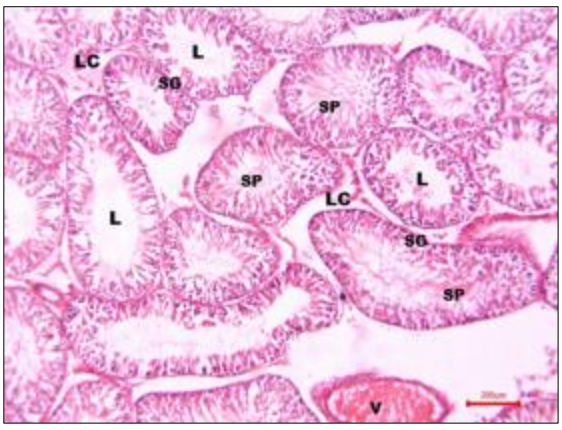

Figure 8 A photomicrograph of a section of the test is presented in this group (Group 8) showed relatively normal seminiferous tubules with moderately active spermatogenesis. Spermatogonia (SG) resting upon the basement membrane, a cluster of spermatozoa (SP) in the lumen (L), interstitial tissue containing Leydig cells (LC) and enlarged blood vessel (V) were observed. (H & E, x100)

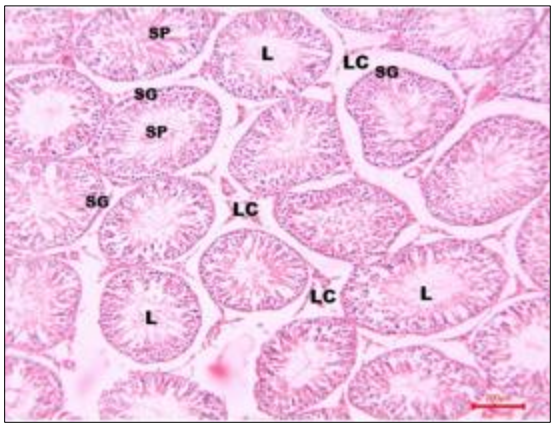

Figure 9 A photomicrograph of a section of the test is presented in this group (Group 9) showed normal seminiferous tubules with moderately active spermatogenesis. Spermatogonia (SG) resting upon the basement membrane, interstitial tissue containing Leydig cells (LC) and a cluster of spermatozoa (SP) in the lumen (L) were observed. (H and E, × 100)

From this present study, we could say that the toxic effects of methylparaben on rat testis can be mitigated by baicalin. Due to the lack of consensus on the safety of methylparaben, further studies are needed to ascertain the conditions under which methylparaben exerts toxic effects. In the meantime, the use of baicalin should be encouraged because of the documented health benefits possessed by this nutraceutical.

5. Conclusion

The results of this study show that methylparaben caused testicular toxicity by increasing oxidative stress, lipid peroxidation and oxidative DNA damage. It also induced considerable degenerative alterations in the histology of the testes. The administration of baicalin resulted in significant protection of the testes from functional and histological damage. We conclude that though methylparaben use is widespread and has previously been considered safe, under certain conditions it may impair fertility in male animals. More studies are needed to clarify the precise conditions in which this toxicity occurs as well as issues such as the power of antioxidants to protect the testis from this damage and the relevance of these findings to humans who use this substance.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

Statement of ethical approval

Experimental procedures involving animals and their care were conducted in conformity with international, national, and institutional guidelines for the care of laboratory animals in biomedical research and the use of laboratory animals in biomedical research as promulgated by the Canadian Council of Animal Care.

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