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Histomorphological assessment of *Brassica oleracea* on male reproductive functions of sprague dawley rats

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Abstract

Brassica oleracea (cabbage) is a significant vegetable with several medicinal potential and health benefits. This study aimed to investigate the effect of cabbage on male reproductive functions in Sprague Dawley (SD) rats. Twenty-eight (28) Adult male Wistar rats, (160-180g), were randomly divided into four groups. Group I (control group) rats received 1.0 ml of distilled water, Group II (HDC-high dose cabbage) received 100 mg/kg body weight crude extract of white cabbage, Group III (MDC-medium dose cabbage) rats received 300 mg/kg crude extract of white cabbage. Group IV (LDC-low dose cabbage) rats receive 500 mg/kg crude extract of white cabbage (LDC-low dose cabbage). The extract was administered orally for four weeks; semen analysis; sperm motility, count, and morphology were observed in the rats. Luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone were assayed. Antioxidants; superoxide dismutase (SOD), catalase (CAT), Glutathione reductase (GSH), and malondialdehyde (MDA), were assayed. The histological examination of the testis was also assessed. Cabbage caused a significant ($p<0.05$) increase in the weight of the testis and epididymis. There was a significant ($p<0.05$) decrease in the serum level of malondialdehyde and a significant ($p<0.05$) increase in the catalase level. Testicular spermatogenic cells were increased in population and proliferation of the germinal epithelium, thereby enhancing spermatogenesis; improving sperm counts significantly, and reducing oxidative stress and lipid peroxidation in a dose-dependent manner. Administration of cabbage may reduce oxidative stress, improve male reproductive fertility, and serve as a potential treatment for male infertility.

Keywords: *Cabbage, Sperm, Fertility, Spermatogenesis, Antioxidant, Rats*

1. Introduction

There are many benefits on the use of medicinal plant in the treatment of diseases. These benefits are backed

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with scientific reports in favor of their biological properties (Shah *et al.*, 2016). However, less information is available on the possible toxicity that these medicinal plants may cause to their consumers. Most times, plants commonly used in traditional medicine are frequently promoted as natural and harmless. This assessment is based on their usage in the treatment of diseases over centuries nonetheless, there are *Brassica oleracea* (cabbage) is among the oldest vegetable and has been used extensively in culinary applications for more than 4,000 years (Abu-Ghannam and Jaiswal, 2015). The plant belongs to the family of Brassicaceae and it is commonly consumed as vegetables, condiments, forages, cauliflower, Brussel sprouts and rape (Talreja *et al.*, 2015).

The presence of glucosinolates in cabbage made it harmful against cancerous cells in the breast, lungs and colon (Gonzales *et al.*, 2026; Oerlemans *et al.*, 2006). It has been reported to possess antimicrobial and antioxidant properties with high levels of flavonoids and anthocyanins (Xu *et al.*, 2014). Thus, it is also important in the treatment of infections (Kavishri *et al.*, 2024; Goralska *et al.*, 2008). Cabbage has high nutritional value and it is a good source of vitamin C and vitamin K^3. The nutritional value possessed by cabbage also promotes anti-inflammatory and formation of new tissues, thereby preventing oxidative damage (Sarandy *et al.*, 2015).

Oxidative Stress (OS) is a major factor in pathological conditions; it is identified as a major cause of infertility (Wang *et al.*, 2023; Agarwal *et al.*, 2014). In male, it causes sperm dysfunction and have the possibility of attacking the DNA, lipids and proteins in the sperm. It also causes a decline in semen parameters by altering the enzymatic system and ultimately causes cell death (Agarwal *et al.*, 2014). OS is the pathological imbalance between Reactive Oxygen Species (ROS) and antioxidants (Wang *et al.*, 2023). Where excessive production of ROS overwhelms the antioxidants (Evans *et al.*, 2021). Although, ROS play an important role in sperm production, an excessive production can also depletes antioxidants which lead to pathological imbalance in ROS and antioxidants. Despite the wide range of the use of cabbage in folklore medicine, its biological effects and antioxidants values, gaps still exist in the outcomes of its excess consumption. This study was therefore designed to investigate the outcomes of cabbage leaf extract on the testicular histoarchitecture and reproductive function of male Sprague Dawley (SD) rats.

2. Materials and methods

2.1. Collection of the cabbage leave

Cabbage leave were collected from cultivated farmland at Igbogbo, Ikorodu, Lagos State, Nigeria. The plant sample was identified and authenticated at Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria, where voucher sample has been deposited in the herbarium.

2.1.1. Plant materials and extraction

The plant materials were thoroughly cleaned with tap water to avoid debris and shade dried to prevent denaturing of its active chemicals. The dried plants were blend and sieved to obtain a fine powder (660 g of fresh cabbage yielded 52 g fine cabbage powder) and the powdered plant was kept in an incubator to further obtain a dry residue. The dried residue was kept in an airtight container and stored in the refrigerator at 4 ^{o}C. The concentration was prepared by using cold water from the tap and allowed for 12 hours in a shaker to get a complete homogenous solution (Raja *et al.*, 2015). After shaking, the liquid part was filtered using a filter paper and the appropriate dose was calculated before administrating to the experimental animals.

2.2. Experimental design

Twenty four male SD rats (160-180 g) obtained from the animal house at Lagos State University College of Medicine (LASUCOM), Ikeja, Lagos State were used for the study. The rats were kept in cages at the animal house under a standard condition of a 12 hour light and 12 hour dark period in a well-ventilated room at 26-27 ^{o}C with food and water provided ad libitum. The use and care of the animals and the experimental protocol were in strict conformity with the laid down guidelines of the National Institute of Health on the use of laboratory animals (NIH, 1985). Before commencement of this study, ethical approval was sought and approved with the ethical approval number. They were allowed to acclimatize for 2 weeks and were randomly divided into four groups (n = 6). Group 1: received distilled water only, Group 2: received 100 mg/kg cold water extract of Low-Dose Cabbage (LDC), Group 3: received 300 mg/kg of Medium-Dose Cabbage (MDC) and Group 4: received 500 mg/kg of High-Dose Cabbage (HDC). The extracts were given orally using oral gauge for 4 weeks.

Blood samples were collected from the retro-orbital plexus following anesthetizing with 0.6 mg/kg ketamine. Tissues sample were collected after cervical dislocation of the animals.

2.4. Collection of serum sample

Blood sample was collected through retro orbital plexus after 0.6 mg/ml ketamine to into a plain bottle using a 5 ml syringe with needle. The blood was allowed to stand at room temperature for 15-30 min. Thereafter, it was centrifuged using a cold centrifuge at 4000 rpm for 15 min. The supernatant was pipette into a plain bottle and stored at -4 °C.

2.4.1. Antioxidant enzymes assay

The blood samples were homogenized in phosphate buffer, Superoxide dismutase activity was determined by its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480 nm as described (Sun and Zigman, 1978). Catalase activity was determined according to the method described (Aebi, 1984). The reduced glutathione (GSH) content was estimated according to the method described (Sedlak and Lindsay, 1968). Malondialdehyde (MDA) which is an index of lipid peroxidation was determined (Buege and Aust, 1978).

2.4.2. Determination of testosterone, follicle-stimulating hormone, and luteinizing hormone

Plasma samples were assayed for Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH) and testosterone using the Enzyme-Linked Immunosorbent Assay (ELISA) as previously described (Ojewale *et al.*, 2014).

2.4.3. Semen analysis

Testes and epididymis were excised after euthanasia and then immersed in normal saline. The caudal epididymis was separated from the testes by anatomic scissors, blotted with filter papers and cut to collect the semen for evaluation of semen parameters (sperm count, motility, and morphology) as described by the modification of (Olaniyan *et al.*, 2021).

2.4.4. Histological analysis

The testes were fixed in 10% bouin's fluid and embedded in paraffin for 48 hr as previously described (Oyeniran *et al.*, 2021). The sample were processed to paraffin sections, 5 µm thick and stained with Hematoxylin and Eosin (H&E). Photomicrograph was shot at × 400 under a microscope where histological sections were examined.

2.5. Statistical analysis

The data collected were expressed as mean ± Standard Error of the Mean (SEM).The analysis was done using one-way analysis of variance (ANOVA) form graphed pad prism 5.0 statistical software. Student Newman-Keuls was used as post-hoc test to identify differences between individual mean. The confidence interval was placed at 95% where p-value < 0.05 was considered significant.

3. Results

3.1. Crude extract of cabbage on relative weight of testis and epididymis

Table 1, the relative weight of the testis was not significantly different ($p > 0.05$) in LDC, MDC and HDC when compared with control. Relative weight of the epididymis was significantly higher ($p < 0.05$) in LDC, MDC and HDC when compared with the control. Some medicinal plants must be used with caution due to the adverse effects showed in the decrease in the weight of rats that arise as a result of their excessive consumption (Talreja *et al.*, 2015).

3.2. Crude extract of cabbage on serum testosterone (ng/ml), FSH (miu/ml) and LH (miu/ml) in male SD rats

In Figure 1, serum level of testosterone was significantly higher ($p < 0.05$) while serum level of LH was significantly lower ($p < 0.05$) in LDC when compared with control. Testosterone was significantly lower ($p < 0.05$) in HDC than in control. When compared with HDC, its level was significantly higher in LDC ($p < 0.001$) and MDC ($p < 0.05$). There was no significant difference ($p > 0.05$) in the serum level of FSH across the group

Table 1: Crude extract of cabbage on relative weight of testis and epididymis in SD rats

Organ Weight	Control	LDC	MDC	HDC
Testis	0.05±0.04	0.009±0.001^{ns}	0.008±0.001^{ns}	0.009±0.001^{ns}
Epididymis	0.003±0.000	0.003±0.000**	0.002±0.000**	0.001±0.000**

Note: Value expressed as mean ± SEM. ** = $p < 0.001$ when compared with the control group; ns = Not significant at $p > 0.05$ when compared with control.

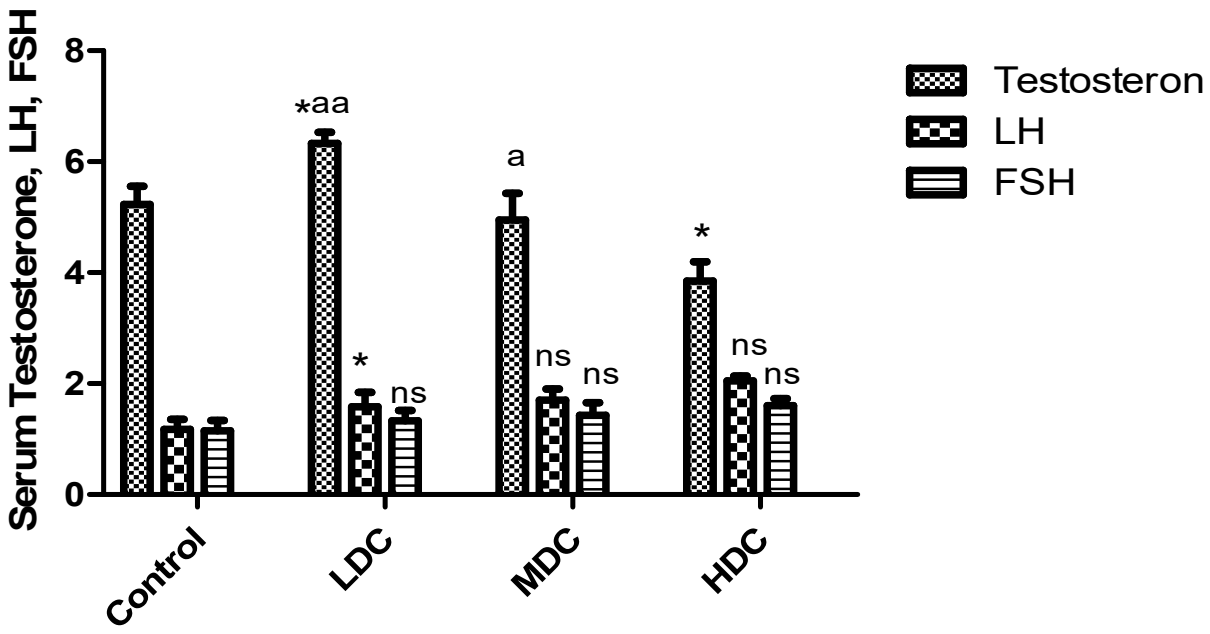

Figure 1: Crude extract of cabbage serum testosterone (ng/ml), FSH (miu/ml) and LH (miu/ml) in male Wistar rats

Note: Value expressed as Mean ± SEM. * = $p < 0.05$; ns = Not significant at $p > 0.05$ when compared with control; ^{a} = $p < 0.05$ when compared with HDC.

and when compared with control. There was no significant difference ($p > 0.05$) in the serum level of LH, FSH in MDC and HDC when compared with the control.

3.3. Crude extract of cabbage sperm motility (%), sperm count (10^6 cells/ml) and morphology in SD rats

In Figure 2, sperm motility was significantly high in LDC ($p < 0.05$) and lower in HDC ($p < 0.001$) than in control. It was not significantly different ($p > 0.05$) in MDC when compared with the control. In LDC and MDC, it was significantly higher ($p < 0.05$) compared with HDC. Sperm count was significantly increased ($p < 0.001$) in LDC and MDC than control. It was also significantly increased ($p < 0.05$) in LDC and MDC compared with HDC. Sperm morphology was significantly increased ($p < 0.05$) in LDC and reduced in HDC compared with the control but not significantly different in MDC when compared with control.

3.4. Crude extract of cabbage on oxidative stress markers in the serum, testis and epididymis of SD rats

Catalase in the serum of LDC, MDC and was significantly higher ($p < 0.05$) compared with control. In HDC rats, catalase was significantly lower ($p < 0.001$) compared with control. The activities of GSH in the serum was not significantly different ($p > 0.05$) in LDC, MDC and HDC compared with control. There was a significant increase ($p < 0.001$) in the serum activities of SOD in LDC compared with control and no significant difference ($p > 0.05$) in MDC and HDC when compared with control. Malondialdehyde in the serum was significantly lower ($p < 0.05$) in LDC and MDC but significantly higher ($p < 0.001$) in HDC compared with control. Its level

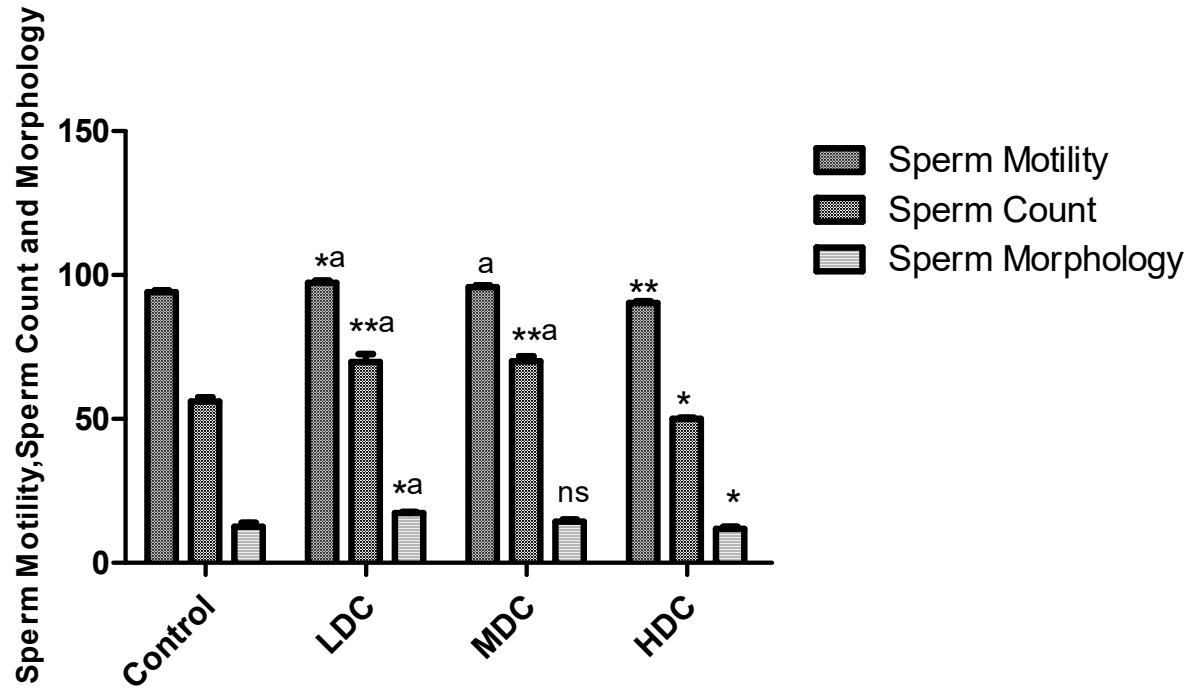

Figure 2: Crude extract of cabbage in sperm motility (%), sperm count (106 cells/ml) and morphology in SD rats

Note: Value expressed as Mean ± SEM. * = $p < 0.05$; ns = Not significant at $p > 0.05$ when compared with control; [a] = $p < 0.05$ when compared with HDC.

in LDC and MDC was significantly lower ($p < 0.001$) compared with HDC (Table 2). Catalase in the testis was significantly reduced ($p < 0.001$) in HDC than in control while its activities in LDC and MDC, it was not significantly different ($p < 0.05$) when compared with control and HDC. The activity of GSH in testis was

Table 2: Crude extract of cabbage on oxidative stress markers in the serum, and testis of Wistar rats

Serum				
Group	**CAT (mg/ml)**	**GSH (µmol/ml)**	**SOD (mg/ml)**	**MDA (nmol/ml)**
Control	6.12 ± 0.55	3.12 ± 0.65	1.19 ± 0.335	1.31 ± 0.07
LDC	11.07 ± 1.23*,aa	5.02 ± 1.25ns,*	2.33 ± 0.11**	0.85 ± 0.15*,aa
MDC	7.99 ± 0.51*,a	4.32 ± 0.39ns	1.84 ± 0.12ns	1.36 ± 0.09*,aa
HDC	4.07 ± 0.67**	2.89 ± 0.27ns	1.85 ± 0.089ns	1.85 ± 0.17**
Testis				
Group	**CAT (mg/ml)**	**GSH (µmol/ml)**	**SOD (mg/ml)**	**MDA (nmol/ml)**
Control	2.92 ± 0.64	2.59 ± 0.37	1.67 ± 0.13	1.07 ± 0.07
LDC	4.23 ± 0.59a	3.80 ± 0.18*	2.0 ± 0.24ns	0.68 ± 0.12*,a
MDC	2.76 ± 0.18a	3.20 ± 0.31ns	1.96 ± 0.24ns	1.04 ± 0.10ns
HDC	2.57 ± 0.14*	3.09 ± 0.27ns	1.55 ± 0.19ns	1.56 ± 0.31*

Note: Value expressed as Mean ± SEM. * = $p < 0.05$; ns = Not significant at $p > 0.05$ when compared with control; [a] = $p < 0.05$ when compared with HDC.

significantly higher in LDC than in control, it was not significantly different in MDC and HDC when compared with control and HDC. There was no significant difference ($p > 0.05$) in the activities of SOD across the group when compared with control and HDC. In rats on LDC, MDA was significantly reduced ($p < 0.05$) when compared with control and HDC, there was no significant difference its activities in MDC when compared with control and HDC (Table 2).

3.5. Histomorphological analysis of the crude extract of cabbage on testis

The photomicrograph of Figure 3 showed histological sections of the control, low and medium dose are essentially the same. However, sections of the highest dose (B) show an increase in the proliferation of spermatogenic cells lineage including the spermatids and spermatozoa. The increase in the spermatids and spermatogenic cell series is observed and this is marked at the peripheral of the tissue (tunica).

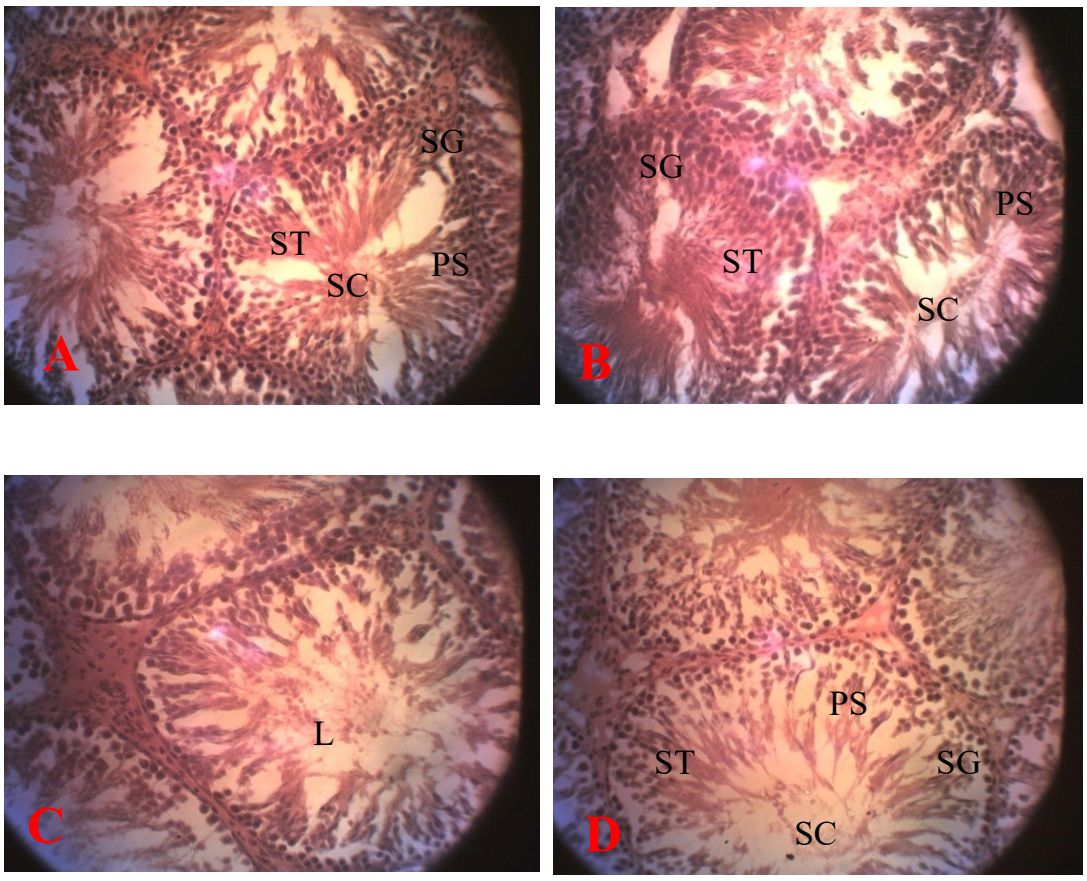

Figure 3: The photomicrograph of the histology of testis of male rat in control (A), compared with high dose (B), medium dose (C) and low dose (D). Hematoxylin and Eosin stain X100, showing the spermatogonia SG, the primary spermatocyte, PS, the spermatids, ST, and the lumen, L containing mature sperm cells SC

4. Discussion

In the present study, administration of a high dose of cabbage extract resulted in a decrease in epididymal weight in rats. The epididymis plays a crucial role in sperm maturation, as spermatozoa leaving the testes are functionally immature. During their transit through the epididymis, spermatozoa undergo morphological, biochemical, and physiological changes that confer motility and the capacity to fertilize an ovum (Das *et al.*, 2023).

The epididymal environment provides a specialized luminal fluid rich in antioxidants and other protective factors that support sperm maturation, protect spermatozoa from oxidative damage, and enhance their concentration during epididymal transit (Juárez-Rojas *et al.*, 2022). The weight of the epididymis is influenced largely by the accumulation and storage of sperm, particularly within the cauda epididymis. Therefore,

persistent accumulation and retention of sperm in the caudal epididymis contribute significantly to increases in epididymal weight and are closely associated with normal testicular function (Hu *et al.*, 2024).

The decrease in epididymal weight observed in the present study may indicate reduced sperm accumulation and storage within the epididymis, suggesting impaired sperm maturation and/or transport. This study also revealed a reduction in serum testosterone levels accompanied by an elevation in Luteinizing Hormone (LH) levels in the High-Dose Cabbage (HDC) group. Testosterone is the principal androgen produced by the Leydig cells of the testes in response to stimulation by LH. Under normal physiological conditions, testosterone exerts negative feedback on the hypothalamus and anterior pituitary, thereby regulating the secretion of Gonadotropin-Releasing Hormone (GnRH) and LH (Li *et al.*, 2024). Consequently, a reduction in testosterone levels may diminish this negative feedback, leading to compensatory increases in LH secretion. Male reproductive function is regulated by the Hypothalamic–Pituitary–Testicular (HPT) axis, which is initiated by the pulsatile release of Gonadotropin-Releasing Hormone (GnRH) from the hypothalamus (Dhole and Kumar, 2017).

The elevated LH levels observed in the present study suggest that the Hypothalamic–Pituitary–Testicular (HPT) axis remained functionally intact, with the endocrine feedback mechanism responding appropriately to the decline in circulating testosterone (Anjum *et al.*, 2017). The increase in LH secretion likely represents a compensatory response aimed at stimulating the Leydig cells to restore testosterone production following the reduction associated with high-dose cabbage administration. However, despite the elevated LH levels, serum testosterone remained reduced, suggesting that Leydig cell steroidogenesis may have been impaired or insufficient to fully compensate for the hormonal deficit.

The reduction in epididymal weight observed in this study further supports the possibility of decreased testosterone bioavailability, which may have adversely affected sperm maturation and storage (James *et al.*, 2020). This interpretation is consistent with our histological findings, which revealed decreased proliferation of spermatogenic cells, reduced numbers of spermatids, and fewer spermatozoa in the testes of rats administered high doses of cabbage extract. Since testosterone is indispensable for the initiation, maintenance, and completion of spermatogenesis, the impaired testosterone response despite elevated LH suggests disruption of the LH–Leydig cell–testosterone signaling pathway required to sustain normal spermatogenesis.

Follicle-Stimulating Hormone (FSH) plays an essential role in regulating spermatocytogenesis and spermatogenesis through its actions on Sertoli cells within the seminiferous epithelium (Adeneye *et al.*, 2019). In the present study, oral administration of 100 mg/kg cabbage extract appeared to enhance spermatogenesis, as evidenced by the improvements in sperm motility, sperm count, and sperm morphology. In contrast, prolonged administration of 500 mg/kg cabbage extract adversely affected spermatogenesis, resulting in deterioration of sperm quality. These findings suggest a dose-dependent effect of cabbage extract on male reproductive function, whereby moderate intake may be beneficial, whereas excessive intake may be detrimental.

Previous studies have reported that doses of cabbage extract above 300 mg/kg may produce toxic effects, while administration of 500 mg/kg has been associated with thyroid hormone deficiency (Olumide *et al.*, 2019). Although Brassica oleracea possesses considerable nutritional value (Posmyk *et al.*, 2009), it is also rich in bioactive phytochemicals, including anthocyanins, flavonoids, glucosinolates, and other antioxidant compounds that have demonstrated antimicrobial, anti-inflammatory, and wound-healing properties (Sarandy *et al.*, 2015). Nevertheless, like many medicinal plants, these beneficial effects are dose dependent, and excessive consumption may result in adverse physiological effects (Talreja *et al.*, 2015).

Oxidative stress has been recognized as a major contributor to male reproductive dysfunction (Aitken, 2017; Ojewale *et al.*, 2014). It is defined as an imbalance between the production of Reactive Oxygen Species (ROS) and the antioxidant defence system (Evans *et al.*, 2021). This imbalance may arise from excessive endogenous or exogenous ROS generation, depletion of antioxidant reserves, reduced antioxidant enzyme activity, or a combination of these factors (Oyeniran *et al.*, 2021; Lushchak, 2014). Physiologically, low concentrations of ROS are essential for normal sperm function, including capacitation, the acrosome reaction, and sperm–oocyte fusion (Mao *et al.*, 2019). However, excessive ROS production induces oxidative damage to spermatozoa because their plasma membranes contain high concentrations of polyunsaturated fatty acids, making them particularly susceptible to lipid peroxidation (Mohideen *et al.*, 2023).

In the present study, administration of 500 mg/kg cabbage extract significantly reduced catalase (CAT) activity while increasing malondialdehyde (MDA) levels, indicating enhanced lipid peroxidation and oxidative stress. Conversely, rats treated with 100 mg/kg cabbage extract exhibited increased CAT and superoxide dismutase (SOD) activities together with reduced MDA levels, suggesting enhanced antioxidant defence. Malondialdehyde is a stable end product of lipid peroxidation and serves as a reliable biomarker of oxidative damage (Mohideen *et al.*, 2023). Catalase is an important enzymatic antioxidant that protects spermatozoa against oxidative injury by decomposing hydrogen peroxide into water and oxygen, thereby preserving sperm viability and function (Rubio-Riquelme *et al.*, 2020; Bansal and Bilaspuri, 2011). Collectively, these findings indicate that moderate cabbage intake enhances antioxidant capacity, whereas excessive intake overwhelms the antioxidant defence system, resulting in oxidative stress and impaired reproductive function.

The biochemical findings are further supported by the histological observations, which demonstrated preservation of testicular architecture in rats administered 100 mg/kg cabbage extract but degeneration of spermatogenic cells and reduced spermatozoa in rats receiving 500 mg/kg. The concordance between the biochemical, hormonal, sperm quality, and histological findings strengthen the conclusion that excessive cabbage consumption adversely affects male reproductive health.

5. Conclusion

The present study demonstrates that excessive consumption of Brassica oleracea (cabbage) adversely affects male reproductive function by reducing testosterone levels, impairing spermatogenesis, decreasing sperm quality, and increasing oxidative stress. In contrast, moderate consumption enhances antioxidant defence and improves sperm characteristics. These findings suggest that while cabbage possesses substantial nutritional and medicinal benefits, its effects on male reproductive health are dose dependent. Moderate intake appears beneficial, whereas excessive consumption may compromise reproductive function through hormonal disruption and oxidative stress.

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