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(RESEARCH ARTICLE)

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Attenuating potential of some antioxidants: Cellgevity, max one, purslane and vitamin C on caffeine induced hormonal and testicular toxicities in male albino rats

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Abstract

Background: Infertility challenges in men, resulting from disturbances in hormonal balance and testicular integrity, stands as a significant health challenge associated with various factors. Consequently, diverse strategies are necessary to tackle this issue. This research explored the attenuating potentials of some antioxidants—Cellgevity (CG), Max One (MX), purslane, and vitamin C (VC)—on caffeine-induced hormonal and testicular toxicities in male albino rats.

Methodology: Sixty sexually matured male albino rats were randomly divided into ten groups consisting of two rats in three replicates using completely randomized design (CRD). Group one served as control and received water and feed only. Group two were given 200 mg/kgBw of CG, group three received 200 mg/kgBW of MX, group four received 100 mg/kgBW of VC, group five received 200 mg/kgBW of caffeine, group six received 200mg/kgBW of purslane, group seven received 200 mg/kgBW of caffeine and 200 mg/kgBW of CG, group eight received 200 mg/kgBW of caffeine and 200 mg/kgBW of MX, group nine received 200mg/kgBW of caffeine and 200 mg/kgBW of purslane, group ten received 200 mg/kgBW of caffeine and 100 mg/kgBW of VC. Administration was done orally and lasted for 65days. The rats were sacrificed after administration using chloroform anaesthesia. The testes were processed for histology while blood sample were obtained for hormonal assay.

Results: The results showed that caffeine significantly ($p<0.05$) reduced the serum levels of testosterone, follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol when compared to the control and other treatments groups. There was testicular toxicity with loosely packed enlarged seminiferous tubules in caffeine treated animals when compared with the control and antioxidants treated animals. However, CG, MX, purslane and VC attenuated the effect of caffeine in all the parameters evaluated by increasing the levels of the hormones and restoring testicular integrity of the animals in the combination groups.

Conclusion: This present study has revealed the toxic effect of caffeine reproductive hormones and testicular integrity of male albino rats. However, the findings of this study provided substantial evidence on the attenuating effects of CG MX, purslane and VC on caffeine-induced hormonal and testicular toxicity in male rats as mammalian models.

Keywords: Antioxidants; Caffeine; Hormonal Toxicity; Testicular toxicity; Attenuating potential.

1. Introduction

Infertility poses a significant challenge to individuals, defined as the inability of a couple to achieve pregnancy after a year of unprotected sexual intercourse. This issue affects approximately 8–12 percent of couples of reproductive ages,

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with male factors contributing to about 50 percent of infertility cases, affecting one in twenty men of reproductive age [1]. Evidence suggests that reactive oxygen species (ROS)-mediated damage to reproductive processes and spermatogenesis play a substantial role in the pathology of infertility, affecting 30–80 percent of infertile men [2].

Free radicals, generated by both external and internal factors, are highly reactive oxygen-derived substances with a short half-life ranging from nanoseconds to milliseconds. These biomolecules significantly impact reproductive parameters and external factors such as lifestyle changes, technological advancements, pollution, alcohol consumption, smoking, stress, and exposure to toxins contribute to the production of reactive oxygen species. Internal factors, including cellular membrane, peroxisomes, mitochondria, and endoplasmic reticulum metabolism processes, also contribute to the generation of internal reactive oxygen species [3-4].

Glutathione, an endogenous antioxidant found in nearly every cell, plays a crucial role in detoxifying drugs and xenobiotics. However, the direct intake of glutathione is ineffective as it gets denatured along the digestive tract before cellular utilization. Riboceine supplements like CG and MX, containing D-Ribose-L-Cysteine, address this issue. The ribose component protects and delivers fragile cysteine molecules, enabling cells to produce glutathione as needed [5]. L-cysteine, a semi-essential amino acid synthesized from methionine, serves as a precursor for glutathione, essential in cellular oxidative stress detoxification. Elevated oxidative stress levels can potentially impair cellular glucose metabolism, leading to redox imbalance, insulin resistance, and reproductive dysfunction [5].

Purslane (*Portulaca oleracea*), also known as mmong mmong ikong mbakara in Efik, demonstrates muscle relaxation, convulsion treatment, pain reduction, anti-inflammatory capabilities, and anti-anxiety properties. Studies indicate its liver-protective effects in rats with liver diseases [6-7]. Purslane is nutritionally rich, providing omega-3-fatty acids, ascorbic acid, b-carotene, a-tocopherols, and glutathione. Its seeds are particularly high in a-linolenic acid, contributing to its antioxidant potential [8]. The total phenolic content (TPC) in *P. oleracea* extracts ranges from 127 ± 13 to 478 ± 45 mg/100 g fresh weight of the plant. Various antioxidant assays, including DPPH scavenging, AEAC, and FRAP, highlight its potent antioxidant capabilities [6-7].

Antioxidants, which attenuate stress by removing free radicals, are abundant in plants, and purslane's effectiveness in antioxidant properties is well-documented. Additionally, it nourishes the kidneys, liver, heart tissues, and testes [10]. VC, a natural antioxidant, plays a crucial role in preventing increased free radical production due to oxidative damage. Its protective effects against oxidative stress are well-established [11].

Caffeine, a widely consumed psychoactive substance, is present in foods, drugs, and beverages. While low to moderate doses offer alertness and positive impacts on the myocardium, excessive intake may lead to undesirable effects, including irritability, nervousness, headaches, sleep disturbances, and heart palpitations [14]. Frequent caffeine intake has been associated with delayed conception, reproductive and developmental toxicity, and an increased incidence of sperm abnormalities [15-19].

2. Material and methods

2.1. Location of the Study

This research was conducted in the Animal House of the Department of Biology, Cross River State College of Education, Akamkpa, Cross River State, Nigeria. The study lasted for 6 months (June 2023-November, 2023).

2.2. Collection of Materials

Caffeine was acquired from Sigma-Aldrich (St. Loius, USA). The antioxidant agents: MX and CG were purchased from Max International, LLC, (Salt Lake City, USA). VC was purchased from Emzor Pharmaceutical Industries Limited, Lagos. Purslane leaves were obtained from the Cross River State College of Education, Akamkpa Botanical Garden and its environs. The leaves were authenticated by Mr. Effa Anobeja of the Herbarium Unit, Department of Plant and Ecological Studies, University of Calabar. The leaves were processed into crude extracts. The leaves were air-dried, pulverized and aqueous extracts obtained using distil water.

2.3. Experimental Animals

Sixty (60) sexually matured male albino rats, twelve weeks old weighing between 160 – 200g was purchased from the Department of Zoology and Environmental Biology, University of Calabar, Nigeria. The animals were kept in steel cages covered with wire mesh under standard laboratory environment. They were given water and commercial feed from

Top Feed Limited (crude protein: 18 percent; metabolizable energy: 2800kcal/kg) *ad libitum* during the study. Animals were allowed to adapt to their environment for two weeks before treatment.

2.4. Experimental Design and Procedure

The 60 albino rats were divided into ten groups consisting of two rats in three replicates using the completely randomized design (CRD). Treatment protocol was as shown in Table 1 and lasted for 65 days [12]. Chloroform fume was used to anesthetize the rats twenty-four hours after administering the last dose. The testes were processed for histology while blood sample were obtained for hormonal assay.

2.5. Histology of testes

The testes were fixed in 10% formol saline. The fixed tissues were transferred to a graded series of ethanol and then cleared in xylene. Once cleared, the tissues were infiltrated in molten paraffin wax in the oven at 58oC. Serial sections of 5µm thickness were obtained from the solid block of tissue, cleared, fixed in clean slide, stained with haematoxylin and eosin stains and examined with the light microscope.

2.5.1. Hormonal Assay

The blood samples were spun at 2500rpm for 10 minutes using Wisperfuge Model 1384 centrifuge (Tamson, Holland) at 10-25^{0}C. Serum samples were assayed for levels of testosterone follicle stimulating hormone (FSH), luteinizing hormone/interstitial cell stimulating hormone (LH/ICSH) and estradiol using the microwell enzyme linked immunoassay (ELISA) technique utilizing the competitive binding principle; with analytical grade reagents from Syntron Bioresearch Inc. USA [19].

Table 1 Protocol for treatment of experimental animal

Treatment groups	Description of treatment
Control	1ml of physiological saline, No Caffeine, purslane, vitamin C, Max one and Cellgevity
C	Caffeine in 1ml of physiological saline, 200mg/kgBW orally by gavage [12]
P	Purslane, 200 mg/kgBW orally
VC	Vitamin C, 100mg/kgBW, orally
MX	Max One, 200mg/kgBW orally
CG	Cellgevity, 200mg/kgBW orally
C+P	Caffeine, 200mg/kgBW and purslane, 200mg/kgBW both orally
C+VC	Caffeine, 200mg/kgBW and Vit. C, 100mg/kgBW both orally.
C+MX	Caffeine, 200mg/kgBW and Max One, 200mg/kgBW both orally
C+CG	Caffeine, 200mg/kgBW and Cellgevity, 200mg/kgBW both orally

2.6. Statistical analysis

Data obtained were analyzed using analysis of variance (ANOVA) on SPSS version 27. Least significant difference was utilized to compare means at $p<0.05$.

3. Results

3.1. Hormonal profile

Results presented in Table 2 revealed that caffeine caused a significant reduction in the hormonal profile of the animals. LH significantly reduced ($P< 0.05$) in caffeine group (0.16± 0.02) when compared to the control (0.31±0.05 IU L^{-1}), CG, MX, VC and PU groups recorded 0.87±0.10, 2.03±0.40, 0.93±0.04 and 3.47±0.65 IU L^{-1}, respectively. The combination groups showed attenuating effects, with C+ CG having the highest value of 0.80±0.04 IU L^{-1}. No significant difference was observed in the serum value of prolactin in the caffeine group when compared to other treatment groups. FSH level reduced significantly in the caffeine group (0.86±0.06 ng L^{-1}) compared to the control (2.22 ng L^{-1}), CG (0.97±0.03 ng L^{-}

^{1}), MX (1.47±0.20 ng L^{-1}), VC (1.06 ±0.03 ng L^{-1}) and PU (1.36±0.09 ng L^{-1}). The level of FSH increase in the combination group when compared to the caffeine group indicating attenuating effect (Table 2). For testosterone, the serum level decreased significantly in the caffeine group (1.16 ± 0.08 ng L^{-1}) when compared to the control (1.86 ± 0.05 ng L^{-1}) and other treatment groups. The estradiol level also reduced significantly in the caffeine group (6.70 ± 0.44 pg L^{-1}) when compared to the control (14.05 ± 0.54 pg L^{-1}), CG (19.58 ± 1.15 pg L^{-1}), MX (10.92 ± 0.03 pg L^{-1}), VC (14.66 ± 0.18 pg L^{-1}) and PU (8.97 ± 0.14 pg L^{-1}). For both testosterone and estradiol, the level increased significantly in the combination groups when compared to the caffeine group as shown in Table 2, indicating attenuating effects.

3.2. Histology of testes

The results on the histological examination of section of the testis of control animals (Figure 1) showed closely packed seminiferous tubules containing proliferating spermatogonia cells at various stage of maturation. The cells are 3 to 5 cell layers thick, round to oval shaped with deeply stained nuclei. The intervening stroma is scanty and contained 3 to 5 Leydig cells per cluster and there lumina cavity contained numerous spermatozoa.

Section of the testis of animals in the CG group showed loosely packed seminiferous filled with proliferating spermatogonia cells in most of the tubules. The cells include spermatogonia A and B, spermatocytes, spermatid and spermatozoa. The cells are 3 to 5 cell layers thick and contained mature spermatozoa within their lumina cavity. The intervening stromal is scanty and contained 3 to 5 Leydig cells per cluster. The Sertoli cell population is greater than 10 per tubules (Figure 2).

Table 2 Effect of some antioxidants on the hormonal profile of rats treated with caffeine

Parameters	Control	CG	MX	VC	Caffeine	PU	C+ CG	C+ MX	C+ PU	C+ VC
LH (IU L^{-1})	0.31 ± 0.05^{b}	0.87 ± 0.10^{d}	2.03 ± 0.40^{e}	0.93 ± 0.04^{d}	0.16 ± 0.02^{a}	3.47 ± 0.65^{f}	0.80 ± 0.04^{d}	0.29 ± 0.01^{b}	0.42 ± 0.07^{c}	0.42 ± 0.07^{c}
Prolactin (ng L^{-1})	1.16 ± 0.17^{a}	0.85 ± 0.01^{a}	1.15 ± 0.66^{a}	0.83 ± 0.01^{a}	0.83 ± 0.01^{a}	0.88 ± 0.02^{a}	1.11 ± 0.26^{a}	1.01 ± 0.24^{a}	0.87 ± 0.01^{a}	1.08 ± 0.25^{a}
FSH (ng L^{-1})	2.22 ± 0.33^{g}	0.97 ± 0.03^{b}	1.47 ± 0.20^{d}	1.06 ± 0.03^{b}	0.86 ± 0.06^{a}	1.36 ± 0.09^{d}	1.72 ± 0.21^{f}	1.10 ±0.08^{b}	1.25 ± 0.03^{c}	1.54 ± 0.13^{e}
Testosterone (ng L^{-1})	1.86 ± 0.05^{b}	1.74 ± 0.03^{b}	1.76 ± 0.02^{b}	2.46 ± 0.21^{c}	1.16 ± 0.08^{a}	3.08 ± 0.32^{d}	1.86 ± 0.03^{b}	1.75 ± 0.02^{b}	1.79 ± 0.03^{b}	1.81 ± 0.03^{b}
Estradiol (pg L^{-1})	14.05 ± 0.54^{d}	19.58 ± 1.15^{e}	10.92 ± 0.03^{c}	14.66 ± 0.18	6.70 ± 0.44^{a}	8.97 ± 0.14^{b}	9.80 ± 0.70^{c}	8.71 ± 0.15^{b}	20.85 ± 0.92^{e}	7.81 ± 0.45^{b}

Values are presented as Mean ± Standard error. Means with similar case letters across horizontal array are not significantly different at P<0.05; Key: CG: Cellgevity; MX: Max one; VC: Vitamin C; C+CG: Caffeine and Cellgevity; C+ MX: Caffeine and Max one; C+ PU: Caffeine and Purslane; C+ VC: Caffeine and Vitamin C

Histological section of the testis of animals in MX group revealed loosely packed irregular shaped seminiferous with thickened basement membrane filled with proliferating spermatogonia cells in few of the tubules. Some of the tubules were atrophic and contained scanty spermatozoa cells within their cavity. The cells include spermatogonia A and B, spermatocytes, spermatid and spermatozoa. The cells are 3 to 4 cell layers thick and contained mature spermatozoa within their Lumina cavity. The intervening stromal is scanty and contained 2 to 4 Leydig cells per cluster (Figure 3).

Animals treated with VC only had section of the testis with closely packed seminiferous with thickened basement membrane filled with proliferating spermatogonia cells. The cells include spermatogonia A and B, spermatocytes, spermatid and spermatozoa. These cells have round to oval deeply stained nuclei. The cells are 3 to 5 cell layers thick and contained mostly late series comprising of spermatid and mature spermatozoa within their Lumina cavity. The intervening stromal is scanty and contained 3 to 5 Leydig cells per cluster and the Sertoli cells consists of greater than 12 per tubules (Figure 4).

The caffeine group histological section of the testes showed loosely packed enlarged seminiferous tubules with intact basement membrane containing proliferating spermatogonia cells at various stage of maturation. The cells are sparsely populated and comprised of spermatogonia A and B, spermatocytes, spermatid and spermatozoa. These were atrophic with round to oval deeply stained nuclei. The cells are 3 to 5 cell layers thick and contained mostly late series comprising

of spermatid and mature spermatozoa within their Lumina cavity. The intervening stromal is scanty and contained 3 to 4 Leydig cells per cluster and the Sertoli cells consists of population greater than 10 per tubules (Figure 5).

Figure 6 shows section of testes of animals in Purslane group with closely packed enlarged irregular shaped seminiferous tubules with intact basement membrane containing moderate amount of proliferating spermatogonia cells at various stage of maturation. The cells comprised of spermatogonia A and B, spermatocytes, spermatid and spermatozoa. These are atrophic with round to oval deeply stained nuclei. The cells are 3 to 4 cell layers thick and contained scanty spermatid and mature spermatozoa within their Lumina cavity. The intervening stromal is scanty and contained 3 to 4 Leydig cells per cluster and the Sertoli cells consists of greater than 10 per tubules.

Section of the testis of animals in the C+CG group indicated loosely packed seminiferous containing moderate amount of spermatogonia cells at various stages of maturation. The cells include spermatogonia A and B, spermatocytes, spermatid and spermatozoa. The cells are 3 to 4 cell layers thick and contained scanty number of mature spermatozoa within their Lumina cavity. The intervening stromal is scanty and contained 2 to 4 Leydig cells per cluster. The Sertoli cell population is greater than 11 per tubules (Figure 7).

C+MX group animals had closely packed seminiferous containing moderate amount of spermatogonia cells at various stages of maturation. The cells include spermatogonia A and B, spermatocytes, spermatid and spermatozoa. The cells are 3 to 5 cell layers thick with round to oval deeply stained nuclei. The intervening stromal is scanty and contained 2 to 4 Leydig cells per cluster. Most of the seminiferous tubules had numerous spermatozoa within their cavity (Figure 8). Histological examination of the animals in C+PU group showed closely packed seminiferous containing moderate amount of spermatogonia cells at various stages of maturation. The cells are 3 to 5 cell layers thick with round to oval deeply stained nuclei and scanty number of spermatozoa seen within their lumina cavity. The intervening stromal is scanty and contained 2 to 4 Leydig cells per cluster. The lumina cavity contains sparsely populated spermatozoa and spermatids within their lumina cavity (Figure 9).

The sections of the C+VC group indicated closely packed seminiferous tubules containing sparsely populated spermatogonia cells. The cells are atrophic and less than 3 cell layers thick. The intervening stromal is scanty and contained 2 to 4 Leydig cells per cluster. There lumina cavity contains sparsely populated spermatozoa and spermatids within their cavity (Figure 10).

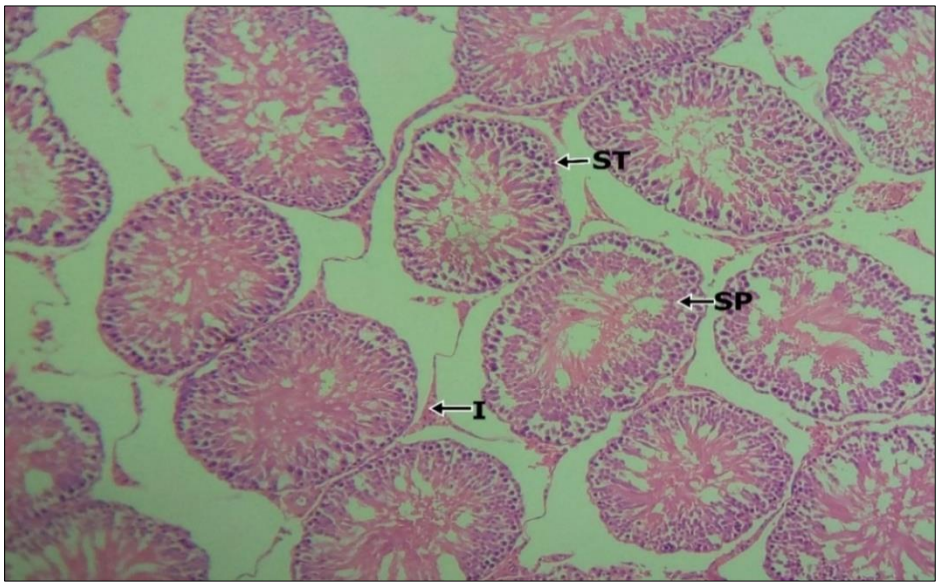
4. Discussion

The findings of this study revealed a significant decrease in hormone levels in animals treated with caffeine alone, compared to the control and combination groups. This aligns with previous studies by Uno *et al*. [20], Karen *et al*. [21] and Anup *et al*. [22], which reported an inverse relationship between caffeine consumption and sex hormones. The decline in testosterone levels in caffeine-treated animals may contribute to the reduced sperm quality. Additionally, FSH and LH, working concurrently with testosterone during spermatogenesis, emphasizes the impact of decreased hormone levels on male fertility [23].

The reduced hormone levels in caffeine-treated animals may be attributed to caffeine-induced increased production of reactive oxygen species (ROS) leading to oxidative stress. This enhanced ROS production can inhibit steroidogenic enzymes and disrupt steroidogenic processes [24]. Normal steroidogenesis also generates ROS, which, produced by mitochondrial respiration and catalytic reactions of steroidogenic cytochrome P450 enzymes, inhibit subsequent steroid production and damage mitochondrial membranes of spermatozoa [25].

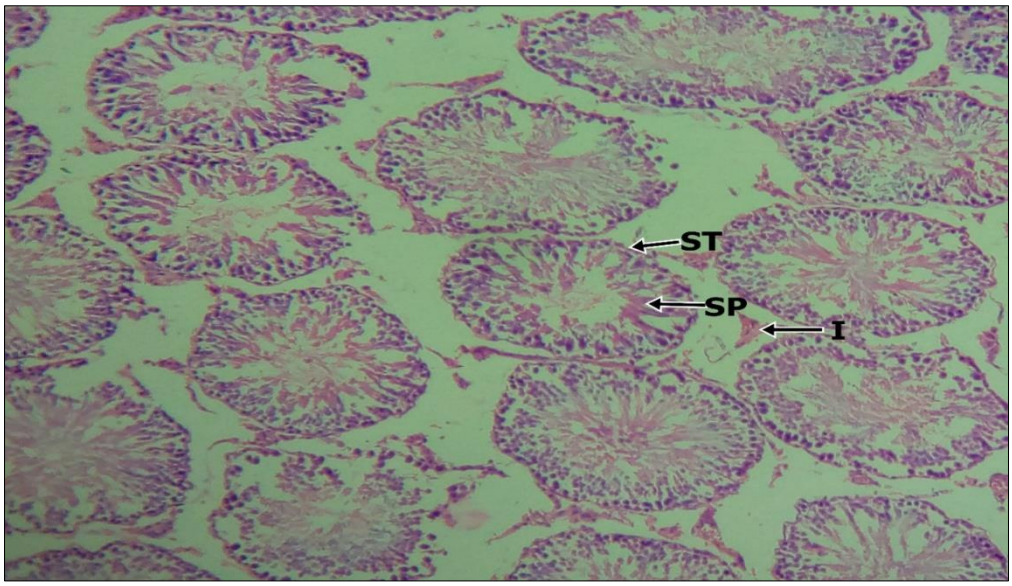
Oxidative stress adversely affects steroidogenesis, as stress-induced changes in autonomic catecholaminergic activities may suppress Leydig cell functions, inhibiting steroidogenic enzyme. activities and testosterone production. Stress-induced elevations of glucocorticoid levels can directly decrease testosterone levels without altering LH levels. In cases of chronic stress, a decrease in LH and gonadotropin-releasing hormone (GnRH) levels becomes apparent, leading to testicular oxidative stress and a reduction in testosterone production [26].

Oxidative stress, associated with an increased number of immature spermatozoa due to its indirect effect on male hormone production correlated with spermatogenesis [27]. This is supported by Wagenmaker *et al*. [26], who noted that oxidative stress adversely affects steroidogenesis by suppressing Leydig cell functions, inhibiting steroidogenic enzyme activity and hormone production. Different types of steroids and peptide hormones are vital for the production and development of mature spermatozoa, regulating the functioning of seminiferous tubules and somatic cells needed for testes development [28-29]. The significant decrease in testicular integrity observed in caffeine-treated animals may be linked to the reduction in sex hormones.



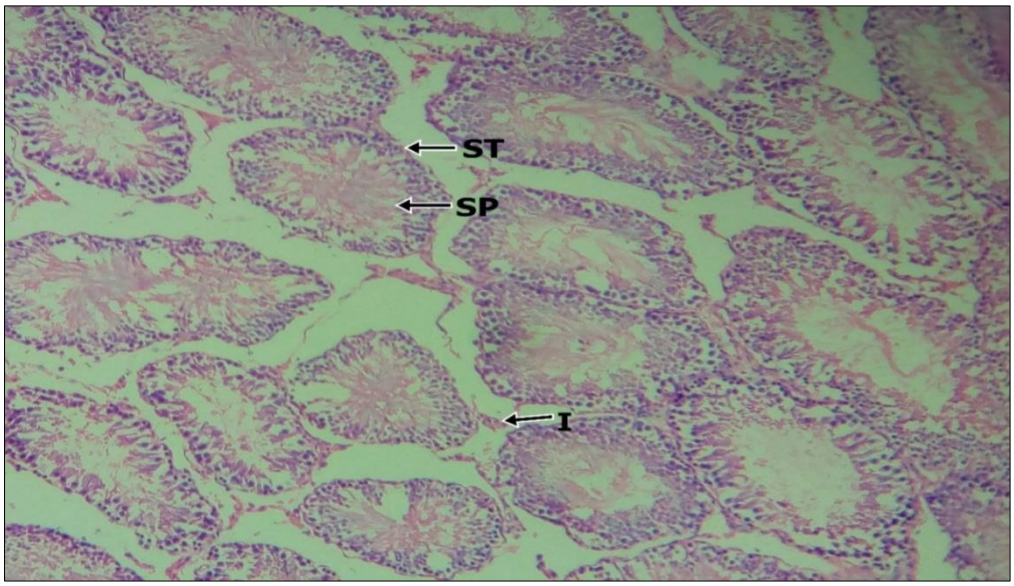
(Hematoxylin and eosin x 100); Key: SZ= spermatozoa; SPT = spermatid; BM = basement membrane; SP = spermatogonia; LE = Leydig cells; I = interstitium containing Leydig cells; ST = seminiferous tubules and SPC = spermatocyte

Figure 1 Section of testis of rats in the control group



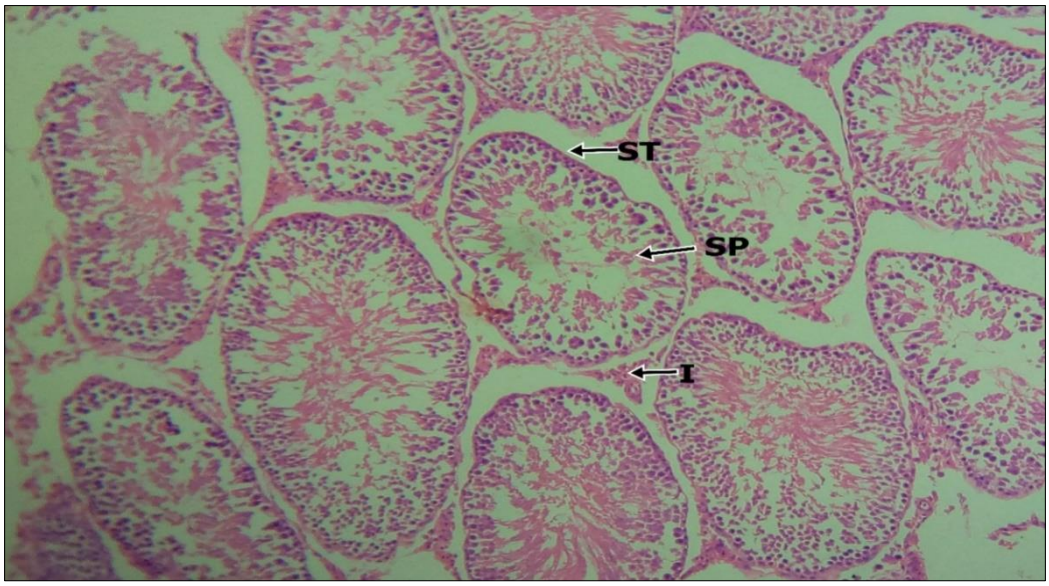
(Hematoxylin and eosin x 100); .Key: SZ= spermatozoa; SPT = spermatid; BM = basement membrane; SP = spermatogonia; LE = Leydig cells; I = interstitium containing Leydig cells; ST = seminiferous tubules and SPC = spermatocyte.

Figure 2 Section of testis showing the effect of CG on male albino rats



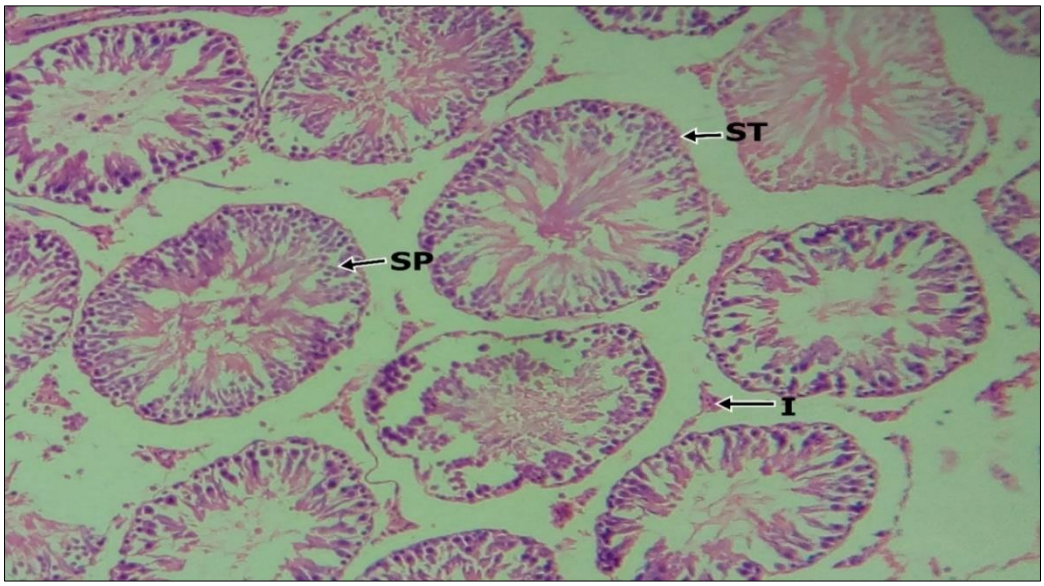
(Hematoxylin and eosin x 100); Key: SZ= spermatozoa; SPT = spermatid; BM = basement membrane; SP = spermatogonia; LE = Leydig cells; I = interstitium containing Leydig cells; ST = seminiferous tubules and SPC = spermatocyte.

Figure 3 Section of testis showing the effect of MX on male albino rats



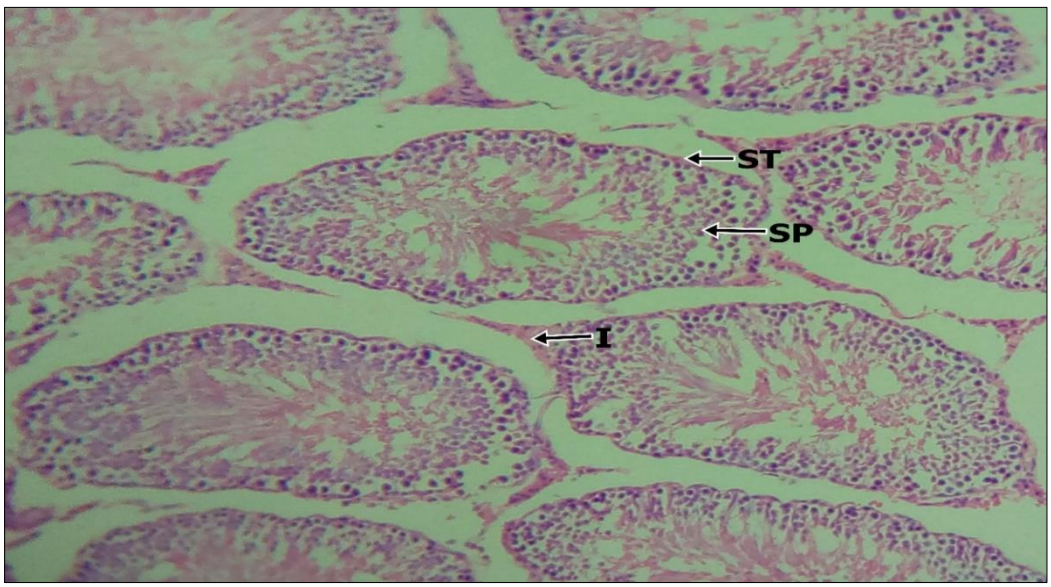
(Hematoxylin and eosin x 100); Key: SZ= spermatozoa; SPT = spermatid; BM = basement membrane; SP = spermatogonia; LE = Leydig cells; I = interstitium containing Leydig cells; ST = seminiferous tubules and SPC = spermatocyte.

Figure 4 Section of testis showing the effect of VC rats on male albino rats



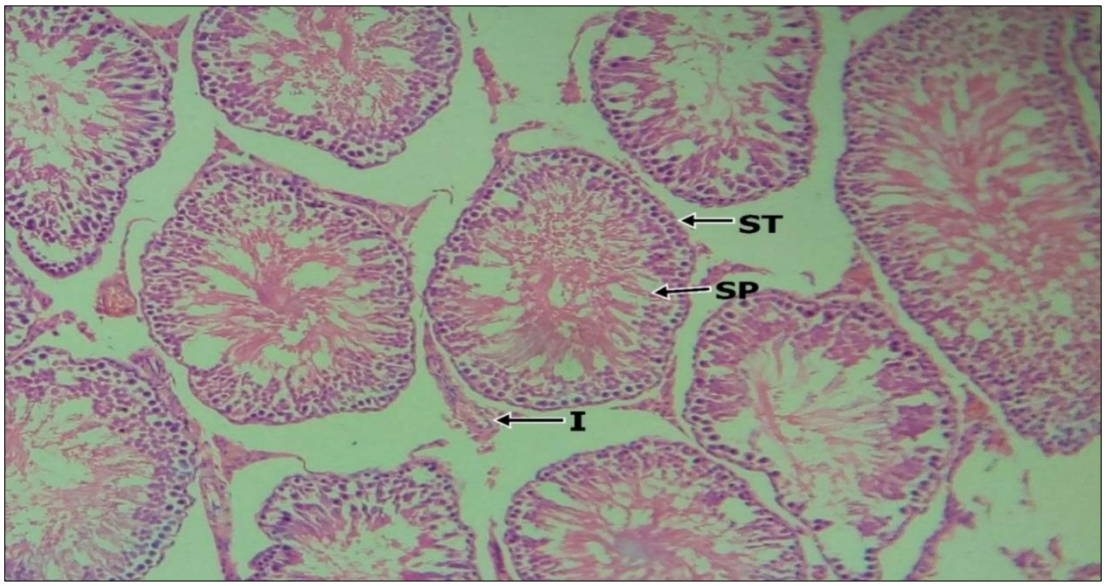
(Hematoxylin and eosin x 100); Key: SZ= spermatozoa; SPT = spermatid; BM = basement membrane; SP = spermatogonia; LE = Leydig cells; I = interstitium containing Leydig cells; ST = seminiferous tubules and SPC = spermatocyte.

Figure 5 Section of testis showing the effect of caffeine on male albino rats



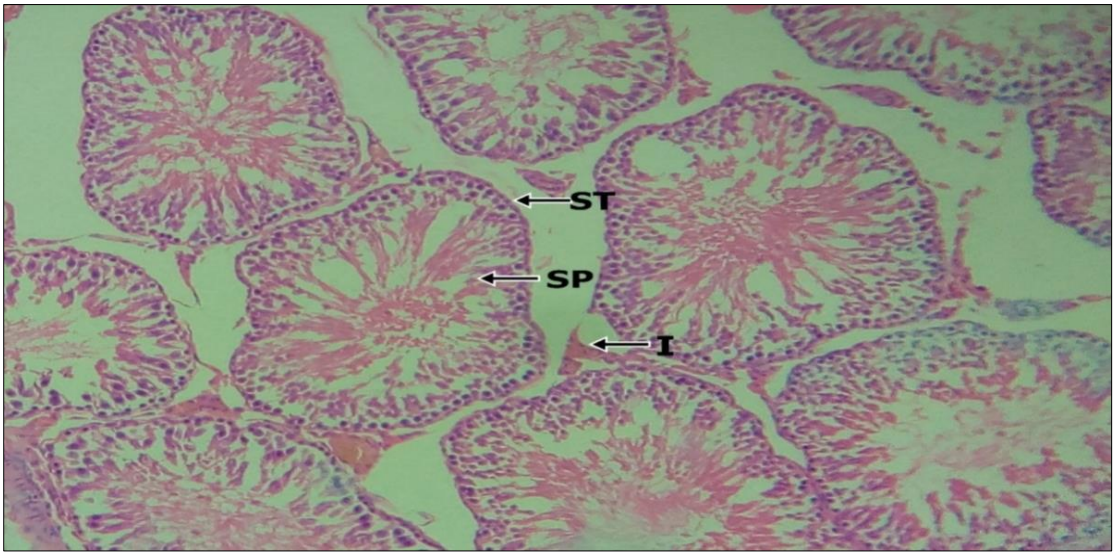
(Hematoxylin and eosin x 100); Key: SZ= spermatozoa; SPT = spermatid; BM = basement membrane; SP = spermatogonia; LE = Leydig cells; I = interstitium containing Leydig cells; ST = seminiferous tubules and SPC = spermatocyte.

Figure 6 Section of testis showing the effect of PU on male albino rats



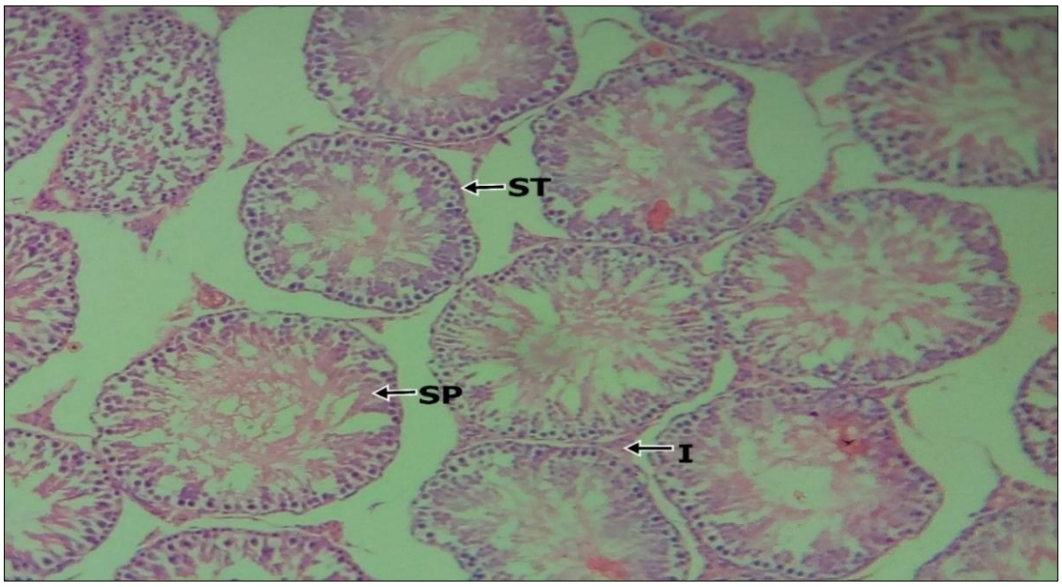
(Hematoxylin and eosin x 100); Key: SZ= spermatozoa; SPT = spermatid; BM = basement membrane; SP = spermatogonia; LE = Leydig cells; I = interstitium containing Leydig cells; ST = seminiferous tubules and SPC = spermatocyte.

Figure 7 Section of testis showing the attenuating effect of CG on caffeine-induced toxicity in male albino rats



(Hematoxylin and eosin x 100); Key: SZ= spermatozoa; SPT = spermatid; BM = basement membrane; SP = spermatogonia; LE = Leydig cells; I = interstitium containing Leydig cells; ST = seminiferous tubules and SPC = spermatocyte.

Figure 8 Section of testis showing the attenuating effect of MX on caffeine-induced toxicity in male albino rats



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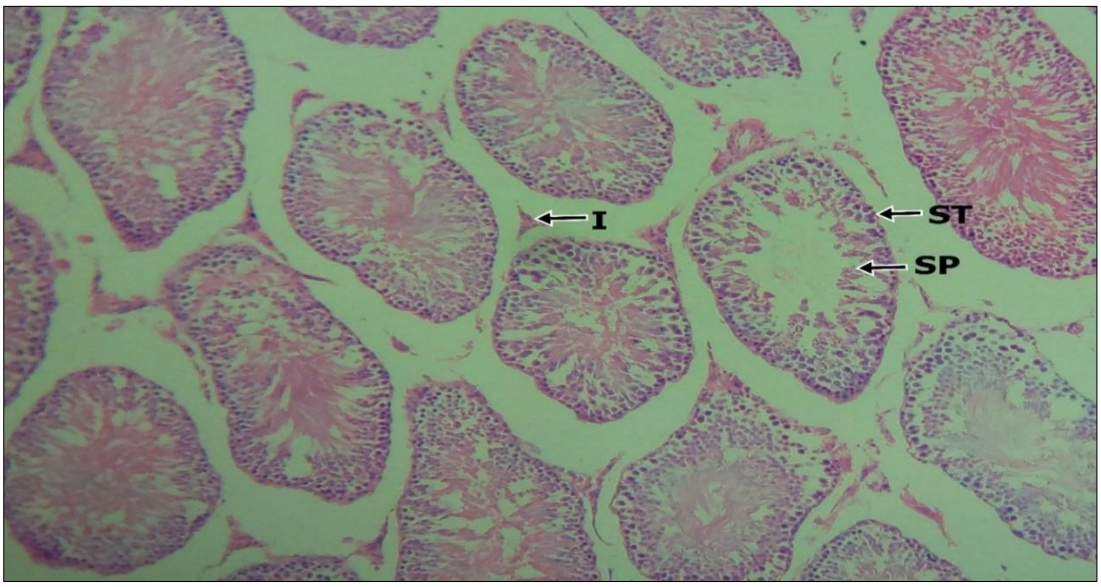
(Hematoxylin and eosin x 100);Key: SZ= spermatozoa; SPT = spermatid; BM = basement membrane; SP = spermatogonia; LE = Leydig cells; I = interstitium containing Leydig cells; ST = seminiferous tubules and SPC = spermatocyte.

Figure 9 Section of testis showing the attenuating effect of PU on caffeine-induced toxicity in male albino rats



(Hematoxylin and eosin x 100); Key: SZ= spermatozoa; SPT = spermatid; BM = basement membrane; SP = spermatogonia; LE = Leydig cells; I = interstitium containing Leydig cells; ST = seminiferous tubules and SPC = spermatocyte.

Figure 10 Section of testis showing the attenuating effect of VC on caffeine-induced toxicity in male albino rats

In contrast, the combination groups showed increased hormone levels compared to the caffeine group, indicating the attenuating effect of CG, MX, purslane, and VC. This suggests that the antioxidant properties of these substances may have protected and sustained steroidogenic enzymes and processes from ROS attacks and oxidative stress.

Histological examinations unveiled loosely packed seminiferous tubules with atrophic spermatogenic cells (Sertoli and Leydig cells) in the caffeine group, aligning with the findings of Bassey *et al*. [30], Mehran *et al*. [31] and Ekaluo *et al*. [32]. Sertoli cells play a crucial role in overall testes development, while Leydig cells are essential for testosterone production and spermatogenesis [33]. The atrophic impact of caffeine is likely attributed to caffeine-induced oxidative stress. This aligns with Zirkin and Chen [34], who reported that testicular oxidative stress can reduce testosterone production due to distortion on Sertoli and Leydig cells or other endocrine structures. This reduction leads to distorted spermatogenesis [35], potentially causing the significant decrease in testicular integrity observed in the caffeine group.

These findings are consistent with reports by Mruk and Cheng [36], and other studies linking increased ROS levels and oxidative stress with testicular toxicity and decreased sperm parameters [37].

However, histological sections of animals in the C+CG, C+MX, C+PU, and C+VC groups showed an absence of atrophy, indicating the potential of CG, MX, PU, and VC to attenuate the effects of caffeine-induced oxidative stress in testicular tissues. This may be attributed to their antioxidant properties [5,10,11], which prevented and/or reduced free radical oxidative damage by caffeine, as suggested by Thakker et al. [38].

5. Conclusion

This present study has revealed the toxic effects of caffeine on reproductive hormones and testicular integrity of male albino rats. However, the findings of this study also provided substantial evidence on the attenuating effects of CG MX, PU and VC on caffeine-induced hormonal and testicular toxicity in male rats as mammalian models.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest

Statement of ethical approval

Ethical approval was obtained for this study.

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